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Research article

Effect of optogenetic modulation on entopeduncular input affects thalamic discharge and behavior in an AAV2-α-synuclein-induced hemiparkinson rat model

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ABSTRACT

Objective: Neuromodulation of the globus pallidus internus(GPi) alleviates Parkinson's disease symptoms. The primate GPi is homologous to the rat entopeduncular nucleus (EP). The aim of the present study was to determine if optogenetic modulation of the EP could alter parkinsonian behavior or thalamic discharge in a hemiparkinson rat model.

Methods: We injected an adeno-associated virus type-2 expressing α-synuclein (AAV2-α-syn) into the substantia nigra pars compacta (SNc) of the right hemisphere and confirmed parkinsonian behavior using an amphetamine-induced rotation test. Then we injected activated or inhibited neurons, using the channelrhodopsin2 (ChR2)/halorhodopsin (NpHR) system in the EP of the hemiparkinson rat model and examined downstream effects *in vivo*. We assessed alterations in parkinsonian behaviors using the stepping and cylinder tests before, during, and after optogenetic stimulation.

Results: Importantly, optogenetic inhibition of the EP improved parkinsonian motor behaviors. When we monitored thalamic neuronal activity following optogenetic neuromodulation *in vivo*, and we observed alterations in thalamic discharge The thalamic neuronal activity is increased for optogenetic inhibition stimulation, whereas decreased for optogenetic activation stimulation.

Conclusions: Taken together, our data demonstrate that optical neuromodulation of the EP can successfully control contralateral forelimb movement and thalamic discharge in an AAV2-α-synuclein-induced hemiparkinson rat model.

1. Introduction

The basal ganglia are subcortical nuclei that controlling voluntary actions and are affected in Parkinson's disease (PD) [7]. Within these nuclei, the neurons that project from the globus pallidus internus (GPi) in primates, or the entopeducular nucleus in rodents, are associated with the motor responses [30]. Inhibitory output from the GPi and substantia nigra (SN) to the thalamus is an important link in the transmission of information from basal ganglia to the cortex [10]. However, recent anatomical and functional evidence called into question the validity of hypothesis that there is inhibitory output from the GPi to the thalamus [12,22,34].

Burst and synchronized basal ganglia output activity are considered a hallmark of parkinsonian pathophysiology [36]. It is thought that integration of cortical and pallidal inputs plays a key functional role in this central node of cortical-basal ganglia interaction. The basal ganglia-recipient thalamus receives inhibitory inputs from the pallidum and excitatory inputs from the cortex, but it is unclear how these inputs interact to produce behavior [6,14,17,26,42].

In this study, we generated a hemiparkinson rat model by injecting adeno-associated virus type 2 expressing alpha-synuclein (AAV2-α-syn) into the SN pars compacta (SNc) of the right hemisphere. After confirming models success, channelrhodopsin2 (ChR2)/halorhodopsin (NpHR) was administered to the EP using optogenetic system to enables

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rapidly bidirectional control of neurons on the timescale of milliseconds [41]. Using this hemiparkinson model, we optogenetically modulated the neuronal activity of the EP and assessed the downstream effects on parkinsonian behaviors and thalamic discharge.

2. Materials and methods

2.1. Animal surgery

For all experiments, we used adult male Sprague-Dawley rats weighing 200–220 g (Daehanbiolink, Eumseong, Korea). All studies were conducted at the Animal Research Center at Chungbuk National University (CBNU). All animal care protocols were approved by the institutional committee of CBNU (CBNUA-1012-16-02). Surgical processes were performed in accordance with guidelines and aseptic techniques. Behavior testing was performed in a randomized, double blind, controlled animal trial, and animals were assigned a group designation on the same day. Rats were kept under standard housing conditions (12h–12 h/light-dark cycle) and were given laboratory food pellets and water.

2.2. AAV_2-CBA-Alpha-synculein viral vector

We obtained the AAV2-CBA-alpha-synuclein viral vector from the Michael J. Fox Foundation (http://genetherapy.unc.edu/MJFF.htm). The exact sample size (n = 11) for required for an independent *t*-tests and a one-way analysis of variance (ANOVA) was determined using the PASS 14 software (NCSS Inc., Kaysville, UT, USA; alpha = 0.05, power = 0.8). A total of 28 rats received right hemisphere received right hemisphere injections of synuclein viral vector into the SNc (coordinates: anterior-posterior [AP], −5.3 mm; medial-lateral [ML], −2.0 mm; dorsal-ventral [DV], −7.8 mm from the dura) to generate a unilateral (right) hemiparkinson model under anesthesia with a combination of 15 mg/kg tiletamine/zolazepam (Zoletil50®; Virbac Laboratories, Carros, France) and 9 mg/kg xylazine (Rompun®; Bayer, Seoul, South Korea) [24]. Virus was injected using a Hamilton syringe and an automated micro-syringe pump (Harvard Apparatus, Holliston, MA, USA) with a flow rate of 0.5 μL/min for 10 min. After viral injection, the needle was in position at target for 5 min to prevent leakage and was then gradually retracted over a subsequent 5-min period. The schematic diagram in Fig. 1 illustrates the injection protocol.

2.3. Injection of AAV-hSyn-hChR2-mCherry and AAV-hSyn-eNpHR3.0-EYFP viruses

The virus package concentrated was 2.8×10^{12}/mL, which is a minimum viral particle.

Four weeks after α-synuclein virus injection, another round of optogenetic virus was injected under general anesthesia with intraperitoneal injection of a mixture of 15 mg/kg tiletamine/zolazepam (Zoletil50®; Virbac Laboratories, Carros, France) and 9 mg/kg xylazine (Rompun®; Bayer, Seoul, South Korea) [40]. The rats were randomly divided two groups: vehicle-control group (n = 9) and AAV-group (n = 15). The vehicle-control group received saline 10 μL into the EP (coordinates: AP, −2.4 mm; ML, −3.0 mm; DV, −7.5 mm from the dura) using the rat brain stereotaxic coordinates (Paxinos & Watson, 1986). The AAV-group received 2 μL of AAV-hSyn-hChR2-mCherry (2.8×10^{13} GC/mL); after 10 min, 2 μL of AAV-hSyn-eNpHR3.0-EYFP (2.23×10^{13} GC/mL) was injected into the EP. The viruses were diluted 1:10 μL in phosphate-buffered saline (PBS) and were delivered following the same injection procedure described above for the AAV2-α-synuclein-viral injections.

2.4. Optical delivery

Optic fibers (200-μm core, 230-μm outer diameter, numerical aperture of 0.48, hard polymer cladding type, Doric Lenses; Québec City, Québec, Canada) were cut to a length of 7.5 mm to optimize EP depth. The optic fiber was implanted using a stereotactic cannula holder into the center of the EP at the same site as the AAV or PBS injection. The optic fiber was firmly fixed with surrounding screws using dental cement (Ortho-ject Pound Package, Lang Dental, Wheeling, IL, USA).

For optical stimulation, we used a laser at wavelengths of 473 nm and 589 nm (ADR-700D, Shanghai, China) with an output power of 10 mW. The laser was controlled by a waveform generator (Keysight 33511B-CFG001, Santa Rosa, CA, USA) that allowed for adjustment of the frequency of the square pulses and pulse widths for optical stimulation. For optical stimuli, the laser intensity was set at 1 mW, light pulses at 40 Hz, and pulse width at 4 ms. To assess the effects of optical neuromodulation, all of the testing was performed prior to (pre), during (laser-on), and after (post) optical stimulation.

2.5. Behavioral testing

2.5.1. Rotation test

The amphetamine-induced rotation test was performed 6 weeks after the AAV2-α-syn viral vector injections to evaluate the extent of the dopaminergic lesion. Rats received 5 mg/kg injections of D-amphetamine sulfate (Lipomed AG, Swiss). All turns were recorded for 40 min using a rotameter (LE902/SR, Barcelona, Spain) equipped with a sensor with a standard sensitivity of four pulses per turn.

2.5.2. Cylinder test

The cylinder test was performed 2 weeks after optic fiber implantation in the pre, laser-on, and post states as previously described [32]. A rat put in a transparent glass cylinder (20-cm diameter, 38-cm height). A rat was placed into a transparent glass cylinder, and the number of spontaneous wall contacts were counted[40]. Twenty wall contacts were analyzed for each animal to assess the number of wall contacts made with the contralateral forelimb, ipsilateral forelimb, or both forelimbs.

2.5.3. Stepping test

To measure akinesia, we carried out stepping tests (pre, laser-on, and post states) 2 weeks after optical fiber implantation. They were performed as previously described with slight modifications [29,39]. The rat was held with one hand by the experimenter to fix the hind limbs, and with the other hand to fix the unmonitored forelimb [2]. The rat was placed on a treadmill (Harvard Apparatus, Holliston, MA, USA)

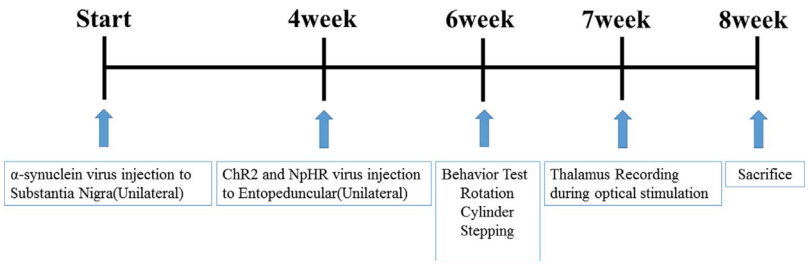

Fig. 1. Schematic diagram illustrating the study protocol.