Journal of Bioinformatics and Neuroscience (JBINS), 3 (3): 134-141, December 29, 2017
e-ISSN: 2432-5422, p-ISSN: 2188-8116
Applied Science and Computer Science Publications

Optogenetics-based Neuromodulation for the Alleviation of hemi-Parkinsonian Motor Asymmetry in Rat

Zeyang Yu, Wael F. Asaad, Arto V. Nurmikko and Ilker Ozden
zeyang_yu@brown.edu, wael_asaad@brown.edu, arto_nurmikko@brown.edu, ozdeni@missouri.edu

School of Engineering
Department of Neuroscience
Brown University
Providence, RI, USA

Department of Neurosurgery
Brown University Alpert Medical School
Rhode Island Hospital
Providence, RI, USA

Department of Bioengineering
University of Missouri
Columbia, MO, USA

Abstract: The development of more effective deep brain stimulation (DBS) paradigms for Parkinson's disease is limited by the non-specific nature of electrical stimulation. Optogenetics, with its spatial and cell-type specificity, is a potential alternative therapeutic approach. In 6-hydroxydopamine-induced hemi-Parkinsonian rats, we investigated the therapeutic values of optogenetic modulation of the subthalamic nucleus (STN) and the motor cortex. Here we report optogenetic inhibition of principal neurons in the STN significantly improved hemi-Parkinsonian motor asymmetry, measured by amphetamine-induced rotations. We also show preliminary results that revealed therapeutic improvement in motor asymmetry by single-site optogenetic excitation of the motor cortex. Although improvement from optogenetic modulations did not exceed the effects of DBS in the STN, our findings suggest that spatially patterned optogenetic stimulation of the cortex, i.e., more precise manipulation of cortical activity over larger area, should be investigated as a therapeutic approach for Parkinson's disease.

Keywords: Parkinson's disease, optogenetics, 6-OHDA-induced hemi-Parkinsonian rats, motor cortex stimulation.

I. INTRODUCTION

Deep brain stimulation (DBS) is an effective neurosurgical treatment for mid/late-stage Parkinson's disease (PD), commonly utilized in conjunction with dopamine replacement therapy. High frequency electrical pulses continuously delivered to basal ganglia nuclei, such as the subthalamic nucleus (STN) [1], [2] and globus pallidus internus (GPi) [3], [4] through an implanted multi-contact electrode, result in marked improvement in motor function and often reduction in medication. However not only are there side-effects associated with the stimulation, such as abnormal tingling sensations (paresthesia), speech problems (dysarthria), involuntary movements (dyskinesia) [5], [6], and impulse control problems (e.g., pathological gambling, compulsive eating) [7]–[10], the mechanisms of its action also remain largely unclear.

Recent endeavors to develop better stimulation paradigms, especially for the purposes of reducing side-effects and conserving battery life, have focused on introducing closed-loop control of stimulus delivery (see [11] for a review). However, with electrical stimulation, ideas for improvement are limited due to the mixed neurophysiological effects of electrical stimulation. For example, electrical stimulation affects nearby axonal elements more strongly than neurons [12], [13], and generates a complex modulation pattern on brain circuits. Therefore, developing more effective stimulation approaches for therapy can benefit from stimulation techniques that provide better spatial and cell-type specificity, such as optogenetics. Optogenetics, by incorporating genetic targeting methods, allows selective modulation (excitation or inhibition) of certain neuronal subtypes by light illumination. We therefore in this work investigated the feasibility of optogenetics as a therapy for ameliorating Parkinsonian motor symptoms. Because the STN has been an effective target in lesion and DBS therapies, we first tested the therapeutic value of optogenetic manipulation, i.e. excitation or inhibition, of the principal neurons in the STN in the well-established 6-hydroxydopamine (6-OHDA) lesion-

Corresponding Author: Ilker Ozden. Department of Bioengineering, University of Missouri, Columbia, MO, USA, Tel: +1 573-884-3686, E-mail: ozdeni@missouri.edu

generated hemi-Parkinsonian rat model. In this model, parkinsonian motor symptoms manifest on one side of animals' body, including akinesia, bradykinesia, and rotational biases. While prior studies that attempted optical inhibition of the STN with halorhodopsin in hemi-Parkinsonian rats showed no improvement in the rotational symptoms [14], [15], we found by contrast consistent reduction in rotational bias by optical inhibition with a new generation inhibitory opsin, though not as potent as electrical stimulation. On the other hand, optical excitation of the STN principal neurons failed to produce therapeutic effects, in agreement with an earlier result [14].

As for electrical DBS in the STN, the therapeutic efficacy of optogenetic inhibition of the STN was limited. Therefore, we turned our attention to other targets in the cortico-basal-ganglia system. Considering the pathophysiology of PD, the role of motor cortex in the motor circuit hierarchy and the effectiveness of optogenetics in controlling functional states of the cortex, here we suggest that motor cortex is a suitable alternative locus for optogenetic stimulation.

Motor cortex not only plays a key role in motor execution through direct corticospinal projections, it also engages tightly in motor regulation through the cortico-basal-ganglia-thalamo-cortical circuitry. In PD, the dynamics in this system shifts into a pathological state where excess power and synchrony in the beta band (13–30 Hz, in humans) in the motor cortex and basal ganglia are thought to be biomarkers of the ongoing (anti-kinetic) pathophysiology [16]–[19]. The anti-kinetic nature of beta-band activity in the motor cortex has been also suggested by studies involving healthy subjects, where beta-band activity in the motor cortex increased during maintenance of motor state (status-quo) [17]. In accordance, it has been demonstrated that both dopamine-replacement drugs [20], [21] and DBS-based therapies [22], [23] decrease the activity in the pathological beta-band. In fact, earlier work also suggested STN-DBS may exert its therapeutic effects through antidromic modulation of the motor cortex via a hyperdirect pathway [14], [24]–[26]. Recently, we have demonstrated that optogenetics is effective in modulating functional network dynamics in the motor cortex, e.g. enhancing gamma band (40-100Hz) oscillations by stimulation of pyramidal neurons in nonhuman primates [27].

Thus, in the latter part of the paper, we provide preliminary evidence supporting therapeutic effects of single-site intracortical optogenetic excitation of motor cortex, optogenetically transduced with a newly developed convection-enhanced delivery method [28].

II. METHODOLOGY

A. Animals

The animals used in this study were seven male Long-Evans rats (Charles River Laboratories, Inc., Wilmington, MA; 250–300 g at the start of the study), housed in the animal care facility at Brown University. The rats were kept on a 12-h light/dark cycle and housed individually with free access to food and water. All procedures were performed according to the National Institutes of Health guidelines and approved by the Brown University Institutional Animal Care and Use Committee (Protocol#: 1402000046, 1703000263).

B. Optogenetic virus constructs

We used recombinant adeno-associated viral vectors (serotype 2 pseudotyped with serotype 5, rAAV2/5) to transduce neurons to express excitatory or inhibitory opsins under the control of the Ca2+/calmodulin-dependent protein kinase II-alpha (CaMKIIα) promoter or the human synapsin 1 (hSyn) promoter. For excitation, we used channelrhodopsin (ChR2) ([rAAV5::CaMKIIα-hChR2(H134R)::EYFP-WPRE-pA], 6.2×10^{12} copies/mL) or its red-shifted variant, C1V1 ([rAAV5::CaMKIIα-C1V1(E122T/E162T)::TS-EYFP], 2×10^{12} copies/mL). For inhibition, we used third-generation halorhodopsin (eNpHR3.0) ([rAAV5::hSyn-eNpHR3.0::EYFP], 3.5×10^{12} copies/mL) or a potent inhibitory variant of channelrhodopsin, SwiChR ([rAAV5::CaMKIIα-$SwiChR_{CA}$::TS-EYFP], 5.6×10^{12} copies/mL). Viral constructs were kindly shared by Dr. Karl Deisseroth at Stanford University and were packaged and distributed through the University of North Carolina Vector Core.

C. Stereotaxic surgeries

Animals underwent stereotactic surgeries using a stereotaxic alignment system (David Kopf Instruments) under isoflurane anesthesia (1-3%). Ophthalmic ointment was applied to prevent eye drying. In one or serial surgeries, animals received virus injection for opsin transduction in STN or motor cortex, unilateral 6-OHDA injection, and implantation of recording and stimulation devices. At the end of the procedures, craniotomies were sealed with silicone elastomer (Kwik-Cast; World Precision Instruments) and the skin was sutured when possible.

1) Virus transfection for opsin expression

Two types of opsin delivery approaches were used. First, for injections in STN (-3.8 mm AP, -2.4 mm ML, -7.8 mm DV), a craniotomy (< 1 mm diameter) was made over the left hemisphere. The depth of STN was confirmed by extracellular recording with a single microwire. Injection was performed with a 25-μL gas-tight syringe with a 32-gauge hypodermic needle (1702SN, 60-° taper; Hamilton Company, NV); 1–2 μL of virus solution was injected at one or two depths within STN (1 μL each) at a speed of 0.1 μL/min. The needle was first inserted 500 μm beyond the target depth, maintained at the depth for 10 min to allow tissue to relax, and then retracted for injection. After injection, we waited another 10 min before retraction to allow for diffusion.

Second, for animals receiving optogenetic modulation in the motor cortex (forelimb area, +1.5 mm AP, -2.5 mm ML, -1.5 mm DV), convection-enhanced delivery (CED) of viruses was employed to enhance spatial spread of opsin expression across the (motor) cortex [28]. In total, 12 μL of virus solution was injected into two cortical sites (+3.0 mm AP, -2.5 mm ML, -1.5 mm DV; +0 mm AP, -2.5 mm ML, -1.5 mm DV) at higher speed (0.25–0.60 μL/min) with a special cannula [28].

2) Unilateral 6-OHDA injection

On the day of injection, 3.4 mg/ml (free-base) 6-hydroxydopamine-Br solution (with ascorbic acid, H116, Sigma Aldrich, MO) was prepared with sterile injectable saline. For protection of noradrenergic and serotonergic neurons [29], 25 mg/kg desipramine hydrochloride (D3900, Sigma Aldrich, MO) solution was injected i.p., 30 min prior to 6-OHDA injections. To achieve complete DA depletion, 3.0 μL (~10 μg free-base) of 6-OHDA was injected into the medial forebrain bundle (MFB) of the left hemisphere (-4.4 mm AP, -1.2 mm ML, -8.0 mm DV) with a 32-gauge hypodermic needle at a speed of 0.1 μL/min.

3) Implantation of optrodes

For STN modulation, an optrode was implanted into STN, following a procedure very similar to that of virus injection. The construct, comprising of a tapered glass optical fiber (0.22 NA, 105/125 μm core/cladding diameters, AFS105/125Y, Thorlabs) glued next to two 100 kOhm stainless steel electrodes (EL30030.1A10, Microprobes), was used to deliver light while recording extracellular activities or deliver bipolar electrical stimulation in the STN. In the optrode, the electrode tips were about 200 μm ahead of the tip of the fiber. For intracortical optical stimulation, a 105/125 μm glass optical fiber was implanted into the center of the motor forelimb area (+1.5 mm AP, -2.5 mm ML, -1.5 mm DV), together with the same optrode implanted in the STN. The implants were secured to stainless steel skull anchoring screws with dental acrylic; and the skin was sutured to close around the implant.

D. Behavior assessment

Hemi-parkinsonian motor symptoms including akinesia/bradykinesia and motor asymmetry were assessed with a video camera during animals' free behavior in a 30.5 cm (12 in) diameter acrylic cylinder with a video camera. Here we report two assessments of motor asymmetry: spontaneous rotations (SR) and amphetamine-induced rotations (Amph-IR). Hemi-Parkinsonian rats in free behavior showed spontaneous rotational bias which was manifested as predominant counter-clockwise (CCW, ipsilateral to the unilateral 6-OHDA injection on the left hemisphere) rotations.

In Amph-IR experiment, D-amphetamine (2.2 mg/kg, free-base, i.p.) blocks dopamine re-uptake and stimulates dopamine release on the healthy hemisphere [30], hence further increases the rotational biases and drives rats into a state of continuous locomotion (rotation) for 30–60 min. After injection, we waited 10 min for rotational behavior to establish and stabilize. 1–2 weeks after 6-OHDA lesion, rats were first tested with amphetamine challenge; only the animals showing peak Amph-IR of more than 6 RPM were included in this study.

In optrode implanted animals, Amph-IR tests were carried out by cabling optical/electrical signals aided by a joint electrical and optical commutator (HRJ-OE, Doric Lenses, Canada). A custom-made gear-based magnetic rotameter was used accurately record the rotational position of the animal, which was synchronized with electrophysiology and the delivery of stimuli. To account for the transient variations in the baseline Amph-IR, each stimulation trial was performed together with two no-stimulation trials (pre-stim and post-stim). Good optical coupling and electrical contacts were confirmed for each stimulation trial based on the modulations on electrophysiology; trials without consistent stimulus-related artifact or LFP modulations were discarded.

E. Electrical and optical stimulation

For electrical stimulation, bipolar high-frequency (125Hz) electrical stimulation was delivered to the STN through the two stainless steel electrodes. Biphasic

charge-balanced pulses (60–240 μsec) were profiled with a pulse generator (Master-8, AMPI, Israel), applied as isolated constant current stimuli (Model 2200, AM systems), and delivered continuously. The current intensities (100–200 μA) used did not trigger direct motor responses.

For optical stimulation, either a 473 nm laser (for ChR2 and SwiChR, MLL-III-473, OptoEngine) or a 561 nm laser (for C1V1 and eNpHR, MLL-FN-561, OptoEngine) was coupled to a patch cable, which was further connected to the optical fiber on the animal's head through an optical commutator. A train of square pulses, 4 msec in duration and 100 Hz in frequency (50% duty cycle), were generated from the pulse generator to control the gating of the laser. In some experiments, a train of light pulses was further organized into several identical bursts, 1.5–9.0 sec burst duration and 0.5–1.0 sec interburst interval. The power of light delivered to the brain was ranged from 3 to 12 mW.

F. Histology and image analysis

For histological analysis, rats were euthanized by a lethal injection of Beuthanasia-D (100 mg/kg, IP) and transcardially perfused with 0.1 M phosphate-buffered saline (PBS) solution, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB) solution. Brain was extracted and sequentially fixed in 4% paraformaldehyde in PB solution (for at least 1 day) and cryoprotected in 30% sucrose solution (until brain fully sunk in solution). The brain was then frozen with dry ice and cut into 50 μm (for confocal imaging) or 100 μm (for fluorescence imaging) coronal slices with a microtome. Slices were examined under a Zeiss LSM 510 Meta confocal laser scanning microscope or a Zeiss Axiovert 200M fluorescence microscope.

In a subset of rats (N = 5), the extent of dopaminergic lesion was evaluated with tyrosine hydroxylase (TH) immunostaining. Slices were sequentially washed in PB (twice, 5 min each) and PBS (three times, 5 min each), and then incubated in block solution for 1 h. The block solution consisted of 0.1% Tween (Sigma–Aldrich, MO), 0.25% Triton-X (Sigma–Aldrich, MO), and 10% normal goat serum (EMD Millipore, MA) in PBS. Slices were then immersed in the primary antibody solution (1:1000 AB152 Anti-TH in block solution EMD Millipore), covered with aluminum foil, and shaked for 2.5 days in a cold room (4°C). Afterward, slides were thoroughly washed in PBS (five times, 5 min each), again immersed in block solution for 1hr, and incubated in secondary antibody solution (1:500, Alexa Fluor ® 594 donkey anti-rabbit IgG in block solution, Life technology, CA) for 2 hr. After the final washing steps with PBS (three times, 5 min each) and PB (twice, 5 min each), slides were mounted on microscope slides with DAPI mounting medium (H-1200, Vector Laboratories, CA).

For characterization of TH immunoreactivity, fluorescence images of slices (containing striatum (STR) or substantia nigra compacta (SNc)) obtained in grayscale were normalized in intensity from 0 to 1. Square regions-of-interest (ROIs) of 200-250 μm size were manually selected from the nucleus at question on each hemisphere. The average normalized fluorescence intensities within these ROIs were calculated (background intensity subtracted), which were used as surrogates for immunoreactivity. The relative difference (% change) of fluorescence on the lesion and control hemispheres was calculated.

I. Statistical analysis

All data are presented as mean ± standard error of mean (s.e.m.). All statistical analyses were performed with MATLAB (MathWorks, MA). A paired-sample, single-tail, unequal variance t-Test was used to compare the rotations with or without electrical or optical stimulation. TH-immunoreactivity was compared with a single-tail, unequal variance t-Test. Two-way analysis of variance (ANOVA) was used to separate effects of treatment and individual variations. Statistical significance was granted for values of $p < 0.01$. For statistically significant comparisons, the effect size of the difference was measured with the Cohen's *d* parameter. In figures, asterisks (*) indicate the level of significance (*p < 0.01, **p < 1E-3, ***p < 1E-4) and palm signs (#) indicate the Cohen's *d* effect size (#d > 0.8, ##d > 1.2, ###d > 2.0).

III. RESULTS AND DISCUSSION

Reliable targeting of the STN for virus injection (Fig.1A-B) and optrode implantation was achieved through electrophysiology-assisted stereotaxic targeting methods (Fig.1C). STN showed characteristic features in extracellular electrophysiology including a high level of background activity and symmetric, large amplitude spikes, which was surrounded by two quiet regions above (zona incerta, ZI) and below (internal capsule, i.c.). Functional opsin expression was also verified with light modulated extracellular recording. Neural responses associated with *in vivo* optogenetic modulation at STN were complex, partially due to the highly interconnected local network. For example,

inhibitory, excitatory, or both inhibitory and excitatory responses were observed during optogenetic activation of an inhibitory opsin, SwiChR (data not shown).

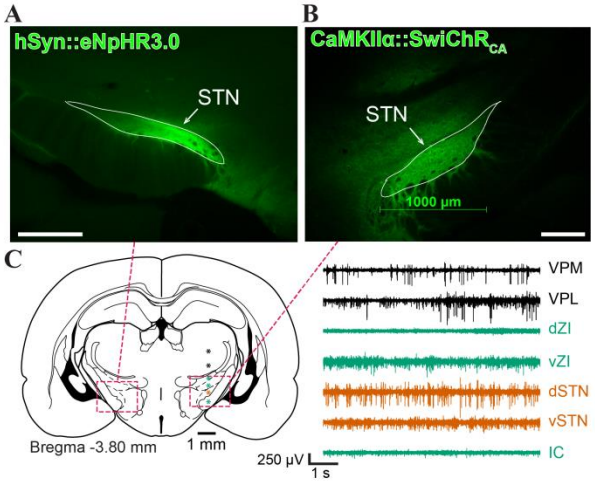

Fig.1: Opsin expression in the STN
A-B. Example STN opsin expression in two animals. Scale bars = 500 µm.
C. Representative electrophysiological features along the track towards STN (VPM, VPL: thalamic nuclei; dZI, vZI: dorsal and ventral zona incerta; IC: internal capsule).

Near-complete dopaminergic depletion is necessary for the establishment of (hemi-)Parkinsonian motor symptoms [31], [32]. Tyrosine hydroxylase (TH) immunostaining showed >90% loss of dopaminergic neurons in the SNc and nucleus accumbens (NAc, data not shown), as well as loss of dopaminergic projection terminals in the striatum, in the hemisphere injected with 6-OHDA (Fig.2). Stereotypical motor symptoms, such as akinesia/bradykinesia and motor asymmetry were observed in all lesioned rats.

Evaluation of spontaneous rotational bias was often confounded by akinesia (animals' tendency to not ambulate). Therefore, we used amphetamine challenge to further increase the rotational biases and drive animals into a state of continuous locomotion (rotation).

Bipolar high-frequency (125Hz) electrical stimulation in the STN with charge-balanced biphasic pulses (60-240 µsec, 100-200 µA) significantly increased animals' movement, alleviated spontaneous rotational biases, and reduced asymmetric Amph-IR by 31.6 ± 4.2% ($p < 1E\text{-}4$, $N = 5$; $F_{1,4} = 63.6$, $p \rightarrow 0$) (Fig. 3A).

Optogenetic excitation of the principal neurons in the STN failed to produce any consistent prokinetic improvement (data not shown), which agreed with the observations in previous literature [14].

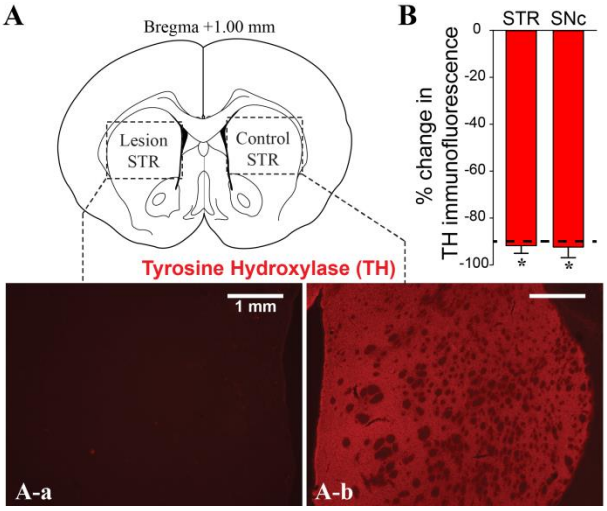

Fig.2: Tyrosine-hydroxylase immunohistology
A. Example confocal images showing TH-immunoreactivity in the striatum (STR) on the lesion (A-a) and control (A-b) hemisphere, respectively. **B.** Near complete (>90%) loss of dopaminergic neurons (in SNc) and their terminals (in STR) in five animals; one-tailed unequal-variance paired t-Test, p→0.

By notable contrast, optogenetic inhibition of the principal neurons in the STN led to marked reduction of Amph-IR by 16.3 ± 4.5% ($p < 0.001$, $N = 3$; $F_{1,2} = 14.87$, $p < 0.001$) (Fig.3B), though not as potent as that of electrical stimulation. While in three animals, behavior improvement occurred only during optical stimulation, in two other rats, the effect of stimulation was long-lasting even after single trial of optical stimulation, i.e. rats started to perform CW Amph-IR even without stimulation for hours or even days. Such persistence of effects limits the formal statistical analysis over multiple trials. In terms of spontaneous behavior (SR or bradykinesia), no noticeable improvement was found with STN inhibition.

In rats, the small size of STN and the rather limited capability to optogenetically interrogate neuronal subtypes in non-transgenic animals constrained the parameter space one can explore for better stimulation outcome. In response, we then investigated the motor cortex as an alternative locus for optogenetic stimulation.

However, studies aiming to use optogenetics-based stimulation in the motor cortex to modulate motor behavior of rats have been so far hampered by the small

volume and insufficient expression level of the opsin transduction. The convection-enhanced delivery (CED) of viruses in the rat cortex, a method recently optimized in our lab [28], enabled us to achieve widespread and functional opsin expression across the motor cortical area.

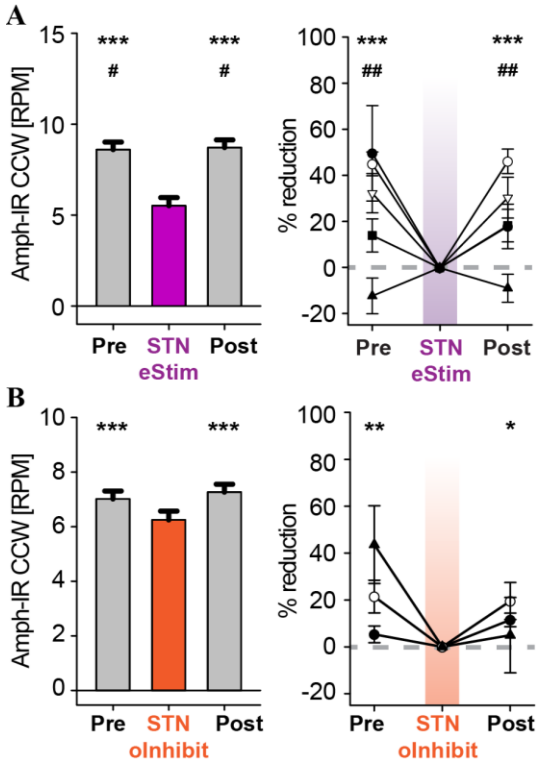

Fig.3: Rotational bias induced by amphetamine (Amph-IR) was reduced by electrical stimulation (**A**) and optogenetic inhibition (**B**) of STN. Both the absolute speed of rotation (left column) and the percentage reduction in rotation (right column) was diagramed. See methods for interpretations of * and #.

Single-site intracortical optical excitation of the motor cortex led to marked reduction in motor asymmetry measured with Amph-IR by 10.4 ± 3.0 % ($p < 1E-4$, N = 2) (Fig.4). Motor cortical excitation from activation of both ChR2 (9.3 ± 3.2 %, N = 1) and C1V1 (13.3 ± 4.8 %, N=1) led to consistent improvements, however while clear, the effect was less potent than STN-inhibition and STN electrical stimulation.

Single-site intracortical optical excitation by a single glass fiber, at the power levels we utilized, is expected to cover a small fraction of the motor cortex (effective light intensity drops to 1% at 1 mm distance from the fiber [33]) and prohibit the intended global activation of motor cortex. We are currently investigating the therapeutic effects of wide-field surface illumination and multi-site intracortical illumination as means of engaging larger areas of the motor cortex to optogenetic excitation. Multi-site cortical modulation is favorable also due to the variable manifestation of Parkinsonian pathophysiology in the motor cortex [34]. An ongoing study, employing multi-electrode array recording in the motor cortex of hemi-Parkinsonian rats, discovered spatial heterogeneity (400 μm resolution) in the power of beta-band activity over the motor cortex: some sites on the lesioned motor cortex showed beta power indistinguishable from the control level [35].

Therefore, spatially targeted delivery of optogenetic stimulation may bring more drastic, yet more specific, behavioral improvements than single-site intracortical or surface illumination, especially, with recently developed optoelectronic probes which can deliver light and record neural activity at multiple locations [36].

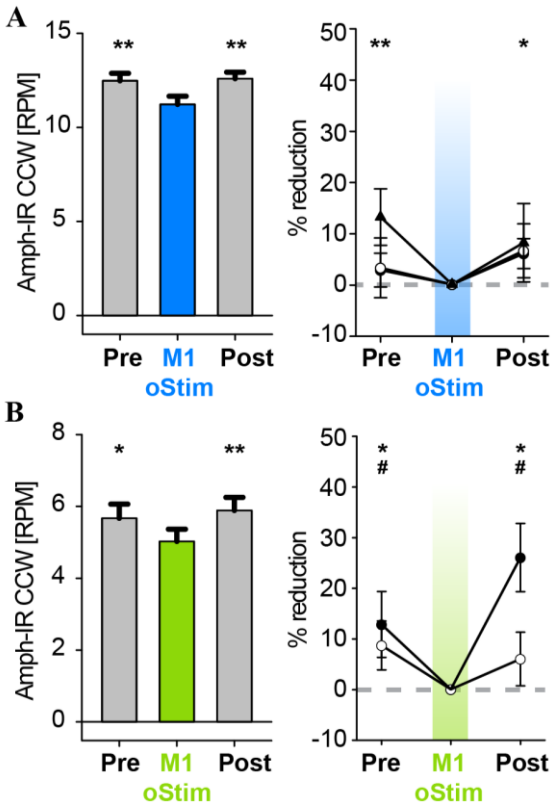

Fig.4: Amph-IR reduced by single-site intracortical optogenetic excitation of the motor cortex with two different excitatory opsins: ChR2 (**A,** N = 1 rat) and C1V1 (**B,** N = 1 rat). Statistical analysis was performed on each animal across sessions.

IV. CONCLUSION

Our results indicate that optogenetic inhibition of STN has therapeutic value as a neuromodulation approach in PD. Both optogenetic inhibition of STN and optogenetic excitation of a small fraction of the motor cortex led to reduction in disease-induced rotational biases in hemi-Parkinsonian rats. However, the efficacy by optogenetic manipulations in both cases was not superior to that of electrical STN-DBS.

Given that optogenetic stimulation has only been delivered at a single-site in the cortex, theoretically limiting excitation volume to a small fraction (a sphere of < 1 mm diameter) of the functional motor cortex, we suggest that further improvements in the therapeutic value of motor cortex stimulation could be achieved by engaging more of the motor cortex to activation and controlling errant cortical dynamics, using spatially and temporally patterned optogenetic stimulation.

ACKNOWLEDGEMENTS

We thank Dr. Saundra Patrick at Brown University for the instructions on the immunohistology, and Dr. Kevin Bath at Brown University Rodent Neurodevelopmental Behavioral Testing Facility for help in behavior assessment. Imaging was performed at Brown University Leduc Bioimaging Facility. This work was supported by the Brown Institute for Brain Science/Norman Prince Neurosciences Institute [New Frontiers Award (I.O. and W.A.) and Graduate Student Research Fellowship (Z.Y.)], and National Science Foundation grants [CBET-1402803 and CBET-1264816 (A.N.)].

REFERENCES

[1] P. Pollak *et al.*, "[Effects of the stimulation of the subthalamic nucleus in Parkinson disease]," *Rev. Neurol.* , vol. 149, no. 3, pp. 175–176, 1993.

[2] A. L. Benabid, "Deep brain stimulation for Parkinson's disease," *Curr. Opin. Neurobiol.*, vol. 13, no. 6, pp. 696–706, Dec. 2003.

[3] J. Siegfried and B. Lippitz, "Bilateral chronic electrostimulation of ventroposterolateral pallidum: a new therapeutic approach for alleviating all parkinsonian symptoms," *Neurosurgery*, vol. 35, no. 6, pp. 1126–9; discussion 1129–30, Dec. 1994.

[4] K. A. Follett *et al.*, "Pallidal versus subthalamic deep-brain stimulation for Parkinson's disease," *N. Engl. J. Med.*, vol. 362, no. 22, pp. 2077–2091, Jun. 2010.

[5] S. Zhang, P. Zhou, S. Jiang, W. Wang, and P. Li, "Interleaving subthalamic nucleus deep brain stimulation to avoid side effects while achieving satisfactory motor benefits in Parkinson disease: A report of 12 cases," *Medicine* , vol. 95, no. 49, Dec. 2016.

[6] H. Akram *et al.*, "Subthalamic deep brain stimulation sweet spots and hyperdirect cortical connectivity in Parkinson's disease," *Neuroimage*, vol. 158, pp. 332–345, Sep. 2017.

[7] T. D. Hälbig *et al.*, "Subthalamic deep brain stimulation and impulse control in Parkinson's disease," *Eur. J. Neurol.*, vol. 16, no. 4, pp. 493–497, Apr. 2009.

[8] M. L. Dodd, K. J. Klos, J. H. Bower, Y. E. Geda, K. A. Josephs, and J. E. Ahlskog, "Pathological gambling caused by drugs used to treat Parkinson disease," *Arch. Neurol.*, vol. 62, no. 9, pp. 1377–1381, Sep. 2005.

[9] M. Broen, A. Duits, V. Visser-Vandewalle, Y. Temel, and A. Winogrodzka, "Impulse control and related disorders in Parkinson's disease patients treated with bilateral subthalamic nucleus stimulation: a review," *Parkinsonism Relat. Disord.*, vol. 17, no. 6, pp. 413–417, Jul. 2011.

[10] A. Merola *et al.*, "Impulse control behaviors and subthalamic deep brain stimulation in Parkinson disease," *J. Neurol.*, vol. 264, no. 1, pp. 40–48, Jan. 2017.

[11] M. Parastarfeizabadi and A. Z. Kouzani, "Advances in closed-loop deep brain stimulation devices," *J. Neuroeng. Rehabil.*, vol. 14, no. 1, p. 79, Aug. 2017.

[12] C. C. McIntyre, M. Savasta, L. Kerkerian-Le Goff, and J. L. Vitek, "Uncovering the mechanism(s) of action of deep brain stimulation: activation, inhibition, or both," *Clin. Neurophysiol.*, vol. 115, no. 6, pp. 1239–1248, Jun. 2004.

[13] E. J. Tehovnik and W. M. Slocum, "Two-photon imaging and the activation of cortical neurons," *Neuroscience*, vol. 245, pp. 12–25, Aug. 2013.

[14] V. Gradinaru, M. Mogri, K. R. Thompson, J. M. Henderson, and K. Deisseroth, "Optical deconstruction of parkinsonian neural circuitry," *Science*, vol. 324, no. 5925, pp. 354–359, Apr. 2009.

[15] H. H. Yoon *et al.*, "Optogenetic inactivation of the subthalamic nucleus improves forelimb akinesia in a rat model of Parkinson disease," *Neurosurgery*, vol. 74, no. 5, pp. 533–40; discussion 540–1, May 2014.

[16] A. A. Kühn, P. Grosse, K. Holtz, P. Brown, B.-U. Meyer, and A. Kupsch, "Patterns of abnormal motor cortex excitability in atypical parkinsonian syndromes," *Clin. Neurophysiol.*, vol. 115, no. 8, pp. 1786–1795, Aug. 2004.

[17] A. Schnitzler, L. Timmermann, and J. Gross, "Physiological and pathological oscillatory networks in the human motor system," *J. Physiol. Paris*, vol. 99, no. 1, pp. 3–7, Jan. 2006.

[18] A. K. Engel and P. Fries, "Beta-band oscillations--signalling the status quo?," *Curr. Opin. Neurobiol.*, vol. 20, no. 2, pp. 156–165, Apr. 2010.

[19] B. Pollok, V. Krause, W. Martsch, C. Wach, A. Schnitzler, and M. Südmeyer, "Motor-cortical

oscillations in early stages of Parkinson's disease," *J. Physiol.*, vol. 590, no. 13, pp. 3203–3212, Jul. 2012.
[20] C. Delaville *et al.*, "Oscillatory Activity in Basal Ganglia and Motor Cortex in an Awake Behaving Rodent Model of Parkinson's Disease," *Basal Ganglia*, vol. 3, no. 4, pp. 221–227, Apr. 2014.
[21] M. Li *et al.*, "The network of causal interactions for beta oscillations in the pedunculopontine nucleus, primary motor cortex, and subthalamic nucleus of walking parkinsonian rats," *Exp. Neurol.*, vol. 282, pp. 27–36, Aug. 2016.
[22] D. Whitmer, C. de Solages, B. Hill, H. Yu, J. M. Henderson, and H. Bronte-Stewart, "High frequency deep brain stimulation attenuates subthalamic and cortical rhythms in Parkinson's disease," *Front. Hum. Neurosci.*, vol. 6, p. 155, Jun. 2012.
[23] V. Litvak *et al.*, "Movement-related changes in local and long-range synchronization in Parkinson's disease revealed by simultaneous magnetoencephalography and intracranial recordings," *J. Neurosci.*, vol. 32, no. 31, pp. 10541–10553, Aug. 2012.
[24] A. Nambu, H. Tokuno, and M. Takada, "Functional significance of the cortico-subthalamo-pallidal 'hyperdirect' pathway," *Neurosci. Res.*, vol. 43, no. 2, pp. 111–117, Jun. 2002.
[25] Q. Li *et al.*, "Therapeutic deep brain stimulation in Parkinsonian rats directly influences motor cortex," *Neuron*, vol. 76, no. 5, pp. 1030–1041, Dec. 2012.
[26] C. de Hemptinne *et al.*, "Therapeutic deep brain stimulation reduces cortical phase-amplitude coupling in Parkinson's disease," *Nat. Neurosci.*, vol. 18, no. 5, pp. 779–786, May 2015.
[27] Y. Lu *et al.*, "Optogenetically induced spatiotemporal gamma oscillations and neuronal spiking activity in primate motor cortex," *J. Neurophysiol.*, vol. 113, no. 10, pp. 3574–3587, Jun. 2015.
[28] Z. Yu, A. Nurmikko, and I. Ozden, "Widespread functional opsin transduction in the rat cortex via convection-enhanced delivery optimized for horizontal spread," *J. Neurosci. Methods*, vol. 291, pp. 69–82, Nov. 2017.
[29] S. L. Thiele, R. Warre, and J. E. Nash, "Development of a unilaterally-lesioned 6-OHDA mouse model of Parkinson's disease," *J. Vis. Exp.*, no. 60, Feb. 2012.
[30] R. J. Carey, "Factors in amphetamine-induced contralateral rotation in the unilateral 6-OHDA lesion rat model during the first-week postoperative: implications for neuropathology and neural grafting," *Brain Res.*, vol. 570, no. 1–2, pp. 11–20, Jan. 1992.
[31] A. D. Smith, M. Amalric, G. F. Koob, and M. J. Zigmond, "Effect of bilateral 6-hydroxydopamine lesions of the medial forebrain bundle on reaction time," *Neuropsychopharmacology*, vol. 26, no. 6, pp. 756–764, Jun. 2002.
[32] R. Deumens, A. Blokland, and J. Prickaerts, "Modeling Parkinson's disease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway," *Exp. Neurol.*, vol. 175, no. 2, pp. 303–317, Jun. 2002.
[33] I. Ozden *et al.*, "A coaxial optrode as multifunction write-read probe for optogenetic studies in non-human primates," *J. Neurosci. Methods*, vol. 219, no. 1, pp. 142–154, Sep. 2013.
[34] J. Hirschmann *et al.*, "Distinct oscillatory STN-cortical loops revealed by simultaneous MEG and local field potential recordings in patients with Parkinson's disease," *Neuroimage*, vol. 55, no. 3, pp. 1159–1168, Apr. 2011.
[35] Zeyang Yu, Wael Asaad, Arto Nurmikko, Ilker Ozden, "Spatiotemporal inhomogeneity of pathological beta activities in the motor cortex of hemi-parkinsonian rats," presented at the Society of Neuroscience Annual Meeting, San Diego, CA, 15-Nov-2106.
[36] J. Lee, I. Ozden, Y.-K. Song, and A. V. Nurmikko, "Transparent intracortical microprobe array for simultaneous spatiotemporal optical stimulation and multichannel electrical recording," *Nat. Methods*, vol. 12, p. 1157, Oct. 2015.