UNIVERSITY OF CALIFORNIA SAN DIEGO

Contributions of Motor Cortex and Thalamus on Striatal Activity and Performance During Motor Learning

A thesis submitted in partial satisfaction of the requirements
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in

Biology

by

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The Thesis of Sun Woo Hong is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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DEDICATIONS

This thesis is dedicated to my family and friends
for their love and support throughout this experience.

This thesis is dedicated to Joshua Roldan
for introducing me to neuroscience and
sharing his love for research with me.

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LIST OF ABBREVIATIONS

Cre- Cre recombinase

D1R- Dopamine-1-Receptor

D2R- Dopamine-2-Receptor

DLS- Dorsolateral striatum

GRIN- Gradient Refractive INdex

ITI- Inter-trial interval

M1- Primary motor cortex

M2- Secondary motor cortex

MSN- Medium spiny neuron

PF- Parafascicular nucleus of the thalamus

ROI- Region of Interest

STN- Subthalamic nucleus

STR- Striatum

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ACKNOWLEDGEMENTS

I would like to acknowledge Takaki Komiyama, the committee chair for my thesis defense, for providing the opportunities and resources that were necessary for the completion of this project.

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This thesis was coauthored with Gjoni, Enida. The thesis author was the primary author of the material.

ABSTRACT OF THE THESIS

Contributions of Motor Cortex and Thalamus on Striatal Activity and Performance During Motor Learning

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Movement is fundamentally essential for our survival in an ever-changing environment. Movement is controlled by the coordinated activity of several interconnected brain regions. Motor regions of the cortex, basal ganglia and thalamus form a major circuit involved in

movement control. The striatum, the input nucleus of the basal ganglia, receives and integrates major excitatory inputs from the motor cortex and the thalamus. I aim to understand how striatal neuronal activity is influenced by these upstream inputs during a motor task that requires training, and whether one of the two inputs has a greater influence on striatal activity and potentially, on movement execution. I combined two-photon calcium imaging of striatal activity via GRIN lens with pharmacological inhibition of the activity in primary (M1) and secondary motor cortex (M2) and the parafascicular nucleus (PF), at early, middle and late stages of motor learning. After M2 inactivation, striatal activity was not significantly altered in beginners, but it was decreased or abolished in expert and master mice. After PF inactivation, striatal activity was absent across all learning stages. In preliminary experiments, M1 inactivation led to complete striatal activity abolishment during early learning. Strong performance deficits were observed after PF inactivation at all stages of learning. M1 inactivation led to significant alterations in beginners' but not in experts' performance. M2 inactivation led to no significant alterations. These results give insight about the contribution of upstream inputs to striatal activity during motor learning and uncover the role of striatum in the execution of a learned task.

Introduction

Movement is a fundamental response to our countless interactions with the environment. All behaviors, ranging from as simple as walking to as complex as playing the piano, can be decomposed into sequences of elementary motor actions. Action selection and planning of action initiation, execution, and termination are examples of movement control enacted by the brain to produce temporally and spatially correct sequences of behavior (Arber & Costa 2018). However, not much is known about how the brain controls movement nor how movement disorders arise from deficits in the brain. As a result, determining how the brain achieves motor control to generate coordinated movements is essential for understanding the basis of behavior and movement disorders.

Basal ganglia, cerebral cortex, and the thalamus are brain regions that are functionally connected in a major neural circuit that is known to control movement (Arber & Costa 2018, Wall et al. 2013). The thalamus and cerebral cortex send excitatory glutamatergic input to the striatum, otherwise known as the main input nucleus of the basal ganglia (Wall et al. 2013; Huerta-Ocampo 2014). The striatum then integrates and projects this information via the direct and indirect projection pathways to basal ganglia output nuclei which influence movement (Klaus et al. 2019) The direct and indirect pathways are innervated by two types of medium spiny neurons (MSNs) in the striatum: Dopamine 1 Receptor-expressing (D1R) MSNs project via the direct pathway onto the substantia nigra pars reticulata (SNr), while Dopamine 2 Receptor-expressing (D2R) MSNs project through multiple brain regions via the indirect pathway onto the same SNr output nucleus (Klaus et al. 2019). Here, we examined the dorsolateral striatum (DLS), because it has been found to be essential for learning motor

sequences as well as performing habitual behaviors which are useful for understanding how multiple actions can be seamlessly arranged (Yin & Knowlton 2006; Wolff et al 2019).

The striatum's control over movement is thought to have contrasting functions, carried out by the D1R and D2R MSNs. D1R MSNs are thought to have a permissive role in movement control because activation of D1R MSNs leads to an increase in movement (Kravitz et al. 2010). In contrast, D2R MSNs may play a role in terminating movement, because activation of D2R MSNs during action initiation leads to a decrease in movement or a switch in behaviors, which requires the cessation of the current behavior before starting a different one (Kravitz et al. 2010; Tecuapetla et al. 2016; Klaus 2019).

However, recent studies have shown that striatal MSNs of both direct and indirect pathways are simultaneously active during movement (Jin et al. 2014; Tecuapetla et al. 2014). Despite the simultaneous activity of D1R and D2R MSNs, their activity patterns - the collective firing of action potentials by specific neurons - are not always similar. It has been found that patterns of activity among D1R and D2R MSNs both increase during movement initiation but differ during movement execution (Jin et al. 2014; Tecuapetla et al. 2014). The differences in activation patterns between the two MSN types indicate that specific ensembles of active D1R and D2R MSNs as well as the correct timing of their activity is required for a precise action to be generated.

Consequently, it is essential to determine how input from different brain regions to the striatum affects striatal activity, specifically the activity pattern of D1R and D2R-MSNs, and consequently, movement itself. Previous research has shown that the cerebral cortex and the thalamus send major input to the striatum (Mandelbaum et al. 2019; Wall et al. 2013). Of the many nuclei in the thalamus, the parafascicular nucleus (PF) appears to most strongly innervate

the dorsolateral striatum (Wall et al. 2013; Mandelbaum et al. 2019). PF has also been shown to be necessary for task execution as well as play a role in action initiation (Díaz-Hernández et al., 2018; Watson et al. 2021). We decided to examine the role of the secondary motor cortex (M2) input onto striatum, because it has been found to be necessary for the execution of learned movement sequences (Makino et al. 2017). Alongside PF and M2, the role of M1 input on the DLS and motor control is also of interest, as a recent study from the Komiyama lab and others suggest that M1 contribution to skilled movements decreases when animals are trained for long periods of time (Hwang et al., 2019; Kawai et al., 2015). The differing influences of M1 and M2 on movement appear to be dependent on learning, which must be studied further to better understand the role of the motor cortex on the basal ganglia-cortex-thalamus neural circuit of motor control.

In this project, I aimed to investigate the functional role of cortical and thalamic inputs on striatal activity during different stages of learning a motor task - ranging from beginner, expert, to master levels of motor performance, and determine whether one input prevails in driving activity in the DLS. I conducted pharmacological inactivation experiments, in which either PF, M1, or M2 would be inactivated during mice performance of a motor task. To inactivate an input region, we injected muscimol, a drug that inhibits neural activity by activating inhibitory GABA receptors (Johnston et al. 1968), into either PF, M1 or M2. The resulting changes in DLS activity as well as in behavioral performance would demonstrate the degree of influence each input has on striatal activity and motor control.

Methods

All procedures were compliant with protocols approved by the University of California, San Diego International Animal Care and Use Committee and guidelines of the National Institute of Health. Mice were housed in cages with standard bedding in a room with a reversed light cycle (12 h-12 h). Experiments were performed during the night cycle. Transgenic D1R-Cre or A2a-Cre mice with genotypes (B6.FVB(Cg)-Tg(Adora2a-cre)KG139Gsat/Mmucd [MMRRC 36158], B6.FVB(Cg)-Tg(Drd1-cre)FK150Gsat/Mmucd [MMRRC 36916]) were used for the experiments.

Behavioral Training

Mice were trained on two behavioral paradigms: a ladder and an wheel task. In the ladder paradigm, head-fixed mice learned to run on a motorized ladder in the shape of a wheel. Each trial began with an auditory cue, followed by 8 seconds of ladder rotation, with a 6-8 second inter-trial-interval. Mice were required to learn how to position their forelimbs and hindlimbs specifically on the ladder rungs without falling between them. In the wheel paradigm, head-fixed mice ran freely on a wheel with a continuous surface. The mice could start and stop by choice and move for as long as they desired, although an airpuff was included to motivate them to initiate movements. Mice were trained daily on two sessions each of ladder and wheel tasks three to four weeks after surgeries. Mice in the beginner stage of learning were trained for 4-5 days before imaging sessions. Mice in the expert stage of learning were trained for 8-12 days before imaging sessions. Mice in the master stage of learning were trained for 6-8 weeks before imaging sessions.

Surgical Procedures: Headplate Implantation

Mice were anesthetized with isofluorane before and during the surgery. Betadine was used to disinfect the scalp. The skull was leveled on all planes with stereotaxic manipulation and

then marked with coordinates for bregma (the intersection between the coronal suture and midline), the dorsolateral striatum (0.5 mm anterior and 2.2mm lateral of bregma), the primary motor cortex (1.0 mm anterior and 1.5 mm lateral of bregma), secondary motor cortex (2.3 mm anterior and 1.2 mm lateral of bregma), and parafascicular nucleus (2.2 mm posterior and 0.85 mm lateral of bregma). A headplate was fixed on top of the skull with glue and dental cement. Following the surgery, mice were injected subcutaneously with buprenorphine and baytril to prevent pain and inflammation or infection. The mice were monitored until they were awake and freely moving.

GRIN Lens Implantation into Dorsolateral Striatum

For the purposes of imaging deep brain structures, a Gradient Index (GRIN) lens with a diameter of 0.5mm was implanted above the dorsolateral striatum. A waiting period of three to four weeks was necessary before imaging DLS neurons with a two-photon microscope.

GCAMP6f Viral Injection into Dorsolateral Striatum

A glass pipette loaded with AAV1.Syn.FLEX.GCaMP6f.WPRE.SV40 was unilaterally injected in the right hemisphere of the mice brains at a rate of 20 nL per minute. The pipette was kept in the brain for 15 minutes after injection to prevent leakage of the virus.

Muscimol Injection into Secondary Motor Cortex or Parafascicular Nucleus

After baseline neural activity of the DLS had been recorded with a two-photon microscope, inactivation experiments with muscimol were performed on mice. Muscimol hydrobromide (5 µg/µL, Sigma) was injected, 25 nL each at depths of 350 µm for M1 and M2, depths of 3,300 µm for PF unilaterally into the right hemisphere, in anesthetized mice. Following the injection, mice recovered from the anesthesia and the same field of view of DLS was imaged, starting 30 minutes after the injection.

Two-photon calcium imaging

Imaging of neural activity was recorded using a commercial two-photon microscope (MOM, Sutter Instrument, retrofitted with a resonant galvanometer-based scanning system from Thorlabs) with a 16x objective (Nikon) and 925 nm excitation light (Ti-Sapphire laser, NewPort) alongside ScanImage (Vidrio Technologies). The objective was submerged in double DI water above the GRIN lens. Field of views with neurons were randomly chosen between 150-350 um from the bottom of the GRIN lens. Imaging rate was at ~30Hz. Mice were imaged for 15 minutes (around 50 trials) for each task of ladder and wheel twice, before and after muscimol injection. Frames from the neural recordings were synchronized with Ephus recordings of behavioral performance.

Data Analysis

A custom MATLAB program was used to align images of fluorescent neurons in each frame of the recorded field of views and to remove motion artifacts (Mitani, 2018). Regions of interest (ROI) were manually drawn to mark the cell bodies determined by the intensity of GCaMP6f fluorescence which could be distinguished from the background. Pixels within 90% of the boundary of the ROI were considered a single neuronal cell body, while pixels extending away from the boundary by 10% were considered the background. dF/F traces were created for each cell within its ROI and analyzed. Neurons identified by ROI boundaries with baseline fluorescent intensities greater than a threshold of 200 a.u. for GCaMP channel were considered for analysis. Another criterion for determining which neurons were active, and would then be analyzed, was to select neurons which had at least two calcium event transients per minute. Neurons without fluorescent calcium transients were not included in this analysis.

DeepLabCut was used to train a deep learning algorithm to detect the position of the four paws of mice during the ladder task. Movement was defined as the change in position of at least two paws between two frames of the imaging session above two standard deviations of the averaged change in movement during baseline. Baseline movement was defined as the averaged change in movement of each paw between subsequent frames for at least 50 frames when the mouse was inactive during the imaging session. From consecutive frames, we selected frames that met either of two conditions: frames with a likelihood of the algorithm correctly identifying the position of the limb being greater than or equal to a threshold value in which outliers were excluded or frames with paw positions that fell between a percentile of the paw position distribution that also removed outliers. These selected frames were considered for analysis. From these frames, we selected frames in which the distance of the paw traveled between two frames was greater than two standard deviations above the average baseline distance between frames of inactivity. We then graphed cumulative histograms for the distances traveled by each paw and pair of forepaw-hindpaw. We also graphed boxplots to examine the distributions of the distances traveled by each paw and pair of forelimbs and hindlimbs.

Histology

Mice were transcardially perfused with 4% PFA and the brains were kept in 4% PFA overnight. The next day, brains were placed in a PBS solution with 30% sucrose for 48-72 hrs. Dry ice and ethanol were used to cool down the microtome (Microm HM 430) platform at ~ -20 degrees Celsius, where the brains were embedded in a cryo-protective fixative O.C.T. Compound Embedding Medium. Brain sections of 40 μm thickness were collected in PBS. The slices were then mounted onto a glass slide, set to dry, and mounting medium was then applied to the slices on the slide before applying a cover slip. The slices were imaged using the wide-field fluorescent

microscopes ApoTome2, Zeiss and Axio Zoom V16, Zeiss, to acquire GCamP6f fluorescent signals. The resulting images of the slices were then saved as TIFF files and edited with the program FIJI (ImageJ).

Results

2-photon imaging of striatal activity and motor performance changes during motor learning

To examine the effects of inactivating an input region on striatal activity, we injected cre-dependent GCaMP6f in DLS neurons of D1R-cre or A2A-cre mice. A GRIN lens was implanted above the DLS to image the calcium activity of these neurons in vivo during imaging sessions. The position of the GRIN lens to the DLS was confirmed by histology slices in which the targeted DLS neurons fluoresced due to the targeted expression of GCaMP6f below the hole left from the lens implantation (Figure 1a). The precise location of the DLS was confirmed using Paxinos Brain Slices (Paxinos & Franklin, 2004). (Figure 1b). Images of DLS activity were successfully acquired in vivo with the two-photon microscope and GRIN lens (Figure 1c).

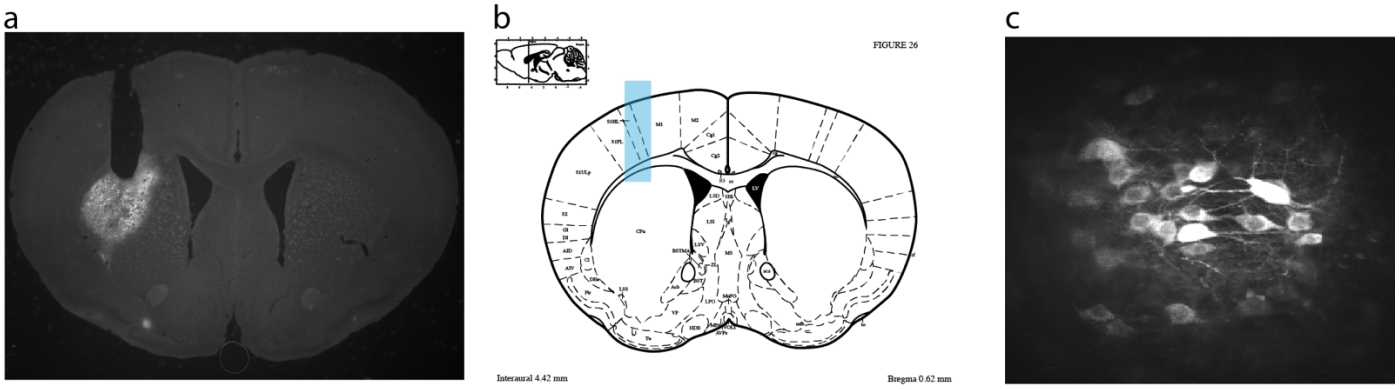

Figure 1. Two-photon calcium imaging of DLS neuronal activity via GRIN lens. a) Histology slice with GCaMP6f fluorescence in DLS and GRIN lens tract directly above it. b) Corresponding brain slice from Paxinos Brain Slices to histology image. c) Max. intensity projection image from two-photon calcium imaging of GCaMP6f-expressing DLS neurons.

Alongside striatal activity imaging, mice behavioral performance was examined. Mice were trained to run on a motorized ladder in a task consisting of a 6-8 second ITI, followed by a a sound cue and after 1 second, finally an 8 second running task on a motorized ladder (Figure 2a). The position of all paws was tracked during each imaging session by a trained deep learning algorithm in DeepLabCut (Figure 2b and c).

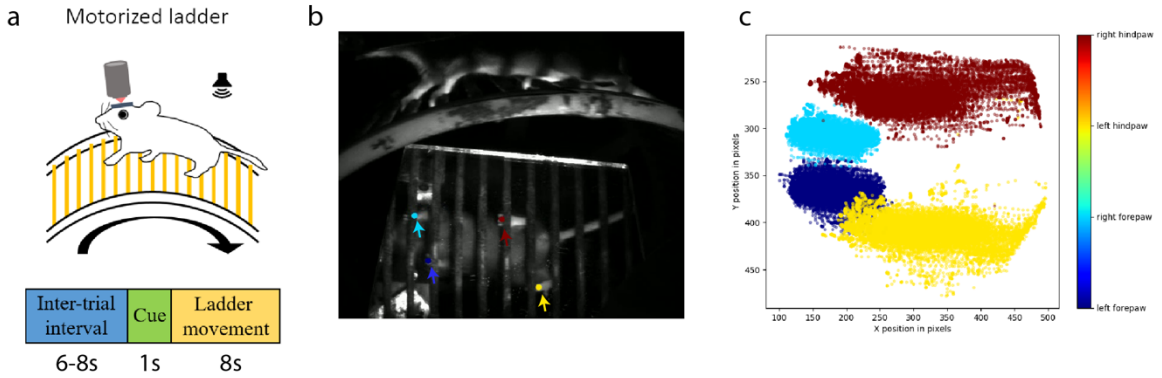

Figure 2. Paw positions labeling during the performance of a ladder task. a) Mice were trained to run on a motorized ladder. b) Still image of a video frame in which all four paw positions were automatically labeled by DeepLabCut. c) All paw positions during one video session.

We first confirmed that mice change their motor performance as they undergo training by generating cumulative histograms of the positions of each paw and pair of forepaw-hindpaw. We observed a pattern in which the distance traveled by individual paws increased from beginner, expert, to master mice (Figure 3), which suggests that mice increase their movement speed as learning progressed. From these results, we interpreted the increase in distance traveled by each paw as an improvement in behavioral performance as training progressed, as beginner mice would travel smaller distances due to inaccurate positioning of paws on the ladder rungs.

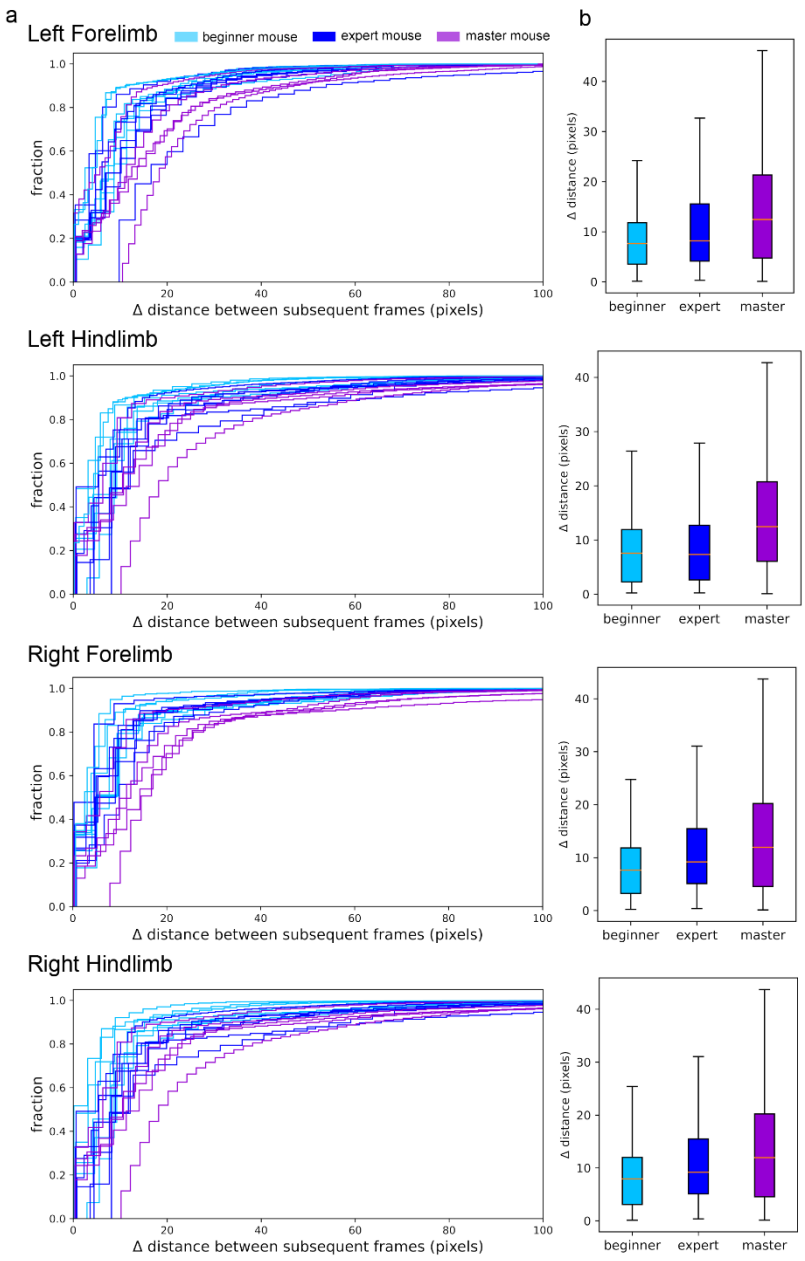

Figure 3. Behavioral performance of mice improved in relation to the training length. a) Cumulative histograms of the distance traveled by each limb during the first imaging session of beginner, expert, and master mice. b) Boxplots of the distance traveled by each limb during the first imaging sessions of beginner, expert, and master mice. Red line indicates median of the distributions.

On the other hand, we observed an inverse pattern for the distance between forelimb and hindlimb pairs, in which the distance between the limb pairings decreased as training progressed. (Figure 4). As mice begin their training, they generally show low coordination between forelimbs and hindlimbs, and they tend to drag their hindlimbs. This observation is reflected in

the bigger forepaw-hindpaw distances in beginners. As mice become better in task performance, they engage all limbs on the motorized ladder. This is reflected in a decrease in the forepaw-hindpaw distances in expert and master mice. With confirmation that training helps mice to learn how to perform the behavioral task, we continued our manipulation experiment to probe the influence of M2, PF, and M1 inputs on DLS activity and behavioral performance.

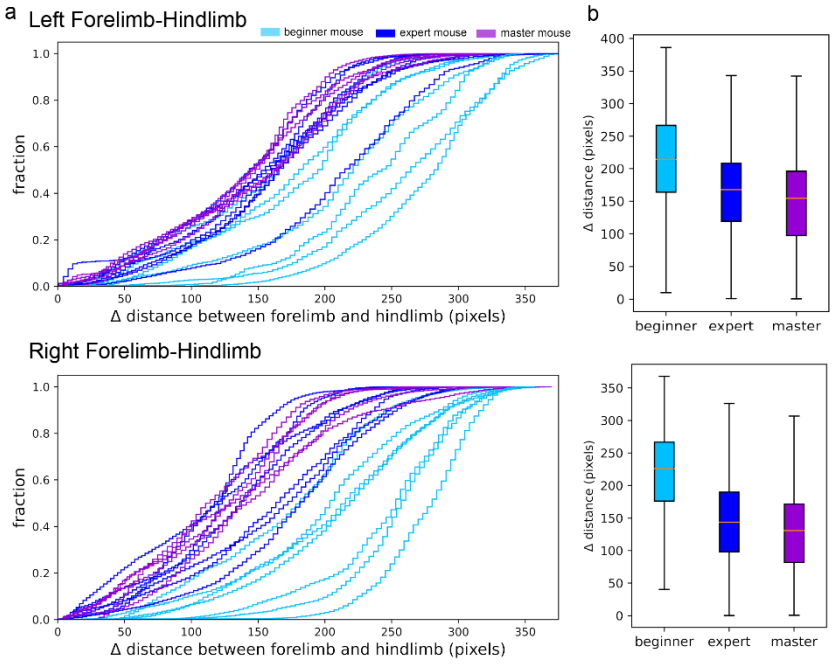

Figure 4. Distances between forepaw and hindpaw decrease with training. a) Cumulative histograms of the distance between forepaw and hindpaw for beginner, expert, and master mice. b) Boxplots of the distances between forelimb and hindlimbs for beginner, expert, and master mice. Red line indicates median of the distributions.

PF Maintains a Strong and Lasting Influence on DLS Activity and Movement Throughout Learning

First a field of view was chosen and the activity of DLS neurons was imaged as mice performed a ladder and a wheel task. The mice were then injected with muscimol in PF. Thirty minutes after surgery, the same field of view was imaged again, during the performance of the

motor tasks. In all mice of all stages of learning (n=11), – beginner (n=3), expert (n=5), and master (n=3) – DLS activity was abolished after inactivation of PF (Figure 5a, b ,c). These results suggest that PF input is largely influential on striatal activity across all stages of learning.

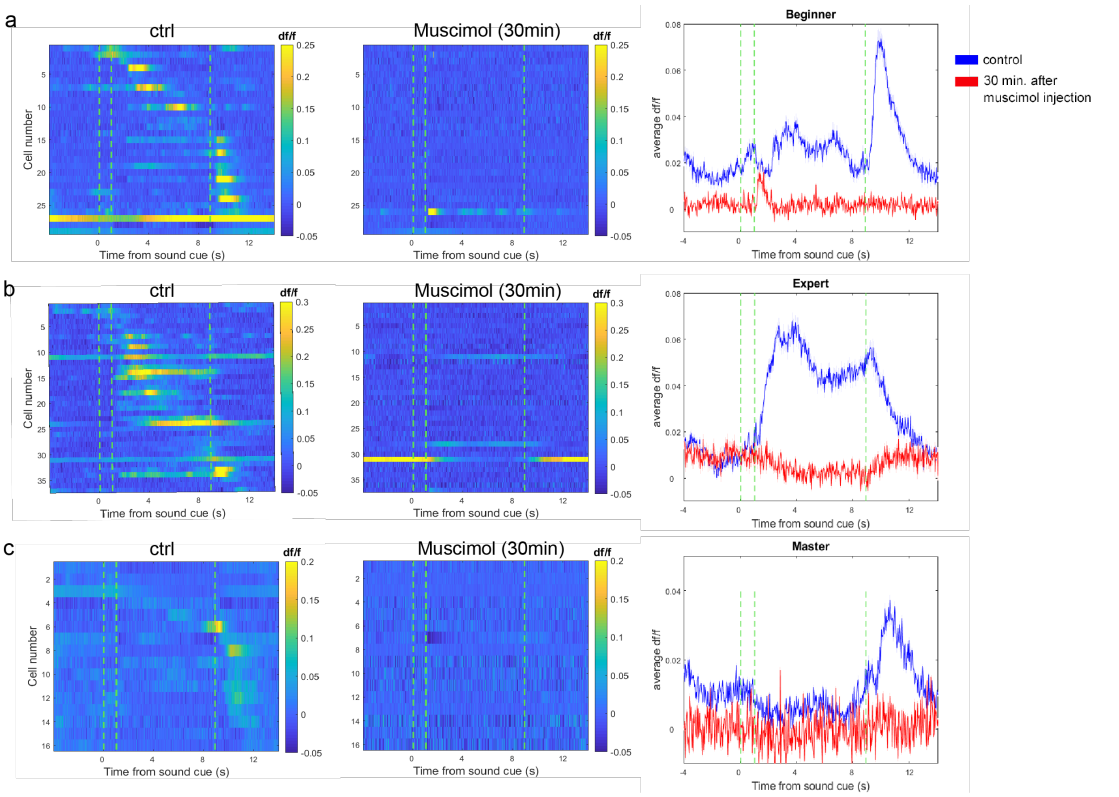

Figure 5. Inactivation of PF abolishes DLS activity. a) DLS neuron activity patterns before and 30 minutes after PF inactivation in beginner mice, b) in expert mice, and c) in master mice. Each row represents the averaged activity across trials for each neuron. On the right, DLS neuron activity averaged across all neurons is plotted.

The behavioral performance of the mice was also recorded on video during imaging sessions before and after inactivation. After inactivation of PF, we found a decrease in distance covered by each individual paw and an increase in distance between forelimb and hindlimbs as compared to before inactivation (Figure 6a-c). We interpreted the decrease in distance traveled by each paw as a decrease in movement speed. We also interpreted the increase in distance between each forepaw-hindpaw pair (Figure 6d-f) as evidence of behavioral deficits, as the distance between forelimb-hindlimbs should decrease when the mice are running, but increase

when the mice are dragging their hindlimbs, as observed in beginner mice when they first learn the motor task. The combined results of DLS activity and behavioral performance in mice after PF inactivation indicate that PF input is strong and stable across all stages of learning, as DLS activity was abolished and strong behavioral impairments were found between beginner, expert, and master levels of trained mice after PF inactivation.

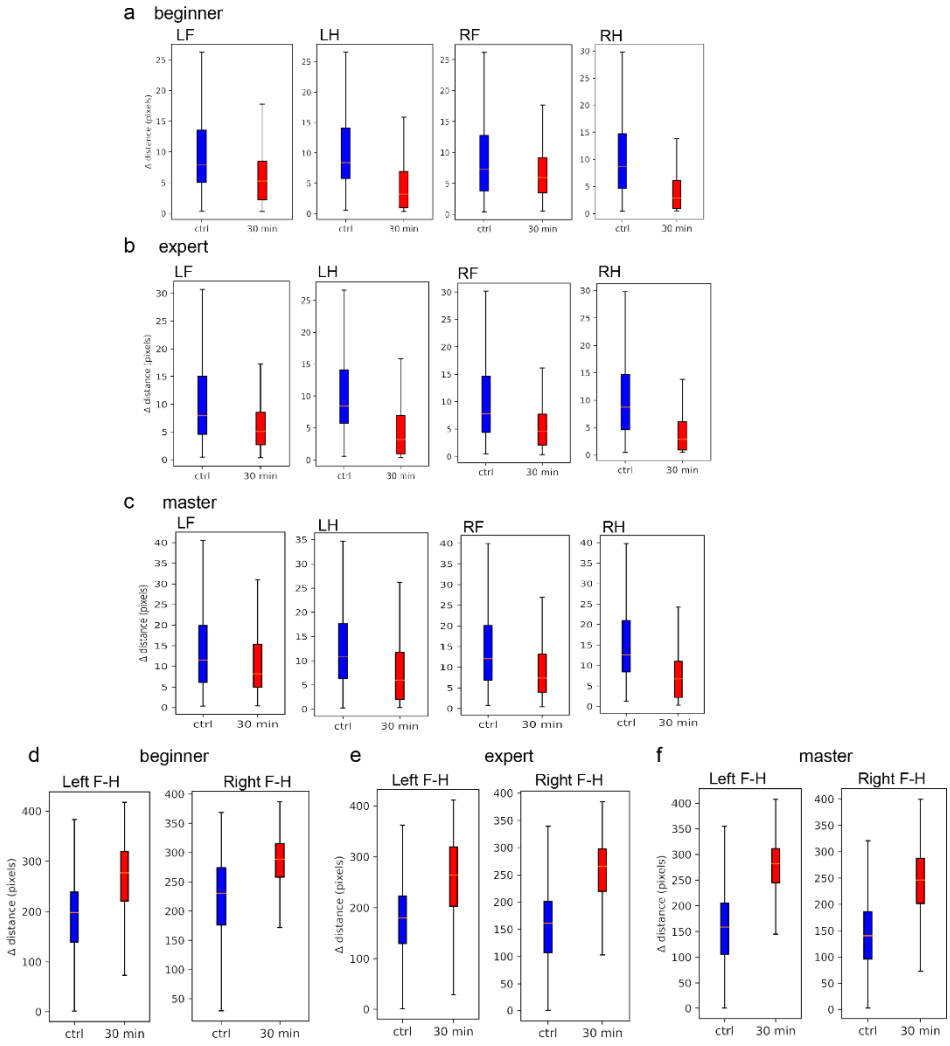

Figure 6. Motor Performance after PF inactivation at different stages of training. Distribution of changes in paw position for LF (left forelimb), LH (left hindlimb), RF (right forelimb), and RH (right hindlimb) before and 30 minutes after PF inactivation in a) beginner, b) expert, and c) master mice. Distribution of distances between left and right F-H (forelimb-hindlimb) pairs before and 30 minutes after PF inactivation in d) beginner, e) expert, and f) master mice.

M2 Influence on DLS Activity and Movement Increases As Learning Progresses

Similar experimental paradigms were used to inactive M2 neurons and simultaneously image DLS activity while recording behavioral performance of mice on video. Inactivation of M2 neurons led to little to no effect on DLS activity of beginner mice (n=4), a decrease in DLS activity in some of the expert mice (n=7), and a complete abolishment of DLS activity in all master mice (n=5) (Figure 7a, b, c). From these results, we observed a pattern in which inactivation of M2 has a greater influence on DLS activity as the stage of learning increases, indicating that M2 plays a conditional and increasing role in influencing movement after it has been learned, rather than when learning has begun. We imaged DLS activity 1 hour after muscimol injection, though somewhat blunted by the presumed spread of muscimol to other brain regions. In beginner mice, we found DLS neurons to still be active after inactivation, though less, as compared to controls. The expert mice were once more imaged, 1 hour post injection, and at this point, DLS activity had completely disappeared.

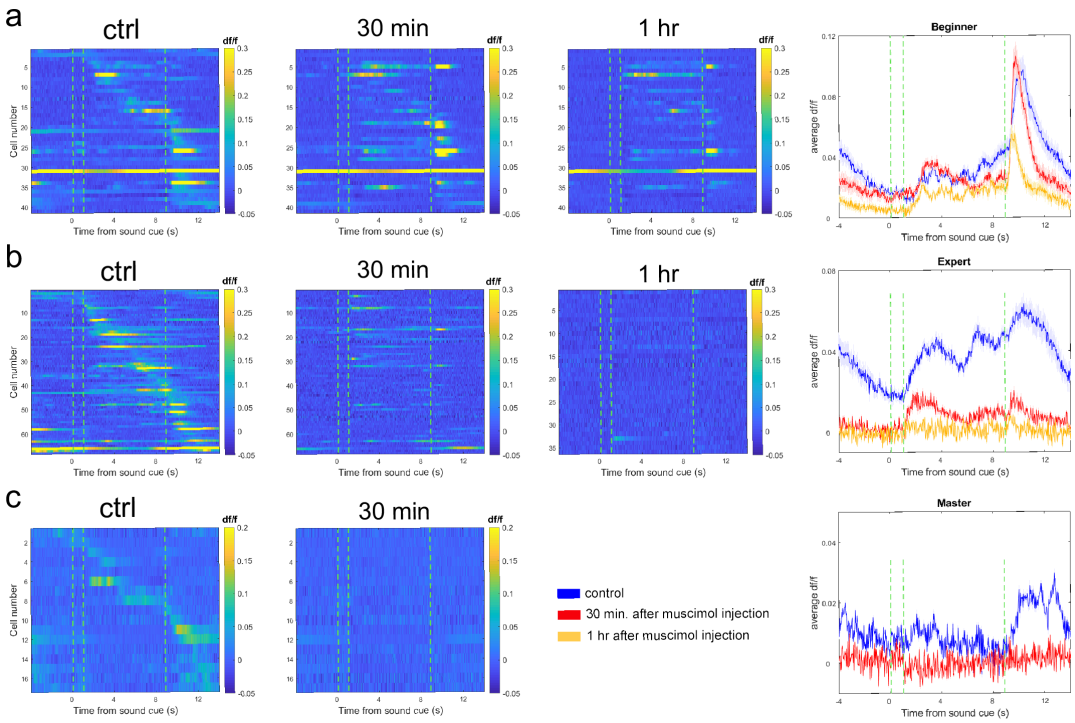

Figure 7. Increasing Influence of M2 on DLS as Learning Progresses. DLS activity patterns before, 30 minutes, and 1 hour after M2 inactivation in a) beginner mice, b) expert mice, and c) master mice.

Alongside the activity patterns in DLS neurons, our analysis of behavioral performance similarly shows a variable change in performance across the three stages of learning. In beginner mice, we found the distance traveled by each limb to increase (Figure 8a-c) and the distance between the forelimb and hindlimbs to decrease after M2 inactivation (Figure 8d-e). We interpreted the increase in distance traveled by each individual limb as an increase in speed, indicating an improvement of behavioral performance. We also interpreted the decrease in distance between forelimb and hindlimbs as an improvement in performance as well, because this distance would be smaller when the mice are successfully running during the motor task rather than dragging their hindlimbs when they first learn the task. Inactivating M2 in beginner or expert mice did not seem to lead to any deficits in DLS activity after 30 minutes, so we imaged again, 1 hour post-injection. Surprisingly, even in this case, we also observed an trend in increase in distance traveled by each paw and a slight decrease or no obvious change in distance between forelimb and hindlimb pairs (Figure 8, orange boxplots). Interestingly, inactivating M2 in master mice led to a stark decline in DLS activity but mice continued to move without impairments. These findings may hint at the possibility of movement to be performed without the DLS, but only in late stages of learning. Nevertheless, across all stages of learning, we find that M2 influence increases with learning, meaning M2 input may not be influential when learning begins, but could potentially play a larger role in motor control after learning has occurred.

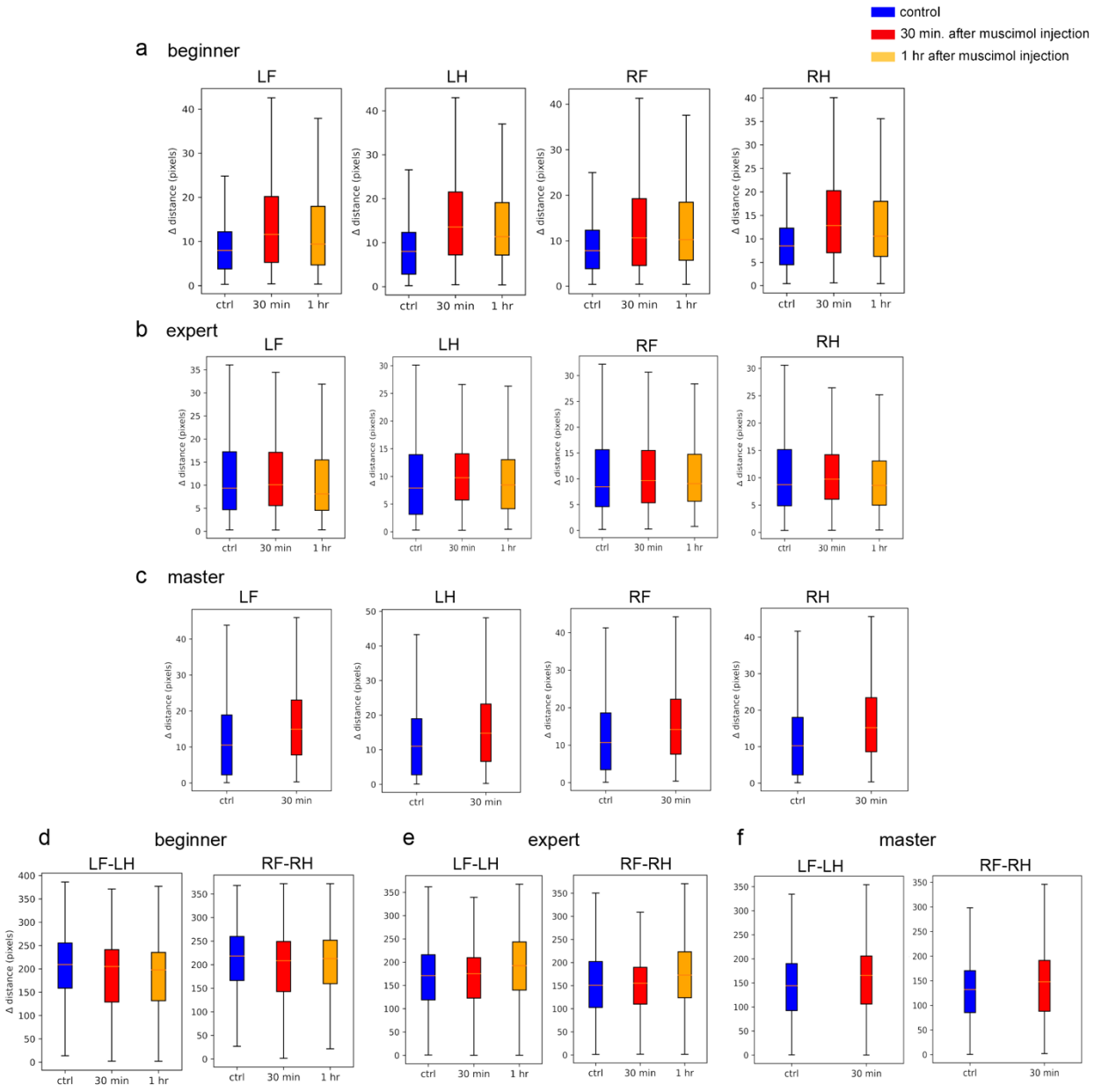

Figure 8. Motor Performance after M2 inactivation at different stages of training. a) Distribution of changes in limb positions for LF (left forelimb), LH (left hindlimb), RF (right forelimb), and RH (right hindlimb) before, 30 minutes, and 1 hour after M2 inactivation in beginner mice, b) in expert mice, and c) in master mice. d) Distribution of distances between LF-LH (left forelimb and hindlimb) and between RF-RH (right forelimb and hindlimb) before, 30 minutes, and 1 hour after inactivation in beginner, expert, and master mice.

Strong Influence of M1 on DLS Activity and Movement At the Start of Learning

The experimental paradigm involving inactivation of an input region paired with imaging of DLS activity and quantification of behavioral performance from video recordings was again

repeated for M1 neurons. We found in beginner mice (n=3), that M1 inactivation leads to abolishment in DLS activity (Figure 9a), similar to the results of inactivating PF in beginner mice. On the other hand, when M1 inactivation was performed in expert mice (n=2), most of DLS neurons became inactive, although a fraction of them still showed activity (Figure 9b). When the same neurons were imaged after 1 hour, no activity was left (not shown).

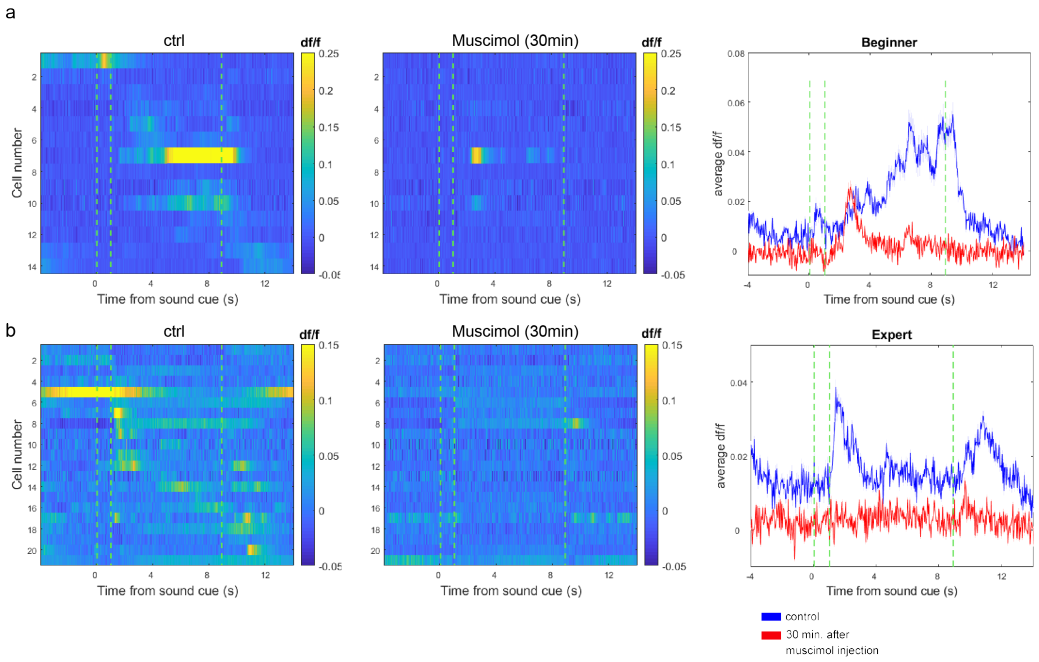

Figure 9. Influence of M1 on DLS activity in beginner and expert mice. a) DLS activity patterns before and 30 minutes after M1 inactivation in beginner mice. b) same as in a), but for expert mice.

Similar to PF inactivation, M1 inactivation leads to decreased distance traveled by each paw and an increase in distance between forelimb and hindlimbs, in beginner mice (Figure 10a). This suggests that M1 has an important role in influencing striatal activity at the early stages of learning, and this might influence the motor performance of the mice, as well. On the other side, in preliminary results, the performance of expert mice did not seem to be obviously affected by M1 inactivation and consequent DLS activity abolishment (Figure 10b), although there seems to be an increase in the distance traveled by each paw and in the distance between forelimbs and hindlimbs as well (Figure 10c, right). These preliminary results need to be confirmed by

additional experiments. As already mentioned in the case of M2 inactivation in expert and master mice, this might uncover a decreasing role of DLS in the execution of the ladder task, once it has been learned. Further studies need to confirm this.

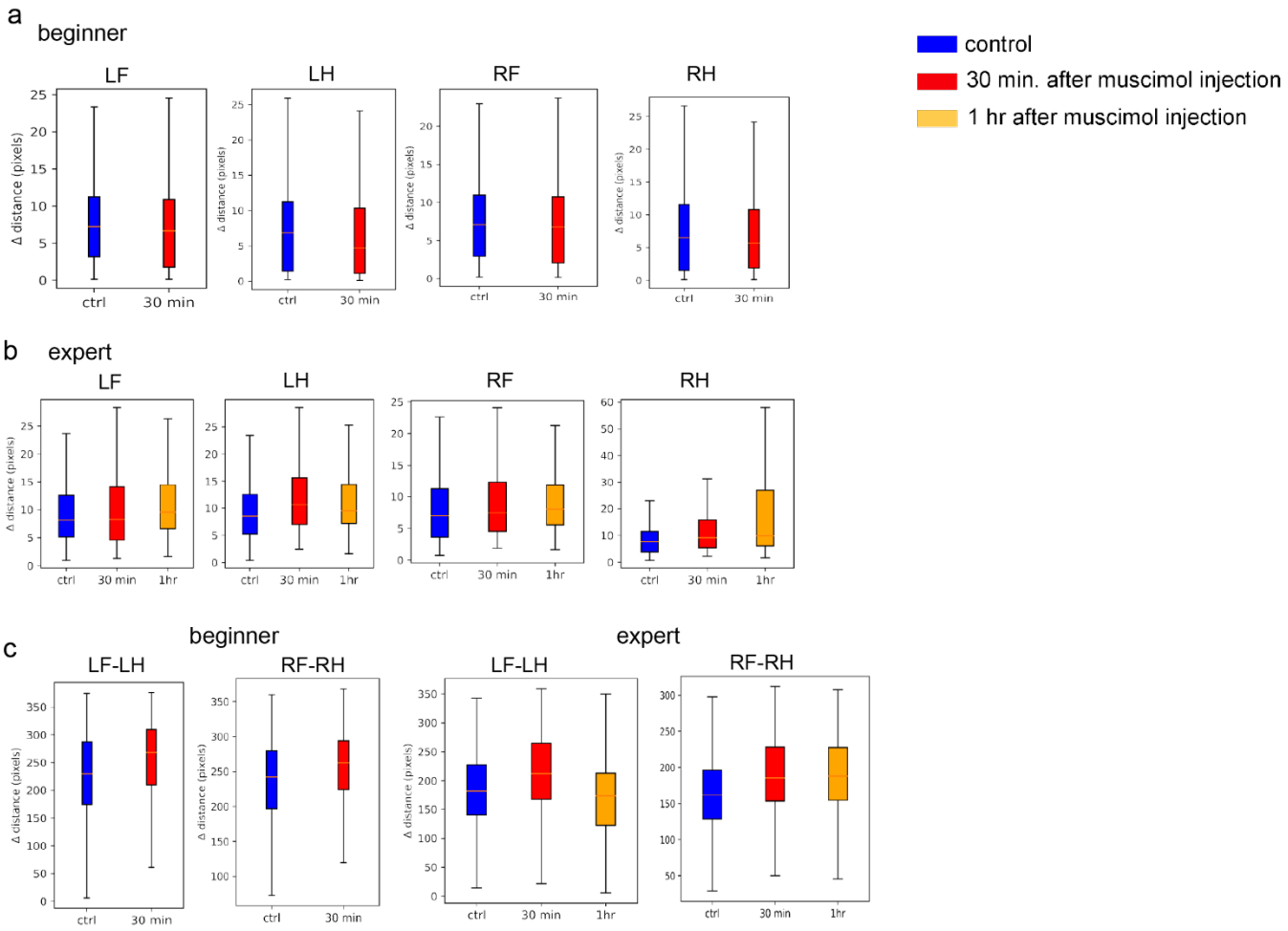

Figure 10. M1 influence on behavioral performance in beginner and expert mice. a) Distributions of changes in distance traveled by each paw, LF: left forelimb, LH: left hindlimb, RF: right forelimb, RH: right hindlimb, before and 30 minutes after M1 inactivation in beginner mice. b) as in a), for expert mice. c) Distributions of distances between forelimb and hindlimbs before and 30 minutes after M1 inactivation in beginner (left) and expert mice (right).

Discussion

We found that M1, M2, and PF neurons differentially influence DLS activity across different stages of learning. After M2 inhibition, we did not observe any changes in DLS activity

nor in movement performance in beginner mice yet observed weakening of DLS activity in expert and master mice as learning progressed. However, we do not see overt deficits in motor performance in mice of all learning stages after M2 inactivation, which contradicts the idea of DLS activity being required for the execution of a learned movement. From these results, we also conclude that M2 plays a larger role in controlling movement after a skilled task has been sufficiently learned and M2 may play a smaller role or none at all when first learning a task. After PF inhibition, we observed a significant decrease in DLS activity and behavioral performance across all beginner, expert, and master mice, which might indicate that PF plays a larger role than M2 in controlling movement regardless of the learning stage. The role of PF input to the DLS may potentially be a basic requirement for movement in general, as learning does not seem to affect this influence on striatal activity nor motor control. After M1 inactivation, we observed similar effects as PF inactivation, as beginner mice also performed poorly on the behavioral task as well as showed an abolishment of DLS activity. However, comparing effects of inactivating M1 versus M2 in beginner mice, we see a difference in both striatal activity and behavioral performance, indicating the two brain regions have differing influences on striatal activity and motor control when learning begins. These results hint to a greater complexity between these two regions and suggest that the two motor cortices should be examined individually rather than grouped together as one region. To fully determine the significance of these results, we aim to continue these experiments with a greater number of mice.

One caveat to our experiment would be our inactivation protocol involving the injection of muscimol into an input region of the striatum, because it impedes our ability to form conclusions regarding the influence of striatal activity on behavior. Muscimol inactivates the all

neurons in a targeted brain area, without any discrimination for neurons that project to specific downstream targets. Without ensuring specific inactivation of striatal-projecting input neurons, we cannot exclude the possibility of inactivating input neurons whose non-striatal projections could indirectly affect behavioral performance. Despite this, the relationship between inactivation of input neurons and DLS activity is comparable, as muscimol would inactivate monosynaptic connections between the input and DLS neurons, which leaves little room for off-target neurons to influence DLS activity. Nevertheless, we recognize that imaging sessions one hour after injection would pose a greater risk for off-target effects, as muscimol spread out to neighboring areas. As a result, we imaged neural activity and recorded behavioral performance thirty minutes post-muscimol injection to minimize the possibility of inactivating off-target neurons. However, in trials where M2 was inactivated in beginner and expert mice, we did not find visible changes in striatal activity thirty minutes post-injection and thus imaged a second time, one hour after the injection of muscimol. In these imaging sessions, we cannot disregard the possibility of off-target inactivated neurons also contributing to the changes in DLS activity and behavioral performance. In considering the time-sensitive effects of muscimol, we propose that we consider the relationship between input region inactivation and the resulting changes in striatal activity as a direct effect. However, in examining the relationship between abolishment of striatal activity and behavioral performance we cannot conclude its direct involvement, as the existence of non-striatal projections from input regions may play a greater role in influencing behavior rather than the activity of striatal neurons.

In specifically examining the role of PF in relation to DLS activity patterns, we find that our results are not sufficient to relate the two, as a recent study has found that PF input to STN may be responsible for an ipsilateral turning behavior (Watson et al. 2021), a specific phenotype

that we also observed in all mice when PF was inhibited by muscimol. It remains a greater possibility that non-specific inactivation of PF neurons led to deficits in off-target and downstream areas to PF, such as STN, which could play a greater role in producing behavioral deficits rather than the inactivation of DLS activity.

Conclusion

The results of our project indicate that PF, M1, and M2 have differential influences on the DLS and motor control than previously known. We suspect PF plays a broader role in influencing DLS activity as well as movement as a basic input required for movement to be initiated, executed, and terminated. M1 also plays a similarly important role at the beginning stages of learning, but its influence might decrease with learning. Our findings also suggest that M2 influence on striatal activity and movement is learning-specific, in which M2 input is important once the behavior has been learned.

This thesis was coauthored with Gjoni, Enida. The thesis author was the primary author of the material.

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