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Striatal D1 medium spiny neuron activation induces dyskinesias in parkinsonian mice

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Abstract

Background—Dyskinesias are a disabling motor complication that arises with prolonged L-dopa treatment. Studies using D1 receptor drugs and genetically modified mice suggest that medium spiny neurons expressing D1 receptors play a primary role in L-dopa-induced dyskinesias. However, the specific role of these neurons in dyskinesias is not fully understood.

Methods—We used optogenetics, which allows for precise modulation of select neurons in vivo, to investigate whether striatal D1-expressing medium spiny neuron activity regulates abnormal involuntary movements or dyskinesia in parkinsonian mice. D1-cre mice unilaterally lesioned with 6-hydroxydopamine received striatal injections of cre-dependent channelrhodopsin2-virus or control-virus. After stable virus expression, the effect of optical stimulation on dyskinesia was tested in L-dopa-naïve and L-dopa-primed mice.

Results—Single pulse and burst optical stimulation of D1-expressing medium spiny neurons induced dyskinesias in L-dopa-naïve channelrhodopsin2 mice. In stably dyskinetic mice, L-dopa injection induced dyskinesia to a similar or somewhat greater extent than optical stimulation. Combined L-dopa administration and stimulation resulted in an additive increase in dyskinesias, indicating that other mechanisms also contribute. Molecular studies indicate that changes in extracellular signal-regulated kinase phosphorylation in D1-expressing medium spiny neurons are involved. Optical stimulation did not ameliorate parkinsonism in L-dopa-naïve mice. However, it improved parkinsonism in L-dopa-primed mice to a similar extent as L-dopa administration. None of the stimulation paradigms enhanced dyskinesia or modified parkinsonism in L-dopa-naïve or L-dopa-primed control-virus mice.

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Conclusion—The data provide direct evidence that striatal D1-expressing medium spiny neuron stimulation is sufficient to induce dyskinesias and contributes to the regulation of motor control.

Keywords

dyskinesia; levodopa; medium spiny neuron; D1 receptor

The striatal direct and indirect pathways play a major role in basal ganglia circuit function and motor control^{1–3}. GABAergic medium spiny neurons expressing D1 receptors (D1 MSNs) are the main striatal output of the direct pathway that projects to the substantia nigra pars reticulata (SNr)^{1, 2, 4}. D1 MSN stimulation inhibits SNr activity, thereby disinhibiting its output nuclei to promote movement^{5–7}. Conversely, activation of the indirect striatopallidal pathway, which comprises GABAergic MSNs expressing D2 receptors, reduces motor activity^{6, 8–10}. The loss of this bidirectional control of motor function makes MSNs primary players in the motor impairments observed in Parkinson's disease (PD) and the dyskinesias that arise with administration of the dopamine precursor L-dopa^{1, 4, 11, 12}.

A role for D1 MSNs in dyskinesias is supported by preclinical evidence showing that dopamine cell loss increases D1 MSN excitability possibly by enhancing the recruitment of D1 receptors to the plasma membrane^{12–19}. As well, the unregulated DA release that occurs with L-dopa administration results in enhanced D1 receptor signaling and abnormal internalization and trafficking^{13, 14, 20}. These alterations may further exacerbate sensitized D1 MSN-mediated transmission and lead to abnormal downstream signaling responses that contribute to LIDs^{1, 3, 11}. Further evidence for a role of D1 MSNs in LIDs comes from studies showing that D1 receptor agonists promote dyskinesias while D1 receptor antagonists reduce them^{21–24}. Additionally, genetically modified mice lacking D1 receptors do not develop LIDs²⁵ and inhibition of D1 MSN activity via chemical ablation or chemogenetic approaches reduces LIDs^{26, 27}.

D2 receptor-mediated signaling also appears to modulate LIDs, although its contribution is less clear^{1, 12, 25, 28–31}. This is because D2 receptor agonists produce only mild or no dyskinesias in L-dopa-naïve lesioned rodents. On the other hand, their administration leads to more severe dyskinesia in L-dopa-primed animals^{21, 32} and D2 receptor antagonists attenuate L-dopa-induced AIMs^{23, 33, 34}, suggesting they are involved. In addition, alterations in D2 receptor-mediated signaling have been shown to contribute to LIDs. For example, increased activity of RGS9-2, a protein that inhibits the interaction of D2 receptors with G proteins, decreases LID severity only after D1 receptor activation³⁵.

The objective of the present work was to directly evaluate the role of D1 MSN activation on dyskinesia development. To approach this, we used optogenetics to precisely modulate D1 MSN activity *in vivo* and assess how it controls dyskinesia expression in parkinsonian D1-Cre mice. The results show that selective striatal D1 MSN activation differentially regulates dyskinesias and parkinsonism in both L-dopa-naïve and L-dopa-primed mice. Altogether, the data provide direct evidence that striatal D1 MSN stimulation regulates dyskinesias and parkinsonism.

Materials and Methods

Mice

Hemizygous male *Drd1a*-Cre BAC transgenic mice (EY262Gsat/Mmucd, B6.FVB(Cg) background) from GENSAT were crossed to wildtype C57Bl/6J. Female and male mice entered the study at ~ 8 wk of age, weighing 18–25 g. They were group-housed in a temperature- and humidity-controlled environment under a 12 h light/dark cycle with free access to food and water.

6-Hydroxydopamine lesion

After a 2–3 d acclimation period, mice were treated as in Fig. 1A. All mice were lesioned by stereotaxic injection of 6-hydroxydopamine (6-OHDA) into the right medial forebrain bundle at the following coordinates relative to Bregma and the dural surface: AP, –1.2 mm; ML, –1.2 mm; DV, 4.75 mm, as described^{36–38}. All procedures were approved by the Institutional Animal Care and Use Committee in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Viral expression

Four wk after 6-OHDA lesioning, mice were stereotaxically injected into the right striatum with cre-inducible AAV vector expressing ChR2 (AAV5.EF1a.DIO.hChR2(H134R)-eYFP.WPRE.hGH) or control vector (AAV5.EF1a.DIO.eYFP.WPRE.hGH) to induce expression in D1 MSNs^{6, 7, 38}. These mice are designated D1-ChR2 and D1-eYFP, respectively, throughout. Virus was obtained from the Vector Core at the University of Pennsylvania, with a viral concentration of $1\text{--}4 \times 10^{12}$ particles/ml. One μ l virus was injected over 2 min at the following coordinates: AP +0.8 mm, ML +2.2 mm, DV –2.7 mm. Immediately after viral injection, the mice were implanted (AP +0.8 mm, ML +2.2 mm, DV –2.5 mm) with optical fiber cannulae (200 μ m core fiber with 0.39 NA and a flat cleave) to allow for *in vivo* optical stimulation during behavioral assessment.

Optical stimulation

We used optical stimulation paradigms consistent with those previously used to modulate D1 MSN activity^{6, 7}. Striatal D1 MSNs were activated using a 473 nm DPSS blue laser (Shanghai Laser, China) with a maximum output power >120 mW. The laser was TTL-controlled by a Master-8 (A.M.P.I., Israel) and its power adjusted such that it was 1.5 mW at the fiber tip of each optical implant (measured with a PM100D optical power meter power with an S130C sensor; Thorlabs). Optical stimulation consisted of a single pulse ranging from 5 ms to 30 s in duration or a burst of 10–50 pulses (20 ms pulse duration) at a frequency of 10–20 Hz. Stimulation was delivered with a 0.5 s off stimulation period between pulses or bursts throughout the entire rating period as detailed in the dyskinesia ratings section.

Assessment of motor deficits

6-OHDA-induced motor impairment was assessed using the cylinder or forelimb-use asymmetry test^{37–39}. Exploratory activity was rated by a blinded rater for 3 min in the

absence and presence of optical stimulation with and without L-dopa. Ratings were done immediately before and 60 min after L-dopa when drug effects are maximal. Wall exploration was expressed in terms of the % use of the impaired contralateral forelimb compared to the total number of forelimb contacts^{37–39}.

Dyskinesia ratings

Abnormal involuntary movements (AIMs) were rated by a blinded rater at least once weekly as described^{36–38, 40–42}. Each AIM subtype (oral, forelimb, and axial) was scored on a frequency scale from 0 to 4 (0= no dyskinesia; 1= occasional dyskinesia displayed for <50% of the observation time; 2= sustained dyskinesia displayed for >50% of the observation time; 3= continuous dyskinesia; 4= continuous dyskinesia not interruptible by external stimuli). Amplitude scores were subdivided as “A”, which indicates oral AIMs without tongue protrusion, forelimb AIMs without the shoulder engaged, and axial AIMs with body twisting <60°, or “B” indicating oral AIMs with tongue protrusion, forelimb AIMs with the shoulder engaged or axial AIMs with body twisting ≥60°. Overall scores for frequency and amplitude of AIMs used for data analysis were calculated as 1A=1, 1B=2, 2A=2, 2B=4, 3A=4, 3B= 6, 4A=6, 4B=8. This allows for scores for any one component (axial, oral or forelimb) ranging from 1 to 8. Total AIM scores were calculated as the sum of all the components, with a maximum possible total score of 24 per time point.

Experiments in L-dopa-naïve mice were initiated five wk after virus injection. AIMs were rated every 5 min for 1 h; 30 min in the absence of stimulation and 30 min in the presence of stimulation in random order. Any one mouse was only subjected to one stimulation paradigm per wk, with the different paradigms tested on a random basis.

After a total of 11 stimulation sessions, the mice were injected subcutaneously with L-dopa (3–5 mg/kg) and benserazide hydrochloride (15 mg/kg) once daily 5 d per week to induce AIMs. For all L-dopa-primed mice, AIMs were scored individually by a blinded rater every 15 min for 1 min for a 2 h period after L-dopa injection.

Tissue preparation

The mice were killed by cervical dislocation and the brains quickly removed and bisected at the mid-striatal level. The anterior portion was post-fixed in 4% paraformaldehyde and cryopreserved in 10–30% sucrose for several days. Fixed brains were sectioned (30 µm) using a cryostat (Leica) for immunofluorescence. The posterior portion of the brain was quick frozen in isopentane on dry ice and stored at –80°C for autoradiography.

Immunofluorescence

Staining for DARPP-32 or p-ERK was done on every 6th section from A1.4–A0.2. Free floating sections were rinsed 3× in 0.3% Triton X-100 in PBS, and then exposed to a blocking solution consisting of 3% normal goat serum in 0.3% Triton X-100 in PBS for 1 h. Sections were then incubated with rabbit anti-DARPP-32 (1:800, Cell Signaling Technology) or rabbit anti-phospho-ERK1/2 (1:250 dilution; Cell Signaling Technology) overnight at 4°C. This was followed by incubation with secondary antibody (goat anti-rabbit Alexa-555; dilution 1:500; Cell Signaling Technology) for 2 h at RT. Sections were then

rinsed in PBS, mounted onto slides with Vectashield mounting medium, cover slipped and viewed under a Nikon fluorescence microscope.

To evaluate colocalization of ChR2-eYFP with DARPP-32 or p-ERK, images were captured at 10× and 40× magnifications to view viral expression in striatal neurons. DARPP-32/eYFP or p-ERK/eYFP double positive cells were counted manually at +A0.8 mm from bregma by a blinded rater using Adobe Photoshop software merged images, as described³⁸. Mean values were calculated for each animal and considered as an individual observation for statistical analyses.

Dopamine transporter (DAT) autoradiography

¹²⁵I-RTI-121 (specific activity, 2200 Ci/mmol; GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK) autoradiography was performed as described⁴³.

Statistical Analyses

Statistical comparisons were done using GraphPad Prism (GraphPad Software Co., San Diego, CA, USA). Since the behavioral data involves integrated or continuous data collected over an extended time frame, that is, the total or area under the time curve (2 h period) of all scores, paired t tests or analysis of variance followed by a Bonferroni multiple-comparison test were used. For the molecular studies, t test was used. A level of 0.05 was considered significant. Values are the mean ± SEM of the indicated number of mice.

Results

Optical stimulation induces oral AIMs in lesioned L-dopa-naïve mice

D1-Cre mice were first lesioned with 6-OHDA to induce nigrostriatal damage and tested for parkinsonism 2 wk later using the forelimb asymmetry test. 6-OHDA lesioning decreased left forelimb use from 51±0.5 % to 33±2.2 %, as described^{37, 38}. Such declines in forelimb use are consistent with the observed 91±13% (n=9) and 98±14% (n=4) loss in dopamine transporter binding in the lesioned striatum of D1-ChR2 and D1-eYFP mice, respectively, in agreement with previous studies^{37, 38, 41, 42}.

We next tested various stimulation paradigms to evaluate whether optical stimulation of striatal D1 MSNs led to AIMs. Optical stimulation consisting of single pulse durations ranging from 0.1–30 s significantly increased total AIMs in D1-ChR2 mice, with no effect of 0.005 s single pulse stimulation (Fig 1B). As previous studies showed that a 1 s optical stimulation causes D1 MSN neurons to fire at a rate ~10 Hz, we also tested the effect of stimulation using 10 or 50 pulses delivered at 10 or 20 Hz^{6, 7}. Burst stimulation increased total AIMs in D1-ChR2 mice to a similar level as single pulses (Fig 1B). None of the stimulation paradigms induced AIMs in D1-eYFP mice (Fig 1B). Thus, enhanced D1 MSN activity induces AIMs only in D1-ChR2 mice.

We also assessed how D1 MSN stimulation influenced the expression of AIM subtypes (axial, oral, forelimb). For the single pulse paradigms, the D1 MSN stimulation-induced increase in AIMs was primarily due to enhanced oral AIMs in D1-ChR2 mice (Fig 1C). Likewise, burst stimulation with 50 pulses at 10 Hz increased oral AIMs to a similar extent

as single pulse stimulation (Fig 1D). The increase in AIMs was observed immediately after the onset of stimulation and lasted throughout the stimulation period as shown in the time courses (Fig 1C,D). As expected, no effect of stimulation was observed in D1-eYFP mice (Fig 1C,D).

D1 MSN stimulation induces a higher level of AIMs in L-dopa-primed compared to L-dopa-naïve lesioned D1-Cre mice

The data in Fig 2 show the AIM scores for L-dopa-naïve unilaterally lesioned D1-Cre mice expressing D1-ChR2 stimulated with a 1s (Fig 2A) or a 10p/10 Hz (Fig 2B) stimulus delivered every 0.5 s. The mice were subsequently treated for 3 wk with daily injections of L-dopa (3 mg/kg) until stably dyskinetic. Total AIMs were similar for the D1-eYFP (32 ± 4.2 , $n=5$) and D1-ChR2 (24 ± 3.3 , $n=11$) mice, as reported³⁷. The L-dopa-primed mice were then optically stimulated (in the absence of an acute L-dopa dose) with a single pulse (1s) or a burst (10 p at 10 Hz) and AIMs rated. No effect of stimulation was observed in D1-eYFP mice. Conversely, stimulation enhanced total AIMs to a significantly greater extent in L-dopa-primed than L-dopa-naïve D1-ChR2 mice (Fig 2A,B). Notably, optical stimulation induced oral and forelimb AIMs in L-dopa-primed mice (Fig 2A,B) while it primarily generated oral AIMs in L-dopa-naïve mice (Fig 1C,D). Such differences may arise from the fact that optical stimulation only activates D1 MSNs within the dorsolateral striatum while L-dopa would have a more generalized effect.

Additive effect of combined D1 MSN stimulation and L-dopa on AIMs expression in lesioned L-dopa-primed D1-Cre mice

We next investigated the level of AIMs obtained with D1 MSN stimulation alone, L-dopa treatment alone or the combination. In D1-eYFP mice, acute L-dopa injection induced oral and forelimb AIMs; however, optical stimulation had no effect as expected (Fig 3A). By contrast, both optical stimulation and L-dopa treatment induced AIMs in D1-ChR2 mice as depicted in the time courses (Fig 3B–D). While AIMs were significantly higher with L-dopa treatment than with the 1s pulse optical stimulation (Fig 3B), burst stimulation-induced AIMs were similar to those obtained with L-dopa administration (Fig 3C). Combined optical stimulation and L-dopa injection resulted in AIMs higher than either condition alone regardless of stimulation paradigm (Fig 3B,C).

We also examined the effect of optical stimulation in the absence and presence of 5 mg/kg L-dopa to evaluate whether the apparent additive effect of optical stimulation and L-dopa was due to a sub-maximal effect of the lower 3 mg/kg L-dopa dose. Five mg/kg L-dopa induced AIMs to a level comparable to that obtained with 3 mg/kg L-dopa for both D1-ChR2 (24 ± 5.5) and D1-eYFP (39 ± 6.3) mice, indicating that maximal L-dopa-induced AIMs had been achieved with the lower L-dopa dose. Optical stimulation alone-induced AIMs were still lower than L-dopa-induced AIMs in D1-ChR2 treated with 5 mg/kg L-dopa (Fig 3D). In addition, an additive effect of L-dopa administration and optical stimulation was also evident (Fig 3D). Altogether, these data suggest that additional adaptive responses extending beyond enhanced D1 MSN activity contribute to the expression of L-dopa-induced dyskinesias.

D1 MSN stimulation improves motor deficits in lesioned L-dopa-primed mice

As D1 MSN activity is associated with the facilitation of movement^{6, 7}, we evaluated whether direct D1 MSN stimulation improved parkinsonism in 6-OHDA lesioned mice. First, we tested the effect of D1 MSN stimulation in L-dopa-naïve D1-ChR2 and D1-eYFP mice. The animals were rated for left forelimb use for 3 min in the absence of stimulation. They were then tested for 3 min in the presence of a 1 s stimulating pulse applied every 0.5s throughout the rating period. Optical stimulation did not improve parkinsonism in either D1-ChR2 or D1-eYFP L-dopa-naïve mice (Fig 4A). The mice were subsequently treated with L-dopa (3 mg/kg) for 3 wk until stably dyskinetic. Parkinsonism was then rated in the absence of L-dopa as well as 1 h after its injection. L-dopa injection improved forelimb use in D1-eYFP mice ($46 \pm 3.4\%$ forelimb use vs $27 \pm 2.7\%$ before L-dopa) (Fig 4B). As expected, optical stimulation did not affect parkinsonism in D1-eYFP mice (Fig 4B). By contrast, optical stimulation improved left forelimb use in ChR2-expressing mice ($36 \pm 3.8\%$ forelimb use) compared to the baseline condition ($26 \pm 3.5\%$ forelimb use) in the absence of L-dopa (Fig 4B). L-dopa administration increased forelimb use ($43 \pm 1.3\%$ forelimb use) to a similar extent as optical stimulation in L-dopa-primed mice (Fig 4B). Combined optical stimulation and L-dopa treatment did not further improve parkinsonism ($46 \pm 1.3\%$ forelimb use) compared to either treatment alone in D1-ChR2 mice (Fig 4B). These results suggest L-dopa priming is required for optical stimulation to ameliorate parkinsonism.

Viral expression in striatal MSNs of lesioned D1-Cre mice

To verify the presence of ChR2 in striatal D1 MSNs, fixed striatal slices containing ChR2-eYFP positive neurons were processed for DARPP-32 immunoreactivity. A representative image (Fig 5A) shows the expression of ChR2 in the dorsal striatum at the level of the injection site (A0.8), with expression extending from A1.4-A0.2. Co-immunostaining of DARPP-32 and eYFP indicated clear co-localization throughout the striatum, with the images in Fig 5B showing the data around the site of injection (A0.8). The spread of D1-ChR2 and D1-eYFP was similar from level A1.4-A0.2. Striatal eYFP+ cell counts showed that variability in viral expression averaged 23.5% among mice (n=11).

Increased p-ERK expression in D1 MSNs of lesioned D1-ChR2 but not D1-eYFP mice

Increased phosphorylation of ERK 1/2 in D1 MSNs has been associated with LIDs^{4, 24, 44–46}. Therefore, we determined if alterations in p-ERK expression are associated with the optical stimulation-induced changes in AIMs expression observed in D1-ChR2 mice. A significant increase in the number of p-ERK⁺ striatal D1 MSNs was observed in D1-ChR2 but not D1-eYFP mice (Fig 5C–E). No significant differences in p-ERK⁺ non-D1 neurons were observed between D1-ChR2 and D1-eYFP mice (Fig 5E). Thus, increased p-ERK may play a role in the changes in AIMs observed with optical D1 MSN stimulation.

Discussion

The present studies are the first to show that selective activation of D1 MSNs in the dorsal striatum induces AIMs in L-dopa-naïve D1-Cre mice expressing ChR2. Our studies also show that L-dopa treatment further enhances the effect of D1 MSN stimulation on AIMs, and that combined L-dopa administration and optical stimulation result in an additive

increase in AIMS. These data strengthen the pivotal role of D1 MSNs on dyskinesias and suggest that other mechanisms, including those related to D2 and possibly D3 receptor activation, may also underlie AIMS^{18, 19, 23, 31, 47, 48}.

We first investigated the effect of D1 MSN stimulation in L-dopa-naïve lesioned animals since enhanced D1 MSN responsiveness is linked to the development of L-dopa-induced AIMS^{1, 4, 30, 46}. Optical stimulation with brief 0.005 s pulses did not induce AIMS consistent with previous studies showing that it requires ~0.04 s of optical stimulation for D1 MSNs to significantly increase their firing rate⁷. We also tested the effect of single pulses ranging from 0.1–30 s in duration since previous work showed that the firing rate of striatal D1 MSNs is increased with these stimulus durations^{6, 7}. In addition, we used burst stimulation paradigms comparable to the firing frequencies previously reported to occur in D1 MSNs neurons after single pulse optical stimulation^{6, 7}. We observed a similar increase in AIMS in L-dopa-naïve mice with single pulses 0.1 s and burst stimulation indicating that AIMS expression is likely due to D1 MSN burst activity. Although previous work had shown that optical stimulation consisting of single pulse durations of 0.1 s did not modify locomotor behavior in normal animals⁶, we observed a worsening of dyskinesias with this stimulation paradigm. These findings agree with studies in lesioned animals showing that D1 MSN excitability increases after DA depletion possibly to compensate for the loss of excitatory D1 receptor stimulation^{12, 18, 19}. Overall, these data provide experimental evidence for a direct role of D1 MSN activation in the development of dyskinesias.

We next examined the effect of optical stimulation in L-dopa-primed lesioned mice. The results show that activation of D1 MSNs led to significantly higher AIMS in L-dopa-primed than in L-dopa-naïve mice. Such findings agree with previous work showing that L-dopa treatment leads to poorly regulated fluctuations in synaptic dopamine levels in the lesioned striatum that contribute to L-dopa sensitization and the emergence of dyskinesias^{1, 3, 4, 28, 49–51}. In addition, L-dopa treatment impairs D1 receptor internalization and trafficking, leading to their increased recruitment at the plasma membrane of D1 MSNs^{13, 14, 20}. Such altered receptor expression has been linked to the coupling of D1 receptors to G α olf proteins which, in turn, increases adenylyl cyclase type 5 expression in D1 MSNs and activates the cAMP/PKA/DARPP-32 and ERK signaling pathways^{1, 3, 4, 14, 30, 45, 51–53}. Altogether, these modifications likely contribute to the enhanced D1 MSN-mediated activity associated with LIDs. In fact, our studies showed enhanced p-ERK labeling only in D1 MSNs of mice expressing D1-ChR2 indicating that activating these neurons potentiates ERK signaling in L-dopa-treated mice. Thus, the enhanced expression of optically-stimulated AIMS in L-dopa-primed mice is consistent with the idea that L-dopa sensitizes D1 MSN-mediated signaling. Future work will examine the effect of D1 MSN activation on FosB in striatal D1 MSNs since increased levels correlate with the intensity of dyskinetic symptoms in lesioned L-dopa-treated rodents^{25, 46, 54, 55}.

Our findings suggest that D1 MSNs are critical to AIMS development; however, other systems also appear to be involved. This possibility stems from the data showing that AIMS were higher with L-dopa treatment than with the 1 s D1 MSN stimulation condition. Since L-dopa derived dopamine activates both D1-like and D2-like receptors, these data suggest that D2 MSNs also contribute to the expression of AIMS.

Unexpectedly, combined L-dopa administration and optical stimulation led to an additive increase in AIMs. This may be due to interactions between the D1 direct and the D2 indirect striatal pathways that lead to changes in overlapping intracellular signaling cascades that modulate transcription factors, gene expression and MSN plasticity to enhance L-dopa-induced AIMs^{1, 3, 4, 30, 31, 45, 52, 53}. For instance, there is evidence that striatal D1 and D2 MSN function is influenced by collateral connections between these two neuronal populations^{56–59}. This synaptic connectivity is significantly downregulated with dopamine depletion, which may contribute to the altered MSN activity observed with motor abnormalities⁵⁶.

D1 MSN activation may not only contribute to dyskinesias, but also modulate parkinsonism. We did not observe an effect of optical stimulation in L-dopa-naïve mice, in contrast to recent optogenetic studies involving D1 MSN activation^{6, 7}. This differential response may have arisen from variations in the lesioning protocol and/or the behavioral tests used. On the other hand, D1 MSN activation improved parkinsonism in L-dopa-primed animals possibly because of long-term L-dopa-induced signaling changes. Lesion-induced parkinsonism may disrupt the bidirectional synaptic plasticity of D1 and D2 MSNs, leading to underactivity of the direct pathway and over-activity of the indirect pathway via long-term depression (LTD) or potentiation (LTP), respectively^{2, 29, 60}. In L-dopa-primed dyskinetic mice, L-dopa administration induces LTD in D2 MSNs and LTP in D1 MSNs²⁹. Thus, chronic L-dopa treatment may modify the outcome of D1 activation by altering the functional interactions between D1 and D2 MSNs. These altered responses may explain both the enhanced AIMs expression and anti-parkinsonian effect of D1 MSN activation on L-dopa-primed animals as well as their increased responsiveness to burst stimulation.

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FIG. 1.

Single pulse and burst D1 MSN optical stimulation induce AIMs in L-dopa-naïve lesioned D1-Cre mice. **A:** Treatment timeline. **B:** L-dopa-naïve unilaterally lesioned mice expressing D1-ChR2 or D1-eYFP were rated for AIMs 1 min every 5 min over a 30 min period in the absence of optical stimulation followed by a 30 min rating session in the presence of stimulation. D1 MSN stimulation increased AIM scores in D1-ChR2 mice [$F(1,24)=36.72$], with no change in D1-eYFP mice. **C:** Continuous single pulse (1 s) stimulation delivered every 0.5 s primarily induced oral AIMs in ChR2-expressing mice [$F(1,32)=74.49$]. **A**

representative time courses is shown in the right panel. D: Burst optical stimulation of striatal D1 MSNs with 50 p delivered at a frequency of 10 Hz increased AIMs to a similar extent as single pulse stimulation in D1-ChR2 mice [$F(1,32)=30.48$]. Each bar is the mean $\pm$ SEM of 5 mice. Significantly different from D1-eYFP, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, using a Bonferroni multiple comparison test.

FIG. 2.

D1 MSN stimulation induces a higher level of AIMs in L-dopa-primed compared to L-dopa-naïve lesioned D1-Cre mice. L-dopa-naïve unilaterally lesioned D1-Cre mice expressing D1-ChR2 or D1-eYFP received no L-dopa; they were then stimulated and AIMs rated. The mice were subsequently treated with L-dopa (3 mg/kg) until stably dyskinetic. AIMs were next rated in the absence of L-dopa in response to a 1s (A) or a 10p/10 Hz (B) stimulus delivered every 0.5 s. As expected, optical stimulation did not induce AIMs in L-dopa-naïve or L-dopa-primed D1-eYFP mice (data not shown). Each bar is the mean $\pm$ SEM of 5 mice. Significantly different from L-dopa-naïve, * p 0.05, ** p 0.01, using a paired student's t -test.

FIG. 3.

D1 MSN stimulation and L-dopa both increase AIMs expression in L-dopa-primed D1-Cre mice. Unilaterally lesioned D1-Cre mice expressing D1-ChR2 or D1-eYFP were treated with L-dopa (3 mg/kg) until stably dyskinetic. The effect of optical stimulation in the absence and presence of L-dopa was subsequently tested. For the combined treatment experiments, optical stimulation was initiated immediately after L-dopa injection and AIMs scored for 2 h. **A:** L-dopa treatment but not optical stimulation significantly induced total [F(1.684, 5.051)=45.73], oral [F(1.299, 3.897)=41.79] and forelimb [F(1.040, 3.121)=33.17]

AIMs in D1-eYFP mice. **B:** In L-dopa-primed ChR2-expressing mice, stimulation alone (1 s pulse every 0.5 s for 2 h) increased AIM scores, while L-dopa injection increased AIMs to a significantly greater extent than stimulation alone. Combined optical stimulation and L-dopa injection led to additive increase in AIM scores. An effect of treatment condition was observed in total [F(1.699, 16.99)=74.71], oral [F(1.935, 19.35)=24.19] and forelimb [F(1.923, 19.23)=38.95] AIMs. **C:** Burst stimulation (10p delivered at 10 Hz every 0.5 s for 2 h) led to AIM scores comparable to those after L-dopa injection, with an additive effect of combined optical stimulation and L-dopa. A significant effect of treatment condition was observed in total [F(1.213, 12.13)=13.23], oral [F(1.181, 11.81)=11.44] and forelimb [F(1.356, 13.56)=11.65] AIMs. **D:** A higher L-dopa dose (5 mg/kg) led to similar changes in AIMs as 3 mg/kg L-dopa, with treatment condition having a significant effect on total [F(1.622, 14.60)=28.56], oral [F(1.407, 12.66)=19.68] and forelimb [F(1.455, 13.09)=28.95] AIMs. Each bar is the mean $\pm$ SEM of 5–11 mice. Significantly different from Stim, *p 0.05, ** p 0.01, *** p 0.001; significantly different from L-dopa, ++ p 0.01, +++ p 0.001, using a using a Bonferroni multiple comparison test.

FIG. 4.

D1 MSN stimulation improves parkinsonism in L-dopa-primed but not L-dopa-naïve mice.

A: L-dopa-naïve lesioned D1-Cre mice expressing D1-ChR2 or control e-YFP were rated for forelimb use in the absence and presence of a 1 s pulse stimulation delivered every 0.5 s for 3 min. Optical stimulation did not affect parkinsonism in L-dopa-naïve mice. **B:** The mice were subsequently treated with 3 mg/kg L-dopa 1× daily, 5 times a wk for 2 mo and parkinsonism rated again in the absence and presence of L-dopa with and without stimulation. Optical stimulation significantly improved forelimb use in L-dopa-primed D1-

ChR2 mice [$F(1,9)=12.42$]. As expected, L-dopa alone also improved motor impairment [$F(1,9)=15.87$] and this effect was not enhanced by optical stimulation. Each bar is the mean $\pm$ SEM of 5–11 mice. Significantly different from own No stim condition, ** $p < 0.01$; significantly different from No L-dopa condition, ++ $p < 0.01$, +++ $p < 0.001$, using a Bonferroni multiple comparison test.

FIG. 5.

Increased p-ERK expression in D1 MSNs of D1-ChR2 mice. **A:** Representative image at 4× magnification depicting the spread of ChR2 (green) immunofluorescence at the site of virus injection and optical probe placement (A0.8). **B:** Representative images at 40× magnification depicting localization of ChR2-eYFP (green), DARPP-32 immunofluorescence (red) and the merge (yellow). **C:** neurons in the boxed area in D at 40× magnification showing cells immunostained with ChR2-eYFP (green, left), p-ERK (red, middle) and the merge (right). **D:** Images of eYFP (green), p-ERK (red) and eYFP+p-ERK

(yellow) positive neurons of D1-ChR2 and D1-eYFP mice at 10× magnification. **E:** Quantitation of the number of p-ERK⁺ D1 and non D1 neurons counted within the dashed boxed area in the image depicting the dorsolateral striatum in A. Each bar is the mean ± SEM of 4 mice. Significantly different from D1-eYFP using a student's t-test, ***p = 0.001. Scale bar = 10 μm for B and C, and 20 μm for D.