Deep brain stimulation of the substantia nigra pars reticulata improves forelimb akinesia in the hemiparkinsonian rat

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Sutton AC, Yu W, Calos ME, Smith AB, Ramirez-Zamora A, Molho ES, Plilitsis JG, Brotchie JM, Shin DS. Deep brain stimulation of the substantia nigra pars reticulata improves forelimb akinesia in the hemiparkinsonian rat. J Neurophysiol 109: 363–374, 2013. First published October 17, 2012; doi:10.1152/jn.00311.2012.—Deep brain stimulation (DBS) employing high-frequency stimulation (HFS) is commonly used in the globus pallidus interna (GPI) and the subthalamic nucleus (STN) for treating motor symptoms of patients with Parkinson’s disease (PD). Although DBS improves motor function in most PD patients, disease progression and stimulation-induced nonmotor complications limit DBS in these areas. In this study, we assessed whether stimulation of the substantia nigra pars reticulata (SNr) improved motor function. Hemiparkinsonian rats predominately touched with their unimpaired forepaw >90% of the time in the stepping and limb-use asymmetry tests. After SNr-HFS (150 Hz), rats touched equally with both forepaws, similar to naive and sham-lesioned rats. In vivo, SNr-HFS decreased beta oscillations (12–30 Hz) in the SNr of freely moving hemiparkinsonian rats and decreased SNr neuronal spiking activity from 28 ± 1.9 Hz before stimulation to 0.8 ± 1.9 Hz during DBS in anesthetized animals; also, neuronal spiking activity increased from 7 ± 1.6 to 18 ± 1.6 Hz in the ventromedial portion of the thalamus (VM), the primary SNr efferent. In addition, HFS of the SNr in brain slices from normal and reserpine-treated rat pups resulted in a depolarization block of SNr neuronal activity. We demonstrate improvement of forelimb akinesia with SNr-HFS and suggest that this motor effect may have resulted from the attenuation of SNr neuronal activity, decreased SNr beta oscillations, and increased activity of VM thalamic neurons, suggesting that the SNr may be a plausible DBS target for treating motor symptoms of DBS.

high-frequency stimulation; 6-hydroxydopamine; whole cell patch clamp; local field potentials; Parkinson’s disease

PARKINSON’S DISEASE (PD) is the second most common neurodegenerative disorder, affecting 0.5–3% of individuals over the age of 60. Moreover, PD will become increasingly common as the affected population is projected to double by 2030 (Dauer and Przedborski 2003; Dorsey et al. 2007; Hirtz et al. 2007). Although levodopa (L-DOPA) remains the gold-standard treatment for PD, 50% of treated patients develop L-DOPA-induced dyskinesias (LIDs) within 5 years, and the incidence increases 10% for each additional year of treatment (Ahlskog and Muenter 2001; Fahn et al. 2004). Furthermore, for many patients, medication can no longer adequately control their symptoms and response to these medications can fluctuate significantly over time. Therefore, once medications can no longer provide reliable efficacy, deep brain stimulation (DBS) becomes a preferred option for treating PD.

DBS applies electrical high-frequency stimulation (HFS) to subcortical brain structures to alter their neuronal patterning and excitability. Over the last 20 years, DBS has become a well-established option for the treatment of PD patients with motor fluctuations and dyskinesias (Benabid et al. 1987; Follett et al. 2010). Thus far, the most successful applications of DBS for PD involve targeting either the subthalamic nucleus (STN), the sole glutamatergic nucleus of the basal ganglia (BG), or the globus pallidus interna (GPI), an output nucleus of the BG. STN and GPI stimulation have been shown to provide comparable benefits on the cardinal features of PD including tremor, akinesia, and rigidity (Deuschl et al. 2006; Diamond and Jankovic 2005; Durif et al. 2002; Follett et al. 2010; Kleiner-Fisman et al. 2003, 2006; Liang et al. 2006; Moro et al. 2010; Rodriguez-Oroz et al. 2005; The DBS for PD Study Group 2001).

Although DBS is an effective treatment for well-selected PD patients, there are some instances when this approach is limited in its efficacy and outcomes. For instance, the motor score improvement can vary significantly among individuals (Kleiner-Fisman et al. 2006), and reports of nonmotor complications and adverse effects are being realized as emerging considerations. In the latter case, some PD patients reported cognitive or mood alterations following STN-DBS (Limousin et al. 1998; The DBS for PD Study Group 2001), whereas others experienced hypomania (Berney et al. 2002), decline in verbal fluency (Castelli et al. 2010; Morrison et al. 2004), cognitive impairment (York et al. 2008), and increased anxiety, aggression, and impulsivity (Temel et al. 2006). Improving DBS efficacy and minimizing nonmotor complications can be achieved with DBS reprogramming and management of medication in some patients, but not in others (Okun et al. 2005). In light of these considerations, we undertook this study to identify a novel target for DBS for treating motor symptoms of PD.

Among potential targets, there is compelling anatomical and functional evidence to suggest that the substantia nigra pars reticulata (SNr) may be a suitable DBS target (DeLong et al. 1983; Hauber 1998; Hedreen and DeLong 1991; Lynd-Balta and Haber 1994; Magarinos-Ascone et al. 1992; Nishino et al. 1991; Parent et al. 1984; Selemon and Goldman-Rakic 1990;
Starr et al. 2000; Szabo 1967). Yet, despite indications that the SNr could be a target for DBS, no study has specifically investigated the utility of the SNr for DBS for akinesia and bradykinesia in PD. A likely reason for this stems from clinical studies which have described hypomania in PD patients receiving SNr stimulation (Bejani et al. 1999). However, more recent studies have reported that SNr-DBS improved axial and gait disturbances in PD patients without hindrance by this complication (Chastan et al. 2009; Weiss et al. 2011a). In fact, a clinical trial is now underway using this approach concurrently with STN-DBS to improve gait disturbance (Weiss et al. 2011b).

In this study, we assessed the impact of SNr-HFS on forelimb function in the 6-hydroxydopamine (6-OHDA)-lesioned (hemiparkinsonian) rat by measuring forelimb akinesia in the limb-use asymmetry test (LAT) (Schallert et al. 2000) and the adjusting step test (Olsson et al. 1995) before, during, and after SNr-HFS. We obtained SNr local field potential (LFP) recordings of freely moving hemiparkinsonian rats with SNr-HFS to evaluate whether dominant beta-frequency (12–30 Hz) oscillations are affected. To elucidate the impact of SNr-HFS on neuronal activity in the SNr and other brain regions, we recorded single-unit recordings from anesthetized hemiparkinsonian rats before, during, and after stimulation. In addition, we employed the whole cell patch-clamp technique to assess whether HFS of the SNr can affect SNr neuronal activity in vitro.

MATERIALS AND METHODS

Animals and Surgery

All animal use was conducted with approval from the Institutional Animal Care and Use Committee at Albany Medical College. Male Sprague-Dawley (SD) rats weighing 225–300 g were anesthetized with 2% isoflurane using an inhalant system (Harvard Apparatus, Natick, MA) in a stereotaxic frame (David Kopf Instruments, Ta- junga, CA). Rats were injected intraperitoneally (IP) with desipramine (25 mg/kg) and pargyline (50 mg/kg) 20 min before craniotomy, and body temperature was maintained at 37°C throughout the surgery (Homeothermic Monitor; Harvard Apparatus). Labufresh P.M. (Major Pharmaceuticals, Livonia, MI) was applied to the eyes to prevent dehydration, and 2% lidocaine gel (Akorn Pharmaceuticals, Lake Forest, IL) was applied to the ear bars to minimize discomfort. An injectable 2% lidocaine solution (Hospira, Lake Forest, IL) was administered subcutaneously (SC) under the shaved scalp to minimize discomfort. Afterward, a burr hole was made in the cranium so that 4 l of 6-OHDA (3 µg/µl, made up in 0.1% ascorbic acid) or saline (0.9% NaCl) could be injected into the right medial forebrain bundle (from bregma: 4.8 mm posterior, 1.2 mm lateral, and 7.7 mm ventral from dura). After injection of 6-OHDA or saline, hemiparkinsonian and sham-lesioned rats that were designated for single-unit recordings had their incisions stapled shut and were given penicillin (80 µg/kg), buprenorphine (0.12 g/kg), and saline (10 ml/kg) SC, as well as topical bacitracin postoperatively; buprenorphine was also given every 12 h for 72 h postsurgery for pain management. Another group of hemiparkinsonian and sham-lesioned rats were designated for freely moving DBS and LFP recording experiments and were implanted with a stainless steel twisted electrode for recording and stimulating (500-µm diameter, <300 KΩ; Plastics One, Roanoke, VA) into the right SNr (from bregma: 5.3 mm posterior, 2.0 mm lateral, and 8.2 mm ventral from dura). Five anchor screws were fastened onto the skull between bregma and lambdoidal sutures, along with a ground screw in the cortex of the left hemisphere at 5.3 mm posterior from bregma and 2.0 mm lateral. Dental cement (Duralay; Reliance Dental, Worth, IL) was then used to fasten the electrodes in position. All animals received the same postoperative care as described above. Naive rats used in this study did not have any injection or implants. All animals were housed in a 12:12-h reverse light-dark cycle (lights on at 7:00 PM) to facilitate greater movement during daytime hours. For experiments during the dark cycle, every effort was made to reduce the amount of light exposure.

Neuronal Recordings

Behavioral testing and in vivo electrophysiological recordings in awake animals. BEHAVIOR. The LAT was used to assess forepaw motor function and akinesia (Schallert et al. 2000). An animal will display akinesia-like immobility in the forepaw contralateral to the 6-OHDA lesion, while forepaw use in the intact, ipsilateral side will remain unimpaired. In brief, the naive, sham-lesioned, or hemiparkinsonian rat was placed individually in an upright Plexiglas cylinder (20 cm in diameter, 30 cm high) and video recorded for 5–15 min while it explored and touched the glass with its forepaws. Forepaw contacts were noted by two experimentally blinded evaluators and later calculated as (no. of right contacts/no. of total contacts) × 100%. A value of 50% was characterized as normal, whereas an animal that touched >80% with its right (ipsilateral) forepaw correlated with >90% striatal dopamine depletion on the ipsilateral side (Schallert et al. 2000). LFPs were recorded during LATs.

We also employed the forelimb adjusting step test (Olsson et al. 1995) as another means to test for changes in forelimb akinesia from SNr-DBS. Briefly, this test requires the experimenter to restrain both hindlimbs and the nontested forelimb while allowing the tested forelimb freedom of motion. The rat is then moved forward and backward horizontally for 10 s along a surface 100 cm long. During this test, the unrestrained forepaw is allowed to contact the surface. The presence of forelimb akinesia is observable when unequal forepaw contacts are made onto the surface. Ipsilateral step percentage is calculated as (no. of ipsilateral steps/no. of total steps) × 100% and averaged from three trials for each animal. Both the step test and LAT were performed 21 days after 6-OHDA or saline injection for hemiparkinsonian or sham-lesioned rats, respectively.

Rats underwent both behavioral tests before, during, and after stimulation. Half of the rats were stimulated with high frequency (HFS), whereas the other half were stimulated with low frequency (LFS); after a 1-wk delay, the frequencies were reversed and the tests were repeated.

LFP RECORDINGS WITH LFS AND HFS PARAMETERS. Stimulation was applied to hemiparkinsonian rats while these animals underwent the LAT. Rats were stimulated using a Grass S44 stimulator (Grass Instruments, Quincy, MA) that was coupled to a current isolation unit (model 2200; A-M Systems, Sequim, WA). The duration of stimulation during behavioral testing varied depending on the activity level of the rat prior to stimulation. More specifically, rats were stimulated for 2–5 min to observe touching behavior in the LAT. If a rat did not make >20 contacts in the first trial, then another 2– to 5-min trial was performed 10 min later. Afterward, the total stimulation duration was compiled from one or both trials. Since all rats made >20 contacts after completing both trials, no more than 2 trials were performed for each animal.

Current for HFS was increased in 50- to 100-µA increments until we noted improvement in contralateral forelimb akinesia. In some cases, current amplitude was increased until we induced contralateral forelimb dyskinesia; immediately afterward, the current amplitude was reduced until the dyskinetic phenotype disappeared and forelimb akinesia improved. SNr-DBS currents averaged 815 ± 160 µA. For LFS, currents up to 1.5 mA were tested; when no effect was observed, the current optimized for HFS was chosen for LFS. These current amplitudes are higher than those typically reported for STN stimulation, but it is unlikely to induce tissue injury since our stimulation

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produced <30 μC/cm² of charge density (Kuncel and Grill 2004). While LATs were conducted, hemiparkinsonian rats received either HFS (150 Hz, 60-μs pulse width) or LFS (50 Hz, 60-μs pulse width), concurrent with LFP monitoring in the SNr. We stimulated the rat SNr at 150 Hz for HFS since this setting is the approximate medium value for stimulation frequencies used in the clinic for DBS (130–185 Hz) and frequencies throughout this range elicit similar efficacy in reducing PD motor symptoms (Kuncel et al. 2006; Moro et al. 2002). Conversely, 50-Hz stimulation was used for the LFS experiments since frequencies from 5 to 60 Hz were ineffective in improving motor function (Benabid et al. 1991; Fogelson et al. 2005; Timmermann et al. 2004). LFPs were acquired from pairs of twisted stainless steel electrodes, sampled at 1 kHz and high- and low-passed at 0.1 and 300 Hz, respectively. LFP analysis was adapted from Kempf et al. (2009). In brief, LFPs were obtained with Axoscope 10 (Molecular Devices, Sunnyvale, CA) and analyzed with MATLAB (R2010b; The MathWorks, Natick, MA). Power spectra were formulated from 30 s of trace taken immediately before and immediately after DBS. To generate these spectra, a 1-s Hamming window with 50% window overlap at 1-Hz resolution was used. To quantify changes in beta-frequency activity, mean beta (12–30 Hz) power was computed from spectrogram data using a 200-ms moving window, smoothed over time, summed (area under the curve), and then represented as arbitrary units (a.u.).

In vivo electrophysiological recordings in anesthetized animals. SNR and Ventromedial nucleus of the thalamus neuronal spiking activity with SNR stimulation. Extracellular single-unit recordings were obtained from six animals anesthetized with urethane (1.5 g/kg dissolved in saline and injected IP) in a stereotaxic frame (David Kopf Instruments); all animals received HFS and LFS at the mean current used to elicit behavior (800 μA). In all cases, no more than four different cells were recorded from each animal in each brain region. Glass- or epoxy-coated tungsten recording microelectodes with resistances ~500 kΩ (FHC, Bowdoin, ME) and platinum-iridium bicentric stimulating electrodes (250 μm in diameter; Plastics One) were lowered into the right SNr with a micropositioner (model 640; David Kopf Instruments). Single-unit recordings were acquired with a multichannel acquisition system (Plexon, Dallas, TX) at 40 kHz, referenced to an anchor screw in the nasal bone, high- and low-passed at 300 Hz and 6 kHz, respectively, and then analyzed with Offline Sorter (Plexon) and NeuroExplorer (Nex Technologies, Westford, MA). Single units were identified using 2:1 signal-to-noise voltage threshold and sorted by principal component analysis (with Offline Sorter). During stimulation, principal components analysis (PCA) was used to discern the stimulus artifacts from single units (see Fig. 3, A1 and B1). The detected events were then confirmed by visual inspection. We also employed stimulus artifact removal software called SARGE (Erez et al. 2010) on two SNr neurons and one ventromedial nucleus of the thalamus (VM) during HFS and found ~10% difference between identified spiking activity using PCA alone or with PCA after SARGE (data not shown). While LFS and HFS was applied to the SNr in hemiparkinsonian rats, single units were also recorded simultaneously from the VM (location from bregma: 2.4 mm posterior, 1.5 mm lateral, and 6.7 mm ventral from dura) before, during, and after SNr stimulation.

In vitro electrophysiological recordings. Consistent with other patch-clamp studies, we used rat pups in our in vitro experiments. Rat pups at postnatal day (P) 12–15 were injected IP with reserpine (5 mg/kg) and α-methyl-paer-tyrosine (AMPT; 300 mg/kg) 24 h before euthanization, respectively, to deplete nerve terminals of dopamine to mimic an acute “parkinsonian” state (Fino et al. 2007). Pups were anesthetized with isoflurane in a bell jar and quickly decapitated. Brains were sliced 400 μm thick in an oblique parasagittal orientation (Beurrier et al. 2006) on a vibratome VT 1200S (Leica, Deerfield, IL) in chilled dissecting solution containing (in mM) 87 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 7 MgCl₂, 0.5 CaCl₂, 24 NaHCO₃, 25 glucose, and 75 sucrose (oxygenated with 95% O₂-5% CO₂). Brain slices were also taken from normal untreated pups, age-matched to the reserpine-treated animals. Afterward, slices were incubated at room temperature in artificial cerebrospinal fluid (aCSF) for at least 1 h before recording. The aCSF in the recording chamber contained (in mM) 125 NaCl, 2.5 KCl, 1 MgCl₂, 1.25 NaH₂PO₄, 1 CaCl₂, 25 NaHCO₃, and 10 glucose (oxygenated as above) at a pH of 7.4, heated to 34°C. SNr neurons were visualized with an Olympus BX51WI upright microscope (Olympus Optical, Melville, NY) equipped with a ×40 water-immersion lens with differential interference contrast optics with infrared (DIC-IR). Whole cell patch-clamp electrodes were pulled from borosilicate capillaries (World Precision Instruments, Sarasota, FL) and filled with intracellular solution containing (in mM) 110 K-glucanate, 8 NaCl, 20 KCl, 1 MgCl₂, 0.0001 CaCl₂, 10 Na-HEPES, 2 Na-ATP, and 0.3 Na-GTP, pH of 7.4.

Brain slices were stimulated in the SNr by a 250-μm-diameter biconcentric electrode (FHC) at a distance of <200 μm from the recorded SNr neuron. Stimulation was monophasic and was applied for 30 s and set to 150 Hz, 60-μs pulse width, and 1.0- to 1.5-mA current with a Grass S48 stimulator (Grass Instruments) that was coupled to a P8106 current isolation unit (Grass Instruments). Recordings were obtained using an Axopatch 200B patch clamp (Molecular Devices), which was low-pass filtered at 5 kHz and sampled at 10 kHz. Only one SNr neuron was recorded from each brain slice.

Immunohistochemistry and Stereology

Rats underwent transcardiac perfusion with 4% paraformaldehyde, and the brain was removed and placed in 30% sucrose. Afterward, 60-μm brain sections were obtained using a freezing microtome HM 400 (Microm; Zeiss, Bern, Switzerland) and stained with anti-rabbit tyrosine hydroxylase (TH) antibody (Vectorstain; Jackson Immunostaining) as described elsewhere (Kitahama et al. 1988). These sections were then mounted on frozen gelatin-subbed slides and digitized with a NanoZoomer Digital Pathology slide scanner (Hamamatsu Photonics KK; Olympus). TH quantification was calculated with ImageJ software (NIH, Bethesda, MD) as described elsewhere (Paille et al. 2011). In brief, regions of interest were selected in the striatum and substantia nigra compacta (SNC) and then pixelated and compared with the same region on the contralateral hemisphere. Percentages represent the right (lesioned) side divided by the left (unlesioned) side. Cresyl violet (CV) Nissl staining was used to identify electrode placement from freely moving animals.

Statistics and Chemicals

All data were statistically analyzed with SigmaStat 10 (Systat Software, San Jose, CA), and unless stated otherwise, a one-way ANOVA (with repeated measures when applicable) was used to find statistical significance at P < 0.05. When appropriate and where noted, two-tailed t-tests were performed. Unless stated otherwise, all chemicals were obtained from Sigma Aldrich (St. Louis, MO).

RESULTS

Confirmation of the Rat Hemiparkinsonian Phenotype and Electrode Placement

The hemiparkinsonian phenotype was confirmed immunohistochemically with anti-TH antibody staining to visualize dopaminergic terminals in the striatum and cell bodies in the SNC. Hemiparkinsonian rats had an 86.9 ± 8.3% decrease in dopaminergic terminals in the striatum ipsilateral to the 6-OHDA lesion compared with the contralateral side (n = 8, P < 0.01, paired t-test; Fig. 1, A and C). Similarly, hemiparkinsonian rats had 62.8 ± 9.9% fewer dopaminergic cell bodies in the SNC...
in hemiparkinsonian rats did not improve forelimb function, and these animals were not included in the analysis. After all single-unit recordings were completed, recording sites in the SNr (data not shown) and VM (Fig. 1F) were confirmed with CV staining.

**SNr-HFS and LFS Effects on Behavior and LFP Activity**

**SNr-HFS** improved forelimb akinesia and decreased beta oscillations in the SNr. Naive (n = 3) and sham-lesioned rats (n = 5) did not show any impairment in the LAT and touched equally with their ipsilateral (right) and contralateral (left) forepaws (unpaired t-test, P > 0.1). Therefore, both groups were pooled together and denoted as the control group (50.8 ± 2.2% right contacts, n = 8; Fig. 2A). In contrast, hemiparkinsonian rats decreased the use of their contralateral (left) forepaw and touched with their right forepaw 92.1 ± 5.5% of the time while exploring in the LAT (n = 6). However, during SNr-HFS, hemiparkinsonian rats touched equally with their right and left forepaws (51.7 ± 1.8% right touches, n = 6, P < 0.001), and this improvement in forelimb akinesia was indistinguishable from forelimb function observed in naive or sham-lesioned rats (Fig. 2A); the current amplitude values that elicited these improvements ranged from 550 to 1,080 μA. SNr-DBS was applied for 240 ± 122 s for LATs. Notably, we found that SNr-HFS in hemiparkinsonian rats with larger current amplitude values from 1,200 to 1,490 μA elicited contralateral (left) forepaw dyskinesia in the LAT (n = 6; data not shown). To further corroborate our LAT findings, we employed the adjusting step test to evaluate forelimb akinesia. Similar to the LAT, naive (n = 3) and sham-lesioned (n = 5) rats did not exhibit any impairment in forelimb function by stepping equally with the right and left forepaw (right stepping 51.6 ± 3.1% of the time, n = 8; Fig. 2B) in the step test (unpaired t-test, P > 0.1); therefore, these groups were pooled together as a control group (Fig. 2A). Conversely, hemiparkinsonian rats exhibited forelimb akinesia with a predominant use of the right forepaw (90.7 ± 4.6%, n = 6). However, SNr-HFS in hemiparkinsonian rats improved the biased right forepaw stepping to 53.5 ± 5.7% (n = 6, P < 0.001), which was similar to the performances seen in control rats (Fig. 2A). When we increased HFS current amplitudes to higher values (1,190 to 1,440 μA), we again observed left forelimb dyskinesias in hemiparkinsonian rats (n = 4; data not shown).

LFPs recorded from the SNr of freely moving hemiparkinsonian rats displayed significant beta-frequency (12–30 Hz) oscillations (n = 6; Fig. 2B), which were not observed in the SNr of sham-lesioned rats (n = 6). When SNr-HFS was applied for 30 s in freely moving hemiparkinsonian rats, HFS significantly suppressed dominant beta oscillations from 0.65 ± 0.04 to 0.34 ± 0.03 a.u.; this suppression lasted 114 ± 28 s (n = 6, P = 0.01; Fig. 2C). Afterward, SNr beta-frequency activity returned to prestimulation values (0.80 ± 0.04, P = 0.48; not shown). HFS in sham-lesioned rats had no effect on beta oscillatory activity (P = 0.31; Fig. 2C).

**SNr-LFS** did not improve forelimb akinesia or beta oscillations in the SNr. Low-frequency DBS applied to the SNr (SNr-LFS; 50 Hz) in the same rats did not improve forelimb akinesia, since hemiparkinsonian rats touched with their right forepaw 91.7 ± 2.3% of the time before SNr-LFS and 88.0 ± 3.7% of the time after stimulation (n = 6, P = 0.4). No difference in the number of contacts was observed.
SNr-DBS improves forelimb akinesia

Fig. 2. SNr deep brain stimulation (DBS) at 150 Hz improved forelimb akinesia in hemiparkinsonian rats to forelimb function similar to that observed in control (naive or sham lesioned) rats. A: in the limb-use asymmetry test (LAT; black bars), group data represent the number of ipsilateral forepaw contacts relative to the total contacts as a percentage and show that forelimb akinesia improved with high-frequency SNr-DBS (HFS; 150 Hz) to values similarly seen in naive and sham-lesioned rats. In contrast, low-frequency SNr-DBS (LFS; 50 Hz) did not improve forelimb akinesia; hemiparkinsonian rats predominantly touched with their ipsilateral, unimpaired forepaw. Contacts/forepaw placements were measured in the stepping test (gray bars). Here, SNr-DBS improved forelimb akinesia to values seen in control (naive or sham lesioned) rats. In both the LAT and step test, forelimb improvement lasted the entire duration of the HFS. Sample sizes are 8 rats for sham and 6 rats for the other 3 groups. *P < 0.05, significantly different from PD groups. #P < 0.05, significantly different from sham groups. †P < 0.05, significantly different from paired groups. SNr-DBS decreased beta-frequency (12–30 Hz) oscillations in the SNr of the freely moving hemiparkinsonian rat. B: a representative power spectrum taken from 30 s of local field potential (LFP) trace before HFS (solid line) shows a dominant beta peak that is markedly decreased after the application of HFS (dotted line). D: a representative power spectrum shows that LFP activity did not differ significantly after LFS. C and E: total beta activity was calculated from the traces used to compile the power spectra and shows that HFS reduced the power in beta oscillatory activity in hemiparkinsonian rats, but not in sham-lesioned rats; furthermore, LFS had no effect on beta oscillations in either group. The data represent a sample size of 6 for hemiparkinsonian rats and 5 for sham-lesioned rats (3 naive rats included in A did not receive implants and were excluded from this evaluation). †P < 0.01.

between any of the groups tested (P = 0.33; data not shown). Consistent with our findings in the LAT, SNr-LFS did not improve forelimb akinesia in the stepping test in the hemiparkinsonian rat, since right-dominated stepping persisted during this treatment (n = 6, P = 0.5; Fig. 2A). The impairment in forelimb function in hemiparkinsonian rats with LFS was also significantly different from the improvement seen with HFS in both the LAT and the adjusting stepping test (P < 0.01; Fig. 2A). HFS and LFS values ranged from 350 to 1,000 μA. LFS applied to the SNr of hemiparkinsonian or sham-lesioned rats did not change beta oscillatory power (n = 6, P = 0.16; Fig. 2, D and E).

Effects of SNr-HFS and LFS on SNr and VM Neuronal Spiking Activity

SNr-HFS attenuated SNr firing and increased VM neuronal spiking activity. SNr-HFS at 150 Hz in anesthetized hemiparkinsonian rats significantly decreased SNr neuronal spiking activity (Fig. 3). More specifically, SNr neuronal spiking activity prestimulation was 28.4 ± 1.9 Hz, and this activity decreased to 0.8 ± 1.9 Hz during the 30 s of HFS (n = 23 units, P < 0.001, repeated-measures 1-way ANOVA; Fig. 3C). After termination of HFS, SNr neurons resumed spiking activity to 29.4 ± 1.9 Hz by 6 ± 5 s later (Fig. 3, A2 and C). Also, before SNr-HFS, VM neurons had a spiking activity of 7.1 ± 1.6 Hz, which was consistent with values reported in other studies (Deniau and Chevalier 1985; Patino and Garcia-Munoz 1985). However, when HFS was applied to the SNr, neuronal spiking activity in the VM increased to 18.6 ± 1.6 Hz (n = 22, P = 0.002; Fig. 3, B2 and C). The increased VM spiking activity persisted
throughout the duration of the 30-s stimulation and returned to prestimulation values (8.2 ± 1.6 Hz) 11 ± 8 s after HFS.

**SNr-LFS increased SNr firing and decreased VM spiking activity.** Thirty seconds of LFS in the right SNr of anesthetized hemiparkinsonian rats increased neuronal spiking activity in most SNr neurons. Specifically, SNr-LFS increased SNr spiking activity from 28.2 ± 3.8 to 38.0 ± 3.8 Hz during LFS in 17 of 23 cells (P = 0.001, repeated-measures 1-way ANOVA; Fig. 4A). The increased SNr spiking activity persisted throughout the stimulation duration and returned to prestimulation values (27.1 ± 3.8 Hz) 18 ± 11 s after LFS was turned off (Fig. 4, A and C). In the other six SNr neurons, LFS did not change SNr spiking activity (27.3 ± 3.3 Hz, P = 0.35). Conversely, when we examined how VM neuronal activity responded to SNr-LFS (50 Hz), we found that stimulation decreased the spiking activity in 15 of 22 VM neurons from 7.0 ± 3.0 to 0.5 ± 3.0 Hz during LFS (P < 0.001, repeated-measures 1-way ANOVA; Fig. 4, B and C) but returned to 8.5 ± 3.0 Hz by 15 ± 9 s after stimulation was terminated (P = 0.12). The other seven VM neurons did not change spiking frequency with SNr-LFS stimulation (7.4 ± 3.0 Hz, P = 0.40).

**HFS in the SNr Attenuated SNr Neuronal Spiking Activity via a Depolarization Block In Vitro**

We recorded from four brain slices taken from naive rats and three taken from reserpine-treated rats. Since SNr neurons from both groups responded to HFS in a similar manner (P > 0.05 for all measurements, unpaired *t*-test), we pooled these groups together. Before stimulation, SNr neurons exhibited tonic spiking activity in vitro. However, HFS in the SNr rapidly depolarized the resting membrane potential from −55.5 ± 2.7 to −36.0 ± 1.9 mV (n = 7, P < 0.001; Fig. 5, A and B), which led to a depolarization block that occurred 1.2 ± 0.7 s after HFS and the abolishment of spiking activity for 288.3 ± 134.4 s (Fig. 5A). After the termination of HFS, the resting membrane potential repolarized to −51.5 ± 2.9 mV, which was not significantly different from the resting membrane potential before HFS (P = 0.50; Fig. 5B). Afterward, SNr neurons resumed tonic spike activity to 6.2 ± 1.6 Hz (n = 7), which was not significantly different from SNr neuronal firing frequency prestimulation (5.6 ± 1.7 Hz, P = 0.78; Fig. 5C).

**DISCUSSION**

Although DBS dramatically improves motor function in advanced PD patients, some symptoms do not improve with DBS and reports of nonmotor, stimulation-induced complications have emerged. Therefore, a DBS target that provides similar efficacy as the STN, but without nonmotor complications, is ideal. As a first step, we have shown that SNr-HFS...
restored forelimb function in hemiparkinsonian rats to activity levels seen in naive and sham-lesioned rats. Moreover, these improvements in motor function are similar to those described in parkinsonian animals treated with STN-HFS in the LAT (Shi et al. 2004) and stepping test (Fang et al. 2010). Therefore, SNr-HFS elicited similar improvements in forelimb function in parkinsonian animals to those reported in the more preferred BG nucleus target. From our data, we propose that the improvement in forelimb function associated with SNr-HFS may have resulted from the attenuation of SNr neuronal spiking activity, through a depolarization block phenomenon; reduced SNr neuronal spiking activity may subsequently disinhibit VM neurons, which results in increased excitability in about 65% of VM neurons.

Dominant beta oscillations in the basal ganglia are emerging as physiological correlates in PD and are thought to represent “akinetic” oscillations (Heimer et al. 2002; Kuhn et al. 2008; Levy et al. 2002; Nini et al. 1995; Sharott et al. 2005; Silberstein et al. 2003; Soares et al. 2004; Wichmann et al. 2002). The dominance in these oscillations has been reported to decrease with L-DOPA or DBS treatment in PD patients and in animal models of PD, which coincide with “prokinetic” movements (Brown and Williams 2005; Eusebio and Brown 2007; Gatev et al. 2006; Gaynor et al. 2008; Hammond et al. 2007; Israel and Bergman 2008). In this study, we report that SNr-HFS in the hemiparkinsonian rat decreased dominant beta oscillations in the SNr, which correlated with improved motor function, and this finding is consistent with the postulate that desynchronizing activity in this frequency range in the basal ganglia coincides with reduced forelimb akinesia. Conversely, SNr-LFS in hemiparkinsonian rats did not improve forelimb function, and this finding is similarly described for PD patients who did not have improved motor scores from STN-LFS (Moro et al. 2002). We speculate that a decrease in VM neuronal spike activity, from enhanced GABA release from LFS-excited SNr neurons, could underlie this effect. Notably, LFS did not exacerbate forelimb akinesia in hemiparkinsonian rats, but this may be due to a ceiling effect. A lack of improvement in forelimb akinesia from LFS may be due to a difference in charge density produced with HFS vs. LFS.

The lateral SNr receives input from the motor-related dorsal putamen of the striatum (Hedreen and DeLong 1991; Lynd-Balta and Haber 1994) and projects to the VM, a thalamic motor region, and then to the supplementary motor area (SMA; Francois et al. 2002), which is an area that is impaired in PD. The dysfunction of the SMA, which is involved in stringing motor sequences together for motor execution, mediates a large part of the motor impairment in PD (Brinkman and Porter 1979; Marsden 1982). Overall, these connections predict an important role of the SNr in modulating motor activity. In addition to anatomic support for the SNr as a potential target, an electrophysiological localization study found that 49% of SNr neurons responded to movement-related activity in the trunk, shoulder, knee, elbow, and wrist (Starr et al. 2000; Wichmann et al. 2001). Other studies report that 70% of recorded SNr neurons responded to arm and wrist movements and motor execution in nonhuman primates (Nishino et al. 1991) and encoded the velocity and direction of arm movements (Magarinos-Ascone et al. 2002). Moreover, amelioration of muscle rigidity coincided with decreases in STN and SNr neuronal spike frequencies (Benedetti et al. 2009), and tremor frequencies (3–8 Hz) have been shown to oscillate in both the Gpi and SNr (Wichmann et al. 1999).

Since the SNr responds to limb velocity, direction, and amplitude, the improvement in motor function from lesioning and pharmacological modulation of this BG output nucleus is not surprising. In particular, SNr lesions improved posture and reaching ability in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated nonhuman primates (Henderson et al. 2005) and improved appendicular akinesia and bradykinesia (Wichmann et al. 2001). Moreover, microinjection of muscimol, a GABA<sub>A</sub> receptor agonist, into the lateral SNr improved akinesia and bradykinesia (Wichmann et al. 2001), whereas an injection of a GABA<sub>A</sub> antagonist induced akinesia (Burbard et al. 1998).

Although the mechanism(s) underlying the therapeutic effects of DBS remain uncertain, a recent study found that both STN-DBS and l-DOPA therapy decreased total GABA content in the ventral anterior/ventral lateral (VA/VL) nucleus of the thalamus in PD patients (Stefani et al. 2011), which may have resulted from increased STN axonal activity to the globus pallidus externa (Gpe) and subsequent inhibition of SNr/Gpi activity (Benazzouz et al. 2000). In our study, we elicited improvement in motor function by directly suppressing SNr neuronal activity, which presumably disinhibited some VM neuronal activity. This finding coincides with predictions derived from the “box and arrow” model (Albin et al. 1989; DeLong 1990) and corroborates the theory that DBS induces a depolarization block-like inhibition of the stimulated brain region in vitro (Beurrier et al. 2001; Bikson et al. 2001; Garcia et al. 2003; Kiss et al. 2002; Lian et al. 2003; Magarinos-Ascone et al. 2002), in animal research in vivo (Benazzouz et al. 2000; Boraud et al. 1996; Meissner et al. 2005; Tai et al. 2003), and from clinical studies in PD patients (Dostrovsky et al. 2000; Filali et al. 2004; Lafreniere-Roula et al. 2010; Welter et al. 2004; Wu et al. 2001). However, our results contrast with studies and models that suggest that DBS inhibits the soma while activating efferent axons (Anderson et al. 2003; Do and Bean 2003; Galati et al. 2006; Hashimoto et al. 2003; Lafreniere-Roula et al. 2010; McIntyre and Grill 2002; Stefani et al. 2005; Windels et al. 2003). Since some recorded VM neurons were affected by SNr-DBS, whereas others were not, it remains to be determined whether a depolarization block perse is solely responsible for the improvement in forelimb akinesia in hemiparkinsonian rats. An alternative model to explain DBS-mediated excitation of the SNr is that the excited neurons serve to inhibit competing motor programs. However, at this time we are unable to substantiate this proposal.

Given that SNr-HFS elicited similar therapeutic effect in the hemiparkinsonian rat as STN-DBS (Fang et al. 2010; Shi et al. 2004), it is plausible that our stimulation of the SNr could have improved limb function by affecting SNr neuronal activity from current spread. It is also possible that SNr-DBS may have increased SNC neuronal activity, since this area is dorsal to, and interdigitated among, the SNr (Fu et al. 2012; Javoy-Agid et al. 1981). If so, HFS-induced excitation of SNC axons (McIntyre et al. 2004; Zheng et al. 2011) could have increased dopaminergic release in the striatum. Although we did not address this possibility, we speculate that the release of dopamine from the remaining 10% of dopaminergic terminals spared from the 6-OHDA injection could not account for the improved motor function in hemiparkinsonian rats.
The depolarization observed in vitro from HFS of SNr neurons demonstrates that SNr cells can respond to HFS by attenuating activity via a depolarization block phenomenon. The sustained depolarization in vitro probably lasts much longer than in vivo because 1) stimulation was more diffuse in the slice chamber and 2) the in vitro preparation may have impaired buffering and homeostatic mechanisms that are normally present in the intact brain. One well-documented effect of stimulation of excitable tissue is an increase in extracellular K+ concentration, which may contribute to a sustained depolarization block (Shin et al. 2007) and prevent any rapid repolarization of SNr neuronal action potentials.

Although we showed that SNr-HFS improved forelimb function in hemiparkinsonian rats, we employed current amplitudes that are larger than those used in STN-DBS (−100–300 μA) in hemiparkinsonian rats (Fang et al. 2010; Li et al. 2007; Spiegels-Engemann et al. 2010; Temel et al. 2005; Walker et al. 2012) and than those used in the human SNr (Laflamere-Roula et al. 2010). In the former, the rat STN (0.8 mm3) is smaller than the SNr (5.5 mm3) (Hardman et al. 2002) and comprises cells that differ architecturally from the SNr. Therefore, the use of higher current amplitude in the SNr to elicit behavioral effects in hemiparkinsonian rats is not altogether surprising. In the latter case, HFS (<10 μA) was applied to the SNr of PD patients through a microelectrode, and a decrease in SNr single-unit activity was reported. However, in this study, microstimulation in the human SNr was not performed to elicit behavioral changes, but instead was intended to attenuate SNr neuronal activity proximal to the stimulating electrode. To elicit any motor responses to SNr stimulation, it is more likely that higher current amplitudes are needed in the human SNr. In addition to the differences in size and cell composition between the STN and SNr, other factors may explain why higher current amplitudes were needed in the latter and not the former BG region. For instance, the STN is the only glutamatergic nucleus in the BG (Albin et al. 1989; DeLong 1990) and therefore has a central role in modulating the BG network. In addition, the STN is part of the hyperdirect pathway (Nambu et al. 2002), and STN-DBS may recruit antidromic activation of the motor cortex via this pathway (Gradinaru et al. 2009). Also, the STN is closely situated to the subthalamic cerebrovasodilator area (SVA), which can significantly influence cerebral circulation (Golanov et al. 2000) and mediate some effects of STN-DBS (Sidits et al. 2012). However, further work is needed to resolve this difference.

If the SNr can be validated as a novel target for DBS, it may offer greater flexibility to better address a patient’s specific needs and conditions. However, there are some concerns regarding the pragmatic use of SNr-DBS that warrant discussion. First, stimulation of the medial portion of the SNr has been shown to induce hypomania (Bejjani et al. 1999). This may be due to the fact that medial SNr receives input from the nonmotor portions of the caudate and the limbic/associative ventromedial STN (Seifried et al. 2012) and projects to the mediodorsal magnocellular nucleus and the VA magnocellular nucleus; these areas project to the anterior, nonmotor regions of the frontal lobe (Schultz 1986). Ideally, the lateral portion of the SNr would be the candidate target for DBS. Yet, in this study, we did not specifically differentiate between electrodes in the lateral and medial SNr. Instead, we placed our stimulating electrodes into the largest portion of the SNr, which corresponds to a more caudal-lateral location (Fig. 1D). However, it is important to note that although we did not explicitly examine nonmotor side effects from SNr-DBS, we did not observe any overt changes in behavior. In fact, the amount of touches that were made by hemiparkinsonian rats undergoing SNr-HFS and nonstimulated rats in the LAT did not differ among the groups. In addition, our behavioral and electrophysiological data demonstrate that SNr-HFS is at least as effective as STN-DBS in improving akinesia in the hemiparkinsonian rat model of PD. However, an examination of the nonmotor complications of SNr-DBS in the hemiparkinsonian rat is needed in the future to validate the stimulation of this area for treating motor symptoms of PD. Second, stimulation of the SNr, like most DBS targets, raises concerns regarding nearby structures, such as the internal capsule (IC). Activation of this white matter tract from DBS-mediated current spread could pose another challenge to SNr-DBS. Last, although the neural circuitry of the SNr in rodents is similar to nonhuman primates (Carpenter et al. 1976; Carter and Fibiger 1978; Clavier et al. 1976; Faull and Carman 1968; Faull et al. 1986; Gerfen 1985; Rinivk 1975), there may be significant differences in the functionality of the SNr between species. Therefore, further studies involving SNr-DBS in the nonhuman primate could provide further validation for the utility of targeting the SNr for PD treatment. Notably, although DBS has not been performed in the nonhuman primate SNr, some studies have shown that modulating SNr neuronal activity in nonhuman primates via lesioning or pharmacological approaches resulted in improved forelimb performance (Burbaud et al. 1998; Henderson et al. 2005; Wichmann et al. 2001).

With regard to clinical information on SNr-DBS, few reports are available since this area is generally avoided for the reasons described above. However, since ventral stimulating contacts of the DBS electrodes are occasionally positioned in the SNr from electrodes implanted for STN-DBS in PD patients, there are rare opportunities to assess the effects of SNr-DBS on motor. For instance, a recent study reported improvement in postural and gait stability in PD patients from SNr-DBS but no improvement in averaged motor scores (Chastan et al. 2009). Yet, on closer examination of the data, SNr-DBS improved motor scores in two of seven patients to values similarly seen with STN-DBS or l-DOPA treatment. Although this finding may seem insignificant, the improvement in these select patients occurred from DBS electrodes that were never optimized or intended for ameliorating PD symptoms from SNr modulation. Instead, the SNr location was a consequence of a STN-DBS electrode implantation. A more thorough targeting of the DBS electrode to the lateral portion of the SNr may result in better motor outcomes in PD patients.

In summary, we are the first to report that SNr-DBS significantly improves forelimb akinesia in hemiparkinsonian rats, presumably from a stimulation-induced decrease in SNr neuronal activity, leading to a disinhibition of VM thalamic neuronal activity and a decrease in beta oscillations in the SNr. Further work will focus on assessing SNr-DBS effects on gait function and on nonmotor complications such as hypomania. From our findings, we posit that the SNr may serve as a novel target for DBS for PD.
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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


