Frequency-dependent effects of electrical stimulation in the globus pallidus of dystonia patients

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DEEP BRAIN STIMULATION (DBS) is used as a treatment for a number of drug-treatment refractory neurological disorders and involves the application of continuous high-frequency stimulation (HFS; ~130 Hz) through indwelling electrodes (Benabid et al. 1987; Perlmutter and Mink 2006; Starr et al. 1998; Wichmann and DeLong 2006). Recently, bilateral globus pallidus internus (GPi) DBS has been shown to improve the tonic abnormal twisting movements and postures referred to as dystonia (Coubes et al. 2004; Hung et al. 2007; Kupsch et al. 2006; Vidailhet et al. 2005). A clinical study in cervical dystonia has shown that low-frequency stimulation of 5 Hz was ineffective, but higher frequencies above 60 Hz produced improvement (Moro et al. 2009). This work demonstrated a clear frequency-dependent effect of GPi DBS on therapeutic outcome, but the effects of different frequencies of electrical stimulation on GP neurophysiology are not well understood.

The mechanisms of DBS action appear to be varied and complex, and which of the specific actions underlie therapeutic benefit remains unclear. Our group previously reported that focal microstimulation leads to inhibition of neuronal firing, and the hypothesized mechanism was synaptic GABA release from the afferent terminals (Dostrovsky et al. 2000; Filali et al. 2004; Lafreniere-Roula et al. 2010; Wu et al. 2001). However, both experimental and modeling studies also indicate that DBS may excite local neuronal cell bodies and/or their efferent fiber outputs, as well as fibers of passage (Bar-Gad et al. 2004; Hashimoto et al. 2003; Johnson and McIntyre 2008; Xu et al. 2008). These excitatory effects of DBS might lead to a more regular pattern of activity, driven at the stimulation frequency in the efferents, which would reduce and replace the irregular pathological neuronal activity (Benazzouz and Hallett 2000; Garcia et al. 2005; Kringelbach et al. 2007).

Recording neuronal activity during stimulation with the macroelectrodes used for clinical HFS is hampered by the presence of large stimulation artifacts and amplifier saturation. Methods have been proposed to overcome these technical issues using artifact removal and data substitution (Hashimoto et al. 2002; Montgomery Jr. et al. 2005; Wichmann 2000), which can recover spiking data during stimulation, but fiber volleys have not been recovered using these methods. We used open filter recordings and detected both spikes and fiber volleys using stimulation trains of biphasic pulses at different frequencies through a nearby microelectrode, which produced narrow artifacts that were removed and substituted with data sampled from within the train. Overall, the data support previous studies of DBS action in showing silencing of spike firing for short periods (Dostrovsky et al. 2000; Lafreniere-Roula et al. 2010; Wu et al. 2001). These previous studies examined the effects of focal stimulation through the same electrode used for recording, and the duration of inhibition was measured after the end of the mostly short (0.5 s), high-frequency (200 Hz) trains. We extended these studies to examine activity during the stimulation trains and the effects of a complete range of different frequencies of stimulation. We compared the decrease in firing rate with the evoked field potentials (EFPs) and measured fiber volleys and antidromic-like activation during the stimulation train. Frequency-response plots were compared before and after short periods of standard tetanizing HFS, which can produce potentiation of the
fEP (Prescott et al. 2009), and found direct evidence of potentiation of the inhibitory synaptic field potentials by HFS, which may be involved in the poststimulation changes in pallidal firing observed here and previously (Bugaysen et al. 2011; Erez et al. 2009). Additionally, we validated these results in a dystonia patient with previously implanted, bilateral GPI-DBS electrodes by observing the effects of DBS stimulation on neuronal activity in the subthalamic nucleus (STN).

**MATERIALS AND METHODS**

A total of 60 GPI sites was investigated during microelectrode-guided placement of DBS electrodes in 14 patients with various types of dystonia. For recording locations and patient characteristics, see Fig. 1B and Table 1, respectively. An additional early-onset primary dystonia (DYT1) patient with bilateral GPI DBS electrodes was studied, and four STN neurons were recorded during bilateral activation of the DBS electrodes. All experiments conformed to the guidelines set by the Canadian Institute of Health Research Policy on Ethical Conduct for Research Involving Humans and were approved by the University Health Network Research Ethics Board. All patients provided written, informed consent prior to the procedure.

**Surgery.** The microelectrode recording and stimulation procedures to map and target the GPI and STN during stereotactic and functional neurosurgery are described elsewhere (Hutchison et al. 1994, 1998; Lozano et al. 1996). Briefly, the patients were under local anesthesia, except in three cases (five cells), where propofol was administered, a stereotactic frame was mounted over the cranium, and burr holes were made to allow access to ventral GPI targets via a guide tube. The target for the lowest contact of the DBS electrode was determined by MRI and was tentatively placed 2.5 mm in front of the midcommissural point, 3–6 mm below the intercommissural (anterior commissure-posterior commissure) line, and 20 mm lateral to the midline (Fig. 1A).

**Data acquisition.** A dual microelectrode assembly was used with independently driven, parylene-coated tungsten microelectrodes (~25 μm tip length, impedance 0.2–0.4 MΩ at 1 kHz, ~600–1,000 μm

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Fig. 1. Experimental recording locations and an example of neuronal recording in human globus pallidus (GP). A: representative electrode track in the GP externus (GPe), GP internus (GPI), and optic tract (OT). The stimulating and recording electrodes are parallel and separated by ~1 mm. Each unit on the electrode track is 1 mm. D, dorsal; AC, anterior commissure; PC, posterior commissures; V, ventral. B: number of recording sites along the electrode track in bins of 2 mm. Gray bars represent the sites sampled, and black bars represent sites included in the analysis. Each bin is exclusive of its lower-bound value and inclusive of its upper-bound value. C: a segment of recording in GPI during the 10-Hz stimulation train, illustrating parameters used for quantitative measures of firing rates, evoked field potential (fEP) amplitude, and fiber volley amplitude. The amplitudes of the fiber volley and fEP were measured from the baseline as indicated. The potentials were detected, distinguished, and measured according to latencies during the stimulation train, and the 1st, last, and average values were recorded. The number of spikes during the stimulation trains was divided by the respective train length to give the average firing rate at each stimulation frequency. Fiber volleys were observed in 11 out of the 25 sites throughout the study. D: timeline of stimulation protocol for the frequency-dependent study. Note that only 1 block of 10 s is shown for the 1st 3 frequencies of 1, 2, and 5 Hz. Intervals between trains were 3 s, and after the set of 4 tetanizing trains, the same ascending series of frequencies was delivered, but only the 1st, 1 Hz, is schematically shown. HFS, high-frequency stimulation.
Table 1. Demographic and clinical characteristics of the dystonia patients

<table>
<thead>
<tr>
<th>Class of Dystonia</th>
<th>Pre-op Motor Scores (Subscores)</th>
<th>Medication Pre-op (Daily Dose in mg)</th>
<th>Number of Sites Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Patient #)</td>
<td>TWSTRS (s/d/p)</td>
<td>BFMDRS (m/d)</td>
<td></td>
</tr>
<tr>
<td>Generalized, tremor (1)</td>
<td>53 (15)</td>
<td>Baclofen (60); Clonazepam (0.5); Trihexyphenidyl (6); Tetrabenazine (50)</td>
<td>3</td>
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<tr>
<td>Generalized, ataxia (2)</td>
<td>70 (15)</td>
<td>None</td>
<td>5</td>
</tr>
<tr>
<td>Cervical (3)</td>
<td>63 (44)</td>
<td>N/A</td>
<td>8</td>
</tr>
<tr>
<td>Segmental, tardive (4)</td>
<td>57 (12)</td>
<td>Anastrozole (1); Pantoprazole (40); Lithium (300); Tamoxifen (20); Citalopram (20); Ramipril (10); Hydrochlorothiazide (25)</td>
<td>6</td>
</tr>
<tr>
<td>Cervical (5)</td>
<td>48 (6)</td>
<td>Fentanyl (0.6); Gabapentin (2.700); Venlafaxine (37.5); Amitriptyline (25)</td>
<td>4</td>
</tr>
<tr>
<td>Generalized (Lubag; 8)</td>
<td>73 (N/A)</td>
<td>N/A</td>
<td>6</td>
</tr>
<tr>
<td>Cervical (6)</td>
<td>77 (61)</td>
<td>Lorazepam (1); Premarin; Hydrochlorothiazide (25); Furosemid (20)</td>
<td>2</td>
</tr>
<tr>
<td>Cervical (7)</td>
<td>52 (17)</td>
<td>Baclofen (15); Trazodone (100); Trihexyphenidyl (14); Tetrabenazine (75); Clonazepam (3.5); Lorazepam (1 PRN)</td>
<td>8</td>
</tr>
<tr>
<td>Neuroacanthocytosis (10)</td>
<td>32 (10)</td>
<td>Trihexyphenidyl (2); Clonazepam (2); Venlafaxine (75)</td>
<td>7</td>
</tr>
<tr>
<td>Cervical (11)</td>
<td>50 (8)</td>
<td>Trihexyphenidyl (24); Olanzapine (7.5); Clonazepam (0.5); Tetrabenazine (75); Venlafaxine (75)</td>
<td>3</td>
</tr>
<tr>
<td>Generalized (12)</td>
<td>62 (6)</td>
<td>Clonazepam (1.5); Gabapentin (1.800); Botox, Nortriptyline (100); Lorazepam (3); Codeine, Lorazepam (4), Tylenol (6-8 tablets PRN)</td>
<td>4</td>
</tr>
<tr>
<td>Cervical (13)</td>
<td>43 (4)</td>
<td>Esomeprazole, Botox, Baclofen (30); Trihexyphenidyl (2); Sertraline (50); Clonazepam (1)</td>
<td>2</td>
</tr>
<tr>
<td>Generalized Dyt 1 (14)</td>
<td>25 (M)</td>
<td>Baclofen (45); Trihexyphenidyl (4)</td>
<td>2</td>
</tr>
</tbody>
</table>

| Mean (SD)                 | TWSTRS: 47 (12)                 | BFMDRS: 27 (27)                      | Total: 62               |

F, female; M, male; BFMDRS, Burke-Fahn-Marsden dystonia rating scale; TWSTRS, Toronto Western spasmodic torticollis rating scale; N/A, not available; d, disability; m, movement; p, pain; s, severity; t, total score; Dyt 1, early-onset primary dystonia; PRN, Pro Re Nata (as needed). Note: Patient 6 was not a patient of the neurologist at Toronto Western Hospital.

separation between tips of the microelectrodes), as described previously (Levy et al. 2007). The two microelectrodes shared a common ground on the stainless-steel intracranial guide tube. Recordings were amplified 5,000–10,000 times and then filtered at 10 Hz-5 kHz (analog Butterworth filters: high-pass, one pole; low-pass, two poles), using two Guideline System GS3000 amplifiers (Axon Instruments, Union City, CA). The recorded signals were digitized at 12.5 kHz with a CED 1401 data acquisition system (Cambridge Electronic Design, Cambridge, UK) and saved to a computer hard-drive running Spike2 software (Cambridge Electronic Design). The acquired neuronal signals were monitored continuously during acquisition by computer display, and the spike signals were band-pass filtered at 300 Hz-5 kHz and monitored on an oscilloscope and loudspeaker.

Recording and stimulation protocol. All microstimulation and recordings were made within the GP of dystonia patients using dual microelectrodes at approximately the same depth in the track but separated by ~1 mm in the mediolateral direction. The pair of electrodes usually started 10 or 15 mm above target so that the recordings from both electrodes often began within the GP externus (GPe) and continued into the GPe. GPe neurons are classified into two types: slow-frequency discharge neurons with pauses and a smaller population of low-frequency discharge neurons with bursts (LFB). Between the GPe and GPi is the internal medullary lamina, an area of white matter, which is identified by an overall decrease in neuronal activity. Neurons of the GPi were identified by their characteristic high firing rate and spiking pattern, with or without short pauses. The ventral border of the GPi was determined based on decreased noise in recordings, as the electrodes passed into white matter, and identification of the optic tract (OT). The OT was identified by microstimulation to elicit visual percepts and/or by recording responses evoked by turning the room lights off and on or by flashing a strobe light (Hutchison et al. 1994; Lozano et al. 1996). A well-isolated spike was recorded initially on one electrode, and stimulation was made with the second electrode with trains separated by 3 s at increasing frequencies (1, 2, 5, 10, 20, 30, 50, and 100 Hz, 10 pulses for the first three low frequencies but 50–60 pulses/train for the remainder; see Fig. 1D). Previous studies from our group have measured responses following short trains at 200 Hz (Dostrovsky et al. 2000), but we examined lower frequencies with similar total electrical energy delivered during the train, i.e., similar number of pulses/frequency train. Stimulation was delivered using an isolated constant-current stimulator (Neuro-Amp1A, Axon Instruments) with symmetric, biphasic pulses, 300 μA in total duration (cathodal followed by anodal, 150 μA each). Data were collected during an initial 20- to 30-s offstimulation (“baseline” or “control”) period, followed by the stimulation trains from lowest to highest frequency, before and after trains of HFS (4 × 100 Hz stimulation blocks, each lasting 2 s, spaced ~10 s apart). We chose the ascending series to avoid possible hysteresis from higher frequency stimulation trains above 20–50 Hz, since stimulation above 20–50 Hz produced IEP potentiation (see RESULTS). In two cases, the order of stimulation train frequency presentation post-HFS was reversed from highest to lowest, and no significant differences in IEP response patterns were seen.

Data were not included for further analysis if the unit being monitored was lost or dropped below a 2:1 signal:noise ratio before completion of the full series of frequency trains. Stimulation currents were usually 100 μA, but if very short latency spikes were observed, currents of 10 μA and below were used to determine thresholds for...
activation. The current density of a 100-μA pulse is \(\sim 0.06 \text{ mA/mm}^2\) at the tip of the electrode, but the current density, 0.6–1.0 mm away, would be \(\sim 0.01 \text{ mA/mm}^2\) (Wu et al. 2001). The current density would exceed the threshold current density for cell somata (\(\sim 0.2 \text{ mA/mm}^2\)) and axons (0.04 \(\text{ mA/mm}^2\)) within 0.3 and 0.5 mm of the tip, respectively (Nowak and Buckler 1999; Ranck Jr. 1975). Therefore, the current density in our study is likely to be below the threshold for directly activating the recorded cells. The equivalent DBS macroelectrode current density is in fact higher, at up to 2.0 mm from the center of contact (Wu et al. 2001). In one additional patient, the GPI DBS electrodes were activated while recording in the STN. Two sites were investigated, with four cells studied in total. Clinically effective stimulation parameters (100 Hz, 4.5 V, 150 μs pulse width, 28 s) and clinically ineffective stimulation parameters (60 Hz, 2.5 V, 150 μs pulse width, 64 s) were tested on the cells. Clinically effective stimulation was determined by an experienced neurologist in the preoperative assessment, and intraoperative monitoring of limb electromyography showed that dystonic contractions were attenuated during application of the higher stimulation intensity.

**Offline analysis of neuronal activity.** The spikes were sorted offline by template-matching software (Spike2, Cambridge Electronic Design). Neurons were accepted for further analysis if they met the following criteria: 1) Spikes were of a consistent, distinct shape that could be separated with a high degree of certainty from the spike waveforms of other neurons and background noise or cardiac pulsations. 2) The unit’s interspike intervals were confirmed to have a minimum duration of 1.5 ms to rule out activity from another unit. Recordings at sites of identified border cells were included in the analysis and showed no difference in their response pattern to stimulation compared with the typical high-frequency discharge cells of the GPi. In this study, the artifacts produced by microstimulation were relatively short lasting (0.6 ms) and were removed from the signal, starting from the onset of the artifact to the end. This dead space was then replaced with an equivalent period of neural data immediately prior to the stimulus artifacts but in a reversed order. This had the advantage of producing a smooth transition in the signal at the site where the artifact was removed. The “cleaned” neuronal recordings were then used for spike sorting with the template-matching algorithm, since spike band-pass filtering without removal of the artifact will increase the duration of the artifacts (Bar-Gad et al. 2004).

**Firing rate and fEP during stimulation.** Changes in GPi firing rate and fEP amplitude were normalized to the baseline pre-HFS amplitude. A further measure of spike inhibition, termed “silent period”, was defined as the time from the beginning of the last stimulus artifact in the train to the time of occurrence of the first spike. The average firing rate during the train was measured for each stimulation frequency and normalized to the baseline firing rate during the prestimulation control period. The results of average fEP amplitude and firing rate were plotted against stimulation frequency before and after HFS.

*Spike train and statistical analysis.* A continuous, complex Morlet wavelet-based transform spectral analysis was used to further characterize the frequency content of fEP during stimulation trains at different frequencies. The spectral power changes for all stimulation frequency combinations were calculated for one site. Spectra were estimated using the continuous wavelet transform, as outlined by Lachaux et al. (1999) and by Le Van Quyen et al. (2001). Records were divided into a number of sections of equal duration, affording frequency resolution of 1 Hz. Morlet wavelet transform is different from the Fourier transform in that the wavelets are localized in time and frequency instead of just frequency. We prefer to use the Morlet wavelet analysis, because the wavelet basis functions have varying windows, and we can balance the resolution at any time and frequency. Therefore, wavelet analysis provides a better representation of the signal at multiresolution analysis.

A custom MATLAB program (MathWorks, Natick, MA) was used to analyze the statistical parameters of the firing rate and pattern during baseline and artifact-removed segments of spike trains during longer 10 or 20 s HFS at 100 Hz with variable intensities. Details of the pattern analysis are described elsewhere (Tang et al. 2007).

**Statistical analysis was performed using SigmaStat software (version 3.00; Systat Software, Chicago, IL).** Changes in normalized firing rate, fEP amplitude, fiber volley amplitude, and silent period in the GPI sites, due to HFS and stimulation frequency, were analyzed by two-way ANOVA with frequency as a repeated measure, followed by Tukey’s post hoc t-tests for all pairwise comparisons. Significance was set at \(P < 0.05\).

**RESULTS**

*Database.* Recordings of well-isolated single units, which met our criteria for location in pallidum, stability, and complete data recorded before, during, and after stimulation of the GP, were obtained at a total of 25 different sites. Of these, 19 neurons were recorded both pre- and post-HFS. Six neurons were lost before post-HFS trains could be applied and were tested with stimulation trains pre-HFS only. The mean baseline firing rate of all neurons before stimulation was 51.2 ± 26.2 (SD) Hz. The average for the six border cells was 45.2 Hz; the five under propofol were 39.9 Hz, and GP neurons not under propofol had an average firing rate of 61.5 Hz. Figure 1A shows the trajectory of a typical electrode penetration through the GPe and GPI. Figure 1B summarizes the number of stimulation and recording sites in each region above the OT. In this study, neuronal response patterns were similar in all sites tested and in patients with various classes of dystonia shown in Table 1. Therefore, the data were pooled together.

*Firing rate and fEP during stimulation.* Our principle analyses focused on the frequency-dependent effects of stimulation on the firing rate of cells and the fEP amplitudes at the same sites. Figure 2 illustrates the change in the firing rate (Fig. 2A) of the neuronal population, plotted with the simultaneously recorded average fEP amplitude (Fig. 2B) at the same GP sites pre-HFS \((n = 25)\) and post-HFS \((n = 19)\) during stimulation trains at different frequencies. During low-frequency stimulation below 5 Hz, there was no change in the average neuronal volley amplitudes were normalized to the baseline pre-HFS amplitude.
firing rate compared with the prestimulus control period. Higher stimulation frequencies above 5 Hz led to significantly lower firing rates ($P < 0.05$) until the cell firing was virtually silenced at 50 and 100 Hz. However, the pre-HFS average fEP amplitude showed a different response from the firing rate and increased significantly at above 10 Hz compared with the 1-Hz baseline ($P < 0.05$) until a maximum was reached at 20 Hz, after which it declined. Note that during stimulation at the highest frequency of 100 Hz, the average fEP amplitude was reduced to only 27% of baseline. Post-HFS, the average neuronal firing rate was decreased significantly compared with baseline but showed the same pattern of decrease and silencing at higher frequencies of 50 and 100 Hz. The average fEP amplitude at 1 and 2 Hz was significantly potentiated following HFS, and no further increase was seen at 20 Hz, after which it declined to a value of 21% of baseline at 100 Hz.

The GP cells showed frequency-dependent decreases in firing rate and were silenced at stimulation frequencies above 50 Hz both pre- and post-HFS. Analysis of firing rate data in Fig. 2A revealed that there was a significant main effect of HFS [$F (1, 335) = 21.066, P < 0.001$] and frequency [$F (7, 335) = 190.137, P < 0.001$] but no significant interaction [$F (7, 335) = 1.903, P = 0.068$]. Significant pairwise comparisons revealed decreased firing rates post-HFS at lower stimulation frequencies (1–20 Hz; $P < 0.05$) and no difference at the higher ones (30–100 Hz).

For the fEP amplitude at the same sites, there was no significant main effect of HFS [$F (1, 335) = 1.052, P = 0.306$] but significant main effect of frequency [$F (7, 335) = 69.736, P < 0.001$] and their interaction [$F (7, 335) = 6.685, P < 0.001$]. Significant pairwise comparisons between pre- and post-HFS revealed an increased average fEP amplitude at 1 and 2 Hz post-HFS and decreased amplitude at 20 and 30 Hz post-HFS ($P < 0.05$).

Potentiation and attenuation of fEP during stimulation. Figure 3A illustrates a rebound bursting-like response after single stimuli of low-frequency stimulation, which was seen in eight of 25 cells. This led to an increase in the firing rate during 2 and 5 Hz stimulation in five of these cases, as illustrated in Fig. 3A.

At low-frequency stimulation, up to 20 Hz, the fEP amplitude remained relatively constant during the stimulation train (Fig. 3B). However, at 30 Hz and above, the fEP amplitude was progressively attenuated during the train, resulting in lower values of average fEP amplitude (Figs. 2 and 3B). Furthermore, Fig. 3B shows that although the fEP amplitudes were strongly attenuated at higher frequencies, the fEP still followed the frequency of each stimulation train, at least for the initial period.

To quantify the temporal evolution of fEP response amplitudes, the population average of the first and last fEP amplitude was determined for each stimulation train (Fig. 4, A and B). ANOVA of the average first fEP amplitudes revealed that there was a main effect of HFS [$F (1, 335) = 11.455, P < 0.001$], indicating that overall, the effect of HFS was to potentiate the first fEP amplitude. There was also a main effect of frequency [$F (7, 335) = 4.717, P < 0.001$] and their interaction [$F (7, 335) = 3.680, P < 0.001$], due to the fact that pre-HFS, a significant increase in the first fEP amplitude compared with 1 Hz baseline was seen with stimulation frequencies above 30 Hz (Fig. 4A; $P < 0.05$), after which it appeared that the potentiation saturated. Post hoc pairwise comparison $t$-tests between pre- and post-HFS revealed significant increases in the post-HFS first fEP amplitude at stimulation frequencies of 1, 2, and 5 Hz ($P < 0.05$).

The population average of the last fEP amplitude in the trains pre- and post-HFS is shown in Fig. 4B. Similar to the average fEP amplitude plots shown in Fig. 2, there was a significant frequency-dependent decrease in fEP amplitude compared with 1 Hz baseline at stimulation frequencies above 50 Hz in both pre- and post-HFS ($P < 0.05$). There was no main effect of HFS [$F (1, 319) = 0.136, P = 0.712$] but a significant main effect of frequency [$F (7, 319) = 42.808, P < 0.001$] and their interaction [$F (7, 319) = 4.824, P < 0.001$], as fEP amplitude is greatly attenuated within short stimulation trains at frequencies above 30 Hz. Post hoc pairwise comparisons between pre- and post-HFS revealed an increased last fEP amplitude post-HFS at 1 and 2 Hz and decreased amplitude at 20 and 30 Hz post-HFS ($P < 0.05$).

Inhibition of cell firing after the end of the train: silent period. We measured the duration of inhibition in cell firing after the end of the stimulation trains (Fig. 4C), similar to that reported in our recent study (Lafreniere-Roula et al. 2010). The average silent period was $\sim 0.05$ s for frequencies up to 10 Hz and then...
increased significantly at 50 and 100 Hz, as revealed by the pairwise post hoc t-test ($P < 0.001$). Preceding HFS increased the average silent period at all stimulation frequencies. There were main effects of HFS [$F_{(1, 319)} = 4.233, P = 0.040$] and frequency [$F_{(7, 319)} = 22.011, P < 0.001$] but no interaction effect [$F_{(7, 319)} = 0.168, P = 0.991$], and post hoc tests did not reveal significant increases at specific frequencies.

**HFS drives GPi fiber volleys.** In a subset of sites in GPi (11/25), a clear fiber volley was present, which was analyzed in a similar way to the synaptic component of the fEP. Unlike the averaged firing rate of the cells and fEP amplitude, which decreased rapidly during HFS, fiber volley amplitudes were reduced only slightly from baseline between 2 and 30 Hz and only started to drop at 50 Hz, and significant attenuation occurred only at 100 Hz compared with 1 Hz baseline (60.3% of baseline; Fig. 4D; $P < 0.001$). Furthermore, fiber volley amplitudes were not altered post-HFS, except at 1 Hz stimulation, where the post-HFS amplitude was reduced to ~80% of pre-HFS ($P < 0.001$; post hoc pairwise comparison). There was a significant main effect of HFS [$F_{(1, 144)} = 13.210, P < 0.001$] and main effect of frequency [$F_{(7, 144)} = 7.916, P < 0.001$] but not their interaction [$F_{(7, 144)} = 1.061, P = 0.392$]. Preceding HFS produced a small transient effect of 20%, discernible only at 1 Hz, possibly due to transient accumulation of extracellular potassium (Shin and Carlen 2008; Shin et al. 2007). When this transient effect was removed from the data set and the ANOVA repeated, there was no significant main effect of HFS [$F_{(6, 108)} = 3.38, P = 0.069$].

**Antidromic-like driving of spikes.** In two cells in GPi and two border cells (16% of cells), we observed very short latency spikes after each stimulus in the train (latencies ranged from 0.1 to 1.2 ms). These spikes reliably followed 100 Hz stimulation with a fixed latency (Fig. 5, A and B) and had clear, all-or-none thresholds in a very narrow range of 8–9 μA.
suggests orthodromic activation. The antidromic-like activation. Follow a 30-Hz stimulation train and only fired after the 1st stimulus. Indicate the position of stimulus artifacts). Short, fixed latency spikes followed the 100-Hz stimulation train, as shown in the bottom trace (gray area is stimulus train), and also suggest antidromic-like activation. The bottom trace shows that the spike did not follow a 30-Hz stimulation train and only fired after the 1st stimulus. Furthermore, offline analysis of the recordings for two cells revealed that when spontaneous spikes occurred immediately prior to the stimuli, the stimulus failed to evoke a spike (Fig. 5A). This is consistent with the collision test for antidromicity, as the absolute refractory period of the spikes propagating in opposite directions leads to their cancellation (Bishop et al. 1962). Since the analysis was performed offline, and we did not attempt more rigorous tests of antidromicity (Fuller and Schlag 1976), we termed these effects “antidromic-like”. Importantly, however, evidence for collision was nevertheless confirmed in two cells, where in every instance, in which there was a spontaneously occurring spike within the collision interval, collision occurred. In contrast to these antidromic-like responses, we also found, in two cases, longer latency (~4.7 ms) excitations with jitter and failure with HFS, one of which is illustrated in Fig. 5C. These responses are most likely due to monosynaptic driving of spikes.

Effects of longer, 100 Hz stimulation on neuronal firing. To further investigate the extent of fEP attenuation at 100 Hz stimulation and its possible effects on cell firing, we applied longer, 100 Hz stimulation trains. In 14 out of 18 cells tested with a longer 10- or 20-s train, we observed the usual, initial inhibition of firing, followed by a return in neuronal firing. Two examples are shown in Fig. 6, A and B, which shows a cell that was silenced for 3.2 s after the start of the train but then returned to firing at a level similar to the baseline firing rate (no significant difference for t-test). Similar results were found for the other 13 cells, in which the return of firing ranged from 0.5 to 10.6 s. The other four cells were inhibited throughout the 20-s train, and the return of firing only occurred 0.8–2.8 s after the end of stimulation. In four of the cases where firing returned during the train, a second train of higher stimulation intensity was given and produced a more prolonged suppression of firing or complete silencing during the whole train. Two examples are shown in Fig. 6, C and D, illustrates the response of a GPi neuron with phase-locked activity during the stimulation train. The overlay traces in Fig. 6C show that the neuron fired at a preferred time after each stimulus pulse in the train with a mean latency ± SD of 4.6 ± 1.6 ms. There was a second time interval of 6.5–7.5 ms, where the neuron tended to fire, but overall, the phase-locking was partial, in that the neuron did not always respond with a spike at these times during the excitation period.

Stimulation with DBS electrodes. In one patient with DYT1 dystonia, which was undergoing implantation of a second set of DBS electrodes bilaterally in STN, we made dual recordings in STN and activated the GPi electrodes bilaterally with clinically effective and clinically ineffective stimulation parameters (Lozano et al. 1997). In all four cells, the lower stimulation intensity had no significant effect on STN firing (96.4 ± 11.6% of baseline; P = 0.514), but at the higher stimulation intensity, which produced some clinical benefit for the patient, the cells’ firing rate was inhibited to 1.4 ± 1.0% of baseline during the whole train (P < 0.05). An example is shown in Fig. 7, A and B.

**DISCUSSION**

The use of dual microelectrodes to microstimulate with one and record from the other allowed assessment of the frequency dependence, as well as the effects of conditioning HFS trains on local somatic spikes, synaptic potentials, and fiber volleys in the human GP. The key findings support several therapeutic
mechanisms of GPi DBS. Consistent with earlier reports (Dostrovsky et al. 2000; Wu et al. 2001), we found that short HFS trains in the GPi can produce an inhibitory effect on local neuronal spiking. We now report that long trains produced synaptic fatigue or depression at frequencies above 50 Hz, but fiber volleys followed each stimulus pulse up to 100 Hz, as observed in cortical slices by Urbano et al. (2002) and Iremonger et al. (2006). We also observed ortho- and antidromic-like driving of action potentials, as well as rebound bursting and short-term plasticity of inhibitory synaptic components. It has been suggested that rebound bursting might play a role in synaptic plasticity (Aizenman and Linden 1999; McElvain et al. 2010; Molineux et al. 2006); however, we observed evidence of synaptic plasticity at all sites, including the sites without bursting.

Potential limitations. One major and important limitation of this study is that effects of HFS could only be examined for short-duration trains, lasting 10s of seconds, compared with...
durations of days and longer in clinical applications. Although some of the clinical effects of DBS are already apparent after such short trains, others may take much longer, especially when considering GPi stimulation for dystonia. Clearly, the findings of this study cannot directly explain the long latency effects of DBS, although they may well be involved in an initial step(s), which leads over time to long onset plasticity. Thus it is important to determine and understand what are the short-term effects of stimulation, as they may be responsible not only for mediating short-onset DBS effects but also be important in initiating and maintaining long-term changes. Another possible limitation is that the microstimulation electrode tip is much smaller than the DBS electrode contacts, meaning that even with the lower stimulus intensities used, the charge density near the tip of the microelectrode is higher than the current densities near the tip of the DBS contacts. However, the charge density at our recording distance is comparable with the charge density produced clinically from DBS electrodes (Wu et al. 2001), and thus our findings should be applicable to understanding the mechanisms that might be involved in DBS stimulation. Limitations compared with studies in animal models are the inability to use various drugs to help elucidate the mechanisms and the fact that the patients in our study were not homogenous in terms of their underlying pathophysiology (Table 1), although it is not clear to what extent this heterogeneity might affect the mechanisms responsible for the therapeutic effects of the DBS. On the other hand, these studies have the advantage over animal studies in that it is not known how well the animal models correspond to the human conditions, and furthermore, we know that the HFS stimulation is therapeutically effective in these patients.

Inhibition of neuronal firing associated with enhanced fEP amplitudes. Since we have found propofol at anesthetic doses to inhibit GPi neurons (Hutchison et al. 2003), the low mean baseline GP firing rate of 51.2 Hz reported here, compared with our previous studies [71.4 Hz in Tang et al. (2007)], is likely due to the inclusion of five cells studied under propofol (44.3 Hz) but also the six border cells (45.2 Hz) and two cells with firing rates, 18–22 Hz, which were likely GPe neurons of the type LFB. With stimulation frequencies from 1 Hz up to 30 Hz, the average firing rate decreased, whereas the fEP amplitudes progressively increased pre-HFS, which supports the involvement of presynaptic GABA release. The inhibition of spike firing rate is consistent with earlier studies from our group (Dostrovsky et al. 2000; Wu et al. 2001) and block of the inhibitory effect with GABA_A receptor antagonist, bicuculline methiodide, as well as synaptic blocker cobalt chloride, in rats (Chin and Hutchison 2008). The increase in size of the fEP with increasing stimulation frequency peaking at 20–30 Hz suggests that there was a maximal amount of GABA in the synapse in this range, perhaps due to saturation of re-uptake from the synapse. Since we occasionally saw rebound bursting at low frequencies, which sometimes increased average firing rates, another possible contribution might be progressive membrane depolarization from an excitatory transmitter released at lower frequencies and resultant increase in the driving force for the inward chloride flux mediating the field IPSP (Eccles 1964). With stimulation frequencies above 30 Hz, a significant decrease in fEP amplitude was observed at all sites during the stimulation trains. This is likely due to synaptic fatigue, since the conduction through GPi fibers was not decreased significantly at frequencies below 100 Hz and even at 100 Hz, was much less decreased than the fEP. In hippocampal in vitro preparations, GABAergic IPSPs are “labile” and attenuate after a period of repetitive stimulation due to reduction of driving force (Huguenard and Alger 1986; McCarren and Alger 1985) and decrease in synaptic release (Thompson and Gahwiler 1989). These results suggest that the short inhibition may be due to an acute release of GABA, and the return in firing may be due to depletion of GABA. However, it remains to be determined whether prolonged inhibition during long trains involves a continuous GABA release, which sustains a postsynaptic inhibition.

At frequencies of >50 Hz, the neuronal firing was silenced, but the average fEP was still present, albeit at a much reduced amplitude, at least during the first 0.5–1 s. This is consistent with our recent findings of maximal post-HFS inhibition of firing (here termed silent period) of 1–2 s and decreased inhibition with increasing, longer train lengths (Lafreniere-Roula et al. 2010). Synaptic fatigue would lead to both low-field amplitudes and loss of further decreased inhibition of spiking with increasing, longer train lengths. During long train stimulation of 10–20 s, most of the cells returned to firing after 0.5–10.6 s, and during this latter period, the fEP amplitude was reduced further. We did not examine the response after many minutes of continuous HFS, but it is likely that the fEP amplitudes would remain at a minimal level. These results are similar to those of Urbano et al. (2002), who used high-resolution calcium imaging and field potential recordings to examine effects of stimulation of thalamocortical fibers on cortical activity. They found a similar sigmoid-shaped, frequency-response curve with marked reduction of synaptic activity over 60 Hz, similar to our firing-rate curve. In addition, microdialysis studies measuring the GABA release/unit time in patients during continuous HFS have found a high initial release of ~1 μM GABA over 10 min, which is followed by a continuous, stable GABA release of ~0.3 μM in the fractions after 30 min of DBS (Kilpatrick et al. 2010). This finding is limited to the temporal resolution of ~10 min collection intervals but suggests that even if some synapses were depressed at the site of stimulation, the efferent fibers surrounding this region, driven by the high frequency, might sustain a postsynaptic inhibition of downstream targets via continuous GABA release. A sustained, regular firing at 100 Hz is physiological for basal ganglia output neurons.

Frequency-dependent potentiation of fEP and silent period shift. The fEP amplitude and silent period in spike firing were both potentiated by HFS. These responses suggest the involvement of short-term synaptic plasticity at inhibitory synapses in human GP. In slice experiments, inhibitory GABAergic synaptic plasticity has been demonstrated in the rat GP (Hanson and Jaeger 2002; Sims et al. 2008), which is the homologue of primate GPe. Sims et al. (2008) showed that the synaptic plasticity at GABAergic synapses was characteristic only of the striatopallidal pathway and not present on the axonal terminals from other GP neurons. Primate GPe differs from rat GP and primate GPe in not having significant, recurrent collaterals (Hazarati and Parent 1992). Also, the GPe-to-GPI projection is sparse and predominately located on the soma, whereas the striatopallidal direct pathway projections are much more numerous but with a tendency to be located on the dendrites (Shink and Smith 1995; Smith et al. 1994).
anatomical features support the notion that the inhibitory synaptic plasticity reported here is predominantly in the direct striatopallidal pathway.

Excitation of Gpi fibers and neurons. We frequently observed a short latency-evoked potential, which followed high stimulation rates. We interpreted the early latency component to be due to a fiber volley, which could include afferent striatopallidal and pallidopallidal fibers; efferent pallidothalamic, pallidohabenular, and pallidolimbic fibers; as well as fibers of passage from GPe to STN (Hazard and Parent 1992; Smith et al. 1994). Excitation of GABAergic afferents, such as those from striatum (~70%) and GPe (~15%), would inhibit Gpi neurons, as has been observed consistently in our previous studies and those of others (Anderson et al. 2003; Moran et al. 2011; Rosin et al. 2011). As reported in this study, we found STN neurons to be powerfully inhibited by clinically relevant Gpi DBS stimulation. This is likely due to driving of fibers of passage from GPe to STN, which pass through Gpi, as predicted by modeling studies (Hahn and McIntyre 2010). The excitation of glutamatergic afferents from STN (~10%) might be responsible for the synaptic driving effect, which was seen occasionally.

We observed cases of neurons faithfully following HFS at short latencies, low-stimulation intensities, and low jitter and manifesting collisions with preceding spontaneous activity, indicative of antidromic activations (Fig. 5A). At these lower intensities, the cells were not silenced completely, and spontaneous spikes observed immediately before the stimulus artifacts allowed us to assess collision-like phenomena with the antidromic spike. Antidromic-like direct driving has also been observed in monkey Gpi recordings in vivo (McCairn and Turner 2009). Since the distance between electrodes was ~1 mm, and the latency response was 0.1–1.2 ms, the propagation velocity of the antidromic-like spikes is 1–10 m/s, and these are thus likely mediated by small diameter-myelinated Gpi axons. The longer latency (4.1 ms; after subtracting synaptic delay of 0.5 ms) orthodromically activated spikes during the long train, 100-Hz, stimulation have a velocity of ~0.2 m/s, which is reasonable for slowly conducting unmyelinated axons of STN neurons (Kita and Kitai 1987). The short latency responses in Gpi further confirm that stimulation can bring about focal inhibition of the neuronal spiking while exciting axonal fibers. Direct and antidromic driving includes aspects of the local effects of DBS, predicted from basic knowledge about the effects of electrical stimulation on neuronal tissue (McIntyre et al. 2004; Ranck Jr. 1975), and has also been reported in human thalamus (Montgomery Jr. 2006) and rat cortex (Li et al. 2007). The significance of antidromic responses to Gpi DBS efficacy is difficult to assess from our study; however, the important significance of the findings is that in addition to the antidromic spike, there would have been an orthodromic spike evoked, which would have propagated to the target nucleus (e.g., thalamus).

Our results showing robust inhibition of Gpi neurons are different from those of Carlson et al. (2010), who found only very brief (1 ms), short latency (6 ms) inhibition of STN neurons when stimulating 1.4 mm away with clinical DBS macroelectrodes and therapeutic stimulation parameters. This difference may have been due to stimulation of STN, which has fewer GABAergic inputs and therefore, both a higher threshold for inhibition and also fewer neurons that respond with inhibition to microstimulation than Gpi (Filali et al. 2004; LaFreniere-Roula et al. 2010). The use of microstimulation might also explain the similarity of our results with the monkey Gpi study of Bar-Gad et al. (2004), who also used microstimulation, but differences with the findings of McCairn and Turner (2009), who used macrostimulation electrodes.

Functional implications. Our study demonstrates inhibition of neuronal firing of GP neurons, driving of fiber volleys and antidromic-like spikes, as well as a return in spontaneous firing with longer trains. Importantly, the findings also show that with longer duration stimulation, even though the spontaneous firing may recover, the FEP remains depressed, indicating that there would be a loss of transmission of information (including pathological signals) to GPi neurons. We also present evidence that therapeutically effective Gpi stimulation is associated with inhibition of neurons in the STN, which is not predicted from rate models of basal ganglia function. The data support the notion that Gpi DBS has complex and varied mechanisms, including increased long-term inhibitory synaptic efficacy.

Previous studies using microelectrodes to infer DBS mechanisms (Erez et al. 2009) have noted that a DBS electrode would stimulate a much larger population of neurons than a microelectrode, since it occupies a much larger volume. The result would be a regular synchronized, high-frequency output to premotor structures in brain stem and thalamus, which lacks any “pattern-sculpting” by basal ganglia input structures. Given that pauses in GP neuron firing are thought to be related to dystonia (Hutchison et al. 2003; Sani et al. 2009), the elimination of pathological inhibitory phasic movement-related signals may help to restore more “normal” voluntary movements and the function of reciprocal inhibition to eliminate overflow. These spatial/volume constraints may explain why microstimulation is usually ineffective, whereas macrostimulation is effective on the target premotor structures. Low basal firing rates in Gpi, purported as “the cause” of dystonia (Nambu et al. 2011), may only serve to prolong pauses, producing an excessive sculpting of Gpi output.

In conclusion, the findings of this study indicate that a major effect of stimulation in Gpi is activation of axons, which produce inhibition of firing and local fEPs, most likely due to release of GABA from terminals on Gpi neurons. However, we have also shown that stimulation activates the axons of the Gpi neurons, as revealed by antidromic activation. The postsynaptically mediated excitations, which we observed in some cases, are probably due to activation of glutamatergic axons from STN, and the recordings in STN during DBS in Gpi revealed robust inhibition of STN neurons, probably due to excitation of GABAergic axons of passage from GPe to STN. Taken together, these findings suggest that during HF DBS in Gpi, the transmission of information through the nucleus is blocked or reduced greatly, due to transmitter depletion and/or receptor desensitization, and the output is replaced by intrinsic activity and stimulation-evoked activity in the axons. Also, activation of GPe-to- STN axons during Gpi must be considered as an additional mechanism.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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