Report

Neuron

Antagonistic but Not Symmetric Regulation of Primary Motor Cortex by Basal Ganglia Direct and Indirect Pathways

Highlights

- The classic model of cortical control by basal ganglia is tested
- Striatal direct and indirect pathways upregulate and downregulate cortex 2-fold within 0.2 s
- Counterintuitively, indirect pathway upregulates a subset of cortical neurons
- Intriguingly, cortical modulation by basal ganglia is weakest during periods of movement

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In Brief

Oldenburg and Sabatini demonstrate the push-pull antagonistic control of cortical activity by direct and indirect striatal pathways. However, additional non-opposing effects on cortex are revealed, whereby subsets of cortical neurons are independently regulated by each pathway in different contexts.

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Antagonistic but Not Symmetric Regulation of Primary Motor Cortex by Basal Ganglia Direct and Indirect Pathways

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SUMMARY

Motor cortex, basal ganglia (BG), and thalamus are arranged in a recurrent loop whose activity guides motor actions. In the dominant model of the function of the BG and their role in Parkinson's disease, direct (dSPNs) and indirect (iSPNs) striatal projection neurons are proposed to oppositely modulate cortical activity via BG outputs to thalamus. Here, we test this model by determining how striatal activity modulates primary motor cortex in awake head-restrained mice. We find that, within 200 ms, dSPN and iSPN activation exert robust and opposite effects on the majority of cortical neurons. However, these effects are heterogeneous, with certain cortical neurons biphasically modulated by iSPN stimulation. Moreover, these striatal effects are diminished when the animal performs a motor action. Thus, the effects of dSPN and iSPN activity on cortex are at times antagonistic, consistent with classic models, whereas in other contexts these effects can be occluded or coactive.

INTRODUCTION

The basal ganglia (BG) are an interconnected group of subcortical nuclei that regulate movements and whose dysfunction contributes to multiple disorders (Albin et al., 1989; DeLong, 1990; Graybiel et al., 1994). Classical models of the motor BG describe a looped architecture in which motor cortex sends glutamatergic inputs to the striatum, the input stage of the BG, and is in turn influenced by the BG through inhibitory output to thalamus. The two output pathways of the striatum, comprised of direct (dSPNs) and indirect (iSPNs) pathway striatal projection neurons, are thought to exert push-pull control over primary motor cortex (M1) by either increasing or reducing its activity to promote or suppress motor action. The anatomical substrates that mediate these antagonistic effects are thought to be the divergent GABAergic striatonigral and striatopallidal projections of dSPNs and iSPNs, respectively (Alexander and Crutcher, 1990; Deniau and Chevalier, 1985). The striatonigral projection inhibits the substantia nigra pars reticulata (SNr), whereas the striatopallidal projection inhibits the external segment of the globus pallidus (GPe). The GPe in turn inhibits SNr, making the net effect of iSPN activity to SNr excitatory (Gerfen et al., 1990). SNr provides GABAergic innervation of the ventrolateral thalamus (VL), which closes the loop via glutamatergic projections to cortex. This anatomical model explains the contributions of the BG to motor control, as well as the mechanisms by which symptoms of Parkinson's disease are ameliorated by deep brain stimulation (Da Cunha et al., 2015) and is supported by lesion and pharmacological (Mink, 1996) as well as genetic and optogenetic (Bateup et al., 2010; Kravitz et al., 2010) studies.

Nevertheless, many features of this model have not been tested and are difficult to predict. The magnitude, kinetics, and homogeneity of a cortical response depend on many factors, including the fraction of cortical activity that is driven by striatum-regulated thalamic inputs, the degree of tonic inhibition in the thalamus from ongoing SNr activity, and the speed with which cascading inhibitory networks disinhibit the thalamus and cortex. Many of these anatomical and functional parameters have not been determined, leaving fundamental aspects of the classic model of BG/cortical interactions untested and unconstrained.

Here we examine the control of cortex by striatum in awake, head-restrained mice. The effects of optogenetic manipulations of dSPN or iSPN firing on primary motor cortex were evaluated as mice performed a simple cued lever-pressing task for water reward. At the level of populations of cortical neurons, our results generally support classic models of BG-cortical interactions. However, individual neurons can have heterogeneous, asymmetric, and context-dependent responses to manipulation of striatal activity, highlighting the existence of BG pathways by which dSPNs and iSPNs can have selective and non-antagonist effects on distinct cortical neurons.

RESULTS

Studies of interactions between BG and cortex require analysis in awake animals as striatal activity is minimal under anesthesia (Mahon et al., 2006; Spampinato et al., 1986). Therefore, mice expressing Cre recombinase in either iSPNs (*Adora-2A-Cre*) or dSPNs (*Drd1a-Cre*) (Figures 1 and S1A) and injected with Cre-dependent adeno-associated virus (AAV) encoding ChR2 were habituated to head restraint. Mice were trained on a cued lever-pressing task in which a motor action carried out shortly after an auditory cue led to a water reward (Figures 1A, S1B, and S1C; see Supplemental Experimental Procedures). In trained

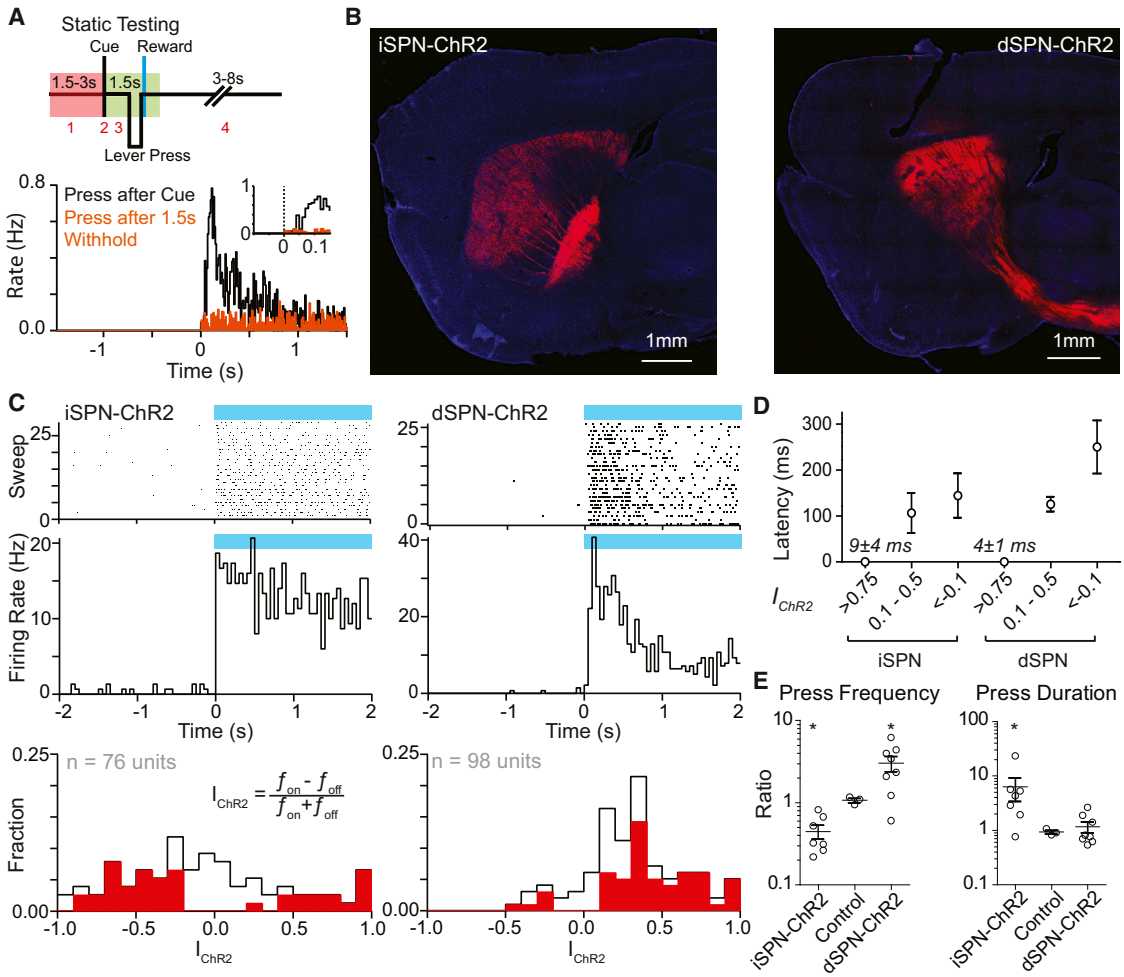

Figure 1. Channelrhodopsin-Mediated Modulation of Striatum

(A) Schematic of task design (top). A trial starts with an uncued 1.5- to 3-s withhold period (red). If the animal does not press the lever during this time, a 10-kHz tone is presented (vertical black line), which is followed by 1.5-s potential reward period (green). If the animal presses and releases the lever during this period, it receives a water reward (blue line). This is followed by inter-trial delay (3–8 s) during which presses are neither rewarded nor punished. (Bottom) Lever press rates during recording sessions (n = 20, eight mice) for periods of 1.5 s without lever presses (t = −1.5 to 0 s) that ended (t = 0) with (black) or without (orange) the cue. (Inset) Finer timescale analysis (10-ms bins) shows that press rates diverge across conditions after ~50 ms.

(B) Sagittal slices showing ChR2 expression (red) following injection of Cre-dependent ChR2-mCherry encoding AAV in mice that express Cre in iSPNs or dSPNs.

(C) (Top/middle) Example raster plots and histograms of activity of highly modulated units from iSPN-ChR2 (left) and dSPN-ChR2 (right) animals. Blue = 473-nm illumination. (Bottom) Histogram of I_{ChR2} for recorded units. Red indicates statistically significantly modulated units (t test, $p < 0.05$, iSPN 35 of 76 units; dSPN 57 of 98).

(D) Latency to modulation of striatal units. $I_{ChR2} > 0.75$: iSPN n = 7 units; dSPN n = 8; I_{ChR2} 0.1–0.5: iSPN 106 ± 44 ms, n = 8; dSPN 125 ± 16 ms, n = 49; $I_{ChR2} < -0.1$: iSPN 144 ± 48 ms, n = 9; dSPN 250 ± 58 ms, n = 3. All units with latency <500 ms are included. Error bars are ± SEM.

(E) ChR2-induced changes in behavior for iSPN-ChR2 (n = 7), dSPN-ChR2 (n = 8), or ChR2-negative control (n = 3) mice. Relative lever press rates (left) and durations (right) are the ratios of each metric with and without stimulation (*$p < 0.05$, Wilcoxon signed rank).

mice, lever presses occurred preferentially after tones with press rate 2.75-fold ± 0.53-fold higher in the reward period compared with similarly structured uncued periods (Figure 1A; $p < 0.01$ Wilcoxon signed rank).

Mice that reached behavioral proficiency were implanted with a fiber optic, and analysis of the effects of ChR2 stimulation was examined on a recording rig. The stimulating laser was on or off continuously for each trial and switched to the opposite state such that transitions occurred in intervals well separated (3–8 s) from the reward and at least 1.5 s before a tone. Multielectrode array recordings in striatum confirmed effective optogenetic manipulation (Figure 1C). The degree of modulation of each unit was calculated as follows:

$$I_{ChR2} = \frac{f_{on} - f_{off}}{f_{on} + f_{off}}, \qquad \text{(Equation 1)}$$

with f_{on} and f_{off} corresponding to average firing rates with the laser on and off, respectively, during a 1.5-s period prior to the delivery of the cue where the animal does not press the lever.

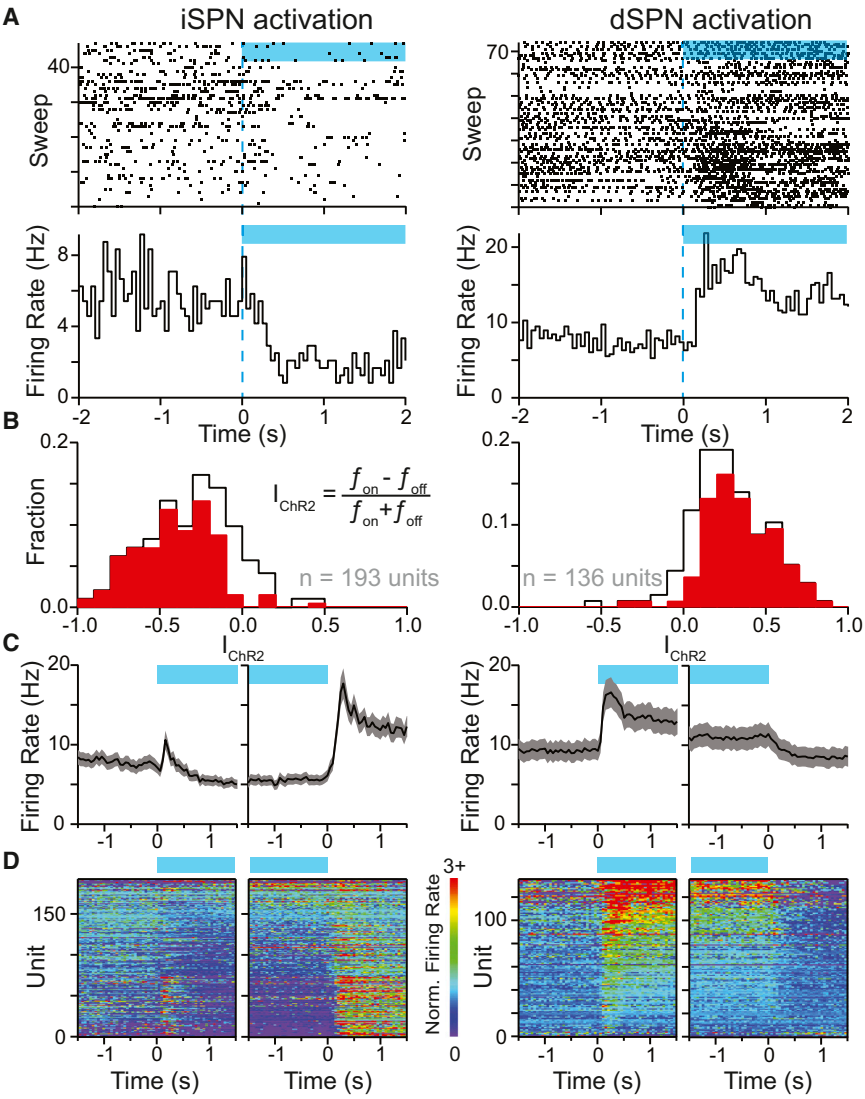

Figure 2. Antagonistic Modulation of Primary Motor Cortex by Direct and Indirect Pathways

(A) Activation of iSPNs decreases (left) and dSPNs increases (right) firing rates in motor cortex. Example raster plots (top) and histograms (bottom) of activity of cortical units prior to and during optogenetic stimulation of striatum (blue).

(B) I_{ChR2} of cortical unit modulation with iSPN or dSPN stimulation. Red indicates statistically significantly modulated units (iSPN 136/193, 4 mice; dSPN 103/136, 4 mice; t test, $p < 0.05$).

(C) Mean firing rate of cortical neurons at the start and end of ChR2-stimulation (blue) of iSPNs (left) and dSPNs (right). Gray is ± SEM.

(D) Pseudo-colored plots of firing of all units normalized to rates in baseline period and ordered by I_{ChR2} (low to high). Blue/purple and yellow/red represent relatively decreased and increased rates.

ChR2 stimulation modulated striatal neurons with I_{ChR2} distributed over most of its −1 to 1 range. Optogenetic stimulation increased firing rates in 39% (30/76) and 87% (85/98) of units when activating iSPNs and dSPNs (Figure 1C), respectively, presumably through a combination of direct activation and network effects. In each condition, ~10% had $I_{ChR2} > 0.75$ (iSPN experiments: seven units; dSPN: nine). These putative ChR2-expressing units had low basal firing rates and responded with short latency to light. Units with intermediate activation had higher basal firing rates and responded more slowly (Figures 1D, S1D, and S1E). Significant inhibition of SPNs was rare following activation of dSPNs (4 units) and more common following iSPN activation (27 units) (Figure 1C). Such inhibition could result from SPN to SPN GABAergic synapses as well as from long-range circuit effects (see below).

SPN activity was modulated by the task. SPNs had high press-related modulation indices (I_{press}), calculated by comparing activity in ±0.25 s around a spontaneous lever press to non-press periods (iSPN experiments: I_{press} = 0.21 ± 0.04; dSPN: 0.26 ± 0.07). Furthermore, stimulation of iSPNs and dSPNs bidirectionally modulated lever press frequency (ratio of frequency with light on versus off: iSPN 0.45 ± 0.09, n = 7 mice, $p < 0.05$; dSPN 3.1 ± 0.66, n = 8, $p < 0.05$; Wilcoxon signed rank), whereas control mice showed no significant modulation (1.1 ± 0.06, n = 3). The duration of lever presses increased with activation of iSPNs but not dSPNs (iSPN: 6.3 ± 2.9-fold change, $p < 0.05$; dSPN: 1.2 ± 0.27, not significant; control: 0.93 ± 0.07, not significant; Wilcoxon signed rank; Figure 1E).

Effects of dSPN and iSPN Activation on Motor Cortex

To determine the effects of striatal activity on cortex, we inserted multielectrode arrays in the forepaw region of primary motor cortex (M1) contralateral to the lever and ipsilateral to the stimulated striatum (Figure S2A). The stereotaxic location of forepaw was confirmed via microstimulation in anesthetized mice (Figure S2B). Furthermore, activity in this area is necessary for the task as focal injection of GABA transiently impaired performance (Figure S2C) and is sufficient, using receiver-operator characteristic analyses, to predict the timing of spontaneous lever presses (area under curve = 0.86 ± 0.02, n = 8 mice).

Firing rates of M1 neurons were compared with and without optogenetic stimulation during a 1.5-s "baseline" period that ended with the tone and lacked lever presses, auditory cues, and rewards. Consistent with classical models, activation of iSPNs reduced the firing rates of ~70% of units (Figures 2A and S2D): of 193 units (n = 4 mice), the firing rates of 136 were significantly changed with 132 inhibited and 4 excited ($p <$ 0.05, two-tailed t tests on alternating trials). The population firing rate was reduced with a modulation index (I_{ChR2}) of −0.31

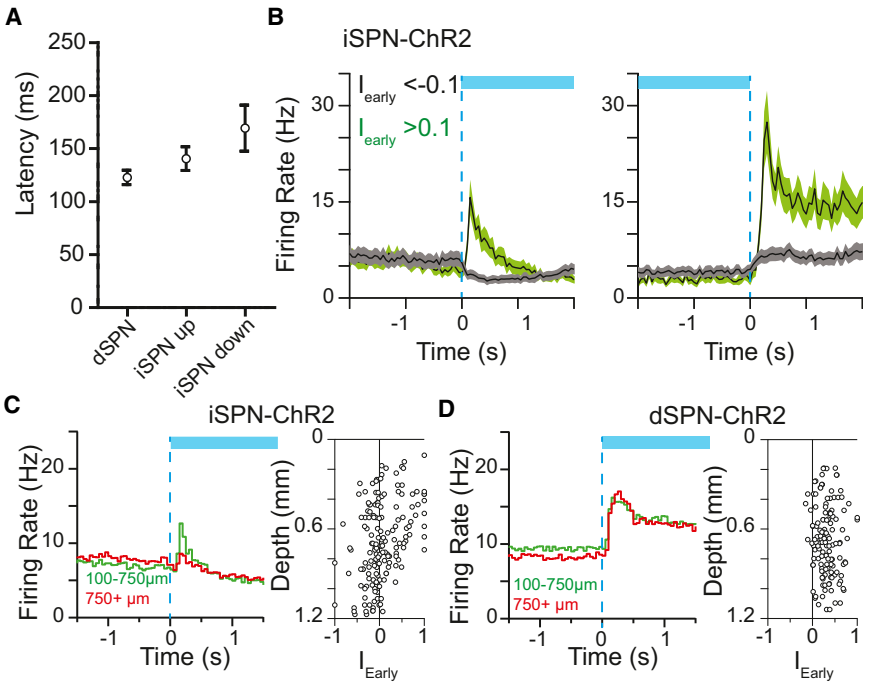

Figure 3. Transient Activation of Motor Cortex by the Indirect Pathway

(A) Latency of cortical response to striatal activation. All units with latency less than 500 ms are included. dSPN: 123 ± 7 ms, n = 125 units; iSPN transient activation: 141 ± 11 (n = 90); iSPNs without transient activation: 169 ± 22 (n = 44). Error bars are ± SEM.

(B) Average firing of cortical units separated into those transiently inhibited (black) or excited (green) by iSPN activation (blue bar). Shaded represents ± SEM.

(C) Average firing of cortical units (left) reveals greater transient activity in superficial (green) than in deeper (red) cortical units. I_{early} plotted as a function of depth from the pia (right).

(D) Similar analysis as in (C) for manipulation of dSPNs.

corresponding to a ~50% decrease (Figure 2B; $p < 0.0001$, matched pairs signed rank).

Conversely, with optogenetic manipulation of dSPNs, activity increased in ~75% of M1 units (Figures 2A and S2D). Of 136 units (n = 4 mice), 103 significantly changed firing rates with 100 excited and 3 inhibited ($p < 0.05$, two-tailed t test on alternating trials). dSPN activation significantly increased the population firing rate with a modulation index (I_{ChR2}) of 0.28 (Figure 2B; $p < 0.0001$), corresponding to an ~80% increase. The average baseline firing rates in M1 were the same for iSPN and dSPN experiments (iSPN 9.6 ± 0.99 Hz, dSPN 9.0 ± 1.3, not significant, Mann Whitney). No robust, consistent change in the pairwise correlations across M1 units was observed due to activation of either pathway (Figure S2E).

Manipulations of iSPNs and dSPNs significantly modulated the majority of M1 neurons recorded. However, in each case, a fraction of neurons was not significantly affected (iSPN experiments: ~30%; dSPN: 25%); percentages were larger than expected from false-negative rates based on power analyses and confidence intervals (Figure S2D; Experimental Procedures), suggesting the existence of intermingled cortical cells whose activity is insensitive to the manipulations delivered to striatum.

Kinetics of Striatal Modulation of Cortex

Modulation of cortex by striatum involves inhibition and disinhibition in a polysynaptic circuit that consists of cascading spontaneously active GABAergic projection neurons. Increasing the activity of downstream structures occurs via relief of tonic inhibition, a process whose kinetics is limited by the firing rates of intermediary neurons. We found that the latency for significant alterations in activity of M1 units by striatal activation was 123 ± 7 and 169 ± 21 ms following activation of dSPNs and iSPNs, respectively.

Unexpectedly, immediately following ChR2-activation of iSPNs average M1 activity increased before decreasing (Figures 2C and 2D), an effect due to a transient increase in firing rates in a subset (59/193) of cells. These units responded at an intermediate latency (140 ± 11 ms; Figure 3A). To identify the transiently upregulated units, we calculated modulation index I_{early} comparing the firing rates 0.5 s before and after laser activation and examined units with $I_{early} > 0.1$ or $I_{early} < -0.1$ (Figure 3B). Units with $I_{early} > 0.1$, found in nearly all recordings, were transiently activated at both light on and off (Figures 3B and S3A–S3C). Within 0.5–1 s, these neurons decreased firing rates, such that the overall I_{ChR2} was negative.

Units with positive I_{early} were detected at electrode sites shallower than those with negative I_{early} (579 ± 29 versus 874 ± 40 µm, $p < 0.0001$, Mann Whitney; Spearman's correlation $r_s = -0.41$, $p < 0.0001$; Figures 3C and S3D). Conversely, average I_{early} was positive (0.19 ± 0.05, n = 93) for shallow units (100–750 µm) and negative (−0.12 ± 0.03, n = 83) for deep units (>750 µm), indicating that transient activation following iSPN stimulation is more likely in superficial cortical layers. Whereas a difference in I_{early} was apparent as a function of depth following iSPN activation, no similar phenomenon was seen with dSPN activation (Figures 3D and S3E–S3H).

Effects of dSPN and iSPN Activation on Motor Cortex during Movements

We separately examined the effects of striatal manipulations on M1 during different aspects of the task, following the tone alone (i.e., when the animal failed to press the lever) and during spontaneous presses (uncued lever presses outside of the reward period). As in the baseline periods, ChR2 activation of iSPNs decreased the firing rates of M1 units at the time of an uncued press or in tone-only trials (Figure 4A; $p < 0.0001$ Wilcoxon matched-pairs signed rank). However, the degree of inhibition was weaker in the ±0.25 s surrounding uncued presses than during the baseline ($I_{ChR2} = -0.31 \pm 0.02$ versus $I_{ChR2\ press} = -0.06 \pm 0.02$,

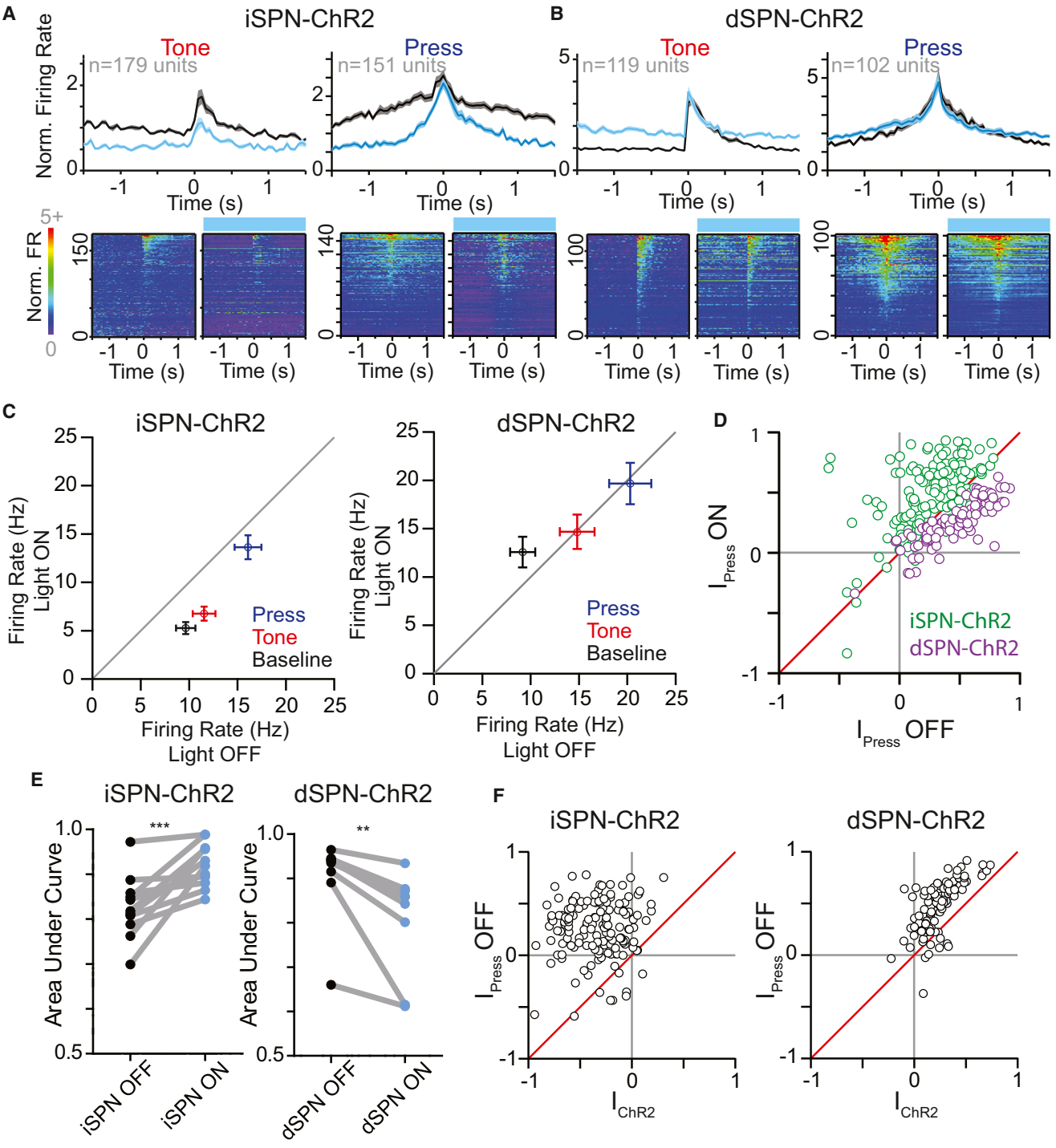

Figure 4. Differential Effects of Striatal Activation on Cortex

(A and B) Average rates of cortical units (top) normalized to basal firing aligned either to the time of a tone in failure trials or of a spontaneous lever press outside of the reward period. Units with >30 spikes in the baseline periods for each event class (press or tone) were included. Trials with optogenetic activation of iSPNs (A) or dSPNs (B) are in blue and without in gray. Shading shows ± SEM. (Bottom) Individual units' normalized firing rates presented as a pseudo-colored plot (as in Figure 2D) and ordered by the press or tone modulation index (low to high), without (left) or with (right) optogenetic activation.

(C) Average firing rates of units during the 1.5 s baseline, ± 0.25 s around a press, or 0.5 s after a tone with (y axis) and without (x axis) iSPN (left) or dSPN (right) activation. Error bars = SEM. iSPN activation (left) decreased firing rates for Baseline (9.6 ± 1.0 Hz off, 5.5 ± 0.6 on, n = 193, $p < 0.0001$), Tone (11.5 ± 1.2 off, 6.8 ± 0.7 on, n = 193, $p < 0.0001$), and Press (16.1 ± 1.4 off, 13.6 ± 1.2 on, n = 179 $p < 0.0001$). dSPN activation (right) increased firing rates for Baseline (9.0 ± 1.3 off,

(legend continued on next page)

$p < 0.0001$, Kruskal-Wallis (KW) statistic: 512.7, KW with Dunn's multiple comparison; 132/193 units inhibited in baseline versus 47 during movement), but unchanged during tone-only trials ($I_{ChR2\ tone\text{-}only} = -0.24 \pm 0.02$, $p > 0.05$; 132 units inhibited during baseline versus 118 during cue; Figure S4D). Trials containing both tones and presses (i.e., success trials) revealed an intermediate response to optogenetic stimulation (Figures S4A and S4B).

Unlike the indirect pathway and in striking contrast to the baseline period, ChR2 activation of dSPNs did not affect firing rates during uncued presses and tone-only trials (Figures 4B and S4E; $I_{ChR2\ press} = 0.02 \pm 0.02$, $I_{ChR2\ tone\text{-}only} = 0.00 \pm 0.02$, $n = 136$ units, $p > 0.05$, Wilcoxon matched-pairs signed rank). The lack of elevated firing rates was not due to a ceiling effect, as the rates during tone-only trials, with or without light, were significantly less than during uncued presses and success trials (Figure 4C; tone-only 14.8 ± 1.8 Hz versus press 22.6 ± 2.4, $p < 0.0001$ Wilcoxon matched-pairs signed rank).

In order to determine the effect of optogenetic manipulation on the dynamic activity of cortical units, indices corresponding to activity during aspects of the task were analyzed. I_{press} was calculated as above, and as expected in primary motor cortex, individual units were strongly modulated during spontaneous presses (iSPN $I_{press} = 0.31 \pm 0.02$; dSPN 0.45 ± 0.03; Figure S4F). Similarly, I_{tone} and $I_{success}$ were calculated for the activity in tone-only (i.e., failure) and success trials, comparing the baseline activity to that in the 0.5-s period after the tone (iSPN $I_{tone} = 0.04 \pm 0.02$, $I_{success} = 0.22 \pm 0.02$; dSPN $I_{tone} = 0.32 \pm 0.02$, $I_{success} = 0.37 \pm 0.04$; each > 0 with $p < 0.05$, Mann Whitney).

In the simplest analysis, the motor character, or tuning, of individual units is unaffected by manipulation of each pathway in the striatum: in both sets of experiments, I_{press} measured without and with striatal activation are correlated (Spearman's r_s: iSPN, 0.49; dSPN, 0.83, $p < 0.0001$; Figure 4D). Thus, units that significantly changed activity at the times of uncued presses without optogenetic stimulation continued to do so with stimulation. Furthermore, I_{press} of individual units generally increased with activation of iSPN and decreased with activation of dSPN, an effect that was also clear at the population level (Figures 4D and S4G).

Such changes suggest that the ability of an observer to predict the onset of a spontaneous movement based on activity in M1 is enhanced by activation of iSPNs and degraded by that of dSPNs. Indeed, a population spike count threshold model revealed such effects when analyzed by receiver-operator characteristics (ROCs). In this model, presses are generated at periods of high population firing above a threshold (Figure S4H; see Supplemental Experimental Procedures) with no time dependence. This model generated good predictions of movement onset with area under the curve (AUC) values of 0.83 ± 0.02 and 0.90 ± 0.03 for iSPN and dSPN experiments. Upon optogenetic activation of iSPNs, AUC increased in nearly every recording (to 0.92 ± 0.01 with iSPN activation, 11 recordings, $p < 0.01$, Wilcoxon matched pairs signed rank). Conversely, upon dSPN activation, AUC decreased in every recording (to 0.81 ± 0.04 with dSPN activation, 9 recordings, $p < 0.001$; Figures 4E and S4I).

Given the observed changes in M1, we examined the possibility that BG exert selective control over distinct cells in motor cortex. For each unit, we compared the modulation of firing during presses (I_{press}) to its modulation by BG activation (I_{ChR2}). The degree of modulation of each unit by activation of the iSPNs was not predictive of the degree of modulation of the unit by spontaneous movements—i.e., I_{press} and I_{ChR2} showed no correlation (Figure 4F; Spearman's $r_s = -0.003$, not significant). In contrast, activation of dSPNs increased the basal activity of neurons in M1 that were more active at the time of the press—I_{press} and I_{ChR2} were highly correlated ($r_s = 0.71$, $p < 0.01$). In effect, dSPN activation preferentially modulates M1 neurons that are active during movements, a specificity that is not seen following activation of iSPNs, suggesting that the motor cortex neurons most sensitive to the activity of iSPNs are not the same as those most highly regulated by dSPN activity.

DISCUSSION

Our results demonstrate that in habituated, head-restrained mice activation of the iSPNs and dSPNs suppresses and enhances, respectively, firing rates of units in motor cortex, consistent with classic models of BG/cortical interactions. However, the effects are spatiotemporally heterogeneous, and three surprising findings emerge that are not immediately predicted by classic models. First, iSPN activation unexpectedly excites a subpopulation of superficial M1 cells such that both dSPN and iSPN activity can be at least transiently excitatory. Second, the task-related activity of neurons that are highly sensitive to dSPN stimulation is different than that of neurons highly sensitive to iSPN stimulation. These two findings indicate that the subsets of neurons in primary motor cortex regulated by each pathway are at least partially non-overlapping, highlighting the existence of separate routes by which dSPNs and iSPNs can modulate cortical activity. Third, trained movements and cues reduce or prevent the effects of dSPN activation on motor cortex activity, but have relatively little influence over the effects of iSPN activation. These differences underlie the non-intuitive result that the ability of an ideal observer to predict the timing of spontaneous movements based on analysis of total activity in primary motor cortex is enhanced by iSPN and degraded by dSPN stimulation.

12.7 ± 1.6 on, $n = 136$, $p < 0.0001$), but not Tone (14.8 ± 1.8 off, 14.7 ± 1.8 on, $n = 136$, $p > 0.05$), or Press (20.3 ± 2.2 Hz off, 19.7 ± 2.1 on, $n = 136$, $p > 0.05$). Wilcoxon matched pairs signed rank.

(D) I_{press} calculated with (I_{press}ON) and without (I_{press}OFF) ChR2 stimulation of iSPNs (green) or dSPNs (purple) are strongly correlated ($p < 0.0001$; Spearman's r_s: iSPN 0.49; dSPN 0.83).

(E) Changes in the ability of an observer to identify movements based on total cortical activity were measured with ROC analysis. Resulting area-under-curve values with and without iSPN (left) and dSPN (right) activation for each recording session.

(F) I_{press}OFF and I_{ChR2} of cortical units are not correlated for iSPN activation (left, $p > 0.05$; Spearman's $r_s = -0.003$) but are highly correlated for dSPN activation (right, $p < 0.0001$; Spearman's $r_s = 0.710$).

Classic Models

dSPN and iSPN activation caused opposite ~3-fold changes in spontaneous lever press frequencies, and iSPN activation increased lever press duration, a freezing-like behavior. Such effects are consistent with classic models of direct/indirect pathway functions (Albin et al., 1989) and of Parkinson's disease (Marsden, 1982), as well as with recent studies in mice (Bateup et al., 2010; Kravitz et al., 2010). Furthermore, activation of dSPNs and iSPNs respectively increases and decreases firing rates in primary motor cortex during periods when the mice are not exposed to any of the overt task features—i.e., no lever presses, cues, or reward. The effects on M1 activity during this period are strong (~2-fold modulation), widespread (>70% units showing significant modulation), and consistent with predicted antagonistic effects of each striatal pathway.

Within the context of classical models, our results provide evidence in favor of assumptions about activity in the cortex-BG-thalamus recurrent loop that are often not directly stated but that are nevertheless assumed. For example, in this model and in order for the dSPNs and iSPNs to bidirectionally modulate cortical activity, it is necessary that SNr output provide tonic inhibition of the thalamus that is significant but not saturated. Although SNr output neurons are tonically active, synaptic depression during maintained high-frequency firing might diminish the inhibitory influence of BG output on thalamus. Furthermore, in order to translate changes in BG output into alterations of basal firing rates in cortex, thalamocortical projection neurons need to both supply sufficient ongoing activity to account for a significant fraction of cortical excitatory drive and be under the control of BG. Thus, when coupled with the behavioral effects described above, bidirectional modulation of basal firing rates in primary motor cortex by dSPN and iSPN activation supports the classic model of BG function and its foundational assumptions.

Beyond Classic Models

The simple classification of dSPNs and iSPNs as pro-kinetic and anti-kinetic pathways, respectively, does not fully account for the activities of these cells in behaving mice since neurons of both classes are active during both the initiation and suppression of movements (Cui et al., 2013; Isomura et al., 2013). Furthermore, in monkeys, BG activity is concurrent or delayed relative to movement initiation, suggesting a function in shaping but not necessarily initiating motor action and associated circuit activity (Aldridge et al., 1980; Hikosaka et al., 1989; Mink and Thach, 1991). Resolving these issues requires knowledge of the kinetics of effects of striatal activity on other brain structures. We find that activation of dSPNs or iSPNs modulates M1 activity with ~150-ms latencies (average ~120 dSPN, ~165 iSPN), with some cells responding in less than 50 ms. This is slower than striatal modulation of SNr (Freeze et al., 2013), consistent with the presence of two additional synapses between SNr and cortex. Given the short latency of cortically evoked action potentials in striatum (Koralek et al., 2012), a complete closed loop interaction from cortex to BG and back likely can occur in less than 200 ms. Such recurrent effects may explain the large fraction of striatal neurons that are inhibited by activation of iSPNs (Figure 1C), which likely indirectly suppresses corticostriatal projections and decreases striatal activity with a delay.

The results presented here reveal a complex dynamic response in cortex to striatal activation that violates the predicted symmetric effects of dSPN and iSPN activity. Although iSPN activation reduces baseline and peak activity in M1 evoked by cues and cued lever presses, activation of dSPNs has no effect on peak firing rates in these periods. This finding cannot be ascribed to a ceiling effect in M1 firing rates, as the peak-firing rate reached during tone-only (failure) trials is well below maximal, yet still unaffected by activation of dSPNs. An alternative explanation is that dSPNs, or circuit elements downstream of dSPNs, are maximally active during the movement such that optogenetic stimulation of dSPNs has no further effect on M1 activity patterns. Such an explanation would also imply that iSPNs are comparatively less active during these periods than dSPNs, which at first may appear in conflict with results observing movement related activity from both iSPNs and dSPNs in vivo (Cui et al., 2013; Isomura et al., 2013). However, this may be reconciled by the greater sustained activity of dSPN compared with iSPNs reported during movement bouts (Jin et al., 2014). Indeed, in motor cortex, we detect larger effects of iSPN activation before the lever press than during the movement itself, indicating that some iSPN activation may be present during the presses.

Two results reveal that not all cortical neurons are equally sensitive to changes in striatal activity, suggesting specificity in either the thalamic target of the BG or of the subcortical to cortical projections. First, although changing iSPN or dSPN activity modulated the vast majority of M1 units, a fraction (8%–20% depending on the statistical model, see Supplemental Experimental Procedures) was insensitive to the optogenetic manipulations of striatum. Although we cannot rule out that different, unstimulated regions of striatum could modulate these cells, such an explanation would still indicate that neighboring cortical cells are differentially sensitive to non-neighboring regions of striatum.

Second, while the majority of cortical neurons monotonically decrease firing following iSPN activation and increase back to baseline levels upon cessation of iSPN stimulation, ~30% of neurons are transiently excited for ~500 ms following stimulation of iSPNs. These units subsequently reduce their firing rate despite maintained iSPN stimulation and rebound strongly upon cessation of iSPN stimulation. We were unable to find an analogous class of units that behaved anomalously to initiation or cessation of dSPN stimulation. It is of particular interest that the transiently excited cells appear in predominantly superficial layers. Thalamocortical axons from the VLo, and that are thus likely modulated by the BG, primarily innervate superficial layers (Kuramoto et al., 2009; McFarland and Haber, 2002) (although see Constantinople and Bruno, 2013).

At the population level, differences between the cortical effects of iSPN and dSPN activation were also evident. Whereas a strong correlation was observed between each neuron's modulation by dSPN activation and its lever press related change in firing rate, no similar correlation was found when iSPNs were activated. This is especially intriguing as the iSPN and dSPN projections target the same neurons in SNr (Smith and Bolam, 1991), and thus, it is difficult to explain differential effects on cortex via a

common output. This may again reflect the existence of functional subsets within the outputs of the BG that are differentially dependent on iSPN and dSPN activity (Saunders et al., 2015).

Conclusions

The results we report here support many predictions of classical models of BG/cortex interactions such that the BG exert strong, push-pull control over motor cortex in behaving mice prior to presentation of a reward-associated cue. However, the classic model fails to account for the effects of striatal manipulations when the animals make spontaneous lever presses and for the asymmetric effects of direct and indirect pathway activation on cortex. Our results suggest the existence of circuitry, either within nuclei downstream of striatum or between the BG and cortex, which allow differential and non-opposing effects of dSPNs and iSPNs on cortex.

EXPERIMENTAL PROCEDURES

Mice expressing Cre recombinase in iSPNs or dSPNs were injected with virus expressing ChR2 in striatum (0.9A, 1.7L, 2.8D) and surgically implanted with a headpost. Mice were trained in an operant task to press a lever after a tone (50 ms, 10 kHz). ChR2 stimulation (continuous 1.5–3 mW) through an implanted fiber optic occurred during extracellular recordings in motor cortex. Full experimental procedures are in the Supplemental Information.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2015.05.008.

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