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# Optogenetic stimulation of cortico-subthalamic projections is sufficient to ameliorate bradykinesia in 6-ohda lesioned mice

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ABSTRACT

Electrical deep brain stimulation (DBS) of the subthalamic nucleus (STN) is effective for ameliorating the motor symptoms of Parkinson's disease (PD) including bradykinesia. The STN receives its main excitatory input from cortex; however, the contribution of cortico-subthalamic projection neurons to the effects of DBS remains unclear. To isolate the consequences of stimulating layer 5 primary motor cortex (M1) projections to the STN, we used a dual virus transfection technique to selectively express opsins in these neurons in mice made parkinsonian by unilateral nigrostriatal 6-OHDA lesioning. AAVs containing WGA-Cre constructs were injected in the STN to retrogradely place Cre in STN afferents, while AAVs containing Cre-dependent ultrafast hChR2(E123T/T159C)-EYFP opsin constructs were injected in M1 layer 5, producing specific opsin expression in M1-STN projections. Under unstimulated conditions, lesioned mice showed bradykinesia and hypokinesia (decreased movement), along with electrophysiological changes similar to those observed in PD patients. Specifically, low frequency power (theta, alpha, low beta) was increased and gamma power was decreased, while M1/STN coherence and STN phase-amplitude-coupling (PAC) were increased. Optogenetic stimulation (100–130 Hz) of STN afferents in these mice ameliorated bradykinesia and hypokinesia and brought the neural dynamics closer to the non-parkinsonian state by reducing theta and alpha and increasing gamma power in M1, decreasing STN PAC, and reducing theta band coherence. Histological examination of the EYFP expression revealed that, in addition to orthodromic and antidromic effects, stimulation of cortico-subthalamic neurons may cause wide-spread increased glutamatergic activity due to collaterals that project to areas of the thalamus and other brain regions.
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## 1. Introduction

Treatment with the dopamine precursor L-DOPA can ameliorate bradykinesia (abnormal slowing of movement) in most Parkinson's disease (PD) patients for 10 years or more. However, many patients eventually require supplemental treatments due to the progressive nature of the disease and the dyskinesias (involuntary movements) that often occur after prolonged L-DOPA use. Deep brain stimulation (DBS) of the STN has been shown to be an effective treatment for bradykinesia in humans (Benabid et al., 2009; DeLong and Wichmann, 2007; Wichmann and DeLong, 2011), and rats (Li et al., 2012), and can prolong the effective window of L-DOPA therapy. However, optimizing DBS treatment to subject-specific neural dysfunction and symptom profiles is often difficult due to a lack of understanding about the cellular and circuit activations (and/or deactivations) that lead to the DBS therapeutic effects. Previous studies have shown evidence that activation of layer 5 cortico-subthalamic projections and antidromic effects may play an important role in the DBS therapeutic mechanism (Gradinaru et al., 2009; Li et al., 2007). Additionally, recent discoveries about the role of STN in motor behavior have emphasized the importance of the STN in movement stopping and initiation. For example, STN activation in inhibiting ongoing movements has been demonstrated (Chu et al., 2015; Mallet et al., 2016), and increased STN neuron spiking has been observed immediately subsequent to stop cues and contralateral movement initiation cues (Schmidt et al., 2013).

Previously, Gradinaru et al., 2009 found that high frequency optogenetic stimulation of cortico-subthalamic projections ameliorated ipsilateral rotations in mice unilaterally lesioned with 6-OHDA. However, these initial studies were performed in Thy1::ChR2 line 18 transgenic mice that expressed first generation ChR2 opsins in many locations including layer 5 cortical neurons across the entire cortex, CA1 and CA3 pyramidal neurons of the hippocampus, and neurons in the thalamus (Arenkiel et al., 2007). The slower response kinetics of the ChR2 opsin and the broad expression of ChR2 under control of the Thy1 promoter led to concerns regarding the specificity and mechanism of action involved in the 2009 results. Motivated in part by these concerns, the study reported here examined whether specific optogenetic stimulation of second-generation ultrafast opsins [hChR2(E123T/T159C)] placed

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selectively in layer 5 motor cortical (M1) projections to the STN using a dual virus retrograde transfection approach in ordinary C57BL/6J mice would ameliorate behavioral signs of parkinsonism.

In addition to observing the effects of specific motor-cortico-subthalamic (M1-STN) stimulation on behavior in normal and parkinsonian mice, this study examined the histology of M1-STN projections and their collaterals, and the electrophysiological signs of parkinsonism. The electrophysiological questions explored include whether increased M1 and STN beta band power (Delaville et al., 2014; Engel and Fries, 2010; Leventhal et al., 2012), increased coherence (Goldberg et al., 2002; Hammond et al., 2007), and changes in phase-amplitude-coupling (Sanders et al., 2013b) observed in other species also occur in mice, and how optogenetic stimulation impacts the M1-STN electrophysiology.

## 2. Materials and methods

### 2.1. Experimental procedures

All experimental procedures were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals, the PHS Policy on the Humane Care and Use of Laboratory Animals, and the American Physiological Society's Guiding Principles for the Care and Use of Vertebrate Animals in Research and Training (updated 2014) and were approved by the Emory Animal Care and Use Committee.

### 2.2. Animals

Male C57BL/6J mice (Jackson Labs) were housed with free access to chow and water in environmentally controlled conditions with a reversed 12 h light/dark cycle (lights on at 7 pm). Mice were gently handled daily for one week prior to surgery and habituated to an open-field arena used for freely moving recording in the study by placing them in the arena for 10–30 min each day. Video was recorded for the purpose of baseline movement assessment. All mice were aged 4–9 months at the time of testing.

### 2.3. Opsin placement

Opsin expression in layer 5 M1-STN projections in C57BL/6J mice (N = 10) was induced by a dual-virus retrograde transfection technique (Fig. 1A) previously used to transfect projections in other brain regions (Gradinaru et al., 2010; Dautan et al., 2014). Two AAVs containing viral constructs (MTA from Karl Deisseroth at Stanford University; purchased from UNC Vector Core) were stereotaxically injected as described in detail in the surgical procedures. 0.2 μl of the solution of AAVs containing WGA-Cre genetic constructs [AAV-Ef1a-mCherry-IRES-WGA-Cre, serotype 2, titer $10^{12}$ vg/ml] were injected in the STN. WGA retrograde transport activity then placed Cre in STN afferents. 0.5 μl of the solution of AAVs containing Cre-dependent ultrafast channelrhodopsin constructs [AAV-EF1a-DIO-hChR2(E123T/T159C)-EYFP, serotype 5, titer $10^{12}$ vg/ml] were injected in layer 5 M1. This led to opsin expression in layer 5 M1-STN projections where Cre-dependent opsin constructs intersected with retrogradely placed Cre.

As described above, the ultrafast opsin used in this study was the ET/TC mutation of ChR2. The newer ET/TC mutation was selected because of its shorter $\tau_{off}$ (8 ms) (Yizhar et al., 2011) and its large stable photocurrents (~1400 pA) (Berndt et al., 2011) as compared to the wild-type ChR2 used in the Gradinaru et al., 2009 study ($\tau_{off}$ = 10 ms; stationary photocurrent = ~950 pA). The decreased $\tau_{off}$ was important for enabling the opsins to respond at a faster stimulation frequency while

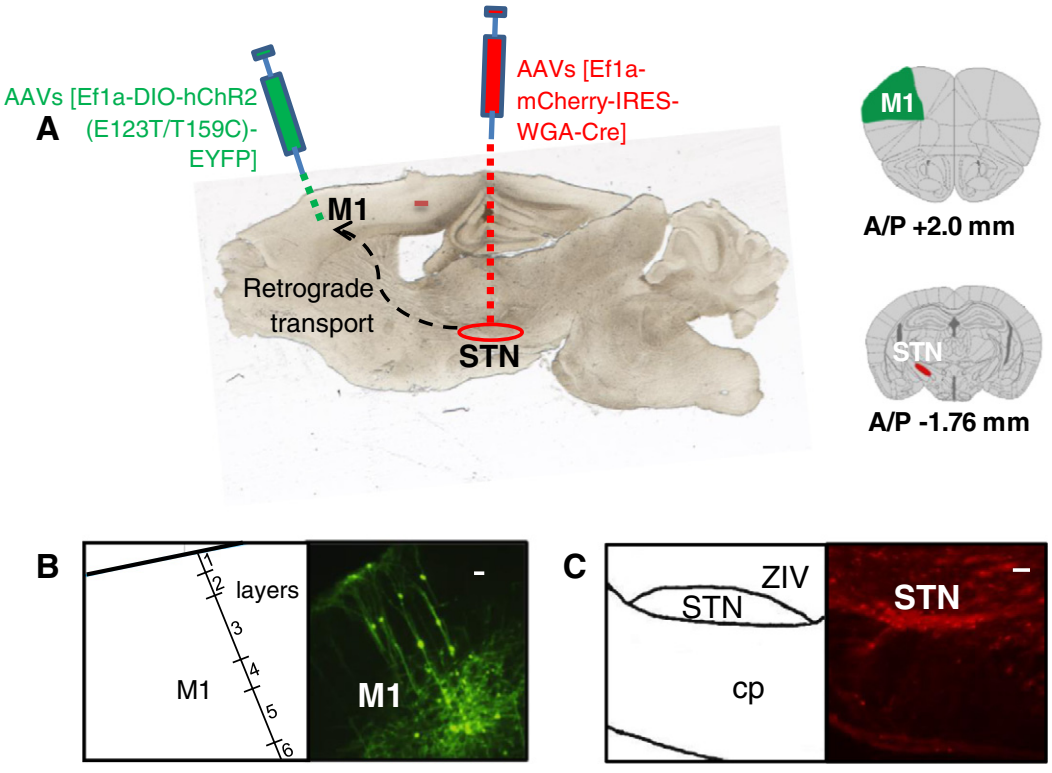


**Fig. 1.** Dual AAV injection strategy to place opsins in layer 5 cortico-subthalamic projection neurons. A) Location of primary motor cortex (M1) and subthalamic nucleus (STN) in sagittal (left) and coronal (right) sections. Ultrafast AAV-EF1a-DIO-hChR2(E123T/T159C)-EYFP AAVs were injected in M1. Ef1a-mCherry-IRES-WGA-Cre AAVs (Cre attached to retrograde labeling agent WGA) were injected in STN during the same surgery. Orange scale bar = 0.5 mm. B) M1 region of sagittal brain section (−1.56 mm) with cortical layers indicated (left); layer 5 EYFP expression marking the location of the M1 layer 5 opsins (right). C) STN region of sagittal brain section (−1.56 mm; left) and corresponding sample of the mCherry (red) expression indicating the location of the Ef1a-mCherry-IRES-WGA-Cre AAV injection (right). White scale bars = 100 μm. cp = cerebral peduncle; ZIV = zona incerta, ventral part.

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