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Input- and Output-Specific Regulation of Serial Order Performance by Corticostriatal Circuits

Highlights

- In a serial order task, secondary motor cortex input to striatum initiates responses
- Striatal direct pathway is necessary for completion of responses in serial order
- Serial order learning strengthens synapses connecting motor cortex and striatum
- Task performance requires a disparity of striatal output favoring the direct pathway

Authors

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In Brief

Many behaviors involve distinct movements performed in a specific serial order. Rothwell et al. show serial order performance is regulated by a monosynaptic pathway linking secondary motor cortex to striatal cells that form the direct pathway through the basal ganglia.

Rothwell et al., 2015, Neuron *88*, 345–356
October 21, 2015
http://dx.doi.org/10.1016/j.neuron.2015.09.035

CellPress

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Input- and Output-Specific Regulation of Serial Order Performance by Corticostriatal Circuits

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http://dx.doi.org/10.1016/j.neuron.2015.09.035

SUMMARY

The serial ordering of individual movements into sequential patterns is thought to require synaptic plasticity within corticostriatal circuits that route information through the basal ganglia. We used genetically and anatomically targeted manipulations of specific circuit elements in mice to isolate the source and target of a corticostriatal synapse that regulates the performance of a serial order task. This excitatory synapse originates in secondary motor cortex, terminates on direct pathway medium spiny neurons in the dorsolateral striatum, and is strengthened by serial order learning. This experience-dependent and synapse-specific form of plasticity may sculpt the balance of activity in basal ganglia circuits during sequential movements, driving a disparity in striatal output that favors the direct pathway. This disparity is necessary for execution of responses in serial order, even though both direct and indirect pathways are active during movement initiation, suggesting dynamic modulation of corticostriatal circuitry contributes to the choreography of behavioral routines.

INTRODUCTION

The ability to learn and adapt to the environment requires experience-dependent plasticity in neural circuits formed by vast networks of synaptic connections. These circuits are both diverse and complex, composed of synapses that originate from a multitude of sources and terminate on many different postsynaptic targets, generating an enormous variety of synaptic connections with distinct properties and functions. Specific experiences are thought to recruit selective and distributed forms of plasticity at synapses connecting particular sources and targets. With the advent of technologies that permit genetically and anatomically defined manipulations of neural circuits (Luo et al., 2008; Yizhar et al., 2011), a major question in modern neuroscience is how the function and plasticity of specific synapses within complex circuits regulates behavioral output (Akil et al., 2010).

This challenge is exemplified by the striatum, which in rodents is analogous to the caudate and putamen of primates (Graybiel, 2008). As the gateway to the basal ganglia, the striatum receives synaptic inputs from many different sources, including a variety of cortical and thalamic subregions (Wall et al., 2013). These presynaptic inputs can terminate on a number of different postsynaptic targets, including a small population of interneurons and a much larger population of medium spiny projection neurons (MSNs) (Kreitzer and Malenka, 2008). MSNs relay information to downstream structures in the basal ganglia through two discrete pathways formed by MSN subtypes that differ in a number of anatomical and cellular properties, including expression of different dopamine receptor subtypes (Gerfen and Surmeier, 2011). Striatonigral MSNs that form the direct pathway (dMSNs) express the *Drd1a* dopamine receptor, whereas striatopallidal MSNs that form the indirect pathway (iMSNs) express the *Drd2* dopamine receptor as well as the *Adora2a* adenosine receptor (Schiffmann and Vanderhaeghen, 1993).

Through its myriad of inputs and outputs, the striatum plays a central role in choreographing movements and behavior. This includes simple movements (e.g., Kravitz et al., 2010) as well as more complex behavioral routines that involve multiple distinct actions performed in serial order (e.g., Aldridge and Berridge, 1998). Serial order is a fundamental aspect of organized behavior (Lashley, 1951), but despite many decades of research on the striatum and basal ganglia, it remains unclear how heterogeneous chains of behavioral responses are regulated by various synaptic inputs to different striatal cell types. While synaptic plasticity in the striatum is important for learning to perform many types of actions (Dang et al., 2006; Jin and Costa, 2010; Koralek et al., 2012; Schreiweis et al., 2014; Xiong et al., 2015), the specific source and target of synapses that undergo plasticity during serial order learning have yet to be identified. We used a combination of retrograde and anterograde viral and optogenetic manipulations in mice to study synapses connecting

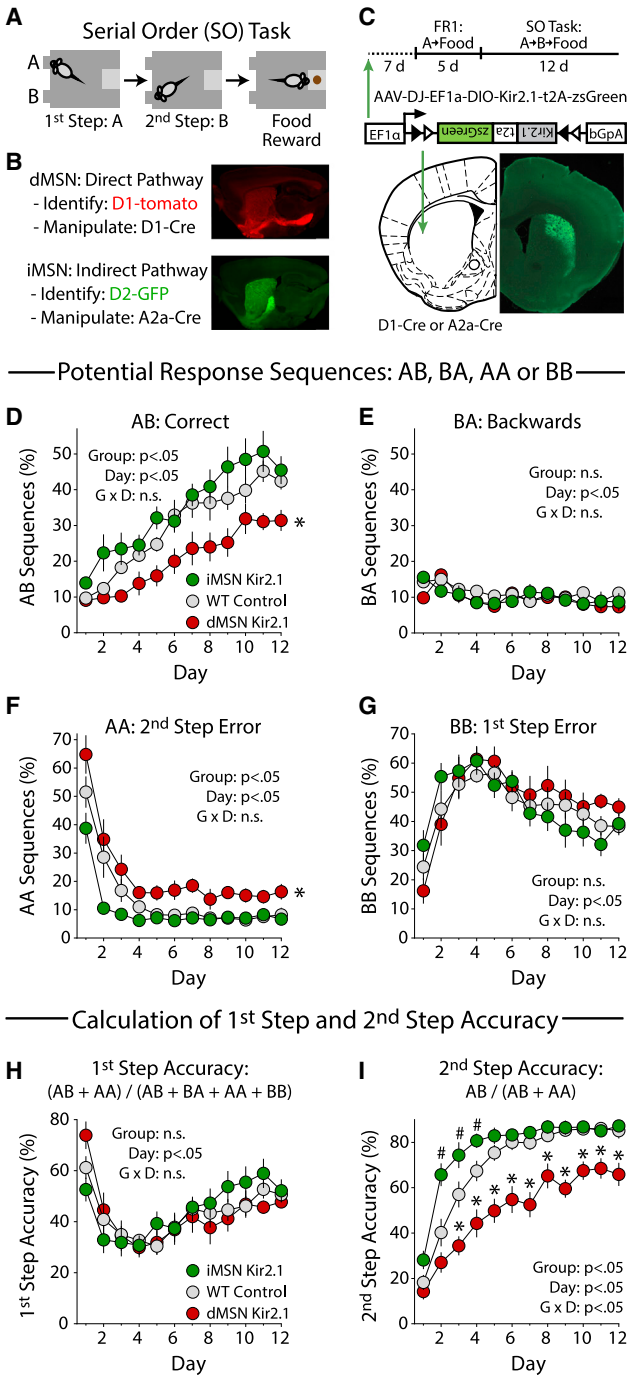

Figure 1. Inhibition of dMSNs Impairs Sequence Completion

(A) The SO task requires two discrete responses to be performed in the correct AB sequence.

(B) MSNs forming the direct and indirect pathways were identified and manipulated using transgenic mouse lines.

(C) AAV-DIO-Kir2.1 was injected into DLS of A2a-Cre (n = 7), D1-Cre (n = 10), or WT mice (n = 13) prior to SO training. Note that virus injections were bilateral.

(D–G) Percentage of sequences completed in each potential order after inhibition of dMSNs or iMSNs through expression of Kir2.1. The percentage of correct AB sequences was significantly decreased by dMSN inhibition (D), with no effect on BA sequences (E). The percentage of AA sequences was increased by dMSN inhibition (F), with no change in BB sequences (G).

(H) First step accuracy was calculated by dividing trials with a correct first step (AB + AA) by the total number of trials, and was not affected by inhibition of dMSNs or iMSNs.

(I) Second step accuracy was calculated by dividing correctly completed AB trials by trials beginning with a correct first step (AB + AA) and was significantly decreased by dMSN inhibition. Note that iMSN inhibition produced a transient but significant improvement in second step accuracy. All data are mean ± SEM; *p < 0.05, worse performance; #p < 0.05, better performance. Also see Figures S1 and S2.

motor cortex and dorsolateral striatum (DLS). We find that the initiation of a simple sequence of two responses is specifically regulated by neurons in secondary motor cortex (M2) that project to the DLS, whereas sequence completion depends on activity of dMSNs in the DLS. The monosynaptic connections between M2 and dMSNs are selectively strengthened by serial order learning, shifting the balance of striatal output in a manner that is critical for performance of a behavioral routine.

RESULTS

Sequence Completion Depends on dMSNs in the DLS

We investigated the role of corticostriatal circuits in behavioral performance of a serial order (SO) task that requires mice to make two sequential responses (do "A" then "B"; Figure 1A). A prior study (Yin, 2010) reported that SO learning was disrupted by excitotoxic lesions of all cells in the DLS. To refine this analysis and manipulate the activity of genetically defined cell types in the DLS, we used D1-Cre and A2a-Cre mouse lines to, respectively, target dMSNs and iMSNs (Gerfen et al., 2013) (Figure 1B) and performed in vivo stereotaxic injection of adeno-associated virus expressing a double-floxed inwardly rectifying potassium channel (AAV-DIO-Kir2.1; Figure 1C). Expression of Kir2.1 introduces a leak current that decreases the input resistance of infected cells (Lin et al., 2010), leading to robust inhibition of spiking in striatal MSNs (Rothwell et al., 2014). To confirm that MSNs expressing Kir2.1 are healthy, we performed whole-cell patch-clamp recordings from infected dMSNs in acute brain slices, using a cesium-based internal pipette solution to block both endogenously and exogenously expressed potassium channels. As expected, infected dMSNs had significantly lower input resistance immediately after break-in, but input resistance normalized to control levels after 5 min of recording (Figure S1A). At this point, the amplitude and frequency of miniature excitatory postsynaptic currents (mEPSCs) were similar in control and infected dMSNs (Figure S1B). These data indicate MSNs infected with Kir2.1 have decreased input resistance, but are otherwise healthy and do not exhibit substantial alterations in the properties of their excitatory synapses.

To test whether activity of either MSN subtype is necessary for SO performance, we initially made bilateral virus injections prior to any behavioral training (Figure 1C). After 5 days of reinforcing the first response ("A") on a fixed ratio one (FR1) schedule (Figure S2A), the SO task conditions were introduced, and food reward was only delivered after completion of the AB response sequence. The pattern of SO learning in our experiments was

similar to previous reports in mice (Yin, 2009, 2010) and reflected complex changes in the repertoire of behavioral responses. The initial percentage of correct AB sequences was low (~10%) but increased monotonically over the course of training (Figure 1D), while the percentage of backward BA sequences was low and decreased slightly during training (Figure 1E). Because response "A" was reinforced in FR1 training, the majority of initial sequences during SO training were AA (Figure 1F). On the small number of trials a mouse completed the correct AB sequence, response "B" immediately preceded reward delivery, and this close proximity between response "B" and reward led to an increase in the number of BB sequences during the first several training sessions (Figure 1G). With further training, mice gradually learned to withhold the initial "B" response and instead began a higher proportion of sequences with the correct "A" response, leading to a decrease in the number of BB sequences. These dynamic changes in SO performance can be decomposed by calculating independent measures of response accuracy on the first and second steps of the sequence, which together determine the overall percentage of correct AB sequences. First step accuracy was defined as the fraction of all trials that begin with a correct first step (Figure 1H), while second step accuracy was defined as the percentage of all trials beginning with a correct first step that were subsequently completed with a correct second step (Figure 1I).

Overall performance of the correct AB sequence was impaired by inhibition of dMSNs, but not iMSNs (Figure 1D). This impairment was caused by failure to complete the second step of the sequence, as inhibition of dMSNs led to a significant increase in the percentage of incorrect AA sequences (Figure 1F). However, the percentages of incorrect BA and BB sequences were not affected by inhibition of dMSNs or iMSNs (Figures 1E and 1G). As a result, second step accuracy was significantly reduced by dMSN inhibition, whereas first step accuracy was not affected (Figures 1H and 1I). While iMSN inhibition did not change overall performance, it did produce a transient but significant improvement in second step accuracy (Figure 1I).

A Specific Role for dMSNs in the DLS in Performance of a Learned Sequence

Although inhibition of dMSNs in the DLS clearly affected performance of the SO task, this could potentially be explained by behavioral impairments unrelated to the choreography of response sequences. For example, general deficits in movement or motivation could also affect SO performance. We have previously shown that inhibition of dMSNs in the DLS with Kir2.1 does not alter spontaneous locomotor activity in an open field test (Rothwell et al., 2014) and therefore does not grossly impair movement. In the present study, inhibition of dMSNs in the DLS did not affect the acquisition of a simple operant response during FR1 training (Figure S2A), demonstrating intact capacities to learn and respond for reinforcement. Although SO performance late in training was robustly impaired by inhibition of dMSNs, there were no corresponding changes in response rate or number of completed sequences (Figures S2B and S2C), suggesting intact motivation to perform the SO task.

An impairment of response switching that leads to perseveration on the initial "A" response could explain both the decrease in AB sequences (Figure 1D) and increase in AA sequences (Figure 1F) caused by inhibition of dMSNs in the DLS. To distinguish a general impairment of response switching from a specific effect on sequence completion, we examined behavior on error trials that began with an incorrect "B" response. Any general change in switching or perseveration should lead to a shift in the probability that these error sequences conclude as BA versus BB. This probability of error trial switching was not affected by inhibition of either dMSNs or iMSNs (Figure S2D), arguing against a generalized behavioral deficit in switching or perseveration.

To further address the role of dMSNs in response switching and perseveration, a separate cohort of D1-Cre mice received bilateral injections of AAV-DIO-Kir2.1 into the DLS (Figure 2A). These mice exhibited an impairment of motor coordination on the accelerating rotarod (Figure 2B), consistent with our previous report (Rothwell et al., 2014) and confirming the efficacy of this manipulation. Next, this cohort was trained to perform a spatial discrimination task in which either the left or right response was active and reinforced, while the other response location was inactive (i.e., responses had no consequences). After acquisition of this task on a variable ratio 2 (VR2) schedule, the spatial rule governing reinforcement was reversed during a test session, such that responses in the previously inactive location were reinforced, and responses in the previously active location had no consequences (Gourley et al., 2010). As expected, the number of non-reinforced responses immediately increased after reversal of task contingencies, and then declined gradually over the course of the reversal session (Figure 2C). This within-session spatial reversal test provides separate behavioral readouts of switching (i.e., responses in the newly reinforced location) and perseveration (i.e., responses in the previously reinforced location). Inhibition of dMSNs in the DLS did not affect the reinforced response rate before, during, or after reversal of task contingencies (Figure 2D), indicating this manipulation does not generally impair response switching. Non-reinforced responses rates were also similar before, during, and after reversal (Figure 2E), indicating inhibition of dMSNs in the DLS does not generally affect perseverative behavior. Response accuracy was also similar between groups (Figure 2F), as expected, given the comparable response rates.

After 2 additional days of training on the reversed VR2 schedule, the same mice were tested on a progressive ratio schedule of reinforcement, with each food pellet requiring a successively increasing number of active responses (Figure 2G). In this assay, the highest response ratio completed (commonly referred to as the "break point") is a sensitive measure of motivational state and effort (Hodos, 1961). Inhibition of dMSNs in the DLS did not affect the total number of active responses, break point, or the number of pellets earned (Figures 2H–2J), providing further evidence that effort and motivation are not impaired by this manipulation. Together, these results indicate that inhibition of dMSNs in the DLS produces a selective deficit in the organization of sequential responses, rather than more general effects on locomotion, motivation, perseveration, or response switching.

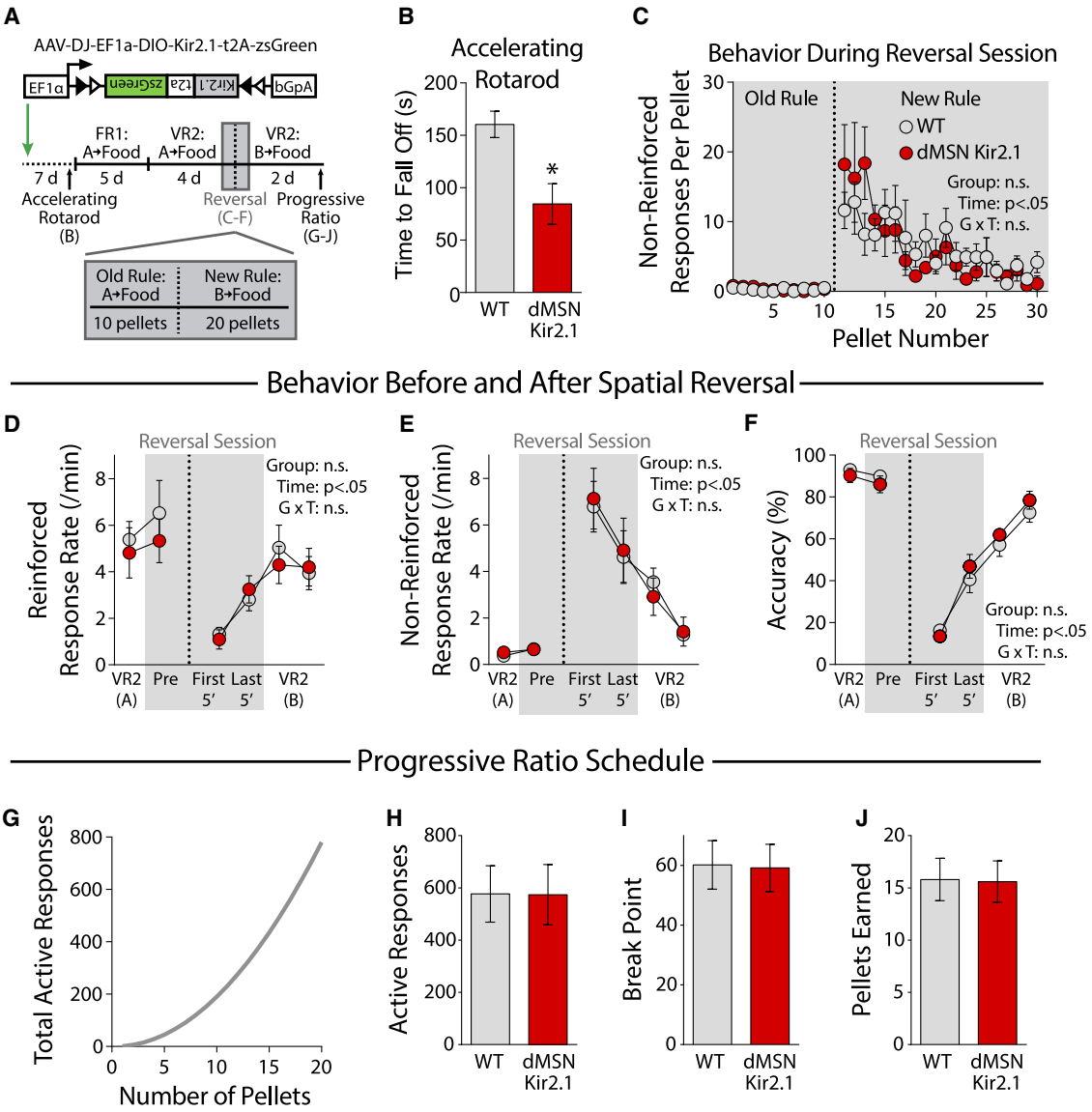

Figure 2. Inhibition of dMSNs Does Not Alter Reversal Learning or Motivation

(A) AAV-DIO-Kir2.1 was injected into the DLS of D1-Cre (n = 9) or WT mice (n = 10) prior to training on a spatial discrimination task with a VR2 schedule of reinforcement.

(B) Performance on the accelerating rotarod was impaired by dMSN inhibition, demonstrating the efficacy of this manipulation.

(C–F) Instrumental performance before and after a within-session reversal of the spatial discrimination. Reversal of task contingencies led to an immediate increase in the number of non-reinforced responses per pellet, which subsequently declined during the reversal session (C). Inhibition of dMSNs did not affect the reinforced response rate (D), non-reinforced response rate (E), or response accuracy (F).

(G–J) Instrumental performance on a progressive ratio schedule of reinforcement (G). The number of active responses (H), break point (I), and number of pellets earned (J) were not affected by dMSN inhibition. All data are mean ± SEM; *p < 0.05, worse performance.

In our original experiment (Figure 1), virus injections were performed prior to behavioral training in the SO task, and the effect of dMSN inhibition was most robust late in training. The observed phenotypes could thus be explained by a change in either learning or performance of the SO task. To distinguish these possibilities, we trained additional groups of mice on the SO task and then made bilateral virus injections after behavioral performance had stabilized. Under these conditions, dMSN inhibition still impaired SO performance by decreasing second step accuracy (Figures S2E–S2G), indicating an effect on task performance rather than learning. The relatively stable impairment of task performance also suggests that accumulation of Kir2.1 protein over time does not influence the temporal pattern of this behavioral phenotype. In subsequent experiments, all manipulations were conducted after training to further investigate SO performance.

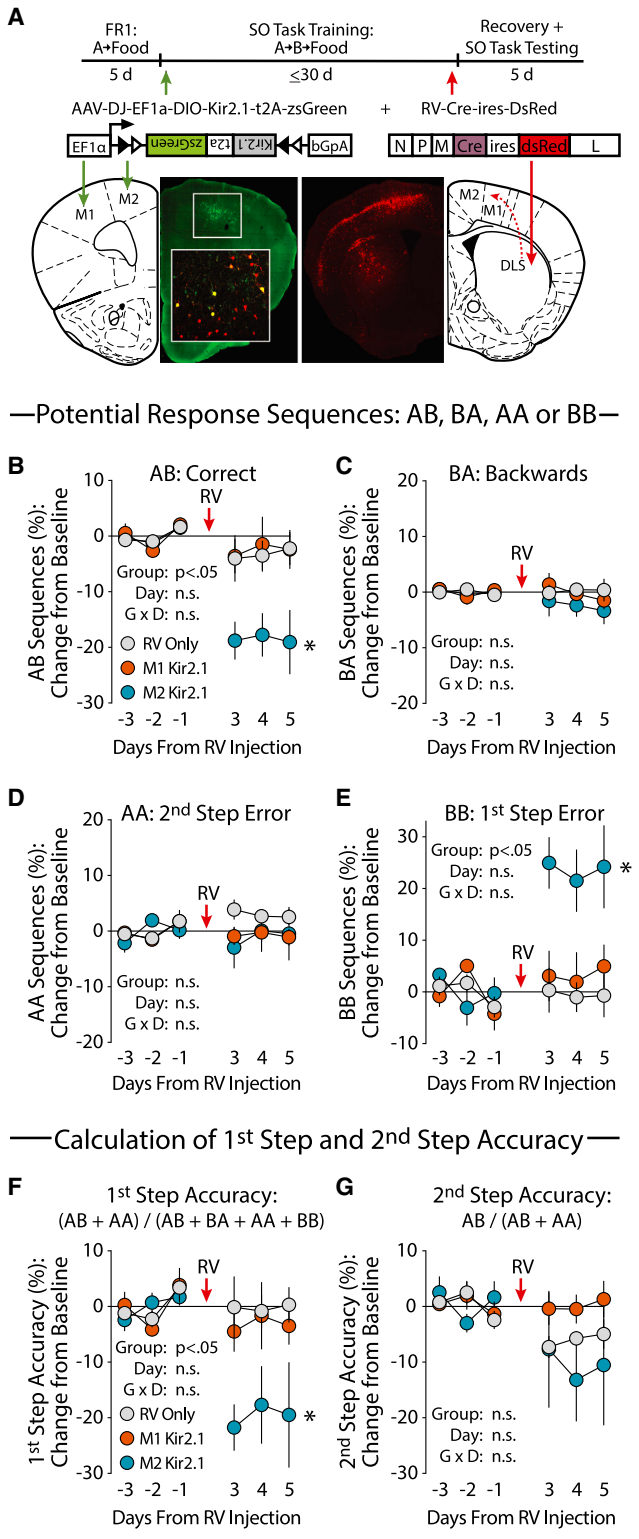

Figure 3. Retrograde Inhibition of M2 Projections to the DLS Impairs Sequence Initiation

(A) Inhibition of specific corticostriatal projections after injection of AAV-DIO-Kir2.1 into M1 (n = 6) or M2 (n = 6), followed by RV-Cre into DLS. A control group received only RV-Cre injection (n = 8), and all virus injections were bilateral.

(B–E) Percentage of sequences completed in each potential order after inhibition of M1 or M2 projections to the DLS with Kir2.1. The percentage of correct AB sequences was significantly decreased by inhibition of the M2→DLS projection (B), with no effect on BA sequences (C) or AA sequences (D), but a significant increase in the percentage of BB sequences (E). Inhibition of the M1→DLS projection had no effect.

(F and G) Inhibition of the M2→DLS projection decreased first step accuracy (F) but not second step accuracy (G). All data are mean ± SEM; *p < 0.05, worse performance. Also see Figure S3.

Sequence Initiation is Specifically Mediated by M2 Projections to DLS

The preceding experiments indicate dMSNs in the DLS play a critical role in completion of a learned sequence. The initiation of this sequence presumably involves one of the many different presynaptic inputs to these cells (Wall et al., 2013). As portions of motor cortex are implicated in action initiation (Erlich et al., 2011; Guo et al., 2014; Murakami et al., 2014) and organization (Cao et al., 2015; Ostlund et al., 2009; Yin, 2009), we next investigated whether synaptic inputs from motor cortex to the DLS are critical for SO performance. To specifically manipulate cells providing monosynaptic input to the DLS, we injected a deletion mutant rabies virus expressing Cre recombinase (RV-Cre), which is taken up by axon terminals and retrogradely transported to the cell bodies from which these axons originate (Dölen et al., 2013). To selectively inhibit distinct cortical projections to the DLS, we used a double-virus strategy in which RV-Cre gates expression of AAV-DIO-Kir2.1, which was injected into either primary motor cortex (M1) or secondary motor cortex (M2) (Figure 3A). A control group received injection of RV-Cre into the DLS alone (i.e., no AAV), and all virus injections were bilateral. Cortical pyramidal neurons infected with both viruses showed decreased intrinsic excitability after only 3 days, as well as a hyperpolarized resting membrane potential that indicated these cells were still healthy (Figures S3A–S3C). These results demonstrate the rapid retrograde transport and expression of deletion mutant RV vectors (Wickersham et al., 2007).

To evaluate SO performance, behavior after double-virus injection was normalized to a 3-day baseline period just prior to injection of RV-Cre. Inhibition of DLS-projecting neurons in M1 had little effect on SO performance (Figures 3B–3G). However, inhibition of DLS-projecting neurons in M2 (M2→DLS) caused a significant decrease in the percentage of correct AB sequences (Figure 3B), accompanied by an increase in the percentage of incorrect BB sequences (Figure 3E). This response pattern led to a significant decrease in first step accuracy, but not second step accuracy, following inhibition of M2→DLS (Figures 3F and 3G). This result points to a role for M2 in sequence initiation and contrasts with the effect of directly inhibiting dMSNs in the DLS, which increased the percentage of incorrect AA sequences and decreased second step accuracy (cf. Figure 1). It should be noted that inhibition of M2→DLS had no impact on the percentage of incorrect AA sequences (Figure 3D), demonstrating that this manipulation does not generally cause response repetition. To quantify this in terms of error trial switching, we examined error trials that ended with an incorrect "A" response and calculated the probability that these sequences began as BA

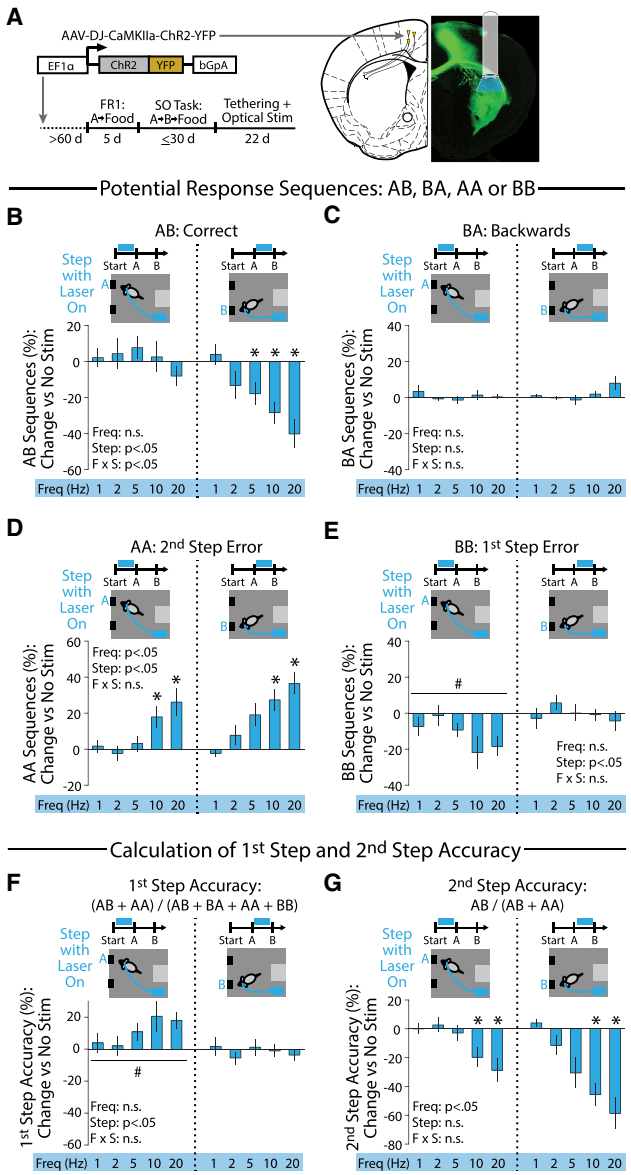

Figure 4. Optogenetic Activation of M2 Projections to the DLS Promotes Sequence Initiation

(A) AAV-ChR2 was injected into M2 (n = 4), with bilateral optogenetic stimulation (473 nm, 1–20 Hz) of axon terminals in DLS.

(B–E) Percentage of sequences completed in each potential order after optogenetic activation of the M2→DLS projection. Bilateral stimulation before the first response (left) had no effect on the percentage of correct AB sequences (B) or BA sequences (C), but increased the percentage of AA sequences (D) and decreased the percentage of BB sequences (E). Bilateral stimulation between responses (right) decreased the percentage of correct AB sequences (B) and increased the percentage of AA sequences (D), with no effect on BA sequences (C) or BB sequences (E).

(F and G) First step accuracy increased with stimulation before the first response (F), while second step accuracy decreased with stimulation before the first response or between responses (G). All data are mean ± SEM; *p < 0.05, worse performance; #p < 0.05, better performance. Also see Figure S3.

versus AA. This general measure of response switching was not affected by inhibition of M2→DLS (Figure S3D).

To further dissect the behavioral function of the M2→DLS projection, we next used optogenetics to activate this pathway in the anterograde direction. We performed bilateral injections of AAV expressing channelrhodopsin-2 (AAV-ChR2) in M2 and implanted bilateral optic fibers in the DLS above axon terminals expressing ChR2 (Figure 4A). After mice had been trained in the SO task, we leveraged the temporal precision of optogenetic stimulation by delivering blue light pulses at varying frequencies during different task segments: from the start of each trial until the first response was made or between the first response and second response. Performance on trials with bilateral optogenetic stimulation of M2→DLS was compared to interleaved control trials with no stimulation.

Stimulation of M2→DLS before the first response did not affect the percentage of correct AB sequences, but a frequency-dependent impairment was observed with stimulation between responses (Figure 4B). This impairment was associated with an increase in the percentage of incorrect AA trials (Figure 4D) but no change in the percentage of incorrect BB or BA trials (Figures 4C and 4E). This overall pattern resulted in a decrease in second step accuracy (Figure 4G) but no change in first step accuracy (Figure 4F) or the probability of error trial switching (Figure S3E). Stimulation before the first step also caused an increase in the percentage of incorrect AA sequences (Figure 4D), but this was associated with a decrease in the percentage of incorrect BB sequences (Figure 4E), demonstrating opposite effects on these two different forms of repeated behavior. This response pattern led to a significant improvement in first step accuracy, while second step accuracy was impaired (Figures 4F and 4G). Thus, stimulation of M2→DLS terminals increases the likelihood of performing the first response of the sequence, which can either improve or impair performance, depending on when stimulation is applied.

Sequence Learning Strengthens Synapses Connecting M2 and dMSNs in the DLS

The preceding experiments indicate that sequence initiation and completion are respectively regulated by M2 and dMSNs in the DLS. The monosynaptic connection linking these corticostriatal circuit elements may thus be a key site that is modified by the experience of SO learning. To evaluate the function of M2→DLS excitatory synapses after sequence learning, we injected AAV expressing ChIEF (Lin et al., 2009) in M2 of D1-tomato transgenic reporter mice. After behavioral training in the SO task, we prepared acute brain slices and performed whole-cell recordings from dMSNs and iMSNs in the DLS (Figure 5A). We used a standard protocol (e.g., Ahmad et al., 2012; Jurado et al., 2013; Schwartz et al., 2014) to measure the ratio of AMPAR- to NMDAR-mediated currents, a surrogate index of synaptic strength that provides a useful readout of synaptic function and plasticity (Kauer and Malenka, 2007). MSNs were first voltage-clamped at a hyperpolarized membrane potential (−80 mV) to measure the amplitude of excitatory synaptic currents mediated by activation of AMPA receptors (AMPARs). We then shifted to a depolarized holding potential (+40 mV) to

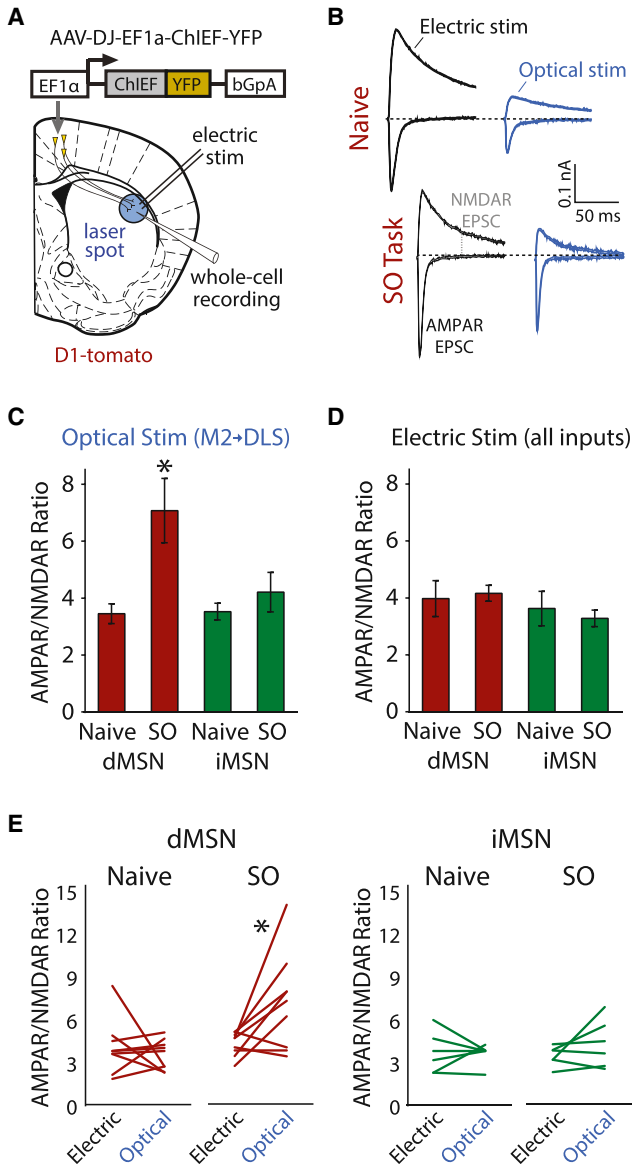

Figure 5. SO Training Causes Pathway- and Cell-Type-Specific Plasticity at M2→dMSN Synapses

(A) Schematic of coronal brain slice showing conditions for synaptic physiology, with whole-cell voltage-clamp recordings and interleaved electric and optical stimulation.

(B) Representative traces show electric (black) and optical (blue) AMPAR and NMDAR currents from mice that were naive (top) or trained in the SO task (bottom).

(C–E) The AMPAR/NMDAR ratio at M2 synapses onto dMSNs was increased by SO training (n = 8 cells) relative to naive control (n = 9 cells), but there was no difference between naive (n = 6 cells) and SO groups (n = 6 cells) for iMSNs (C). No differences were observed with electric stimulation of all synapses (D), with a significant difference between electric and optical AMPAR/NMDAR ratios in dMSNs after SO learning (E). All data are mean ± SEM; *p < 0.05, increased AMPAR/NMDAR ratio. Also see Figure S4.

relieve magnesium blockade of NMDA receptors (NMDARs) and measured the amplitude of NMDAR-mediated synaptic currents 50 ms after stimulation (Figure 5B).

When optically evoking EPSCs from M2 terminals expressing ChIEF, we observed an increase in the AMPAR/NMDAR ratio at M2→dMSN synapses after SO training (Figure 5C). This change was not observed in iMSNs and was also absent in control groups trained on operant tasks that do not involve SO learning (Figures S4A–S4D). Importantly, there were no changes in the AMPAR/NMDAR ratios generated by interleaved electrical stimulation, which indiscriminately activates all synaptic inputs to MSNs (Figure 5D). Our experimental design permitted within-cell comparisons of the electrical and optical AMPAR/NMDAR ratios, which were significantly different only for dMSNs after SO training (Figure 5E).

We also assessed several biophysical properties of NMDARs and AMPARs that can influence measurement of the AMPAR/NMDAR ratio. For example, changes in NMDAR subunit composition can alter the decay kinetics of NMDAR currents and affect the NMDAR EPSC amplitude 50 ms after stimulation. However, we detected no change in the half-width of NMDAR EPSCs with either optical or electric stimulation (Figure S4E). The rectification properties of AMPAR currents are also dictated by subunit composition, though we intentionally measured the AMPAR EPSC at −80 mV so that its amplitude would be relatively independent of changes in rectification. Direct measurement of the current-voltage relationship and reversal potential of AMPAR EPSCs did not reveal any alterations following SO training (Figure S4F). There were also no changes in mEPSC frequency or amplitude (Figure S4G), and because these events are generated at all excitatory synapses onto MSNs, this result is consistent with anatomical evidence that M2 inputs represent <10% of all monosynaptic inputs to dMSNs (Wall et al., 2013). These findings illustrate that input-specific forms of synaptic plasticity, like the one we observe at M2 synapses onto dMSNs, can be obscured in electrophysiological assays that measure the aggregate function of all synaptic inputs.

Sequence Completion Requires a Disparity of Striatal Output

The preceding experiments suggest dMSNs are critical for sequence completion, because they are driven to spike by potentiated excitatory input from M2. This enhanced firing of dMSNs would increase striatal output through the direct pathway relative to the indirect pathway—a disparity that may be critical for sequence completion (Figure 6A). To test this hypothesis, we performed bilateral injections of AAV-DIO-ChR2 in the DLS of D1-Cre and A2a-Cre mice and implanted bilateral optic fibers above infected cell bodies in the DLS (Figure 6B). We initially attempted to enhance the disparity of striatal output by conducting bilateral stimulation of dMSNs in the DLS during performance of the SO task. However, this manipulation produced mild dyskinesia that disrupted ongoing behavior, leading to an increase in response latency during the period of stimulation (Figure S5A). This dyskinesia may result from the simultaneous activation of multiple competing motor programs. As an alternative strategy, we attempted to boost output through the indirect pathway by stimulating iMSNs in the DLS. This manipulation did not disrupt behavior in the same fashion as dMSN stimulation, as response latencies in the SO task were not significantly affected (Figure S5B).

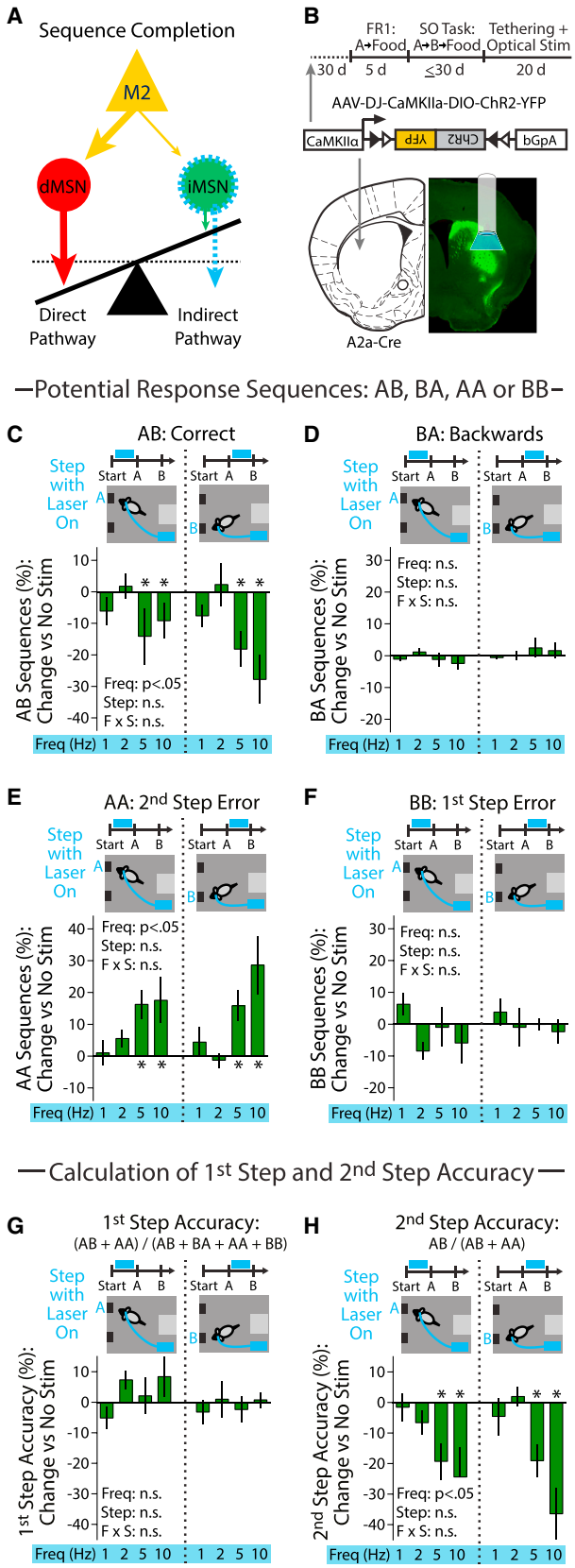

Figure 6. Optogenetic Activation of iMSNs Impairs Sequence Completion

(A) Schematic model of activity in corticostriatal circuits during sequence completion, with a disparity in striatal output that favors the direct pathway. Dotted blue lines indicate expression of ChR2 in iMSNs and subsequent optogenetic stimulation to normalize this disparity.

(B) AAV-DIO-ChR2 was injected into DLS of A2a-Cre mice (n = 4), with bilateral optogenetic stimulation (473 nm, 1–10 Hz) after SO training.

(C–F) Percentage of sequences completed in each potential order after optogenetic activation of iMSNs in the DLS before the first response (left) or between responses (right). Bilateral stimulation during either task segment decreased the percentage of correct AB sequences (C) and increased the percentage of AA sequences (E), with no effect on BA sequences (D) or BB sequences (F).

(G and H) Bilateral stimulation had no effect on first step accuracy (G), but stimulation during either task segment decreased second step accuracy (H). All data are mean ± SEM; *p < 0.05, worse performance. Also see Figures S3, S5, and S6.

Stimulation of iMSNs in the DLS led to a frequency-dependent decrease in the percentage of correct AB sequences, which was apparent when stimulation was applied either before the first step or between steps (Figure 6C). This impairment was associated with an increase in the percentage of incorrect AA sequences, but no change in the percentage of incorrect BB or BA sequences (Figures 6D–6F). This response pattern led to a significant decrease in second step accuracy (Figure 6H), but no change in first step accuracy (Figure 6G), indicating an impairment of sequence completion. Stimulation of iMSNs in the DLS did not alter the probability of error trial switching (Figure S3F) and did not impair response latency, rate, or accuracy during reversal of a spatial discrimination in a separate cohort of mice (Figures S6A–S6F). These latter results indicate perseveration and response switching are not generally affected by stimulation of iMSNs in the DLS.

We further analyzed the nature of incorrect AA sequences by measuring response latencies during 10-Hz stimulation, which produced the most robust impairment of SO performance (Figure S5C). Simulation before the first step did not affect latency of the initial "A" response, further demonstrating that this type of optogenetic stimulation of iMSNs in DLS does not cause a general slowing of behavior (Figure S5D). In fact, on trials with an incorrect AA sequence, the second erroneous "A" response was emitted very quickly after the first response (<1 s), much more rapidly than on control trials when the second step response latency was >2 s (Figure S5E). As optogenetic stimulation was delivered prior to the first "A" response and then terminated, the accelerated performance of second incorrect "A" response must reflect a carryover effect of the stimulation that rapidly disrupts sequence completion. In marked contrast, stimulation of iMSNs immediately following the first "A" response tended to prolong the second step response latency (Figure S5E) while still increasing the probability of a second erroneous "A" response (Figure 6E). Thus, optogenetic stimulation during these two different task segments caused errors to be committed with different latencies but led to the same net reduction in second step accuracy. Together, these results are consistent with the notion that sequence completion requires a disparity in striatal output that favors the direct pathway over the indirect pathway (Figure S6G).

DISCUSSION

Neural circuits are formed by diverse networks of synaptic connections linking specific sources of presynaptic input with specific postsynaptic targets. In this study, we combined viral and optogenetic manipulations of corticostriatal circuits with behavioral assays and electrophysiological readouts of synaptic function, to dissect the source and target of a specific synaptic connection that regulates the performance of a SO task. A previous report showed SO learning was disrupted by excitotoxic lesions of all striatal cells in the DLS (Yin, 2010). We extended this finding by manipulating the activity of genetically defined MSN subtypes in the DLS and found that activation of dMSNs was necessary for completion of the response sequence, as decreasing the activity of these cells through expression of AAV-DIO-Kir2.1 caused a reduction of second step accuracy. In contrast, lesions of all striatal cells were reported to impair performance of the first step of the SO task (Yin, 2010). As recent evidence suggests dMSNs and iMSNs are both active during action initiation (Cui et al., 2013; Jin et al., 2014), and both are capable of positively modulating motor cortex (Oldenburg and Sabatini, 2015), activity in either pathway alone may be sufficient for sequence initiation, whereas sequence completion specifically requires sustained activity of dMSNs.

To identify the excitatory synaptic inputs that are critical for activating MSNs during the SO task, we focused on regions of the motor cortex that are known to provide major inputs to DLS (Wall et al., 2013). Our retrograde and anterograde manipulations provided convergent evidence that synaptic projections from M2 to the DLS are critical for sequence initiation. Inhibition of M2 neurons projecting to DLS impaired first step accuracy, while activation of M2 terminals in the DLS increased the probability of performing the first sequential response. The latter phenotype manifested as an increase in the percentage of AA sequences—the same phenotype produced by the manipulations of the DLS, which was interpreted as an impairment of sequence completion. The limited repertoire of response sequences available in the SO task (AB, BA, AA, and BB) gives rise to this ambiguity between impaired sequence completion and repetition of the first response, but we were able to resolve this ambiguity by examining cases where our manipulations actually improved SO performance. Inhibition of iMSNs in the DLS with Kir2.1 produced small but significant improvements in second step accuracy, thus pointing to a role in sequence completion. Conversely, stimulation of the M2→DLS projection before the first response caused a small but significant improvement in first step accuracy, which points to a role in sequence initiation. In rodents, M2 is also known as the medial agranular cortex, and its numerous projection targets are similar to the premotor cortex, supplementary motor cortex, and frontal eye fields of primates (Reep et al., 1987; Stuesse and Newman, 1990). Our results demonstrate the specific involvement of direct monosynaptic connections from M2 to the DLS in SO performance and complement previous studies implicating M2 in action initiation and organization (Cao et al., 2015; Erlich et al., 2011; Guo et al., 2014; Murakami et al., 2014; Ostlund et al., 2009; Yin, 2009).

To determine if behavioral training in the SO task alters the function of synapses connecting M2 and DLS, we used optogenetics to stimulate synaptic inputs arising from M2 in a brain slice preparation and performed whole-cell recordings from identified dMSNs and iMSNs. We found that the AMPAR/NMDAR ratio, a surrogate measure of synaptic strength (Kauer and Malenka, 2007), was significantly increased after SO learning at synapses between M2 and dMSNs. This form of experience-dependent plasticity was highly specific, as it was not detected at synapses between M2 and iMSNs, or with bulk electrical stimulation of all excitatory inputs to dMSNs. The increase in AMPAR/NMDAR ratio at M2→dMSN synapses was not associated with major changes in the biophysical properties of AMPARs or NMDARs, and was only observed after training in a behavioral task that required SO learning. Previous studies show the organization of sequential actions is impaired by genetic deletion of NMDARs from striatal MSNs (Jin and Costa, 2010; Jin et al., 2014), leading us to speculate that activation of NMDARs on dMSNs triggers the strengthening of synaptic input arriving from M2 and that this highly selective synaptic change may be a critical neural circuit modification that mediates the choreography of a behavioral routine. However, additional work will be needed to test this hypothesis and determine if plasticity of the M2→dMSN synapse is specifically necessary for SO performance.

The Nature of SO Performance Impairments

Our SO task requires performance of a heterogeneous chain of two distinct responses, so repetition of either response is considered an error. It was therefore important to evaluate whether our manipulations of M2 and DLS had any impact on general tendencies to repeat or switch responses, which would confound interpretation of SO performance. We assessed this possibility in a different behavioral context by training mice to perform a spatial discrimination and then reversing task contingencies within a test session. Performance of this within-session reversal was not affected by inhibition of dMSNs with Kir2.1, or by optogenetic activation of iMSNs, providing evidence that SO performance impairments are not a secondary consequence of more general changes in perseveration or response switching. These findings are consistent with evidence that excitotoxic lesions of the entire DLS do not impair spatial reversal in rodents (Castañé et al., 2010).

In the spatial reversal task, switching from one response to the other takes place within a single session, but over the course of minutes. This contrasts somewhat with response switching during a single trial of the SO task, which takes place within seconds. It is thus possible that different mechanisms are involved in switching responses on these two different timescales. To further investigate response switching in the context of the SO task, we examined the percentage of error trials that involve a response switch (BA), which was not affected by any experimental manipulation. We also calculated the probability of error trial switching by comparing the percentage of BA error sequences with the percentage of error sequences that involve repetition of the same response (AA or BB). Again, the probability of error trial switching was not affected by any experimental manipulation, providing further evidence that SO performance

impairments are not caused by the inability to switch from one response to another.

Other alternative explanations for the behavioral phenotypes we observed in the SO task also merit consideration. For example, a shift in motivational state could affect the effort expended to switch from one response to another, versus repeating the same response. However, inhibition of dMSNs in the DLS did not affect instrumental performance on a progressive ratio schedule, arguing against a shift in motivational state. We also found that optogenetic stimulation of iMSNs in the DLS did not impact response latencies in the SO task, which might seem to contradict reports that iMSN stimulation in the dorsal striatum impedes movement (Kravitz et al., 2010). However, there are a number of technical differences between these studies, including the location, pattern, intensity, and duration of optogenetic stimulation. Perhaps most importantly, Kravitz et al. (2010) performed bilateral optogenetic stimulation of the dorsomedial striatum, whereas all of our experiments involved bilateral stimulation of the DLS. These two different striatal subregions are embedded in distinct cortico-basal ganglia circuits that are known to mediate different behavioral functions (e.g., Castañé et al., 2010; Durieux et al., 2012; Lerner et al., 2015; Thorn et al., 2010; Yin et al., 2009).

When we performed bilateral optogenetic stimulation of dMSNs in the DLS, we observed mild dyskinesia that interfered with ongoing behavior, which again differs from the locomotor activation produced by a similar manipulation in the dorsomedial striatum (Kravitz et al., 2010). However, general motor function does not appear to be affected by inhibition of dMSNs in the DLS with Kir2.1, as evidenced by intact instrumental performance under a variety of task conditions (FR1, VR2, reversal, and progressive ratio), as well as normal levels of open field activity (Rothwell et al., 2014). We do find that this manipulation impairs performance on the accelerating rotarod, consistent with our previous report (Rothwell et al., 2014). This impairment of rotarod performance may represent another example of a response sequence—i.e., the series of foot steps required to maintain balance on the rotarod—that depends on activity of dMSNs in the DLS. Recent evidence that manipulations of M2 also impair rotarod performance (Cao et al., 2015) suggests common corticostriatal circuit elements may be involved in many different types of sequential movements and actions. This includes performance of the SO task and other learned sequences of self-initiated responses (e.g., Desrochers et al., 2015; Lu et al., 2002), as well as longer action sequences guided by instructive cues (Bailey and Mair, 2006, 2007)

Encoding of Response Sequence by Corticostriatal Circuits

Our data are consistent with patterns of single unit activity recorded in motor cortex and DLS during homogenous sequence learning tasks involving multiple repetitions of the same response (Jin and Costa, 2010; Jin et al., 2014) and provide a direct test of hypotheses generated by these studies while also generalizing the results to heterogeneous response sequences. Action initiation is correlated with increased firing of neurons in motor cortex (Erlich et al., 2011; Fujii and Graybiel, 2003; Guo et al., 2014; Jin et al., 2014; Murakami et al., 2014), which is thought to precede and drive the activity of both dMSNs and iMSNs in the DLS (Cui et al., 2013; Jin et al., 2014; Koralek et al., 2013). However, as an action sequence unfolds, most iMSNs exhibit a reduction in firing rate, while most dMSNs exhibit sustained activity, leading to divergent firing patterns in downstream basal ganglia targets (Jin et al., 2014). Our data suggest the sustained activity of dMSNs during sequence completion is driven, at least in part, by strengthened excitatory synaptic input from M2. Conversely, the quiescence of iMSNs could involve collateral inhibition by dMSNs (Taverna et al., 2008) or feed-forward inhibition by fast-spiking GABAergic interneurons (Burguière et al., 2013; Gittis et al., 2011), which also exhibit sustained activity during sequence completion (Jin et al., 2014). Our optogenetic stimulation experiments demonstrate that quiescence of iMSNs is necessary for accurate sequence completion—a notion further supported by the small but significant improvements in second step accuracy caused by iMSN inhibition with Kir2.1. This disparity in striatal output that favors the direct pathway may positively modulate motor cortex via basal ganglia outputs to the thalamus (Oldenburg and Sabatini, 2015), thereby enabling responses required to complete a behavioral sequence.

In summary, our experiments have identified the specific source (M2) and target (dMSNs in the DLS) of a synaptic circuit that regulates SO performance and may be generally involved in other types of sequential actions. Our data demonstrate that the M2→dMSN synapse becomes stronger over the course of behavioral training, which may contribute to the chunking or concatenation of individual responses into a sequence, as well as persistent activation of the direct pathway as behavioral routines are performed. The ability to dissect these complex corticostriatal circuits is not only important for understanding the learning and performance of movement patterns under normal conditions but should also advance our understanding of movement disorders that impair sequential actions (Agostino et al., 1992; Benecke et al., 1987), as well as psychiatric diseases involving repetition of maladaptive routines and habits (Belin and Everitt, 2008; Burguière et al., 2015).

EXPERIMENTAL PROCEDURES

Animals

All procedures conformed to NIH Guidelines for the Care and Use of Laboratory Animals and were approved by the Stanford University Administrative Panel on Laboratory Animal Care. Experiments were performed on male littermate mice, at least 40 days old and maintained on a C57Bl/6J background; see Supplemental Experimental Procedures for additional details.

Behavioral Assays

Behavioral testing took place between 8 am and 6 pm, and all assays except the accelerating rotarod took place in standard mouse operant chambers (Schwartz et al., 2014). Food-restricted mice were first trained to retrieve 14 mg chocolate pellets from the food magazine. Operant training commenced on a fixed ratio one (FR1) schedule, with each instrumental response causing delivery of one pellet. For the SO task (Yin, 2009, 2010), mice had to perform two distinct and sequential responses ("A" then "B"), with four potential outcomes: AB (correct), BA (backward), AA (repeat first response), and BB (repeat second response). Correct AB sequences led to delivery of one pellet, and both correct and incorrect trials were followed by an 8-s inter-trial interval. Behavioral performance on the accelerating rotarod

(Rothwell et al., 2014), spatial discrimination/reversal (Gourley et al., 2010), and progressive ratio (Schwartz et al., 2014) was tested using published protocols; see Supplemental Experimental Procedures for details. Optogenetic stimulation in vivo was delivered with a 473 nm, 100 mW laser fitted to a 1 × 2 intensity splitter/rotary joint. Light intensity at cannula tips was verified at 3–5 mW prior to and following every session. Bilateral light pulses (5 ms) were delivered at frequencies ranging from 1 to 20 Hz, triggered during specific segments of the behavioral task.

Virus Production and Stereotaxic Surgery

Adeno-associated virus and rabies virus were produced using standard procedures (Dölen et al., 2013; Rothwell et al., 2014); see Supplemental Experimental Procedures for details. Stereotaxic surgery was performed under general ketamine-medetomidine anesthesia. A small volume of virus (0.5–0.75 μl) in a glass needle was infused at 0.1 μl/min through a craniotomy, and the injection needle was left in place for at least 5 min after the end of infusion before being withdrawn. For optogenetic stimulation, dual ferrule fiberoptic cannulas were lowered into position above the DLS and fixed in place with dental acrylic. Behavioral testing did not commence until at least 3 days after surgery.

Electrophysiology

Whole-cell voltage-clamp and current-clamp recordings from coronal brain slices (250 μm) containing the DLS or M2 were performed using standard procedures (Rothwell et al., 2014); see Supplemental Experimental Procedures for details. Pharmacologically isolated EPSCs were evoked at 0.1 Hz with a bipolar electrode or 1 ms pulses of blue light (~5 mW). AMPAR/NMDAR ratios were determined by comparing peak amplitude of the AMPAR EPSC at −80 mV, and the average NMDAR EPSC recorded 50–60 ms after stimulation at +40 mV.

Data Analysis

Experimental data were always compared to control data collected from littermate animals during the same time period. Data were analyzed using factorial ANOVA; between-subject factors included genotype, virus injection site, and training condition, while repeated-measures included session, stimulation frequency, and step with laser on. Significant main effects were followed by SNK post hoc tests, with symbols denoting statistical significance placed to the side of the last data point in the series or in parallel above corresponding data points in separate series. Significant interactions were decomposed using one-way ANOVA to test for simple effects, with symbols denoting statistical significance placed above individual data points. Type I error rate was set at $\alpha = 0.05$ (two-tailed) for all statistical comparisons, and all data are presented as mean ± SEM. Sample sizes are indicated in figure legends; pairwise comparisons that are not significant are not identified.

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2015.09.035.

AUTHOR CONTRIBUTIONS

P.E.R., S.J.H., and R.C.M. designed experiments. P.E.R. and S.J.H. performed and analyzed all experiments with contributions from G.L.S. for behavioral assays and M.V.F. for slice electrophysiology. B.K.L. provided important reagents. P.E.R., S.J.H, and R.C.M. wrote the paper with editorial input from all authors.

ACKNOWLEDGMENTS

We thank Dr. Elizabeth Steinberg for advice and all members of the Malenka lab for helpful discussions. This work was supported by CIHR and NSERC Postdoctoral Fellowships (to S.J.H.) and grants from the NIH (DA037279 to P.E.R., MH099243 to M.V.F., and DA008227 to R.C.M.).

Received: April 7, 2015
Revised: August 14, 2015
Accepted: September 11, 2015
Published: October 21, 2015

REFERENCES

Agostino, R., Berardelli, A., Formica, A., Accornero, N., and Manfredi, M. (1992). Sequential arm movements in patients with Parkinson's disease, Huntington's disease and dystonia. Brain *115*, 1481–1495.

Ahmad, M., Polepalli, J.S., Goswami, D., Yang, X., Kaeser-Woo, Y.J., Südhof, T.C., and Malenka, R.C. (2012). Postsynaptic complexin controls AMPA receptor exocytosis during LTP. Neuron *73*, 260–267.

Akil, H., Brenner, S., Kandel, E., Kendler, K.S., King, M.C., Scolnick, E., Watson, J.D., and Zoghbi, H.Y. (2010). Medicine. The future of psychiatric research: genomes and neural circuits. Science *327*, 1580–1581.

Aldridge, J.W., and Berridge, K.C. (1998). Coding of serial order by neostriatal neurons: a "natural action" approach to movement sequence. J. Neurosci. *18*, 2777–2787.

Bailey, K.R., and Mair, R.G. (2006). The role of striatum in initiation and execution of learned action sequences in rats. J. Neurosci. *26*, 1016–1025.

Bailey, K.R., and Mair, R.G. (2007). Effects of frontal cortex lesions on action sequence learning in the rat. Eur. J. Neurosci. *25*, 2905–2915.

Belin, D., and Everitt, B.J. (2008). Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. Neuron *57*, 432–441.

Benecke, R., Rothwell, J.C., Dick, J.P., Day, B.L., and Marsden, C.D. (1987). Disturbance of sequential movements in patients with Parkinson's disease. Brain *110*, 361–379.

Burguière, E., Monteiro, P., Feng, G., and Graybiel, A.M. (2013). Optogenetic stimulation of lateral orbitofronto-striatal pathway suppresses compulsive behaviors. Science *340*, 1243–1246.

Burguière, E., Monteiro, P., Mallet, L., Feng, G., and Graybiel, A.M. (2015). Striatal circuits, habits, and implications for obsessive-compulsive disorder. Curr. Opin. Neurobiol. *30*, 59–65.

Cao, V.Y., Ye, Y., Mastwal, S., Ren, M., Coon, M., Liu, Q., Costa, R.M., and Wang, K.H. (2015). Motor Learning Consolidates Arc-Expressing Neuronal Ensembles in Secondary Motor Cortex. Neuron *86*, 1385–1392.

Castañé, A., Theobald, D.E., and Robbins, T.W. (2010). Selective lesions of the dorsomedial striatum impair serial spatial reversal learning in rats. Behav. Brain Res. *210*, 74–83.

Cui, G., Jun, S.B., Jin, X., Pham, M.D., Vogel, S.S., Lovinger, D.M., and Costa, R.M. (2013). Concurrent activation of striatal direct and indirect pathways during action initiation. Nature *494*, 238–242.

Dang, M.T., Yokoi, F., Yin, H.H., Lovinger, D.M., Wang, Y., and Li, Y. (2006). Disrupted motor learning and long-term synaptic plasticity in mice lacking NMDAR1 in the striatum. Proc. Natl. Acad. Sci. USA *103*, 15254–15259.

Desrochers, T.M., Amemori, K., and Graybiel, A.M. (2015). Habit Learning by Naive Macaques Is Marked by Response Sharpening of Striatal Neurons Representing the Cost and Outcome of Acquired Action Sequences. Neuron *87*, 853–868.

Dölen, G., Darvishzadeh, A., Huang, K.W., and Malenka, R.C. (2013). Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. Nature *501*, 179–184.

Durieux, P.F., Schiffmann, S.N., and de Kerchove d'Exaerde, A. (2012). Differential regulation of motor control and response to dopaminergic drugs by D1R and D2R neurons in distinct dorsal striatum subregions. EMBO J. *31*, 640–653.

Erlich, J.C., Bialek, M., and Brody, C.D. (2011). A cortical substrate for memory-guided orienting in the rat. Neuron *72*, 330–343.

Fujii, N., and Graybiel, A.M. (2003). Representation of action sequence boundaries by macaque prefrontal cortical neurons. Science *301*, 1246–1249.

Gerfen, C.R., and Surmeier, D.J. (2011). Modulation of striatal projection systems by dopamine. Annu. Rev. Neurosci. *34*, 441–466.

Gerfen, C.R., Paletzki, R., and Heintz, N. (2013). GENSAT BAC cre-recombinase driver lines to study the functional organization of cerebral cortical and basal ganglia circuits. Neuron *80*, 1368–1383.

Gittis, A.H., Hang, G.B., LaDow, E.S., Shoenfeld, L.R., Atallah, B.V., Finkbeiner, S., and Kreitzer, A.C. (2011). Rapid target-specific remodeling of fast-spiking inhibitory circuits after loss of dopamine. Neuron *71*, 858–868.

Gourley, S.L., Lee, A.S., Howell, J.L., Pittenger, C., and Taylor, J.R. (2010). Dissociable regulation of instrumental action within mouse prefrontal cortex. Eur. J. Neurosci. *32*, 1726–1734.

Graybiel, A.M. (2008). Habits, rituals, and the evaluative brain. Annu. Rev. Neurosci. *31*, 359–387.

Guo, Z.V., Li, N., Huber, D., Ophir, E., Gutnisky, D., Ting, J.T., Feng, G., and Svoboda, K. (2014). Flow of cortical activity underlying a tactile decision in mice. Neuron *81*, 179–194.

Hodos, W. (1961). Progressive ratio as a measure of reward strength. Science *134*, 943–944.

Jin, X., and Costa, R.M. (2010). Start/stop signals emerge in nigrostriatal circuits during sequence learning. Nature *466*, 457–462.

Jin, X., Tecuapetla, F., and Costa, R.M. (2014). Basal ganglia subcircuits distinctively encode the parsing and concatenation of action sequences. Nat. Neurosci. *17*, 423–430.

Jurado, S., Goswami, D., Zhang, Y., Molina, A.J., Südhof, T.C., and Malenka, R.C. (2013). LTP requires a unique postsynaptic SNARE fusion machinery. Neuron *77*, 542–558.

Kauer, J.A., and Malenka, R.C. (2007). Synaptic plasticity and addiction. Nat. Rev. Neurosci. *8*, 844–858.

Koralek, A.C., Jin, X., Long, J.D., 2nd, Costa, R.M., and Carmena, J.M. (2012). Corticostriatal plasticity is necessary for learning intentional neuroprosthetic skills. Nature *483*, 331–335.

Koralek, A.C., Costa, R.M., and Carmena, J.M. (2013). Temporally precise cell-specific coherence develops in corticostriatal networks during learning. Neuron *79*, 865–872.

Kravitz, A.V., Freeze, B.S., Parker, P.R., Kay, K., Thwin, M.T., Deisseroth, K., and Kreitzer, A.C. (2010). Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. Nature *466*, 622–626.

Kreitzer, A.C., and Malenka, R.C. (2008). Striatal plasticity and basal ganglia circuit function. Neuron *60*, 543–554.

Lashley, K.S. (1951). The problem of serial order in behavior. In Cerebral Mechanisms in Behavior, L.A. Jeffress, ed. (Wiley), pp. 112–146.

Lerner, T.N., Shilyansky, C., Davidson, T.J., Evans, K.E., Beier, K.T., Zalocusky, K.A., Crow, A.K., Malenka, R.C., Luo, L., Tomer, R., and Deisseroth, K. (2015). Intact-Brain Analyses Reveal Distinct Information Carried by SNc Dopamine Subcircuits. Cell *162*, 635–647.

Lin, J.Y., Lin, M.Z., Steinbach, P., and Tsien, R.Y. (2009). Characterization of engineered channelrhodopsin variants with improved properties and kinetics. Biophys. J. *96*, 1803–1814.

Lin, C.W., Sim, S., Ainsworth, A., Okada, M., Kelsch, W., and Lois, C. (2010). Genetically increased cell-intrinsic excitability enhances neuronal integration into adult brain circuits. Neuron *65*, 32–39.

Lu, X., Matsuzawa, M., and Hikosaka, O. (2002). A neural correlate of oculomotor sequences in supplementary eye field. Neuron *34*, 317–325.

Luo, L., Callaway, E.M., and Svoboda, K. (2008). Genetic dissection of neural circuits. Neuron *57*, 634–660.

Murakami, M., Vicente, M.I., Costa, G.M., and Mainen, Z.F. (2014). Neural antecedents of self-initiated actions in secondary motor cortex. Nat. Neurosci. *17*, 1574–1582.

Oldenburg, I.A., and Sabatini, B.L. (2015). Antagonistic but Not Symmetric Regulation of Primary Motor Cortex by Basal Ganglia Direct and Indirect Pathways. Neuron *86*, 1174–1181.

Ostlund, S.B., Winterbauer, N.E., and Balleine, B.W. (2009). Evidence of action sequence chunking in goal-directed instrumental conditioning and its dependence on the dorsomedial prefrontal cortex. J. Neurosci. *29*, 8280–8287.

Reep, R.L., Corwin, J.V., Hashimoto, A., and Watson, R.T. (1987). Efferent connections of the rostral portion of medial agranular cortex in rats. Brain Res. Bull. *19*, 203–221.

Rothwell, P.E., Fuccillo, M.V., Maxeiner, S., Hayton, S.J., Gokce, O., Lim, B.K., Fowler, S.C., Malenka, R.C., and Südhof, T.C. (2014). Autism-associated neuroligin-3 mutations commonly impair striatal circuits to boost repetitive behaviors. Cell *158*, 198–212.

Schiffmann, S.N., and Vanderhaeghen, J.J. (1993). Adenosine A2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. J. Neurosci. *13*, 1080–1087.

Schreiweis, C., Bornschein, U., Burguière, E., Kerimoglu, C., Schreiter, S., Dannemann, M., Goyal, S., Rea, E., French, C.A., Puliyadi, R., et al. (2014). Humanized Foxp2 accelerates learning by enhancing transitions from declarative to procedural performance. Proc. Natl. Acad. Sci. USA *111*, 14253–14258.

Schwartz, N., Temkin, P., Jurado, S., Lim, B.K., Heifets, B.D., Polepalli, J.S., and Malenka, R.C. (2014). Chronic pain. Decreased motivation during chronic pain requires long-term depression in the nucleus accumbens. Science *345*, 535–542.

Stuesse, S.L., and Newman, D.B. (1990). Projections from the medial agranular cortex to brain stem visuomotor centers in rats. Exp. Brain Res. *80*, 532–544.

Taverna, S., Ilijic, E., and Surmeier, D.J. (2008). Recurrent collateral connections of striatal medium spiny neurons are disrupted in models of Parkinson's disease. J. Neurosci. *28*, 5504–5512.

Thorn, C.A., Atallah, H., Howe, M., and Graybiel, A.M. (2010). Differential dynamics of activity changes in dorsolateral and dorsomedial striatal loops during learning. Neuron *66*, 781–795.

Wall, N.R., De La Parra, M., Callaway, E.M., and Kreitzer, A.C. (2013). Differential innervation of direct- and indirect-pathway striatal projection neurons. Neuron *79*, 347–360.

Wickersham, I.R., Finke, S., Conzelmann, K.K., and Callaway, E.M. (2007). Retrograde neuronal tracing with a deletion-mutant rabies virus. Nat. Methods *4*, 47–49.

Xiong, Q., Znamenskiy, P., and Zador, A.M. (2015). Selective corticostriatal plasticity during acquisition of an auditory discrimination task. Nature *521*, 348–351.

Yin, H.H. (2009). The role of the murine motor cortex in action duration and order. Front. Integr. Nuerosci. *3*, 23.

Yin, H.H. (2010). The sensorimotor striatum is necessary for serial order learning. J. Neurosci. *30*, 14719–14723.

Yin, H.H., Mulcare, S.P., Hilário, M.R., Clouse, E., Holloway, T., Davis, M.I., Hansson, A.C., Lovinger, D.M., and Costa, R.M. (2009). Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. Nat. Neurosci. *12*, 333–341.

Yizhar, O., Fenno, L.E., Davidson, T.J., Mogri, M., and Deisseroth, K. (2011). Optogenetics in neural systems. Neuron *71*, 9–34.