Hyung Ho Yoon, MS*‡
Jin Hoon Park, MD, PhD§*
Yong Hwan Kim, BS¶
Joongkee Min, PhD‡
Eunmi Hwang, PhD||
C. Justin Lee, PhD||
Jun-Kyo Francis Suh, PhD#
Onyou Hwang, PhD**
Sang Ryong Jeon, MD, PhD‡

‡Department of Neurological Surgery and **Department of Biochemistry and Molecular Biology, University of Ulsan College of Medicine, Seoul, Republic of Korea; §Department of Neurological Surgery, Gangneung Asan Hospital, University of Ulsan College of Medicine, Gangneung, Republic of Korea; ¶Department of Computer Science and Engineering, Bioengineering Graduate Program, University of Notre Dame, Notre Dame, Indiana; ||Center for Neural Science and WCI Center for Functional Connectomics, Korea Institute of Science and Technology, Seoul, Republic of Korea; #Center for Bionics of Korea Institute of Science and Technology, Seoul, Republic of Korea

*These authors contributed equally to this work.

Correspondence:
Sang Ryong Jeon, MD, PhD,
Department of Neurological Surgery,
Asan Medical Center,
University of Ulsan College of Medicine,
Pungnap-2 dong, Songpa-gu,
Seoul 138-736, Korea.
E-mail: srjeon@amc.seoul.kr

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Optogenetic Inactivation of the Subthalamic Nucleus Improves Forelimb Akinesia in a Rat Model of Parkinson Disease

BACKGROUND: The inhibition of neuronal activity by electrical deep brain stimulation is one of the mechanisms explaining the therapeutic effects in patients with Parkinson disease (PD) but cannot specifically activate or inactivate different types of neurons. Recently, a new technology based on optogenetics has been developed to modulate the activity of specific neurons. However, the therapeutic effects of optical inactivation in the subthalamic nucleus (STN) have not been fully investigated.

OBJECTIVE: To perform various behavioral tests to evaluate changes in motor functions in a PD rat model after optogene expression and, unlike previous studies, to assess the therapeutic effects of direct optogenetic inactivation in the STN.

METHODS: 6-Hydroxydopamine-induced hemiparkinsonian rats received injections of hSynapsin1-NpHR-YFP adeno-associated virus or an equivalent volume of phosphate-buffered saline. Three weeks after injection of adeno-associated virus or phosphate-buffered saline, the optic fiber was implanted into the ipsilateral STN. A stepping test, a cylinder test, and an apomorphine-induced rotation test were performed in 3 sequential steps: during light-off state, during light stimulation, and again during light-off state.

RESULTS: Stepping tests revealed that optical inhibition of the STN significantly improved 6-hydroxydopamine-induced forelimb akinesia. PD motor signs, as assessed by cylinder and apomorphine tests, were not affected by optical inhibition. Immunofluorescence revealed that halorhodopsin was highly expressed and colocalized with vesicular glutamate transporter 2 in the STN.

CONCLUSION: Optogenetic inhibition in the STN may be effective in improving contralateral forelimb akinesia but not in changing forelimb preference or reducing dopaminergic receptor supersensitivity. These findings are useful as a basis for future studies on optogenetics in PD.

KEY WORDS: Deep brain stimulation, Forelimb akinesia, Halorhodopsin, hSynapsin1, Optogenetics, Parkinson disease, Subthalamic nucleus

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Abbreviations: AAV, adeno-associated virus; **DBS,** deep brain stimulation; **PBS,** phosphate-buffered saline; **PD,** Parkinson disease; **6-OHDA,** 6-hydroxydopamine; **SN,** substantia nigra; **STN,** subthalamic nucleus; **TH,** tyrosine hydroxylase; **VGLUT2,** vesicular glutamate transporter 2

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Deep brain stimulation (DBS) is a surgical treatment for advanced Parkinson disease (PD), resulting in remarkable amelioration of motor function and a reduction in the need for medication.[1-3] However, the mechanisms underlying DBS are not clear. One hypothesis is that the inserted electrode may lead to an elevated extracellular potassium level, resulting in inhibition of neuronal activity in the target area in the PD rat model.[4,5] However, it is difficult to activate or inactivate a specific neuron because of the nature of nonspecific electrical stimulation.[6] Recently, a new technology using

optogenes and illumination has been developed, leading to the modulation of specific neurons.[7,8]

To investigate the therapeutic effects of this new optogenetic stimulation method, the subthalamic nucleus (STN), which is the most common target of electrical DBS in PD patients, was used to assess optogene expression. Optical stimulation of the STN with halorhodopsin has previously been performed in a hemiparkinsonian rat model generated by 6-hydroxydopamine (6-OHDA) injection.[6] After optical inhibition with halorhodopsin in the STN, no therapeutic effects, as assessed by rotational behavior, were observed in the rats, whereas high-frequency electrical stimulation produced therapeutic effects.[6] Unfortunately, drug-induced rotation tests in PD rats do not fully mimic the symptoms of PD seen in humans.[9-11] Thus, more adequate behavioral evaluation methods are needed to determine the efficacy of optical STN inhibition.

In our present study, we provide therapeutic evidence of STN inhibition using optical stimulation with halorhodopsin. The stepping test has previously been used to monitor unilateral motor deficits in a rat model of PD.[10,12,13] By performing this test, we were able to clarify whether the motor signs of animals improved during light stimulation.

METHODS

Experimental Animals

Seventeen male Wistar rats (Orient Bio Inc, Seongnam, Korea) weighing 250 to 300 g at the beginning of the experiment were housed in a room with a 12 hour/12 hour light/dark cycle and free access to food and water. All procedures complied with the guidelines of the Institutional Animal Care and Use Committee of the Asan Institute for Life Sciences (Seoul, Korea).

6-OHDA Lesion Surgery

Surgical procedures were performed under general anesthesia induced by intraperitoneal injection of a mixture of 35 mg/kg zoletil (Virbac S.A., Carros, France) and 5 mg/kg rompun (Bayer, Leverkusen, Germany). A total of 17 rats received unilateral injections of 8 μg 6-OHDA (Sigma, St. Louis, Missouri) in 4 μL of 0.9% saline with 0.1% ascorbic acid into the right medial forebrain bundle at the following coordinates: AP −2.2 mm, L +1.5 mm relative to the bregma, and V −8.0 mm from the dura by the Paxinos rat atlas, with the tooth bar set at +4.5 mm.[14] The toxin was delivered at a rate of 1 μL/min with a 33-gauge Hamilton syringe and an automated microsyringe pump (Harvard Apparatus, Holliston, Massachusetts). After injection, the needle was kept in place for 5 minutes to prevent the solution from flowing backward and was then retracted over a subsequent 5 minutes.

Preparation of hSynapsin1-NpHR-YFP Adeno-Associated Virus

The adeno-associated virus (AAV) vector plasmid pAAV2-hSynapsin1-NpHR-YFP was constructed by removing the CMV promoter from pAAV2-CMV (Stratagene, La Jolla, California) and inserting the hSynapsin1 promoter and NpHR-YFP sequence (plasmid 26775, Addgene). pAAV-RC plasmid harbors the AAV replication and capsid genes encoding the replication and virus capsid structure protein, respectively. The pHelper plasmid contains the essential subset of adenovirus genes, VA, E2A, and E4, necessary for AAV production in HEK293 cells. For AAV2/2 production, 3 AAV plasmids (10 μg of each plasmid) were cotransfected into HEK293 cells by use of a polyethylenimine transfection kit (polyplus, Illkirch-Graffenstaden, France).

Injection of hSynapsin1-NpHR-YFP AAV

The minimum number of viral particles was 1.0×10^{10} per 1 mL, which is a concentrated virus package. Three weeks after injection of 6-OHDA, viral injections were performed under general anesthesia induced by intraperitoneal injection of a mixture of 35 mg/kg zoletil and 5 mg/kg rompun. The rats were randomly separated into 2 groups. The NpHR group (n = 10) received injections of 2.3 μL hSynapsin1-NpHR-YFP AAV targeted at the ipsilateral STN (coordinates: AP, −3.8 mm; L, +2.5 mm relative to the bregma; and V, −7.8 mm from the dura[15]). The control group (n = 7) received an equivalent volume of phosphate-buffered saline (PBS) injections. The AAV and PBS were delivered at a rate of 0.5 μL/min with a Hamilton syringe and an automated microsyringe pump in the same way as for the procedure of 6-OHDA injections.

Optic Fiber Implantation and Light Stimulation

Optic fibers (200-μm core, 245-μm outer diameter, numerical aperture of 0.53, RM3 type, and flat tip; Doric lenses; Québec City, Québec, Canada) were cut to the length of 8.5 mm to optimize STN depth. At least 3 weeks after injection of AAV or PBS, the animals were positioned in a stereotactic frame under general anesthesia. After scalp incision, 4 burr holes were drilled to fix screws around the insertion site of the optic fiber. The optic fiber was implanted with the use of a stereotactic cannula holder into the center of the STN at the same site of AAV or PBS injection. The optic fiber was firmly fixed with surrounding screws with dental cement (Vertex; Zeist, Nederland). For light stimulations, the optic fiber was connected to the fiberoptic rotary joint and LED (light-emitting diode) fiberoptic light source (Doric lenses) that produces amber (590 nm) light. Spectral bandwidth was 17-nm full-width half-maximum, and output power with a fiber with a 200-μm core and a numerical aperture of 0.53 was 1.1 mW by the manufacturer's measurement for amber (590 nm).

Stepping Test

The stepping test was performed twice at 1 and 4 to 6 weeks after optic fiber implantation. Each stepping test was performed in 3 sequential steps: during light-off state (prelight test), during light stimulation, and during light-off state (postlight test). The stepping test was performed as previously described with slight modifications.[13,16] Briefly, both hind limbs were firmly fixed in 1 hand of the experimenter, and 1 of the forelimbs was fixed in the other hand. The test was repeated with both the contralateral and ipsilateral forelimbs. The rostral part of the rat was lowered onto a treadmill (Jeung Do Bio & Plant Co, Seoul, Korea) that was moving at rate of 0.9 m/5 s. The rat's body remained stationary while the unilateral forelimb was allowed to spontaneously touch the moving treadmill track for 10 seconds. All of the experiment sessions were video recorded to allow the number of adjusted steps taken in the backward direction to be counted. Every rat performed the stepping test twice in each session, and the number of steps taken was averaged across the 2 trials. Data are expressed as a percentage of contralateral forelimb

touches from combined forelimb touches. When the rats used both forelimbs symmetrically, the contralateral forelimb touch rate was 50%.

Cylinder Test

The cylinder test was performed at 2 to 3 weeks after optic fiber implantation. Each cylinder test was performed in 3 sequential steps: during light-off state (prelight test), during light stimulation, and during light-off state (postlight test). The cylinder test was performed as previously described.[17] Briefly, a rat was placed in a transparent glass cylinder (20-cm diameter, 35-cm height) until he explored the walls vertically with his forelimbs >20 times. All the tests were recorded to count the number of spontaneous wall contacts. The first 20 wall contacts were included for data analysis and are expressed as the number of wall contacts in the contralateral forelimb, ipsilateral forelimb, and both forelimbs.

Apomorphine-Induced Rotation Test

The apomorphine-induced rotation test was performed at 2 to 3 weeks after optic fiber implantation. The test was performed in 3 sequential steps: during light-off state (prelight test), during light stimulation, and during light-off state (postlight test). Apomorphine (0.25 mg/kg, Sigma) in sterile water was administrated subcutaneously, and rats were immediately connected to the fiberoptic rotary joint (Doric lenses). Rotational behavior was recorded for a total of 30 minutes (prelight test for 10 minutes, light stimulation for 10 minutes, and postlight test for 10 minutes). Data are expressed as the net (contralateral − ipsilateral turns) average rotations per min.

Tissue Processing

For tissue fixation, rats were transcardially perfused with 0.9% saline containing 10 000 IU heparin (Hanlim Pharm, Seoul, Korea), followed by 4% paraformaldehyde in PBS. Brains were extracted and postfixed overnight in the same fixative, followed by dehydration in 30% sucrose until they sank. Coronal sections (40-μm thickness) of the STN (AP, −3.36 to −4.08 mm) and substantia nigra (SN; AP, −4.8 to −6.0 mm) were collected with the use of a cryotome (Thermoscientific, Waltham, Massachusetts) and preserved under free-floating conditions in 0.08% sodium azide (Sigma) in PBS at 4°C.

Histology

For the detection of YFP expression, serial coronal sections of the STN were washed in 0.5% bovine serum albumin in PBS (pH 7.4) and incubated with DAPI (KPL, Gaithersburg, Maryland) in PBS. Coverslipped slides were then observed by confocal microscopy (Carl Zeiss, Oberkochen, Germany; Leica, Wetzlar, Germany). Immunostaining and immunofluorescence procedures were performed as previously described.[18] STN sections were incubated overnight with a rabbit antivesicular glutamate transporter 2 (VGLUT2; 1:300; Synaptic Systems, Göttingen, Germany) in 0.5% bovine serum albumin in PBS. They were subsequently incubated for 2 hours with Alexa Fluor 555 goat anti-rabbit IgG (Invitrogen, Carlsbad, California). Fluorescent-labeled tissues were coverslipped with DAKO fluorescent mounting medium (DAKO, Glostrup, Denmark). SN sections (rostral section, −4.8 mm; medial section, −5.4 mm; caudal section, −6.0 mm) were selected and incubated overnight with a mouse anti-tyrosine hydroxylase (TH; 1:2000; Sigma) antibody in 0.5% bovine serum albumin in PBS (pH 7.4). They were subsequently incubated for 2 hours with biotinylated anti-mouse IgG (1:200; Vector Laboratories, Burlingame, California) in the same solution. The complexes of primary and secondary antibody were visualized with a diaminobenzidine (R&D Systems, Minneapolis, Minnesota) colorimetric reaction.

Imaging and Nigral Cell Counting

Fluorescent images were obtained with confocal microscopes (Carl Zeiss or Leica) with the ZEN microscope software (Carl Zeiss). For nigral cell counting, TH-immunopositive cells on both sides of the SN were observed in 3 coronal sections with a Nikon 80i microscope (Nikon, Tokyo, Japan) with NIS-Elements F3.0 software at ×100 magnification. Data are expressed as the percentage of cell loss in the ipsilateral compared with the contralateral SN.

Statistical Analysis

All data are presented as mean ± SD. All statistical analyses were performed with SPSS (version 12.0.1; SPSS Inc, Chicago, Illinois). A 1-way analysis of variance with a Tukey post hoc test was used to analyze behavioral parameters between 2 groups. A repeated measures analysis of variance was conducted to analyze differences in time-dependent patterns in the stepping tests. TH-positive cells in the groups were compared by use of independent-samples *t* tests. A value of $P < .05$ was regarded as statistically significant.

RESULTS

Behavioral Analysis for Forelimb Akinesia

Before halorhodopsin injection, stepping tests showed that all rats in the halorhodopsin from the *Natronomonas pharaonis* (NpHR) group and the control group had similar contralateral forelimb akinesia induced by 6-OHDA unilateral injection (data not shown). After NpHR expression, the ratio of contralateral forelimb touches in the prelight test was not different between the NpHR and control groups (15.0 ± 6.3% and 14.5 ± 6.1%, respectively; $P = .88$). During light stimulation, the NpHR group showed significant improvement in the use of the contralateral forelimb compared with the control group, which showed a continuous decrease in contralateral forelimb touches (38.8 ± 8.1% vs 9.8 ± 6.3%; $P < .001$). In the NpHR group, there was also a significant increase in contralateral forelimb touches during light stimulation compared with the light-off states (prelight, 15.0 ± 6.3%; postlight, 17.5 ± 10.1%; $P < .001$) at 1 week after optic fiber implantation (see Figure 1A and **Video**, **Supplemental Digital Content 1**, http://links.lww.com/NEU/A614, which demonstrates the stepping test of contralateral forelimb touches). The improvement in contralateral forelimb akinesia during light stimulation persisted significantly in the NpHR group at 4 to 6 weeks after optic fiber implantation (34.3 ± 10.9%; $P = .002$ vs light-off states). In the control group, there was no significant change in contralateral forelimb touches during light stimulation compared with light-off states at 4 to 6 weeks after optic fiber implantation (prelight, 21.9 ± 6.3%; light, 13.7 ± 8.8%; postlight, 16.4 ± 5.7%; $P = .22$). There was also no significant change in time-dependent patterns between 1 and 4 to 6 weeks after optic fiber implantation in the NpHR group ($P = .93$; Figure 1B).

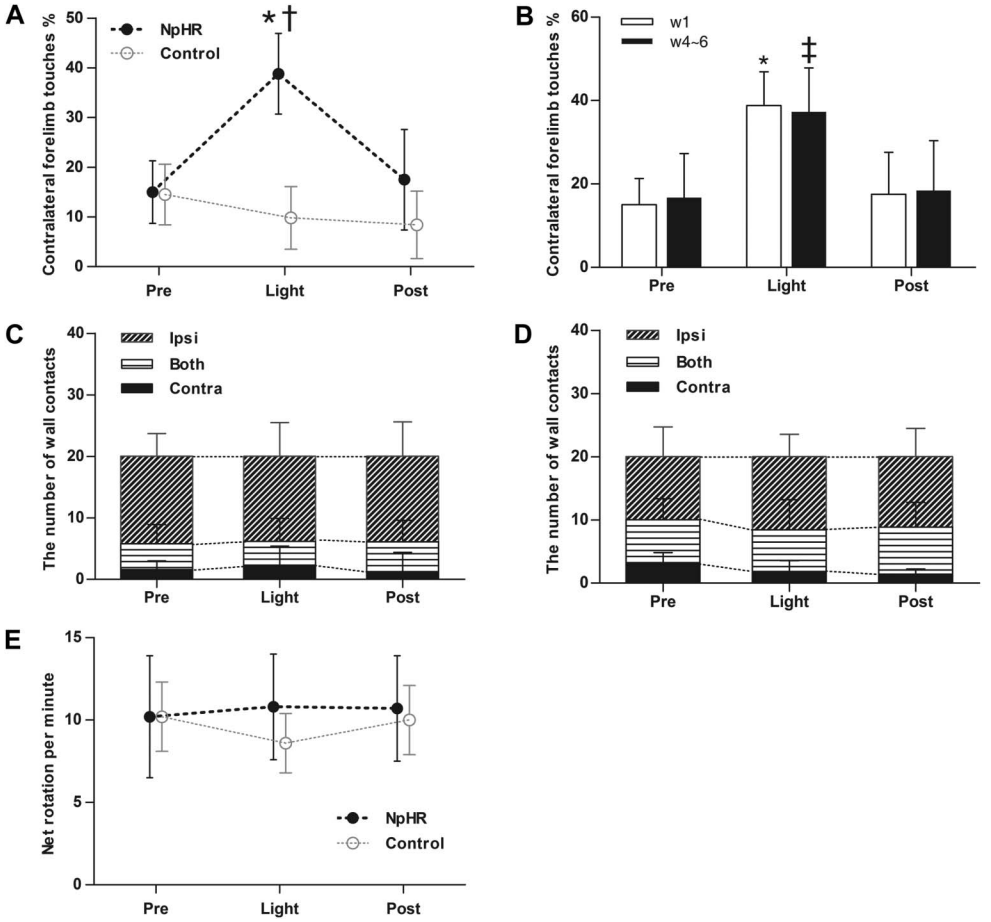

FIGURE 1. *Optical inactivation of the subthalamic nucleus (STN) induces improvements in contralateral forelimb akinesia.* **A**, *stepping tests were performed to evaluate whether contralateral forelimb akinesia could be improved by light stimulation of NpHR in the STN. Data are shown as the rate of contralateral forelimb touches (%) in both forelimb touches. In the NpHR group, the contralateral motor deficit of hemiparkinsonian rats was significantly improved during light stimulation compared with light-off states and the control group (*P < .001, †P < .001).* **B**, *the NpHR group showed persistent improvement during light stimulation compared with the light-off states at 1 and 4 to 6 week after optic fiber insertion (*P < .001, ‡P = .002).* **C**, *the preference of forelimb use in the cylinder test was not affected by optical stimulation of NpHR in the STN.* **D**, *the control group showed no significant change in the preference of forelimb use during optical stimulation compared with the light-off state.* **E**, *apomorphine-induced rotation tests were not affected by optical stimulation in either the NpHR or the control group. Data are expressed as the net (contralateral−ipsilateral turns) average rotations per min.*

In the cylinder test, all rats in the NpHR and control groups showed remarkable motor deficits in the use of their contralateral forelimbs in the light-off states. The number of contralateral forelimb contacts in the prelight test was 1.6 ± 1.4 in the NpHR group and 3.0 ± 1.8 in the control group. Both the NpHR and control groups showed no significant change in the number of wall contacts by contralateral forelimb during light stimulation compared with the light-off states (Figure 1C and 1D). The number of contralateral forelimb contacts in the light stimulation test was 2.1 ± 3.3 in the NpHR group and 1.9 ± 1.7 in the control group. These effects persisted in the postlight test for both groups (NpHR group, 1.2 ± 3.2; control group, 1.4 ± 0.8).

In the apomorphine-induced rotation test, all rats in the NpHR (10.2 ± 3.7) and control (10.2 ± 2.1) groups showed contralateral rotations after apomorphine administration. These rotations were persistent during light stimulation in both the NpHR (10.8 ± 3.2) and control (8.6 ± 1.8) groups. This effect also persisted in the postlight test (NpHR group, 10.7 ± 3.2; control group, 10.0 ± 2.1). There was no significant change induced by light stimulation in either group (NpHR, $P = .91$; control, $P = .25$; Figure 1E).

NpHR-YFP Expression Patterns and Immunofluorescence for VGLUT2 in the STN

Confocal imaging revealed that YFP-expressing cells were observed largely in the STN but were not detected in the zona incerta or internal capsule, which is adjacent to the STN. The tip of the optic fiber reached only the restricted area in the STN (Figure 2A). Immunofluorescence for VGLUT2 revealed that the majority of YFP-expressing cells were VGLUT2 positive (Figure 2B). Colocalization of YFP and VGLUT2 signals is shown in detail in Figure 2C.

TH Immunohistochemistry

Three sections through the SN (rostral section, −4.8 mm; median section, −5.4 mm; caudal section, −6.0 mm from bregma) showed considerable loss of TH-positive cells in the lesion (NpHR group, 93.1 ± 1.6%; control group, 93.6 ± 4.7%; Figure 3A-3C),

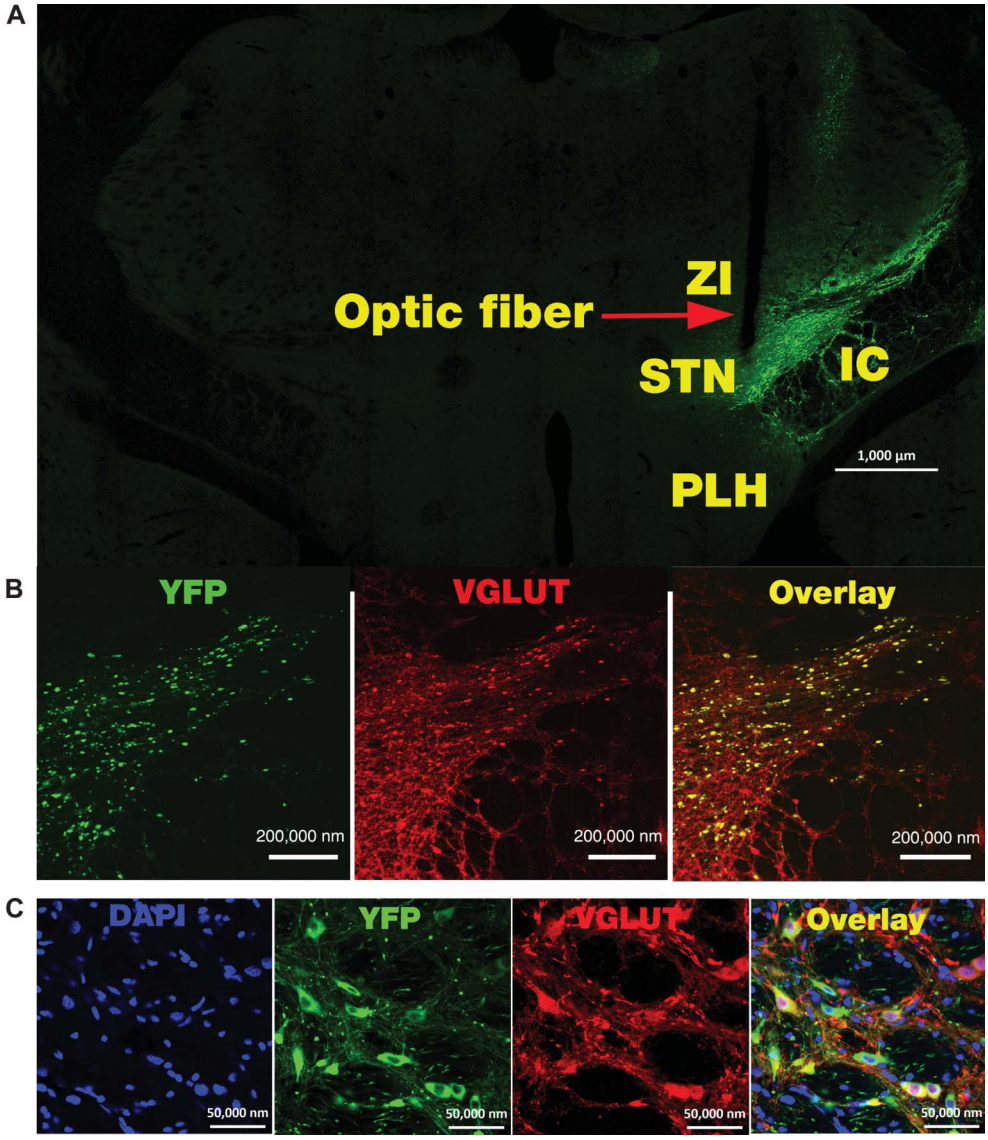

FIGURE 2. *Confocal images showing glutamatergic neurons expressing NpHR-YFP in the subthalamic nucleus (STN).* **A**, *NpHR-YFP was expressed in the target of the STN, and the end of the optic fiber (red arrow) was located exclusively in the STN.* **B**, *vesicular glutamate transporter 2-(VGLUT2; a glutamatergic marker) positive cells were overlaid with NpHR-YFP expression in the STN.* **C**, *NpHR-YFP was found to be expressed in the plasma membrane rather than in the nucleus (***B**, *×100;* **C**, *×400 magnification). IC, internal capsule; PLH, peduncular part of the lateral hypothalamus; ZI, zona incerta.*

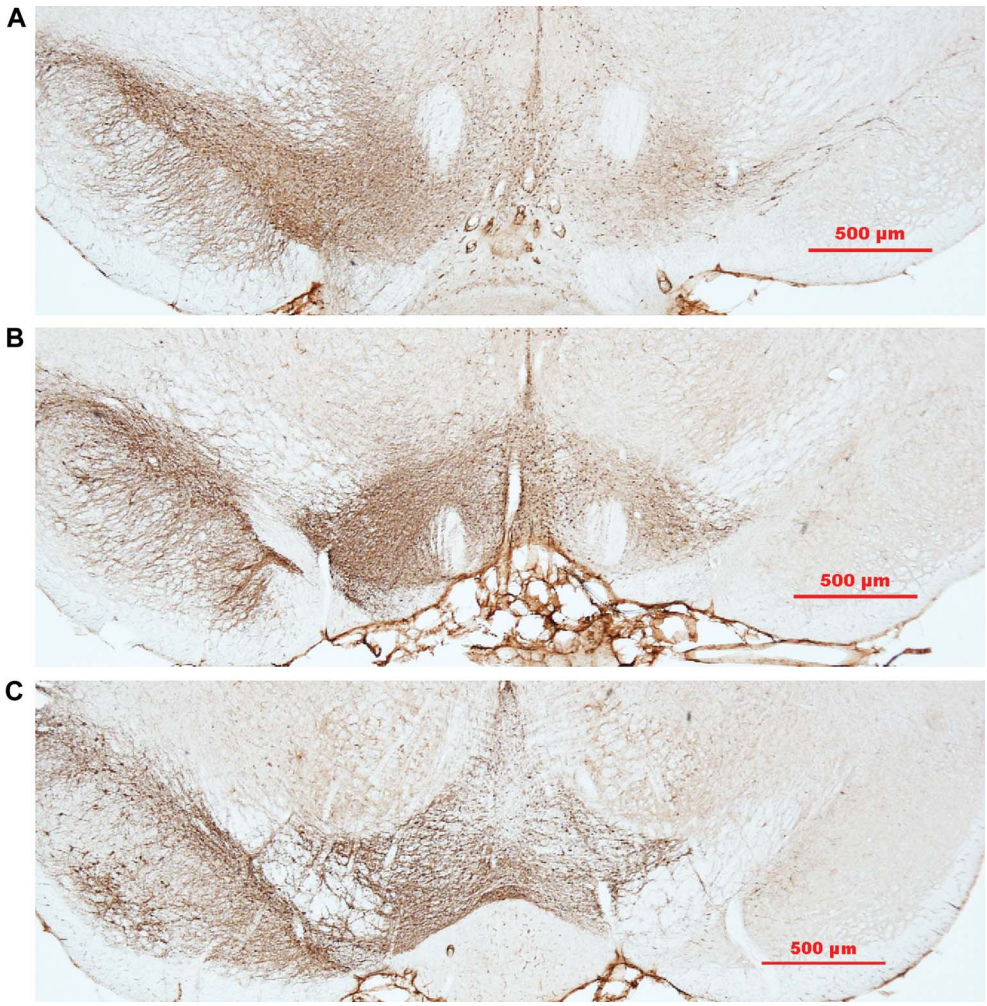

FIGURE 3. *Tyrosine hydroxylase immunohistochemistry results showing unilateral complete lesions of the substantia nigra in rats used in this study.* **A**, *rostral.* **B**, *median.* **C**, *caudal sections.*

indicating that the animals that were used in the present study had complete lesions.

DISCUSSION

The mechanism of DBS action in humans is known to be related to the generation of orthodromic and antidromic trains of pulses that modulate the basal ganglia oscillations.[19] In addition to this mechanism, the inhibition of glutamatergic neurons in the STN is another hypothesis regarding the underlying mechanisms for the therapeutic effects of STN DBS.[4-6] This inactivation of the STN may decrease the firing pattern entropy of inhibitory neurons in such areas as the globus pallidus interna and substantia nigra pars reticulate, thereby ameliorating motor signs in PD.[20-24] Recently, based on this idea, clinical trials of AAV2-glutamic acid decarboxylase injection into the STN to add an inhibitory GABA outflow were conducted. This resulted in a decrease in excessive excitatory glutamatergic output to the globus pallidus interna and substantia nigra pars reticulate, indicating positive results.[25,26] NpHR is a membrane protein that can pump chloride ions inwardly when illuminated with yellow light (590 nm), resulting in inhibition of the action potential.[6,27-29] Therefore, application of NpHR into STN and light stimulation might exert therapeutic effects by STN inhibition. A previous study showed that optical activation of afferent fiber to STN by channelrhodopsin, which has neuronal activation effect by blue light (wavelength, 470 nm), was effective in eliciting therapeutic effects on motor signs as measured by the rotation test, but optogenetic inhibition of excitatory neurons in the STN itself via NpHR was not effective.[6] We consider that rotation tests appear insufficient to evaluate proper motor signs of PD because asymmetrical dopamine release or supersensitivity of the dopaminergic receptor is poorly related

to human PD.[10,30,31] In our present study, we performed spontaneous behavioral tests for unilateral forelimb akinesia to assess the efficacy of optical stimulation in the STN for optogenetic inhibition using halorhodopsin.

To evaluate forelimb akinesia, the stepping test and the cylinder test were used, which can assess changes in motor deficit of the contralateral forelimb and indicate the preference of either forelimb, respectively.[17] The rats that received AAV-NpHR injection into the ipsilateral STN showed significant improvement in contralateral forelimb akinesia during light stimulation in the stepping test but did not show any changes in the preference of forelimb use in the cylinder test. These results indicate that transient inactivation of neurons in the STN by optical stimulation was effective for contralateral forelimb akinesia but may not be sufficient to improve asymmetrical preference in using the ipsilateral forelimb. This finding supports our notion that a variety of behavioral tests should be performed to analyze improvements in motor deficits in a unilateral rat model of PD and that the cylinder test may be inappropriate in this model because rats cannot alter their preferable forelimb during a short period of optical stimulation. An evaluation using various optic illumination parameters would be needed to elucidate optogenetic effect in the cylinder test. The therapeutic effect seen in the stepping test was maintained for 1 month (Figure 1B). Even though the response to the light stimulation in the second test seemed to be less sensitive than that of the first test, there was no statistical significance (P = .85). Besides, the PD control group showed no significant changes in contralateral forelimb touches during prelight, light, and postlight stimulation, suggesting that the therapeutic effect of forelimb akinesia was caused not by a lesion or surgical effect but by optical inactivation of neurons in the STN.

We conducted apomorphine-induced rotation tests for 30 minutes (prelight for 10 minutes, light stimulation for 10 minutes, and postlight for 10 minutes). During rotation tests, the amount of time sufficient for optic stimulation is not well known, but we believe 10 minutes is sufficient to compare results between groups. In this study, no significant changes in rotation were observed during light stimulation in either group, suggesting that inactivation of STN neurons by halorhodopsin stimulation did not affect postsynaptic changes in the dopaminergic neurons induced by presynaptic dopaminergic depletion. However, inhibition of STN neurons by halorhodopsin stimulation modulated the output to the globus pallidus interna or substantia nigra pars reticulate as shown by the motor improvements during the stepping test.

The hSynapsin1 promoter used in the present study has previously been used to express optogenes in neurons with the exception of glial cells.[28,32-34] Synapsin1 plays an important role in activity of vesicles in glutamatergic and GABAergic terminals,[35] indicating that NpHR-YFP could be expressed in glutamatergic neurons of STN using the hSynapsin1 promoter. In addition, a previous study showed that the AAV-hSynapsin1 combination could transduce either excitatory or inhibitory neurons.[33] However, cell bodies of the STN are strongly immunoreactive to glutamate but not to GABA, indicating that neuronal cell bodies in STN are glutamatergic neurons,[20,36] and the AAV-hSynapsin1 combination could be selectively localized in STN by injection. In our present study, NpHR-YFP was expressed in the cell bodies of STN under the control of hSynapsin1. Immunofluorescence for VGLUT2, which is a specific marker of glutamatergic neurons, revealed colocalization between YFP-expressing cells and VGLUT2-positive cells, indicating that NpHR is expressed in glutamatergic neurons of the STN. In addition, it was reported that selective glutamatergic inactivation could be achieved by strict insertion of optic fibers into the STN.[27] In our study, the tip of the inserted optic fiber was located exclusively in the STN area, which could increase the selectivity of the optic inhibition effect of STN.

We did not perform in vivo electrical recordings of neuronal activity, but it is well established that expressed halorhodopsin with light stimulation inhibits action potential occurrence.[29] Besides, the present study showed that NpHR is expressed in glutamatergic neurons of the STN. Thus, it is reasonable to infer that inactivated STN neurons by NpHR expression and light illumination resulted in the improvement of stepping test.

TH immunohistochemistry revealed that all rats in this study had considerable cell loss in the lesions of the SN and that the loss was sufficient to cause contralateral forelimb akinesia. This indicates that the therapeutic effect of forelimb akinesia was induced not by spontaneous improvement but by optical inactivation in the STN.

CONCLUSION

The results of our present study reveal that inhibition of glutamatergic neurons in the STN using optogenetics may be effective in improving contralateral forelimb akinesia but not in changing forelimb preference or reducing dopaminergic receptor supersensitivity. These findings suggest that it is important to assess the effect of optogenetic stimulation using appropriate behavioral methods, which may be useful for future studies of optical modulation in PD.

Disclosure

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COMMENTS

Although deep brain stimulation provides substantial relief of the motor signs of Parkinson disease, the mechanism of deep brain stimulation is unclear. This article provides insight into possible mechanisms for the treatment of Parkinson disease by modulating neuronal activity with optogenetics. The authors demonstrate the ability to improve contralateral forelimb akinesia with the use of optogenetics while emphasizing the importance of selecting appropriate methods for examining the therapeutic benefits of treatments in animal models. The authors are the first to effectively demonstrate the therapeutic benefit of optical inhibition of the subthalamic nucleus by halorhodopsin in the rat model. Previous studies reported improvement in rotation tests in mice using channelrhodopsin but saw no improvement in rotation tests using halorhodopsin.[1]

Sara J. Hanrahan
Adam O. Hebb
Englewood, Colorado

1. Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K. Optical deconstruction of parkinsonian neural circuitry. *Science*. 2009;324(5925):254-259.

This is an interesting article in a dynamic and promising area of research. The authors have studied the effects of optogenetic inhibition of the subthalamic nucleus in a rat parkinsonian model. They have

been able to demonstrate a behavioral effect on the modeled symptoms through targeted inhibition of glutamatergic subthalamic nucleus neurons. This article is important for a number of reasons: It further advances the relatively recent field of optogenetic methodology; it demonstrates a direct symptomatic benefit in parkinsonian rats via glutamatergic subthalamic nucleus inhibition; and most important, it offers further insight into the pathophysiology of motor parkinsonian deficits with relevance to therapeutic approaches, particularly deep brain stimulation and gene therapy.

Codrin Lungu
Bethesda, Maryland