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DEEP BRAIN STIMULATION IN THE GLOBUS PALLIDUS EXTERNA PROMOTES SLEEP

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Abstract—The basal ganglia, a network of subcortical structures, play a critical role in movements, sleep and mental behavior. Basal ganglia disorders such as Parkinson's disease and Huntington's disease affect sleep. Deep brain stimulation (DBS) to treat motor symptoms in Parkinson's disease can ameliorate sleep disturbances. Our series of previous studies lead the hypothesis that dopamine, acting on D_2 receptors on the striatopallidal terminals, enhances activity in the globus pallidus externa (GPe) and promotes sleep. Here, we tested if DBS in the GPe promotes sleep in rats. We found that unilateral DBS (180 Hz at 100 μA) in the GPe in rats significantly increased both non-rapid eye movement and rapid eye movement sleep compared to sham DBS stimulation. The EEG power spectrum of sleep induced by DBS was similar to that of the baseline sleep, and sleep latency was not affected by DBS. The GPe is potentially a better site for DBS to treat both insomnia and motor disorders caused by basal ganglia dysfunction.

Key words: deep brain stimulation, globus pallidus externa, sleep, insomnia.

INTRODUCTION

One of the most common basal ganglia disorders is Parkinson's disease, which is characterized by muscle rigidity, tremor and bradykinesia. These motor symptoms mostly result from the loss of substantia nigra pars compacta (SNc) dopamine signaling to the basal ganglia. Parkinson's disease also has prominent non-motor symptoms, including sleep abnormalities like insomnia, sleep fragmentation, and daytime sleepiness. The neural substrate for these sleep symptoms in Parkinson's disease has recently explored in animal studies. MPTP (1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine) lesions of the SNc in primates reduce total sleep amounts and flatten circadian rhythm (Belaid et al., 2014). SNc dopamine signaling in the basal ganglia promotes sleep in rodents (Qiu et al., 2010, 2014). We postulate that dopamine from the SNc acts on D_2 receptors on striatum inputs to the globus pallidus externa (GPe), disinhibiting the GPe and promoting sleep. The loss of this SNc dopaminergic input in Parkinson's disease may disrupt sleep via these basal ganglia connections.

Deep brain stimulation (DBS) effectively treats parkinsonian motor symptoms, and DBS can also improve sleep dysfunction such as insomnia (Amara et al., 2012). The most common sites targeted for DBS in Parkinson's disease are the globus pallidus interna (GPi) and subthalamic nucleus (STN). How DBS in these sites improves sleep problems is unclear, but an intriguing possibility is DBS in the STN and GPi may improve sleep and motor disorders through the GPe. Both STN and GPi project to the GPe, and electrophysiological studies have shown an increased activity in STN target neurons in the GPe during STN DBS (Hashimoto et al., 2003; Kita et al., 2005; Miocinovic et al., 2006; Hahn et al., 2008). Lesions of the GPe in monkeys exacerbate parkinsonian symptoms (Zhang et al., 2006), while DBS in the GPe works as effectively as DBS in the GPi for motor symptoms (Hahn et al., 2008; Vitek et al., 2012).

The GPe is a major basal ganglia hub that has the most extensive efferent connections of basal ganglia structures, including a unique direct projection to the frontal cortex (Chen et al., 2015; Saunders et al., 2015). The interconnectivity of major DBS target sites and the GPe raises the possibility that direct GPe stimulation with DBS may also be an effective treatment for sleep dysfunction in Parkinson's disease. We hypothesized that DBS directly in the GPe may promote sleep. We examined the effects of DBS in the GPe on sleep in rodents and found that it increased both non-rapid eye movement (NREM) and rapid eye movement (REM) sleep.

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Abbreviations: DBS, deep brain stimulation; EEG, electroencephalogram; EMG, electromyographic; GPe, globus pallidus externa; GPi, globus pallidus interna; LC, locus coeruleus; NREM, non-rapid eye movement; PBS, phosphate-buffered saline; REM, rapid eye movement; SNc, substantia nigra pars compacta; STN, subthalamic nucleus; TMN, tuberomammillary nucleus; VLPO, ventrolateral preoptic area.

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EXPERIMENTAL PROCEDURES

Animals

Experiments were performed on male Sprague–Dawley rats (300–325 g, Harlan). Animals were individually housed with *ad libitum* access of food and water, and under light-controlled conditions (12-h light/12-h dark cycle, with light on at 07:00 h; 100 lux) in isolated ventilated chambers maintained at 20–22 °C. All protocols were approved by the Institutional Animal Care and Use Committees of Beth Israel Deaconess Medical Center and these experiments were carried out in accordance with guidelines laid down by the U.S. National Institutes of Health regarding the care and use of animals for experimental procedures. Every effort was made to minimize the number of animals used and any pain and discomfort experienced by the subjects.

Surgery

Under anesthesia (ketamine, 100 mg/kg; xylazine, 10 mg/kg), rats were implanted with electrodes for recording electroencephalogram (EEG) and electromyographic (EMG) as described previously (Lu et al., 2000; Qiu et al., 2010). In brief, four EEG screw electrodes were implanted into the skull (two in the frontal and two in the parietal bones on each side) and two flexible EMG wire electrodes were placed into the nuchal muscles. And a twisted bipolar stimulation electrode (part# MS303/1-B/SPC, Plastics One, Roanoke, VA, USA) was targeted to the GPe (coordinate: AP = −0.92 mm, ML = +2.9 mm, DV = 6 mm) unilaterally, as per the atlas of Paxinos and Watson (2009). The free ends of the EEG/EMG electrodes leads were soldered into a head socket, together with the stimulation electrode, were then affixed to the skull with dental cement. Rats were allowed 10 days for recovery after the surgery.

EEG recording and stimulation

Each rat was transferred from the holding room to a recording chamber and habituated to the flexible EEG/EMG and stimulation electrode connection cables and conditions for two days. Following this habituation period, the EEG/EMG cables were connected to the amplifier (CED, Micro1401) and the stimulation electrode cable was connected to the stimulator (GRASS Technologies, Model S88) through a commutator (Plastics One). The stimulation signals were performed through a photoelectric stimulus isolation unit at a constant current output mode. The stimulation conditions were: frequency 180 Hz, duration 100 μs, stimulus intensity 100 μA. Each animal received a 180 Hz at 1.0 μA stimulation as baseline control and a 180 Hz at 100 μA of DBS from 19:00 to 22:00 in two consecutive days.

The EEG and EMG signals were amplified, digitized at a sampling rate of 256 Hz and recorded using Spike 2 (CED, Cambridge, UK). When complete, EEG/EMG recording data were transformed to EDF data format, filtered (EEG: 0.1–40 Hz; EMG: 10–100 Hz), and automatically scored with SleepSign (Kissei Comtec, Nagano, Japan) by 10-s epochs as wake, NREM sleep, and REM sleep, according to previously established criteria (Lu et al., 2000, 2001). After automatic scoring, sleep–wake stages were examined and corrected manually. The amount spent in wake, NREM sleep and REM sleep, sleep stage transitions, bouts and mean duration of each stage were determined from the scored data. In addition, EEG power spectra for NREM and REM sleep epochs were analyzed offline using FAST Fourier Transformation (512 point, Hanning window, 0.5–24.5 Hz with 0.5 Hz resolution using SleepSign).

Histology

Rats after 2-h DBS were deeply anesthetized with chloral hydrate (500 mg/kg) and perfused with saline followed by 500 ml 10% formalin through the heart. The brains were removed, post-fixed for 4 h in 10% formalin, and then equilibrated in 20% sucrose in phosphate-buffered saline (PBS) overnight. The brains were sectioned in the coronal plane on a freezing microtome into four series at 40 μm. One series of sections were processed for Nissl staining to verify the location of the tip of the stimulate electrode in the GPe. One series of sections were processed for c-Fos immunocytochemistry to observe whether GPe DBS would affect the activity of the neurons in the nucleus which are related with sleep and wake, such as ventrolateral preoptic area (VLPO) and tuberomammillary nucleus (TMN). Immunohistochemistry was performed in accordance with the free floating method described previously (Qiu et al., 2010). Sections were incubated with 0.3% H_2O_2 for 15 min to quench the endogenous peroxidase activity. After washing in 0.1 M PBS (pH 7.4), the sections were incubated with a rabbit polyclonal primary antibody against c-Fos (Ab5, Oncogene Research Products) at a 1:10,000 dilution in PBS containing 0.25% Triton X-100 for 24 h at room temperature. On the second day, the sections were washed in PBS and incubated in biotinylated donkey anti-rabbit secondary antiserum (Jackson ImmunoResearch Laboratories, PA, USA; 1:1000 dilution) for 1 h followed by a 1:1000 dilution of avidin–biotin-peroxidase (Vector Laboratories, CA, USA) for 1 h at room temperature. The peroxidase reaction was visualized with 0.05% 3,3-diaminobenzidine tetrahydrochloride (Sigma, MO, USA) in PBS and 0.01% H_2O_2 and strengthened with 0.002% Ni, 0.001% $CoCl_2$. After terminating the reaction by PBS-azide, sections were mounted, dehydrated and cover slipped. As controls, adjacent sections were incubated without the primary antibody to confirm that non-specific staining had occurred.

Statistical analysis

The quantitative data were presented as mean ± standard error of mean (SEM). Time course of the hourly amounts of each stage, histograms of sleep/wake amounts, sleep/wake stage transition number, number and duration of sleep/wake bouts were analyzed by pared *t*-test, with each animal serving as its own control. In all cases, differences were considered statistically significant at the 95% level ($p < 0.05$).