Deep brain stimulation of the globus pallidus internus in the parkinsonian primate: Local entrainment and suppression of low frequency oscillations.

Kevin W McCairn2,3 and Robert S. Turner1,2

1 Departments of Neurobiology and Bioengineering and The Center for the Neural Basis of Cognition, University of Pittsburgh, Pittsburgh, Pennsylvania 15261
2 Department of Neurological Surgery, UCSF Box 0520, San Francisco, California 94143
3 The Open University, Milton Keynes, Dept of Biological Sciences, MK7 6AA, UK

Running Title: Local effects of Gpi DBS

Address correspondence to:
Robert S Turner, Ph.D.
Department of Neurobiology
University of Pittsburgh
4047 BST-3, 3501 Fifth Avenue
Pittsburgh, PA 15261
Phone: 412-383-5395
Fax: 412-383-9061
e-mail: rturner@pitt.edu

| Abstract | 245 words |
| Figures | 11 (plus 2 supplementary figures) |
| Tables | 3 |
ABSTRACT

Competing theories seek to account for the therapeutic effects of high-frequency deep brain stimulation (DBS) of the internal globus pallidus (GPi) for medically-intractable Parkinson’s disease. To investigate this question, we studied the spontaneous activity of 102 pallidal neurons during GPiDBS in two macaques rendered parkinsonian by administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Stimulation through macroelectrodes in the GPi (≥200-µA at 150-Hz for 30-s) reduced rigidity in one animal and increased spontaneous movement in both. Novel artifact subtraction methods allowed uninterrupted single-unit recording during stimulation. GPiDBS induced phasic (78% of cells) or sustained (22%) peristimulus changes in firing in both pallidal segments. A subset of cells responded at short latency (<2-ms) in a manner consistent with antidromic driving. Later phasic increases clustered at 3-5ms latency. Stimulation-induced decreases were either phasic, clustered at 1–3-ms, or sustained, showing no peristimulus modulation. Response latency and magnitude often evolved over 30-s of stimulation, but responses were relatively stable by the end of that time. GPiDBS reduced mean firing rates modestly and only in GPi (−6.9-sp/s). Surprisingly, GPiDBS had no net effect on the prevalence or structure of burst firing. GPiDBS did reduce the prevalence of synchronized low-frequency oscillations. Some cell pairs became synchronized instead at the frequency of stimulation. Suppression of low-frequency oscillations did not require high-frequency synchronization, however, or even the presence of a significant peristimulus response. In summary, the therapeutic effects of GPiDBS may be mediated by an abolition of low-frequency synchronized oscillations as a result of phasic driving.
Deep brain stimulation (DBS) is a well-recognized treatment option for a range of neurological disorders (Agid 1999; Benabid et al. 1987; Cooper et al. 1980; Perlmutter and Mink 2006; Starr et al. 1998). Medically-intractable Parkinson’s disease (PD), dystonia, essential tremor, and neuropsychiatric disorders (Houeto et al. 2005; Mayberg et al. 2005) are candidates for DBS. Most cardinal signs of PD are ameliorated by DBS of the subthalamic nucleus (STN) or globus pallidus internus (GPi) (Pollak et al. 1993; Rodriguez-Oroz et al. 2005). DBS in GPi (GPiDBS) has similar therapeutic efficacy to that of the more commonly-targeted STN (Rodriguez-Oroz et al. 2005; Vitek 2002a).

Despite the general consensus that DBS for PD works by suppressing pathologic neuronal activity that is a hallmark of the disease, the local effects that mediate this suppression remain a topic of debate. Similarities to the clinical effects of ablative procedures led many to infer that DBS works by inactivating nearby neurons (i.e., the "inactivation/suppression hypothesis," Benabid et al. 1991; Benazzouz and Hallett 2000). Indeed, several groups have reported that DBS suppresses impulse generation in neuronal somata within the stimulated nucleus (Benazzouz et al. 1995; Boraud et al. 1996; Dostrovsky et al. 2000; Meissner et al. 2005; Shi et al. 2006; Wu et al. 2001). Other results suggest, however, that DBS excites local somata and axons (Anderson et al. 2003; Bar-Gad et al. 2004; Garcia et al. 2003; Hashimoto et al. 2003; Montgomery and Gale 2008), thereby implying that DBS replaces pathological activity with spiking time-locked to the stimulation frequency (Galati et al. 2006; Stefani et al. 2005). Therefore, research has focused more intensely on discovering the specific effect(s) of DBS on neuronal firing that mediate clinical benefit.
Abnormalities in GPi activity observed in the parkinsonian state include increased mean firing rates, an increased propensity to fire action potentials in bursts, and low frequency oscillatory (LFO, <40 Hz) activity synchronized across neurons (Filion and Tremblay 1991; Miller and DeLong 1987; Raz et al. 2000; reviewed in Utter and Basso 2008). A causal relationship between any of these firing abnormalities and parkinsonian signs has been brought into question by recent studies however (Boraud et al. 1998; Wojtecki et al. 2006). We focused on DBS-induced changes in neuronal activity that were present after 20 s of continuous stimulation because effects during this period were most relevant to the slow timecourse of some of the therapeutic effects of DBS (Temperli et al. 2003, though minutes/hours of continuous stimulation may well reveal additional stimulation effects). Effects in GPi neurons were of greatest interest, given that these neurons form the principle output pathway for the BG motor circuit. Effects in GPe provided information about potential pathways mediating the local effects of GPiDBS.

A major impediment to studying local neuronal effects of DBS has been the presence of large stimulation-induced electrical artifacts, which can occlude neuronal recording for significant fractions of the short (6–8 ms) inter-stimulus intervals. Previous studies used artifact subtraction methods that shortened the occlusion period (Bar-Gad et al. 2004; Hashimoto et al. 2003; Meissner et al. 2005; Montgomery et al. 2005; Shi et al. 2006), but those reports acknowledged that short latency effects of stimulation were obscured by artifact-induced saturation of the recording system. Here we use several methodological refinements that allowed verifiably-continuous neuronal recording within millimeters of the site of GPiDBS during clinically-relevant stimulation.
METHODS

Two mature female (3 kg & 4 kg) cynomolgus macaques (*M. fascicularis*) were used for this study. Both animals were used previously for experiments investigating cortical stimulation (Wu et al. 2007). All aspects of animal use were approved by the UCSF Animal Care and Use Committee and adhered to National Institutes of Health guidelines for the care and use of animals.

*Surgery*

After an animal was acclimatized to the recording environment, the animal underwent aseptic surgery under Isoflurane anesthesia following ketamine induction. A cylindrical titanium recording chamber (19 mm ID) was implanted over a craniotomy to allow access to the right GP via a coronal approach (Szabo and Cowan 1984) (35° from vertical; Horsley-Clark anterior 15 mm, lateral 13 mm, depth 18 mm). The chamber and head fixation hardware were fixed to the skull using bone screws and methyl-methacrylate cement. Prophylactic antibiotics and analgesics were administered post-surgically.

*MPTP administration*

Experimental parkinsonism was induced using the overlesioned hemi-parkinsonian model (Oiwa et al. 2003). Animals received initial infusions of MPTP via the right intracarotid artery (0.55 mg/kg) followed by variable numbers of systemic doses (0.4 mg/kg IV, 0.35–0.75 mg/kg, IM) over the course of several weeks. Symptom severity was rated regularly according to a scale developed by Schneider et al. (2003). Both animals showed stable severe parkinsonian symptoms during the period of recording reported here (Schneider rating averages = 37, 38).
When necessary during the acute phase of MPTP intoxication, animals were maintained by nasogastric feeding and subcutaneous fluids.

*Indwelling HF-DBS macroelectrode*

The methods used to target and implant stimulating electrodes are described in detail elsewhere (Turner and DeLong 2000). Briefly, we used standard microelectrode mapping to identify the chamber coordinates for electrode placement. A custom electrode was assembled from two Teflon-coated Pt-Ir microwires (50 $\mu$m diameter) glued inside a 5 mm length of 30 ga stainless steel cannula. The cut ends of the microwires extended below the cannula tip by 0.8 and 1.5 mm. Insulation was stripped from $\sim$0.5 mm of the distal ends of each microwire (surface areas $\sim$0.08 mm$^2$, impedance 5-10 kOhm at 1 kHz, $\sim$0.2 mm separation between contacts). The electrode assembly was implanted transdurally via the chronic recording chamber using a protective guide cannula (28 ga ID) and stylus mounted in the microdrive. During implantation, the location of the electrode tip was confirmed using multiunit activity recorded from the distal electrode contact. On reaching the target location for implantation, the guide tube and stylus were withdrawn, and the electrode assembly was left floating in the brain with only the proximal ends of the microwires exiting the dura. The proximal ends were led through a port in the side of the recording chamber (subsequently sealed with cyanoacrylate glue) and soldered to a head-mounted connector.

*Data acquisition and artifact subtraction*

The extracellular activity of multiple single neurons in the globus pallidus was sampled using a 4-electrode microdrive and glass-insulated tungsten microelectrodes (0.5-1.0 MOhm impedance; MT, Alpha Omega Engineering, Nazareth, Israel). Data were passed through a low-gain
headstage (gain = 4×, 2 Hz to 7.5 kHz bandpass) and then digitized at 24 kHz (16 bit resolution, Tucker Davis Technologies, Alachua, FL). The digitized signals were filtered (300 Hz – 6 kHz; First order Butterworth) and saved to disk either as continuous data or as 38 sample-long snippets of the continuous data stream aligned on experimenter-adjusted threshold crossings. The acquired neuronal data were monitored continuously during acquisition both by computer display and by digital-to-analog conversion and playback on a digital oscilloscope and audio monitor.

During periods of GPiDBS, online signal processing (Tucker Davis Technologies, Alachua, FL, USA) was used to eliminate the large stimulation-induced electrical artifacts in the neuronal recordings (Fig. 1A). We used a method similar to the template subtraction algorithms described previously (Bar-Gad et al. 2004; Hashimoto et al. 2002; Montgomery et al. 2005; Wichmann 2000). Our method differed from previous implementations in the following ways: (1) The timing of stimulus delivery, analog-to-digital conversion, and artifact subtraction were all synchronized to the same microsecond-accurate clock, thereby eliminating shock-to-shock variation in the shape of the artifact caused by temporal jitter. (2) The low gain of the headstage and preamplifier and 16-bit A/D conversion prevented artifact-induced saturation of the analog electronics. (3) The artifact "template" (which was subtracted from the data stream at the time of any one shock) was computed as a 6.1 ms-long moving average of the previous 100 shocks. Thus, the template was allowed to change over time in response to gradual changes in artifact shape (e.g., due to changes in an animal's posture). (4) The algorithm was applied independently and in parallel to up to four channels of neuronal data. And (5) the algorithm was applied in real-time during acquisition so that its efficacy could be monitored. Valid operation of the subtraction method was readily apparent during data collection in that stimulation-related voltage
transients were absent from the signals played back on oscilloscope and audio monitors. Off-line analyses were used to verify that stimulation-related voltage transients were not included in the action potentials of sorted spikes and that the subtraction method did not distort the shapes of spike waveforms (see below for details). The basic efficacy of the artifact subtraction algorithm is illustrated in Fig. 1A. Note that the recording arrangement in Fig. 1A, in which the same signal was acquired on two channels in parallel, with and without artifact subtraction (Fig. 1Ai and Aii, respectively), was used only to illustrate the method. During the collection of actual datasets, artifact subtraction was always applied to all microelectrode channels. Fig. 4 illustrates the efficacy of artifact rejection during collection of a typical dataset.

Recording and stimulation protocol

An animal was seated in a primate chair and placed in a sound attenuating booth to which it had been acclimatized. One or more microelectrodes were lowered transdurally into the pallidum. Recording tracks were always placed >0.5 mm away from the indwelling stimulating electrode so as to avoid mechanical collisions between recording and stimulating electrodes. As the recording electrodes were lowered into the basal ganglia (BG), neurons of the globus pallidus were identified by their characteristic high mean firing rates and short duration action potentials (DeLong 1971; Turner and Anderson 1997). As soon as the action potentials of one or more single neuron was isolated on the recording electrode(s), high frequency stimulation was delivered for <5 s to train artifact templates into the artifact rejection system. Stimulation was delivered using a isolated constant current stimulator (Model 2100, A-M Systems, Carlsborg, WA) with symmetric biphasic pulses (cathodal followed by anodal) 200 µs in duration delivered at 150 Hz. (Only one stimulation frequency was used in the results reported here.) Data were then collected during an initial one minute off-stimulation (“baseline” or “control”) period
followed by multiple 30 s blocks of stimulation delivered approximately once every 70 s. Our goal was to collect data from a set of neurons during five such stimulation blocks, but data collection was halted early if unit isolation deteriorated. Stimulation currents ranged from 200 µA to 1000 µA (mean current 400 µA, SD =120 µA). Charge densities were approximately 100 µC/cm²/phase, which fell within the range for safe stimulation (as discussed in McCreery et al. 1990; Testerman et al. 2006). The distribution of currents used over time in the two animals is plotted in Supplementary Figure 13A. The current used for each recording session was determined primarily by whether artifact-free neuronal recording was possible at that current. Currents never exceeded 0.8× the threshold current for short latency corticospinal-like muscle activation (determined by direct observation of muscle contraction in response to 10 biphasic pulses at 300 Hz). The activity of one set of neurons was studied at only one stimulation current.

Animals remained seated and at rest throughout each recording session. Animals occasionally changed posture or moved their limbs during a recording session, but such movements were relatively infrequent. Animals were observed continuously during recording sessions, but no formal record was kept of those observations.

Off-line analysis of neuronal activity

The action potentials of individual neurons were sorted off-line by drawing contours around waveform clusters in principal components space (Offline Sorter, Plexon Inc., Plano, TX). Neurons were accepted for further analysis if they met the following criteria: (1) The recording was from a location within the globus pallidus. Locations were inferred from microelectrode mapping of nuclear boundaries and alignment of those boundaries with known neuroanatomy (Szabo and Cowan 1984). (2) A unit's action potentials 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. (3) The unit's interspike intervals were confirmed to have a minimum refractory period of 1.5 milliseconds. (4) Stable neuronal data were available from the unit for at least 30 seconds under the control “no stimulation” condition and at least one complete 30 second period of stimulation. Recording stability was judged by inspection of spike density functions [500 ms sigma; see (Szucs 1998) for method]. Sections of a recording session were rejected from further analysis if they showed an abrupt change in rate not attributable to the onset of GPiDBS (Elias et al. 2007). The longest continuous period of recording with stable firing rate was selected for analysis. (5) We found no evidence that single unit isolation was corrupted by shock artifacts. We tested for artifact-related corruption or distortion by determining if the shapes of action potentials during stimulation deviated significantly from the mean shape of action potentials recorded during non-stimulation periods (Fig. 1Bi). A “control mean” and 95% confidence interval (CI) for action potential shape was computed from all of the action potentials that occurred during non-stimulation periods. Action potentials from periods of stimulation were collected into one of four test means according to the timing of the spikes relative to the time of stimulation (i.e., test means collected spikes from four non-overlapping 1.67 ms-long bins across the 6.67 msec long interstimulus interval; horizontal brackets Fig. 1Bi). If any part of any of the four test means deviated outside of the 95% CI of the control mean, then artifact rejection was deemed incomplete and the neuron was rejected. We applied two exceptions from this rule. One was if spike waveforms were altered uniformly across the four interstimulus interval (e.g., if all four test means increased or decreased in size equally ). This exception allowed the inclusion of neurons that showed long-lasting stimulation-induced changes in action potential size, similar to those reported previously (Hashimoto et al. 2003). The second exception was if an inhibitory
effect of GPiDBS was so profound as to prevent all spiking activity during one or more peristimulus interval. In this case, we tested for consistent waveform shape in the peristimulus intervals that did contain spikes. In three cases, so few action potentials were emitted during GPiDBS, it was not possible to perform valid statistical tests for consistent waveform shape. Nonetheless, even in these cases, the few spikes detected during stimulation fell within the 95% CI of the control mean.

The primary tests for effects of DBS on a neuron’s firing were performed on a peristimulus change histogram (PStH) constructed from the last 10 seconds of all valid 30 second-long stimulation blocks (bin size=0.2 msec, 35 bins; Fig. 5Cv.). In this way, we focused on the relatively long-lasting effects of DBS that were present after at least 20 seconds of stimulation. Matching control histograms ("peri-control change histograms", PCtHs) were constructed from 10 second periods during no-stimulation periods (Fig. 5Ci.-ii.). PCtHs were constructed around a series of “sham stimulation” time points that were aligned at 6.67 ms intervals (e.g., at 150 Hz) arbitrarily set across control periods. The 10 second period immediately following the offset of a stimulation block was excluded from PCtHs so as to exclude any slow post-stimulation changes from the PCtHs (Bar-Gad et al. 2004). A neuron's baseline “control” firing rate was defined as the grand mean across all PCTHs. This baseline was subtracted from all histograms (PCtHs and PStHs) so that histograms reflected stimulation- (and sham-) induced changes from baseline firing rates. (Baseline rates are summarized in Table 2.) Areas of deviations from baseline firing were used as the fundamental statistic for tests of significance. The area statistic allowed small-magnitude long-duration effects to hold equal significance with large-magnitude short-duration effects (Fig. 5D). This statistic was particularly useful for detecting decreases in firing, which, due to floor effects, often went undetected using
other measures of response magnitude. Deviations from baseline firing rate (i.e., transient increases or decreases in firing) were detected in PSThS and the areas of those deviations were converted to z-scores relative to the population of control deviation areas (i.e., the areas of all deviations in all PCtHS for a neuron). The threshold for significance was adjusted to compensate for multiple comparisons [alpha=0.05/(mean number of deviations detected per PCtH)].

Significant phasic responses were examined for changes in timing and magnitude across the 30 s blocks of stimulation. These analyses were applied to phasic increases and decreases in firing that were significant according to the primary test for effects of DBS. Thirty mean peristimulus histograms were computed (bin size=0.2 msec, 35 bins), one histogram for each 1 s section of the mean stimulation block. The timing of response peaks (i.e., maxima for peristimulus increases and minima for decreases) were determined in each histogram. The 30 timing points for each response were then modeled both as linear and piece-wise linear functions according to least-squares regression. The piece-wise linear function allowed an initial linear slope up to a discrete break-point, after which response timing remained constant (see Fig. 8A for example). A response was considered to have a significant shift if the slope of the linear regression was significant at $P<0.01$ and the total shift across 30 s of stimulation exceeded 0.5 ms. (These thresholds were used so as to exclude extremely small shifts in response latency.) If the linear regression was significant, but the piecewise function fit the data significantly better ($P<0.01$, F-test), then slopes and total shifts were taken from the piecewise fit. Peak response magnitudes were subjected to a parallel analysis. (Statistical thresholds in the peak response analysis were $P<0.01$ and a total shift $>10$ sp/s.)

Burst discharges were detected using the Legendy surprise method (Legendy and Salcman 1985) which is described in detail by Wichmann et al. (2006). Bursts were defined as
groups of three or more spikes whose inter-spike intervals (ISIs) were unusually short compared with other ISIs of a spike-train. Following Wichmann et al., we used a surprise threshold of 3, which equates to an alpha <0.05 that the candidate burst would occur as part of a Poisson-distributed sequence of spikes. Spike-trains were concatenated across all control periods and across the last 10 seconds of all stimulation blocks. The concatenated control and stimulation trains were analyzed separately. A neuron’s spike-trains were subjected to burst analysis only if both control and stimulation trains contained >1000 spikes. Two fundamental measures of the prevalence of bursts were used: (1) the fraction of time within a spike-train spent in bursts; and (2) the fraction of spikes contained within bursts. In addition, effects of DBS on burst morphology were analyzed by constructing burst-triggered averages and 95% confidence intervals for a cell’s peri-burst instantaneous frequency of firing. Separate averages were computed around the onset of bursts detected in control and stimulation trains. The mean pre-burst firing rate (-50 – 0 ms before burst onset) was subtracted from each burst-triggered average to aid comparison between stimulation conditions. Comparing burst-triggered averages provided a way to detect potential effects of DBS on the general structure of bursts without having to extract a potentially-large number of burst parameters.

Oscillations in neuronal activity were detected using methods adapted from Rivlin-Etzion et al. (2006). First, spectral power estimates were computed separately for control periods and the last 10 seconds of all stimulation blocks. The discrete Fourier transform was applied to non-overlapping 1024-point segments of the spike delta function (1 kHz resolution) smoothed with a Hanning window of the same length. This yielded power spectral density estimates for frequencies between 0 and 500 Hz with a resolution of 1 Hz. Distortions of these estimates attributable to a neuron's refractory period and slow changes in firing were compensated for by
normalization using spectra computed from the same data but after local shuffling of the interspike intervals (Rivlin-Etzion et al. 2006). The normalizing spectra were the mean of spectra computed from each of 1000 random shufflings of the same interspike intervals. Shuffling was performed locally on 150-to-200 ms long sections of the spike train. Peaks in the normalized spectra between 2 Hz and 160 Hz were tested for significance relative to the SD of the normalized spectra in the 340-500 Hz “control” range (Rivlin-Etzion et al. 2006). Frequencies up to 160 Hz were included so as to detect the fundamental spectral correlates of phasic responses to DBS. High frequency driving occasionally introduced narrow peaks in the 340-500 Hz “control” range of a spectrum (i.e., at higher harmonics of the stimulation frequency). To prevent these peaks from biasing the detection of low-frequency oscillations (LFOs), we trimmed outliers from the control ranges of all spectra before computing a standard deviation. Trimming used a robust outlier detection method based on the median absolute deviation (MAD) scaled to approximate the standard deviation (Rousseeuw 1990). Values >3*MAD away from the control median were excluded from the computation of the control SD. The threshold for significance applied to each peak in a spectrum was corrected for multiple comparisons (Bonferroni correction based on n=161 spectral points in the tested range 2–160 Hz) to yield an omnibus alpha of 0.01. Autocorrelograms were inspected to verify the qualitative accuracy of the spectral analysis (1 ms resolution, maximum lag = 1 s, smoothed with a $\sigma = 3$ ms gaussian).

Clinical testing

The severity of parkinsonian signs was measured during multiple hour-long observation periods with the animal in a sound-proofed cage under video-tape observation. The frequency of whole-body movements was recorded using an accelerometer-based logging device fixed to the
animal’s backpack (Actitrac, IM Systems). To confirm the animal’s parkinsonian status we administered L-DOPA methyl ester (IM, 5 mg/kg with 10 mg/kg benserazide). L-DOPA was administered on a maximum of 5 days per animal, all at times at least 1 month prior the recordings reported here. Video-tapes were reviewed by one researcher blinded to the time and conditions of taping. The reviewer rated the severity of the animal’s parkinsonism using a clinical rating scale designed by Schneider et al. (2003).

To measure the therapeutic effects of GPiDBS, transient changes in posture and rigidity were measured at the elbow joint using a servo-controlled torque motor (Aerotech, Pittsburgh, PA). This testing was performed as a part of cortical recording experiments that will be reported elsewhere. Because of this, torque testing and GPi neuronal recording were always performed on different days. During torque testing, the animal's left arm (contralateral to the DBS electrode) was held in the horizontal plane and secured in a padded splint with the elbow joint aligned with the vertical axis of rotation of the motor. The motor moved the elbow through a sinusoidal displacement of ±20 deg around the elbow's neutral position at a cycle rate of 1 Hz (Fig. 3A). Reactive torque was measured using a torque sensor sampled at 1 kHz. Rigidity was computed cycle-by-cycle as the integrated resistive torque (i.e., "work") required to move the elbow joint through a cycle (Prochazka et al. 1997) (Fig. 3B and C). Transient changes in elbow posture (e.g., Fig. 3E) were detected as significant (>3 SD) deviations in cycle-by-cycle mean torque away from a record’s overall mean torque. Direct observation indicated that these transient changes in limb posture were associated reliably with spontaneous movements of one or more limb and/or adjustments of posture. Based on these observations, we used torque transients as surrogate markers for the occurrence of spontaneous movements. For each testing session, we computed the mean fraction of torque cycles that contained significant postural...
transients during baseline (20 – 0 s prior to DBS onset) and stimulation (10 – 30 s after DBS onset) conditions. The influence of GPiDBS on postural transients was summarized as the difference in means between stimulation and baseline conditions [i.e., (mean fraction of stimulation cycles containing transients) – (mean fraction of baseline cycles containing transients)]. Stimulation blocks that contained significant postural transients were excluded from the analysis of rigidity described above. Postural transients prevented rigidity analysis in some testing sessions, especially those in Monkey E.

Histology

At the completion of the study, animals were anesthetized deeply and perfused transcardially using phosphate buffered saline with 10% formalin. Stimulating electrodes were left in place until after perfusion. The tissue was blocked in the coronal plane, cut in 40 µm sections, mounted, and stained with cresyl violet. To verify depletion of dopaminergic terminals in the striatum, selected sections were stained using tyrosine hydroxylase (TH) immunohistochemistry (Fig. 2B). These sections were incubated for 24 hours with a mouse anti-TH monoclonal antibody, followed by 1 hour of incubation with a biotinylated horse anti-mouse antibody (Chemicon international, Temecula, CA). Sections were then incubated with Streptavidin for 1 hour and then revealed using DAB-Vector SK-400 (Vector Laboratories, Burlingame, CA). The locations of stimulating electrodes within GPi were clearly visible based on the locations of defects left in the histologic sections by the electrode shaft.
RESULTS

Clinical status and behavioral effects of GPiDBS

Following MPTP administration, both animals showed moderate-to-severe parkinsonian signs bilaterally, which included a paucity of spontaneous movement, bradykinesia, limb rigidity, action tremor, and stooped posture. The left limbs were affected more severely because of the initial right-intracarotid infusions of MPTP. Mean scores on the Schneider scale for parkinsonism (Schneider et al. 2003) were 37 and 38 for the two animals, compared with a mean of 8 prior to MPTP treatment. Both animals had very low rates of spontaneous movement in their home cages (mean 9.0 movements/minute) compared with the >50 movements/minute rate typical for neurologically-intact macaques. Administration of L-dopa methyl ester (5 mg/kg IM) reduced clinical scores in both animals and increased the rates of spontaneous activity significantly (mean = 17.3 movements/minute). Postmortem histology from both animals revealed widespread depletion of TH immunolabeling throughout the dorsal striatum and the lateral substantia nigra compacta (Fig. 2B).

GPiDBS reduced rigidity at the elbow joint in Monkey C (Fig. 3A-D). The torque necessary to move the arm through a ±20 deg sinusoidal displacement was reduced markedly within 1 s of the onset of GPiDBS (150 Hz, 400 µA; Fig. 3A). This reduction in mechanical resistance was evident as a reduction in the “work” (Nm-degrees; i.e., the area delimited by each angle-torque excursion; Fig. 3B) required to move the elbow joint through a displacement cycle. The reduction in rigidity was present consistently across repeated blocks of stimulation (n=8 in Fig. 3C) and the reduction persisted across 30 s epochs of GPiDBS. Rigidity recovered slowly to pre-stimulation levels after the offset of stimulation (Fig. 3C). The magnitude of the reduction in
rigidity correlated with stimulation current across the range of currents tested (0-600 µA; Fig. 3D). Similar effects of GPiDBS on rigidity were observed on 16 separate days during the period of GPi neuronal recording in Monkey C. GPiDBS-induced reductions in rigidity were not observed consistently in Monkey E.

GPiDBS also increased the occurrence of transient changes in postural tone at the elbow. During rigidity testing, occasional transients were noted in the records of reactive torque. (Figure 3E shows results from a testing session in which transients were particularly common.) These “postural transients” were detected as significant (>3 SD) deviations in mean reactive torque. (Actual movements of the elbow joint always followed the sinusoidal displacements determined by the servo-motor; Fig. 3A.) Direct observation indicated that postural transients were typically accompanied by frank movement of other body segments. The latency, magnitude, and timecourse of postural transients varied substantially across repeated blocks of stimulation on a single day (compare blocks 1, 3, and 4 in Fig. 3E) and across days of testing. Due to this variability, it is unlikely that the transients reflected involuntary activation of muscles (e.g., via direct stimulus-induced cortico-spinal driving). Therefore, postural transients likely reflected volitional postural adjustments. The frequency of postural transients was measured as the mean fraction of torque cycles that contained significant postural transients around the time of GPiDBS onset (black histogram, Fig. 3F). Note, as in the example shown, GPiDBS reduced rigidity (mean cycle-by-cycle “work”) during stimulation blocks that did not contain significant postural transients (i.e., during stimulation blocks #4 and 7, Fig. 3E; mean plotted as dark gray line in Fig. 3F). The effects of GPiDBS on the occurrence of postural transients varied substantially across sessions in both animals (note size of ±SEM error bars for the cross-session means plotted in Fig. 3G). Nonetheless, GPiDBS at currents ≥200 µA increased the mean
frequency of postural transients in both animals \( (P<0.05, \text{T-statistic}>2.1 \text{ for both animals individually}) \). Although the clinical effects of GPiDBS were not studied on the same days as the neuronal recordings reported on below, clinical effects were stable over the time periods of clinical testing, which partially overlapped the time periods when GPi neuronal recordings were obtained (Supplementary Fig. 13D).

In both animals, the indwelling macroelectrode targeted the GPi and was located approximately 3 mm posterior to the anterior commissure (Fig 2A). The most distal stimulating contact was positioned in the dorsal-medial GPi in monkey E and in the approximate center of GPi in monkey C.

**Neuron Database**

Recordings from a total of 102 cells met our criteria for location, stability and artifact rejection efficacy \( (n=69 \text{ and } 33 \text{ from GPe and GPi, respectively}) \). These neurons were sampled during stable parkinsonian signs, 416–607 days after the last administration of MPTP. L-dopa was not administered during this period. The mean duration of recording was 335 s \( \text{(range: 81–647 s)} \). During these recordings, 30 s-long blocks of GPiDBS were delivered a mean of 3.3 times \( \text{(range: 1–6 times)} \).

**Uninterrupted pallidal recording during GPiDBS**

Because of the technical challenges involved, it is important first to demonstrate our ability to sample pallidal activity continuously during GPiDBS at clinically-relevant levels \( (200–1000\mu\text{A}; 150 \text{ Hz}) \). Figure 4 illustrates data from a typical recording, in which the only evidence of stimulation-related artifacts was one brief voltage transient \( (0.5 \text{ ms}, 340 \mu\text{V peak-to-peak}) \) at the first shock in a 30 s long train of stimuli \( \text{(marked by * in Fig. 4B and C)} \). Action potentials
that overlapped the exact times of stimulation were of similar magnitude and shape as action potentials from other peristimulus periods and from control non-stimulation periods (↓ in Fig. 4Cii and Dii). All neurons accepted for analysis demonstrated similar preservation of action potential isolation and shape during GPiDBS. The only exception was 9 neurons in which action potentials attenuated >10% peak-to-peak (range: −12.5% to −20%; e.g., Fig. 6B), likely due to partial inactivation of voltage-sensitive channels during high frequency driving. Action potential attenuations were always consistent across peristimulus intervals (<4% variation across the interval) and were always associated with stimulation-induced increases in mean firing rate.

**GPiDBS evoked polyphasic responses**

Our principal analyses focused on the quasi-steady state effects of GPiDBS 20–30 s after the onset of stimulation. During this period, stimulation affected the discharge rate of 85% of cells (Table 1). Responses tended to be more common in GPe though the difference was not significant ($\chi^2=5.6, P>0.05$; Table 1 “Any effect”). In both pallidal segments, stimulation-evoked decreases more often than increases ($\chi^2=28.2, P<0.0001$; Table 1). We show below, however, that the net effects of stimulation on mean firing rates were minimal.

The most common type of response was a phasic peristimulus modulation in firing (78% of cells; Table 1 “Response Type”), which often included a combination of increases and decreases in firing (35% of cases; e.g., Fig. 5 and Fig. 6B). Phasic effects were equally common across pallidal segments ($\chi^2=3.8, P>0.1$). Figure 5 shows an example of a polyphasic type of response in a cell from GPe. A short-latency (0–1.4 ms) increase in firing was followed by two distinct decreases at 1.4–3.2 and 4.0–6.7 ms following stimulation. These effects were relatively consistent across the four repeated blocks of GPiDBS (Fig. 5B), but their form evolved over each
30 s block of stimulation. Inspection of the average response (collapsed across the four blocks; Fig. 5C) provided clear evidence that the short-latency increase declined in magnitude across a stimulation block while a longer-latency increase emerged at 3.4–4.6 ms. The phasic peristimulus modulations of this cell (measured from peristimulus histograms, “PStHs”; Fig. 5Ci. – v. and Di. – v.) were highly significant relative to a population of control modulations (changes in firing rate detected in two peri-control histograms; Fig. 5Ci. and ii., and Di. and ii.).

Another common form of peristimulus response (composing 50% of all phasic responses) was an isolated increase (e.g., Fig. 6A) or decrease in firing. For the example in Fig. 6A, the magnitude of the response declined across the stimulation block, but onset latency remained fixed at ~3 ms.

Fifteen percent of phasic increases occurred at short fixed latencies (<2 ms) with very brief durations (<0.5 ms). For example, in Figure 6B, the brief epoch of spiking at ~1 ms latency did not shift in latency across the stimulation block, unlike the longer-latency phasic responses. The possible antidromic nature of these responses is addressed in a separate section below.

The repetitive nature of GPiDBS often led to a “wrap around”-like phenomenon in long-latency responses. For instance, the decrease marked j. in Fig. 5Cv. began at ~4.8 ms and persisted past the next stimulation delivery (time 0). Another example of a wrap-around type effect can be seen in the late (latency ~6.4 ms) increase in Fig. 6B (onset indicated by ↓ in i.-iii.). The latency of the increase began progressively later across the stimulation block so that it eventually merged with the fixed-latency response at ~1 ms.

A minority of cells showed sustained stimulation-induced changes that did not vary across the peristimulus interval [Fig. 6C; Table 1 “Sustained”; peristimulus modulations <3×...
SD of peri-control histograms (PCTHs)]. Sustained responses were more common in GPi (32% of neurons vs. 18% in GPe; Table 1) though the difference from GPe did not reach significance ($\chi^2=3.8, P>0.1$). As exemplified in Fig. 6C, nearly all sustained changes were decreases in firing (95% of cases) in which firing rates were reduced dramatically but not blocked completely. In all cases, the few action potentials detected during a sustained decrease were indistinguishable from action potentials from non-stimulation periods (Fig. 6C, right top).

To examine the net effect on tonic firing rates of these disparate response types, we computed mean stimulation-induced changes in firing rate across the 20–30 s period of mean stimulation blocks. For the population as a whole, GPiDBS had no significant effect on firing rates ($-2.2 \pm 20.0$ sp/s, mean $\pm$SD). Restricting analysis to cells that responded to GPiDBS, mean rates declined modestly but significantly in GPi ($-6.9$ spikes/s; Table 2 “Any Effect”). This overall reduction reflected the substantial decreases in sustained-type GPi responses (Table 2 “Response Type”) combined with a zero net change in phasic-type GPi responses. No differences were found between cells from GPe and GPi for any of the sub-categories identified in Table 2 ($P>0.1$, two-sample t-test). The nominal effects of GPiDBS on mean firing rates are illustrated as box-plots at the right of each panel in Fig. 7A.

Figure 7 provides a summary of all peristimulus responses observed in GPe and GPi. Population mean histograms (Fig. 7A, averaged across all phasic-type PSTHs) showed increased firing at 0–1 ms and 3–5 ms. Decreases intervened between the two periods of increased firing. The relatively large standard errors (gray shading) reflected the fact that individual responses (Fig. 7B) differed substantially from the mean response pattern. Nonetheless, ANOVA confirmed that mean firing rates were elevated 3–5 ms post-stimulus ($P<0.0001$; $F=4.8$; main
Local effects of GPi DBS McCairn & Turner 23
effect of time in ANOVA of PSTH time×nucleus; \( P<0.05 \) Tukey’s HSD). The early increase at 0–1 ms did not reach significance in post hoc testing (\( P>0.1 \) Tukey’s HSD). Though inspection suggested stimulation effects were more pronounced in GPi (Fig. 7A), this impression was not borne out in the ANOVA (\( P>0.3, F<0.9 \) for both main effect of nucleus and time×nucleus interaction). A comparison of latency distributions confirmed that phasic increases and decreases peaked at different times (Fig. 7C; \( P<0.0005 \), two-sample K-S statistic = 0.38). Latency distributions were similar, however, for cells from GPe and GPi (\( P>0.5 \) for between-nucleus comparisons of maxima and minima distributions; two-sample K-S statistic < 0.15). Increase maxima clustered at 0–1 and 3–5 ms post-stimulus, whereas decreases clustered at 1–3 ms. Sustained responses (thick dashed lines, Fig. 7A) did not vary significantly across the peristimulus interval or between the two pallidal segments (\( P>0.4, F<1 \) for all effect in ANOVA of PSTH time×nucleus).

The different response types (increases versus decreases, phasic versus sustained) showed no obvious anatomical organization relative to either the location of the stimulating electrode or the location of recording within internal or external pallidum (Fig. 2A). To address this question more rigorously, we tested for significant changes in response sign or form as a function of the estimated three dimensional distance between a recording location and the active contact of the stimulating electrode (Supplementary Fig. 12A). The ratio of stimulation-induced decreases to increases showed no significant change as a function of distance from the stimulating electrode (\( \chi^2=0.3, P>0.5; \) Supplementary Fig. 12A). Sustained-type responses tended to be most common in close proximity to the stimulating contact, but this trend was also not significant (\( \chi^2=2.8, P<0.2; \) Supplementary Fig. 12B).
We found no obvious relationship between current and response type (i.e., ratios of increases vs. decreases and phasic vs. sustained responses did not correlate with stimulation current; \( P > 0.3 \); Spearman’s rho < 0.1; Supplementary Fig. 13). This was not surprising given that the effects of stimulating at different currents was not explored systematically. Note also that during neuronal recordings stimulation was always at currents \( \geq \) 200 \( \mu \)A (i.e., at currents shown to have therapeutic effects; Supplementary Fig. 13). Similar distributions of response types were observed throughout the duration of the GPi recording experiments in both animals (Supplementary Fig. 13; statistical results summarized in caption).

Temporal evolution of responses

We investigated whether response timings and magnitudes were stable by the end of the 30 s block of stimulation. Significant latency shifts were found in 45% of the responses analyzed (39 of 86), most consisting of slowly increasing latencies with increasing stimulation duration [i.e., latency versus block-time slopes were positive in 85% of cases (33 of 39 responses)]. As illustrated for one neuron in Fig. 8A, longer-latency responses (i.e., those peaking > 2.0 ms post-stimulus) were more likely to show significant latency shifts (\( P < 0.001 \); \( \chi^2 = 13.1 \), Fig. 8B). Longer-latency responses also had steeper slopes (\( P < 0.02 \); \( F = 11.3 \); main effect of latency in ANOVA of latency×response sign×nucleus) and greater total shifts in latency (mean = 0.7 ms versus 0.2 ms for short-latency responses; \( P < 0.05 \); \( F = 4.28 \); latency main effect). However, most latency shifts asymptoted within the 30 s stimulation block (79%, 31 of 39 responses; e.g., \( \downarrow \) in Fig. 8A; black filled symbols in Fig. 8B) at times distributed uniformly across the block (\( P > 0.2 \); one-sample K-S statistic = 0.17 versus a uniform distribution). For the few responses that continued to shift after 30 s (red in Fig. 8B) the slopes were evenly balanced around zero (mean slope = 0.01; \( P > 0.9 \), 1-sample t-statistic < 0.04 versus zero). The prevalence of latency
shifts and distributions of their slopes were similar between pallidal segments and between phasic increases and decreases in discharge \((P>0.3\) for all interactions; ANOVA of latency×response sign×nucleus).

Response magnitude seldom changed as a function of the duration of stimulation \((24\%\) of responses; 21 of 86). Responses that did change were primarily phasic increases in firing \((20\%\) of 21 cases; \(\chi^2=21.9, P<0.0001\)) that decreased in magnitude over the course of stimulation \((76\%\) of cases; \(\chi^2=8.2, P<0.02\); Fig. 8C). Unlike the results for response timing, significant magnitude shifts were not restricted to longer latency responses \((P>0.3\); \(\chi^2\) and ANOVA of latency×response sign×nucleus). Similar to the results for response timing, however, most shifts in response magnitude reached an asymptote within the 30 s stimulation block \((67\%, 14\) of 21 responses; black in Fig. 8C). Notably, for the 7 changes that did not asymptote \((red\) in Fig. 8C), the mean slope did not differ significantly from zero \((mean+1.2\) sp/s/s; \(P>0.5\), 1-sample t-statistic = 0.3). In summary, the latencies and magnitudes of most responses were stable by the end of 30 s of continuous stimulation. In the minority of responses that continued to evolve at the end of 30 s of continuous stimulation, latencies and magnitudes were balanced near zero net change.

*Antidromic-like driving was a component effect of GPiDBS*

The brief duration and fixed timing of some short latency responses suggested antidromic or direct activation. We examined short latency responses for evidence of collision, a key test for antidromic driving. Figure 9 illustrates two examples in which collision-like phenomena were apparent. Both cells responded to stimuli at a fixed latency (\(Ai\). and \(Bi\).). When spontaneous spikes occurred immediately prior to stimulation, however, the cells failed to respond (\(Aii\). and
Because this analysis was performed offline and we did not apply definitive tests for antidromicity (e.g., testing for a fixed collision interval), we termed these effects “antidromic-like.” Brief fixed latency driving was observed in ten cells, 8 in GPe and 2 in GPi (12% and 6% of cells, respectively; \( P>0.3; \chi^2=1.9 \)). Evidence for collision was confirmed in all 4 cells for which continuous data acquisition was used. Latencies ranged from 0.2 ms to 1.2 ms. Failure rates were high for all antidromic-like responses, ranging between 73% and 94%, similar to those reported for direct activation during STN DBS (Garcia et al. 2003).

It is important to note that other short latency excitations were not considered likely effects of antidromic activation because the response lacked the tight temporal synchrony that is a hallmark of antidromic activation. For instance, Fig. 4D and Fig. 5 illustrate a short latency excitation that was considered unlikely to be a product of antidromic activation because of its variable latency. Such responses may instead represent long-latency (>6.67 ms) responses that have “wrapped around” from previous (n-1) stimuli.

**GPiDBS did not suppress burst firing**

One mechanism hypothesized for GPiDBS is through suppression or blockade of burst firing. Inspection of individual spike-trains during stimulation suggested this was not the case, however (Fig. 10A). In many cases, burst firing persisted or even increased in prevalence during GPiDBS, even in neurons that had robust peristimulus responses. For example, the same neuron shown in Fig. 10A to have bursty spike-trains during GPiDBS also had a significant peristimulus response (Fig. 6B). The seemingly incongruous combination of phasic peristimulus driving and burst firing was explained by the relatively high failure rate for spike responses to any one
stimulation (Fig. 10Aiii.). In other words, neuronal responses to stimulation occurred episodically in burst-like clusters of spikes.

We found no DBS-induced change in the overall prevalence of bursts across GPe or GPi populations (Table 3). GPiDBS did not alter the mean fraction of time spent in bursts or the mean fraction of spikes in bursts ($P>0.4$ for main effect of stimulation and interaction for both time and spike count measures; $F<1.0$ in ANOVAs of stimulation×nucleus). GPiDBS did alter burst prevalence in individual cells, but nearly equal fractions of cells showed increases and decreases in burst prevalence (51% vs. 49% and 58% vs. 42% for time and spike fractions, respectively; $P>0.1$, $\chi^2<3.6$ for both and for comparisons between nuclei). The burstiness of a neuron under baseline conditions (i.e., “OFF” stimulation) was a strong predictor for how bursty the neuron appeared during GPiDBS (compare position and orientation of 50% confidence ellipses with unity lines in Fig. 10B and C; $P>0.2$ for linear regression slopes differing from slope = 1). This observation held true independently for GPe and GPi neurons and for neuronal sub-populations categorized according to their responses to GPiDBS (Fig. 10B and C). One interaction that approached significance was a propensity for neurons with sustained responses to stimulation to spend less time in bursts during stimulation (10 neurons decreased % time in bursts versus 4 increased; $P=0.05$, $\chi^2=5.9$), although the mean change in that metric was small ($-1.3%$; $P>0.2$, 1-sample t-statistic = 1.2). No other interaction between measures of burstiness and effects of stimulation approached significance (% of time or % of spikes in bursts versus response type, response sign, or change in mean firing rate; $P>0.2$, $\chi^2<3.6$ for all comparisons).

GPiDBS altered the structure of bursts in the spike-trains of some individual neurons (e.g., altering intraburst firing rate or burst duration), but these changes were equally balanced
across cells such that no net change was found in the population. For the example in Fig. 10A-B, GPiDBS shortened burst duration and increased intraburst firing rate. These effects are clearly evident by comparison of burst-triggered averages from control and stimulation periods (Fig. 10D). Figure 10E provides an example in which the intraburst firing rate was reduced in a GPi neuron that was driven phasically by GPiDBS (see inset PSTH). Across GPe and GPi populations, however, absolutely no DBS-induced differences in burst structure were evident (Fig. 10F and G). Similarly, mean burst structures were indistinguishable on- and off-stimulation for neuronal sub-populations categorized according to their responses to GPiDBS (results not shown). Thus, although GPiDBS affected the prevalence and structure of bursts in some individual neurons, these effects yielded no net change in burst-firing when averaged across populations of pallidal neurons.

**GPiDBS suppressed low frequency oscillatory activity**

Many neurons in both pallidal segments displayed LFO firing (Fig. 11A; 39 of 102 cells, 38%; equally prevalent in both nuclei: \( P>0.5, \chi^2=1.2 \)). GPiDBS frequently altered the oscillatory pattern, significantly reducing the power of LFOs in 82% of the cells that had significant peristimulus responses to stimulation (27 of 33 cells; Fig. 11A). Significant suppressions in LFO were equally common in cells that had phasic- and sustained-type peristimulus responses \( (P>0.5, \chi^2=0.6) \). Interestingly, GPiDBS also reduced LFO in 4 of the 6 oscillatory cells that had no significant peristimulus response. Figure 11B shows an example in which a marked oscillation at 13.7 Hz was attenuated significantly during GPiDBS despite the absence of a significant peristimulus modulation in firing (inset, Fig. 11B). These few cases are noteworthy because they show that oscillation-suppressive effects of GPiDBS may extend beyond the population of cells whose firing rate was affected by stimulation.
Across the population, GPiDBS reduced the prevalence of oscillatory firing in all frequency bands except >70 Hz (Fig. 11D; $P<1\times10^{-5}$, $\chi^2=26.5$). The suppression was greatest between 3 and 12 Hz, which was the frequency range with the highest prevalence of oscillatory activity off stimulation ($P<0.005$, $\chi^2=11.3$). During stimulation, spectral peaks at high frequencies (>70 Hz) were either at the frequency of stimulation (38 peaks at 150±7.5 Hz, allowing multiple peaks per cell; 84%) or at its half-harmonic (6 peaks at 75±7.5 Hz; 13%). Although phasic-responding cells were the most likely to have high frequency spectral peaks (e.g., see Fig. 11A), nearly half of the phasic-responding cells did not (47%, 32 of 68 cells). This point is germane to the coherence results below.

GPiDBS also reduced oscillations that were coherent (i.e., synchronized) between neuron pairs. Eleven recording sessions yielded 30 neuron pairs that were recorded from simultaneously on different electrodes. Coherent LFO activity was found in 63% of the pairs. Figure 11C shows an example in which, prior to stimulation, a pair’s firing was highly synchronized at 10.7 Hz ($R^2 = 0.18$). GPiDBS completely abolished this synchronized oscillation. Notably, despite the phasic peristimulus responses in both neurons (inset, Fig. 11C), their activity was not synchronized at ~150 Hz (i.e., the coherence spectrum was flat at that frequency). One of the units (unit 2c) belonged to the group of phasic-responding cells mentioned above that did not have high frequency spectral peaks.

GPiDBS reduced the overall prevalence of LF synchronized firing assessed across cell pairs (Fig. 11E; $P<0.05$, $\chi^2=6.5$). Unlike the effects observed on oscillatory firing in individual neurons, the largest effect on coherence was in the beta frequency range (13 – 35 Hz; $P<0.05$, $\chi^2=6.0$). Coherence in the low gamma frequencies (35 – 70 Hz) increased during GPiDBS,
although this effect was not significant ($P<0.2$, $\chi^2=3.7$). Off stimulation, coherent oscillations were surprisingly common at frequencies $>70$ Hz (8 of 30 pairs, 27%). High frequency coherence was only slightly more common during GPiDBS, but coherence frequencies became tightly coupled to the frequency of stimulation (8 of 11 peaks, 72% at either $150\pm7.5$ Hz or $75\pm7.5$ Hz).

In summary, GPiDBS reduced the prevalence of LFOs both in the firing of individual neurons and in the synchronized firing of neuron pairs. Though these changes often were associated with phasic peristimulus responses and the emergence of spectral peaks coupled to the frequency of stimulation, the associations were not obligatory.

**DISCUSSION**

The present results are inconsistent with the concept that suppression of local somatic spiking is a principal therapeutic mechanism of GPiDBS. The local effects of GPiDBS included combinations of direct driving, phasic facilitations and suppressions of firing, and, in a minority of cells, tonic suppression. GPiDBS reduced mean GPi firing rates only slightly. Our findings support the alternative hypothesis that GPiDBS works by suppressing the LFO activity associated with PD. Interestingly, GPiDBS did not modify the overall prevalence of burst firing or other burst characteristics.

*Potential confounds and limitations*

It is important to acknowledge that DBS can have different effects on neuronal somata and efferent axons (McIntyre et al. 2004a; McIntyre et al. 2004b). Therefore, the present results may not provide an accurate representation of pallidal *efferent* signaling during GPiDBS.
Nevertheless, because GPi somata form a critical bottleneck for the pathologic neuronal activity associated with parkinsonism (Baron et al. 2002; Lonser et al. 1999), stimulation-induced reductions in abnormal somatic firing are likely to reflect true network-wide suppressions of those abnormalities. The effects of stimulation on nearby somata and fiber tracts are influenced by electrode geometry and stimulation parameters (Miocinovic et al. 2006). Although we used low-impedance macroelectrodes and clinically-relevant frequencies and currents, our electrodes did not replicate the exact geometry of clinical electrodes and we used 0.2ms pulse-widths (versus <0.1ms used clinically). Thus, our results may not extrapolate perfectly to the clinical situation.

The present study did not address possible relations between clinical efficacy and the anatomical locations of stimulation and neurophysiologic effects. GPiDBS had different therapeutic effects in the two animals, increasing mobility in both animals, but consistently reducing rigidity in only one (monkey C). It is possible that this difference was due to the different locations of stimulating electrodes in the two animals. In monkey C, the electrode was placed in the middle of the posterior GPi, a region known to project to primary motor and premotor thalamocortical circuit (Akkal et al. 2007; Hoover and Strick 1993). In monkey E, the more dorsomedial electrode placement was likely to have a greater impact on premotor and associative thalamocortical circuits and less of an effect on the primary motor circuit. Similar neurophysiologic effects were observed in the two animals, however, and those effects were distributed across similar regions of the GPi (see Fig. 2A).

Neuronal data were not collected during clinically-ineffective stimulation, thus preventing a correlative analysis of specific neurophysiologic effects of GPiDBS versus therapeutic efficacy (e.g., Hahn et al. 2008; Hashimoto et al. 2003). The present results do, however, reveal the
dominant changes in pallidal activity that are present during clinically-effective GPiDBS. It is also important to recognize that the present results, collected in animals at rest, may have limited relevance for other behavioral contexts [e.g., during performance of a behavioral task (Montgomery and Gale 2008; Shi et al. 2006)]. Finally, because this study was performed in animals with severe neurotoxin-induced parkinsonism, the results may have incomplete relevance to the mild-to-moderate parkinsonism typical of the idiopathic disease in humans.

**GPiDBS seldom silenced GPi activity**

Contrary to predictions of the inactivation/suppression hypotheses (Benabid et al. 1991; Benazzouz and Hallett 2000; Dostrovsky et al. 2000), we found nominal effects on mean firing rates. Profound sustained reductions in firing were observed in a minority of neurons, but those reductions were offset by net increases in the more common phasically-responding neurons. This result is qualitatively consistent with previous observations made during brief trains of pallidal microstimulation (Bar-Gad et al. 2004). Previous studies may not have detected pallidal action potentials emitted immediately following shock delivery due to artifact-induced occlusion of neuronal recordings. Also, microelectrodes (which were used in some studies) and macroelectrodes (used here and in clinical DBS) are likely to recruit different neuronal elements (McIntyre et al. 2004a; McIntyre et al. 2004b). Corroborating evidence against the inactivation hypothesis comes from reports of DBS-induced phasic driving in efferent-recipient nuclei (Anderson et al. 2003; Hashimoto et al. 2003) and observations of DBS-induced changes in neurotransmitter concentrations (Galati et al. 2006; reviewed in Perlmutter and Mink 2006; Stefani et al. 2005; Windels et al. 2000). These results are also consistent with the mounting evidence that altered tonic firing rates are not a principal causative factor in the pathophysiology of parkinsonism.
The profound rate reductions seen in a minority of neurons may contribute to the efficacy of GPI-DBS by disinhibiting a subset of GPI-recipient neurons. These reductions were probably not a product of depolarization-block or voltage-gated current inactivation (Beurrier et al. 2001), because the action potentials detected during sustained reductions showed none of the amplitude attenuation that should accompany such effects (e.g., Fig. 6B). Sustained reductions more likely arose from activation of local GABAergic afferents that collateralize to both pallidal segments (Nambu and Llinas 1997; Parent et al. 1995; Sato et al. 2000a).

**GPI-DBS evoked polyphasic responses**

We found abundant polyphasic driving in both pallidal segments during GPI-DBS. Although Bar-Gad et al. (2004) reported similar effects, the present work linked those results to the clinical context by applying longer trains (30s, versus their 1.3s) of demonstrably-therapeutic stimulation using low-impedance macroelectrodes (versus their microelectrodes). Despite the methodologic differences, both studies found phasic responses in a majority of neurons (67% here versus 70% in Bar-Gad et al.) and sustained suppressions in a minority (19% versus their 12%). The modest differences between studies (e.g., in response latencies) may arise from our ability to detect responses at <1ms latency, our use of longer-duration trains of stimulation, and the observation that response latencies lagged progressively across a stimulation block.

Many short-latency responses may have reflected direct or antidromic activation based on their short fixed-latency (Fig. 6B) and the absence of significant latency shifts (Fig. 8). Although off-line analysis prevented strict tests for antidromicity (Bishop et al. 1962), we observed collision-like phenomena in all short-latency datasets amenable to analysis (Fig. 9). Previous in vivo studies were unlikely to have observed such effects because of artifact-induced occlusion of the short-latency spikes (Benazzouz et al. 1995; Boraud et al. 1996; Dostrovsky et al. 2000;
Meissner et al. 2005; Wu et al. 2001). Direct driving has been observed in intracellular recordings from an STN slice preparation (Garcia et al. 2003). The short-latency responses in GPe are consistent with known GPe-to-GPi connectivity and the recognized ability to drive GPe neurons antidromically from their projection targets (Kita et al. 2005). The substantial (>73%) failure rates found here are consistent with in-slice observations (Garcia et al. 2003) and predictions from modeling studies (McIntyre and Grill 2000). Direct and antidromic driving are aspects of the local effects of DBS predicted from basic knowledge about the effects of electrical stimulation on neuronal tissue (McIntyre et al. 2004a; Ranck 1975), but seldom observed in vivo until now (Li et al. 2007; Montgomery 2006).

Later phasic response components may be mediated by a variety of mechanisms and pathways. Firing rate reductions that immediately followed phasic increases may reflect refractory periods. Decreases may also result from activation of GABAergic afferents from striatum (Hedreen and DeLong 1991), local axon collaterals (Parent et al. 2001) and afferents from the other pallidal segment (Nambu and Llinas 1997; Sato et al. 2000a). Longer-latency increases (3-5ms) in both pallidal segments may result from “axon-reflex”-type activations of collateralized glutamatergic afferents from STN (Sato et al. 2000b) and pedunculopontine nucleus (Gonya-Magee and Anderson 1983; Scarnati et al. 1988). Post-inhibition rebound may also contribute to some long-latency increases (Nambu and Llinas 1994). Multi-synaptic reentrant pathways are also likely to contribute [e.g., via thalamostriatal, corticostriatal, cortico-subthalamic, and brainstem pathways (DeLong and Wichmann 2007)]. The presence of long-latency responses reinforces the concept that GPiDBS alters neuronal firing patterns throughout an interconnected network of brain regions and that the critical site of therapeutic action, if any one site exists, may be distant from the site of stimulation. The observation that long-latency
responses to one stimulus often “wrap-around” to influence a neuron’s response to the next stimulus pulse is consistent with the concept that multi-synaptic resonant effects play a role in the effects of DBS (Li et al. 2007; Montgomery and Gale 2008).

Temporal evolution of responses

Longer latency (>2.0ms) response components often shifted timing and magnitude across stimulation blocks. The multi-second course of shifts and their prevalence in longer-latency (presumably, synaptically-mediated) responses suggested they reflect forms of synaptic plasticity. Though plasticity has been demonstrated in pathways that may contribute to the responses observed here (Calabresi et al. 1996; Shen et al. 2003; Wang et al. 2006), plasticity is seldom considered in mechanistic models of DBS (but see Tass and Majtanik 2006). The similarity of response evolution across stimulation blocks (e.g., Fig. 5C), suggested the plastic changes were short-term and able to recover over the ~40s interval between stimulation blocks. A slow evolution of responses, as seen here, could also be explained by a build-up of network-level resonance or precession-like interactions between multiple oscillators of different frequencies (Montgomery and Gale 2008). The mechanisms mediating these changes are a matter of speculation, however, due to the number of potential pathways involved and incomplete knowledge of their physiology.

Despite the prevalence of response evolution, 83% of responses were stable by the end of 30s of stimulation. Therefore, the results reported here may approximate the pallidal firing patterns present during long-term continuous application of GPiDBS. Our results do not rule out the possibility that additional effects arise after minutes/hours of continuous stimulation, as predicted from the slow timecourse of some therapeutic benefits (Temperli et al. 2003). There is
little question, however, that the neuronal effects described here were associated with clinical effects within seconds of GPiDBS onset (see Fig. 3).

*GPiDBS did not suppress burst firing*

We found no stimulation-induced change in the overall prevalence of burst discharges or in the characteristics of their structure. This finding brings into question the concept that GPiDBS reduces parkinsonian signs by suppressing pallidal burst firing (Rubin and Terman 2004; Shi et al. 2006; Vitek 2002b). The result is counterintuitive because phasic responses, as seen in 78% of our cells (e.g., Fig. 6A-B), might be predicted to regulate spike generation to the degree that it reduced bursting significantly. The unexpected conjunction of phasic responses and sustained burst-firing was made possible by the high failure rate for responses to any one shock. Trains of stimulation-induced responses were aggregated into burst-like clusters separated by periods of response failure (Fig. 10Aiii.). A similar conjunction of DBS-induced phasic responses and increased bursting was observed in intra-cellular recordings from STN slices (Garcia et al. 2003). The concept that DBS does not alleviate parkinsonism by reducing burst firing is supported by the recent work of Hahn and colleagues (2008) who showed that the clinical efficacy of STN-DBS did not correlate with its ability to reduce pallidal burst firing.

*GPiDBS suppressed low frequency oscillatory activity*

Parkinsonian signs have been associated repeatedly with the presence of synchronized LFOs in the BG-thalamocortical network (Hammond et al. 2007; Kuhn et al. 2008). It is important to recognize, however, that some signs may appear before LFOs emerge (Leblois et al. 2007; Mallet et al. 2008). GPiDBS reduced the prevalence of LFOs both in the firing of individual neurons and in the synchronized firing of neuron-pairs. Similar local reductions in LFOs have
been reported for STN-DBS in non-human primates (Meissner et al. 2005) and have been implied from studies in DBS patients (e.g., Brown et al. 2004; Marsden et al. 2001). Reductions in LFOs were often associated with phasic peristimulus responses and spectral peaks at stimulation frequency, but those associations were not obligatory. GPiDBS also suppressed LFOs in 2/3 of cells that had no peristimulus response (Fig. 11B). The low fidelity of neuronal driving resulted in a general desynchronizing effect of GPiDBS on pallidal firing despite the fact that most neurons responded phasically to stimulation (Fig. 11E). These results suggest the LFO-suppressive effects of GPiDBS extended beyond the neuronal circuits that mediated phasic peristimulus responses, and that GPiDBS may have dampened the large-scale network reverberations that are thought to generate LFO firing. Of the three hallmark abnormalities in GPi firing in PD, only synchronized LFOs showed a highly significant suppression during GPiDBS.

Acknowledgements: Mr. Eric Schaible designed and built the data acquisition system used in this study.

Grants: This research was supported by NIH grant NS15017.
REFERENCES


Boraud T, Bezard E, Bioulac B, and Gross C. High frequency stimulation of the internal Globus Pallidus (GPI) simultaneously improves parkinsonian symptoms and reduces the firing frequency of GPI neurons in the MPTP-treated monkey. *Neuroscience letters* 215: 17-20, 1996.


Dostrovsky JO, Levy R, Wu JP, Hutchison WD, Tasker RR, and Lozano AM.


Meissner W, Leblois A, Hansel D, Bioulac B, Gross CE, Benazzouz A, and Boraud T.


Miocinovic S, Parent M, Butson CR, Hahn PJ, Russo GS, Vitek JL, and McIntyre CC.


Montgomery EB, Jr., and Gale JT. Mechanisms of action of deep brain stimulation (DBS).


Schneider JS, Gonczi H, and Decamp E. Development of levodopa-induced dyskinesias in parkinsonian monkeys may depend upon rate of symptom onset and/or duration of symptoms. *Brain research* 990: 38-44, 2003.


FIGURE LEGENDS

Figure 1. Validation of artifact subtraction method.  

Ai. A neuronal signal acquired during GPiDBS but with artifact subtraction disabled showed large long-duration voltage transients time-locked to delivery of each shock (vertical gray lines; 150 Hz, 400 µA, 200 µs pulsewidth). Note that extreme voltage values are clipped in this figure to aid comparisons with the processed traces illustrated below.  

Aii. The same microelectrode signal acquired on a parallel acquisition channel with artifact subtraction working shows no evidence of shock artifacts while action potentials and recording noise are preserved. Action potentials that were completely obscured by artifacts in the un-subtracted data stream were easily detected in the processed data stream (↓).  

Aiii. Action potential waveform snippets aligned on the times of spiking of an individual neuron based on spike sorting of the signal shown in Aii. The snippets are stretched ×2 in time to make waveform shape more apparent.  

B. Offline test of artifact subtraction efficacy. The mean waveforms of spikes during stimulation (thick black lines; ±SEM indicated by gray shading) were required to fall within the 95% confidence interval (CI) for all spikes from non-stimulation periods (dotted lines). The test was performed separately for four adjacent peristimulus intervals (indicated by time intervals and horizontal brackets in Bi). Numbers below each mean indicate the number of action potentials contributing to each mean.  

Bi. For the same neuron, a peristimulus rate histogram (gray bars) is compared with an equivalent peri-event rate histogram from non-stimulation periods (thick black line). Note that action potentials approximately coincident with the time of stimulation (time zero, vertical gray line) had mean shapes, SEMs, and mean rates of occurrence that were indistinguishable from those during other peristimulus intervals and from those during non-stimulation periods.
**Figure 2.** Histologic confirmation of recording locations and dopamine depletion.  
A. Approximate recording positions (circles) were collapsed across 2mm in the coronal plane and projected onto atlas-derived line drawings of pallidal nuclear boundaries at two positions relative to the anterior commissure (AC). The approximate locations of stimulating electrodes are indicated by diagonal black lines. Results are plotted separately for monkeys E and C (top and bottom rows, respectively). The color and fill of each symbol indicate, respectively, the sign and form of DBS-induced peristimulus responses (see caption and Table 1). Clusters of symbols indicate recording sites where >1 neuron was sampled. The locations of some symbols have been shifted slightly to improve visibility.  
B. Tyrosine hydroxylase (TH-DAB) immunoreactivity in an adjacent coronal section illustrates the MPTP-induced depletion of TH-reactivity in dorsolateral regions of the caudate and putamen (open arrow) and relative preservation of TH-reactive fibers in the ventral medial regions of both nuclei (filled arrowhead).

**Figure 3.** GPIDBS reduced elbow rigidity and increase postural transients.  
A. The elbow was moved through a constant ±20 degree sinusoidal displacement at 1 Hz (thin line) with a servo-controlled torque motor. The torques required to move the arm (thick line) were reduced within 1 s of the onset of GPIDBS (gray shading; 150 Hz, 400 μA, 200 μs pulsewidth).  
B. Torque-angle plots from the same dataset illustrated in A. GPIDBS (gray line) reduced the slope of the torque-angle relationship (i.e., elastic stiffness) and reduced areas defined by the torque-angle hysteresis loop (i.e., work).  
C. Elbow rigidity (measured as cycle-by-cycle work) was reduced consistently during GPIDBS. The plot shows mean cycle-by-cycle work averaged across eight 30s-long presentations of GPIDBS (gray shading). Rigidity was reduced for the duration of GPIDBS and recovered slowly after stimulation ended.  
D. The GPIDBS-induced reduction in rigidity scaled with the intensity of stimulation (current). Mean values (±SEM) are shown for 13
stimulation sessions collected on one day in Monkey C. (All but one error bar fell within the bounds of the filled symbols.)

**E.** Postural transients (*) occurred more frequently during GPiDBS. Raw reactive torque records (thick black traces) are plotted aligned on the onset of GPiDBS stimulation blocks (gray shading) in an exemplar dataset from monkey C. The shape and timing of the transients varied substantially between stimulation blocks. No significant transients occurred during blocks # 4 and 7. **F.** For the dataset shown in **E,** the mean fraction of torque cycles containing significant postural transients (black histogram) was zero prior to stimulation onset, but increased markedly during GPiDBS. Rigidity (mean cycle-by-cycle work; dark gray line) was reduced during the two stimulation blocks that did not contain significant postural transients (i.e., during blocks 4 and 7, **E**). **G.** GPiDBS at currents ≥200 µA increased the frequency of postural transients in both animals. Symbols reflect the mean (±SEM) change in occurrence of postural transients attributable to GPiDBS during stimulation at different currents in Monkey C and E. (Data points with no error bars indicate currents that were used one time.)

**Figure 4.** Exemplar neuronal recording during GPiDBS. **A.** A raw microelectrode signal is shown as acquired during 400 s of collection including four 30-second long episodes of GPiDBS (gray shading; 150 Hz, 1000 µA, 200 µs pulsewidth). There was no obvious effect of GPiDBS on the quality of recording (i.e., stimulation artifacts were not evident). The compressed timescale of the figure obscures individual action potentials. **B.** An expanded representation of 5 s of the record resolves individual action potentials and illustrates how the onset of one block of GPiDBS (*) was associated with a modest reduction in mean spike rate. There was no apparent stimulation-induced change in spike amplitude or deterioration in recording quality. One short-
duration artifact coincided with the onset of GPiDBS (*). The times of individual GPiDBS stimuli are marked by vertical gray lines. **Ci.** Further expansion around the onset of GPiDBS (*) confirmed that recording quality was similar before and during GPiDBS. Action potentials had similar shapes before and during GPiDBS, even when spikes were coincident (↓) with the times of individual stimuli (vertical gray lines). **Ci.** The waveforms of individual action potentials are shown in expanded format. **Di.** Unprocessed microelectrode recording aligned on 1000 successive stimuli. (Data are from the period indicated by the horizontal gray bar at the bottom-right of *A.*) The times of stimulus delivery are indicated by vertical gray bars and *. (Two stimuli were delivered within the 8ms-long epochs plotted.) Action potentials tended to cluster at 0–1.5 ms and 3.5–4 ms after stimulus delivery. **Dii.** From the whole 400s-long data record, the shapes of spike waveforms during GPiDBS (black lines and gray areas showing mean ±SEM, respectively) fell within the 95% CIs for spike waveforms sorted from control (non-stimulation) periods (dotted lines). Spike waveforms were also virtually identical across the four peristimulus epochs. Horizontal brackets above indicate the peristimulus interval from which each waveform mean (±SEM) is derived. Numbers below indicate the number of sorted waveforms contributing to each mean. Voltage scales indicate 0.1 mV throughout the figure.

**Figure 5.** A polyphasic response to GPiDBS. **A.** Raw microelectrode signal during four blocks of GPiDBS (conventions as in Fig. 4A). **B.** Each block of GPiDBS induced a moderate sustained reduction in mean firing rate (spike density function, SDF; top), which was the product of a polyphasic pattern of increases and decreases in firing (bottom). Time-resolved peri-event histograms (0.2 ms bins), constructed from consecutive 2 s epochs around either sham events (during control periods) or real stimulation events (gray shading), are plotted versus the time from the beginning of recording (0 – 400 s). The color scale (right) represents firing rate. The
horizontal gray line indicates peri-event time zero (i.e., the time of stimulation delivery during GPiDBS blocks). **C left.** A mean time-resolved peri-event histogram summarizes the time-dependent nature of the short-latency responses to GPiDBS. (Color scale same as in **B.**) **C right; i. – v.** Peri-control and peristimulus change histograms (PCtH and PSTH, respectively), formed from 10 s epochs of the mean stimulation block (indicated by brackets labeled *i.* – *v.*, in **C left** bottom), were used for quantitative assessment of response magnitude, timing, and significance. The histograms illustrate peri-stimulus changes from the cell’s baseline firing rate (60.2 sp/s). **D.** Changes in firing induced by stimulation were highly significant relative to the deviations detected in control histograms. The areas of all deviations from baseline firing are plotted for two PCtHs (*i.* and *ii.* ) and three PSTHs (*iii.* – *v.*). A threshold for significance (horizontal black line) was derived from the mean and SD (black circle and error bar, respectively) of all PCtH areas. Red and blue symbols indicate areas of increases and decreases, respectively. For significant deviation areas (filled circles), character labels correspond with matching labels next to the PSTHs in **C right.**

**Figure 6.** Characteristic responses to GPiDBS. **A.** A monophasic increase in firing in a neuron sampled from GPe. The only significant stimulation-induced change began 3 ms after stimulation and lasted ~3.5 ms. The latency of the response did not shift significantly, although its magnitude declined across the 30 s block of stimulation. (Baseline rate = 64.3 sp/s.) **B.** A polyphasic response that included brief fixed-duration driving at ~1 ms latency. (Baseline rate = 47.4 sp/s). **C.** A typical sustained-type response in a neuron sampled from GPe. (Baseline rate = 44.1 sp/s.) Each panel of the figure shows: **Left** – a mean SDF and mean time-resolved peri-event histogram aligned on stimulation onset (*) across multiple 30 s blocks of GPiDBS (gray shading). Color plots in panels **A – C** use the scale found at the right of the time-resolved
histogram in \( A \) (in spikes/s). Text to the upper right of the SDF identifies the neuron and the number of GPiDBS blocks contributing to the data shown. \textit{Upper right} – Results from analysis of waveform isolation across the peristimulus interval. Horizontal and vertical scales indicate 1 ms and 0.1 mV, respectively. \textit{Lower right} – Mean PSTHs derived from the indicated epochs of the time-resolved histogram (\( i. – iii. \)). Otherwise, the figure follows the conventions outlined for Fig. 5.

\textbf{Figure 7.} Summary of effects of GPiDBS on pallidal firing. \textit{A.} Population mean PSTHs averaged across all phasic- and sustained-type cells from GPe and GPi (\textit{left} and \textit{right} panels, respectively). Gray shading and thin dotted lines indicate \( \pm \)SEM for phasic- and sustained-type responses, respectively. Box-and-whisker plots (to the \textit{right} of each population PSTH) indicate the median and range of GPiDBS-induced changes in mean firing rate. The horizontal ends of each box indicate upper and lower quartile values. Whiskers extend to the most extreme value \( 1.5 \times \) the interquartile range. Outliers are displayed as \(+\)'s. Notches in the sides of each box display the 95\% confidence interval of the median.

\textit{B.} Color plot of all PSTHs (one row per cell) classified as \textit{phasic} (top) or \textit{sustained} (bottom). Colors along each horizontal band indicate the significant changes in firing rate of one cell induced by stimulation (red–yellow = increases; blue–cyan = decreases; firing rate scale at \textit{far right}). \textit{Black} = no significant change in a PSTH. Individual phasic PSTHs are sorted top-to-bottom by a response’s score on the first principal component across all phasic PSTHs. \textit{C.} Peristimulus distributions of increase maxima (above zero) and decrease minima (inverted below zero) as a percent of all phasic-type cells. Times of individual maxima and minima were collected into 1 ms bins to aid visualization. (Statistical comparisons were performed on
cumulative distributions of the raw latency values.) Scales are the same for right and left
columns of the figure unless noted otherwise.

**Figure 8.** Latency and magnitude shifts in responses.  *A.* An example of gradual shifts in
response latency across the stimulus block in a cell recorded from GPe. Results from the timing
analysis are overlaid on a time-resolved peristimulus change histogram [30 histograms (0.1 ms
bins), one histogram for each second of the mean stimulation block]. Dashed lines plot the
latencies of peak changes across the 30 histograms (green and magenta for increases and
decreases, respectively). Solid lines show the best piece-wise linear fit to each dashed line.
Color scale (*right*) in spikes/second: red–yellow = increases; blue–cyan = decreases, both from
a baseline firing rate of 47.4 sp/s.  *B.* Slopes of temporal shifts (ms shift per second of
stimulation, derived from the best-fit function) are plotted versus the initial latency of all
responses. Filled symbols indicate responses with significant latency shifts that asymptoted
within the stimulation block (black) or did not asymptote (red). A histogram (right) summarizes
the distribution of slopes.  *C.* Slopes of shifts in response magnitude (measured as spikes/s shift
per second of stimulation) are plotted versus the initial latency of responses. Filled symbols
indicate magnitude shifts that asymptoted within the stimulation block (black) or did not
asymptote (red). Note that the ordinates of the plots in *B* and *C* are split to provide greater
resolution for points clustered close to a slope of zero.

**Figure 9.** Two examples of antidromic-like activation.  *Ai.* and *Bi.* action potentials at a short
fixed latency following stimulation delivery (vertical dotted line).  *Aii.* and *Bii.* collision-like
phenomenon in which stimulation failed to evoke action potentials when spontaneous spikes
occurred immediately prior to stimulation delivery.
**Figure 10.** Burst firing during GPiDBS.  
A	extit{i}. Raster representation of a typical 4 s period of neuronal activity off-GPiDBS. (The peristimulus response of this neuron is illustrated in Fig. 6B.) Black vertical ticks indicate times of individual action potentials. Horizontal blue bars show times of bursts as determined by the Legendy surprise method (Legendy and Saleman 1985; Wichmann and Soares 2006).  
A	extit{ii}. An exemplar spike train from the same neuron during GPiDBS (gray shading). The incidence of bursting increased during GPiDBS and burst duration decreased.  
A	extit{iii}. An expanded section of the same spike train during GPiDBS illustrates how bursts persisted during GPiDBS because of multi-stimulus periods of response failure. Gray vertical ticks show times of individual GPiDBS shocks.

**B and C.** A summary of measures of neuronal burstiness under control and GPiDBS conditions (“OFF” and “ON” stimulation respectively, plotted on abscissae and ordinates). Sub-panels plot two measures of burstiness (left: fraction of total time spent in bursts, right: fraction of total spikes found in bursts). Each sub-panel plots one symbol for each neuron recorded from GPe and GPi (B and C, respectively). The shape of the symbol reflects the neuron’s general response to GPiDBS (see legend at left). Neurons with identical measures of burstiness OFF and ON stimulation have symbols on the line of unity (diagonal dotted line). Confidence ellipses (computed to encompass 50% of the points assuming gaussian distributions) illustrate the similarity of the population’s burst measures under OFF and ON conditions.

**D and E.** Mean spike density functions (“burst-triggered averages”; ± 95% confidence interval) aligned on burst onset times for all bursts detected off- and on-GPiDBS (black and red traces, respectively). Mean pre-burst firing rate has been subtracted from each average to aid comparison of the burst characteristics. In B (from the same GPe cell as used in A), GPiDBS significantly increased intra-burst firing frequency but reduced burst duration. (Baseline rate =
47.4 sp/s). C compares the burst characteristics of a GPi cell that responded phasically to stimulation (inset peristimulus histogram). (Baseline rate = 58.6 sp/s).

F and G. Mean burst-triggered averages for all GPe and GPi cells, respectively. The mean burst characteristics were remarkably similar during off- and on-GPiDBS conditions for both GPe and GPi populations.

**Figure 11.** Effects of GPiDBS on oscillatory firing. A. Autocorrelation (top) and power spectrum (bottom) from a GPe neuron that responded phasically to GPiDBS. *Left column:* detail for short lags and low frequencies. The autocorrelation from control periods (*thin black line*) showed distinct peaks and valleys indicative of oscillatory firing. Oscillatory firing was confirmed by the presence of significant peaks in the power spectrum at 5.9 and 12.7 Hz (*). *Horizontal dotted line:* threshold for a significant elevation of spectral power. During GPiDBS, low frequency oscillations were replaced by a highly significant peak at the frequency of stimulation (*†, right*). Note the different scales for top and bottom halves of the spectra ordinates. *Inset:* The same neuron’s phasic peristimulus response following the conventions of Fig. 6. (Baseline rate = 40.8 sp/s). B. DBS-induced suppression of oscillatory activity did not require a peristimulus response. Spectra are shown for a GPi neuron that had no significant peristimulus response to stimulation (*inset*). The peak at 13.7 Hz was reduced significantly during GPiDBS (*). (Baseline rate = 52.6 sp/s) C. DBS-induced suppression of synchronized oscillations. Coherence spectra are shown for a pair of GPe neurons, both of which had significant polyphasic responses to stimulation (*inset*). The coherence peak at 10.7 Hz was suppressed completely during GPiDBS (*). (Baseline rates = 45.8 and 68.4 sp/s) D. GPiDBS reduced the prevalence of low frequency oscillations. The bar plot indicates the fraction of cells with one or more significant peaks in the indicated frequency ranges during control periods (*Off*).
DBS) and after 20 seconds of stimulation (On DBS). E. GPiDBS reduced the prevalence of LF synchronized oscillations. The bar plot indicates the fraction of cell pairs with one or more significant coherence peaks in the indicated frequency ranges during control periods (Off DBS) and after 20 seconds of stimulation (On DBS). * = P<0.05; ** = P<0.005 (χ² test).

**Supplementary Figure 12.** The effects of GPiDBS varied modestly as a function of distance from stimulating contact. A. Stimulation-induced decreases in firing became slightly more common at greater distances from the stimulating contact. B. Sustained neuronal responses were most common in close proximity to (<1.5 mm away from) the stimulating contact, but this trend was not significant.

**Supplementary Figure 13.** Summary of stimulation currents used and response types observed over time in the two animals (left and right columns). The results presented in A – C are divided into four time periods (quarters), each containing nearly equal numbers of consecutive recording sessions. (The number of recording sessions contributing to each quarter is indicated at the top of the figure.) A. The distribution of currents used in each period is indicated. The size of each symbol reflects the fraction of recordings during the quarter period that used the indicated current. B. The relative prevalence of stimulation-induced increases and decreases is indicated for each of four recording periods. No significant differences were found in either animal (χ²<3.0, P>0.1). C. The relative prevalence of stimulation-induced phasic and sustained responses is indicated for each of four recording periods. No significant differences were found in either animal (χ²<2.5, P>0.2). D. The effects of GPiDBS on the frequency of postural transients (mean ±SEM change from baseline) is plotted for all clinical testing sessions that
occurred within each of the indicated quarter periods. $N/A$ indicates that clinical testing was not performed during the quarter period.
**TABLES**

*Table 1 – Prevalence of effects of GPeDBS and effects on mean rate*

Percents in *Decrease(s)* and *Increase(s)* categories sum to >100% because some cells had both increases and decreases.

<table>
<thead>
<tr>
<th>Any effect</th>
<th>Response sign</th>
<th>Response type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Decrease(s)</td>
<td>Increase(s)</td>
</tr>
<tr>
<td><strong>GPe</strong> (n=69)</td>
<td>62 (90%)</td>
<td>47 (76%)</td>
</tr>
<tr>
<td><strong>GPi</strong> (n=33)</td>
<td>25 (76%)</td>
<td>19 (76%)</td>
</tr>
<tr>
<td><strong>Total</strong> (n=102)</td>
<td>87 (85%)</td>
<td>66 (76%)</td>
</tr>
</tbody>
</table>
Table 2 – Effects of GPiDBS on mean firing rate

Mean (SD) changes from baseline firing rates (sp/s). Results in each cell are from cells that matched the categories indicated in row and column headings. Numbers are the same as those indicated in Table 1. * $P<0.05$, ** $P<0.005$ – one sample t-test versus zero change from baseline.

<table>
<thead>
<tr>
<th></th>
<th>Baseline rate (sp/s)</th>
<th>Any effect (change re baseline)</th>
<th>Response sign</th>
<th>Response type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Decrease(s)</td>
<td>Increase(s)</td>
<td>Phasic</td>
</tr>
<tr>
<td>GPe (n=62)</td>
<td>60.8</td>
<td>−0.9</td>
<td>−6.7*</td>
<td>+12.9**</td>
</tr>
<tr>
<td></td>
<td>(21.7)</td>
<td>(23.4)</td>
<td>(22.1)</td>
<td>(22.7)</td>
</tr>
<tr>
<td>GPi (n=25)</td>
<td>61.7</td>
<td>−6.9*</td>
<td>−11.0*</td>
<td>+2.6</td>
</tr>
<tr>
<td></td>
<td>(23.6)</td>
<td>(16.1)</td>
<td>(16.2)</td>
<td>(14.1)</td>
</tr>
<tr>
<td>Total (n=87)</td>
<td>61.1</td>
<td>−2.6</td>
<td>−7.9**</td>
<td>+10.2**</td>
</tr>
<tr>
<td></td>
<td>(22.2)</td>
<td>(21.6)</td>
<td>(20.5)</td>
<td>(21.1)</td>
</tr>
</tbody>
</table>
Table 3 – Effects of GPiDBS on burst firing

Mean fractions (SEM, as percentages) of time spent in bursts (left) and spikes in bursts (right) off stimulation (Control) and during GPiDBS. Results in each cell are from cells that matched the categories indicated in row and column headings. GPiDBS had no significant main effects or interactions (stimulation×location ANOVA).

<table>
<thead>
<tr>
<th></th>
<th>Time in burst</th>
<th></th>
<th>Spikes in burst</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>GPiDBS</td>
<td>Control</td>
</tr>
<tr>
<td>GPe</td>
<td>8.15 ( .58 )</td>
<td>8.12 ( .58 )</td>
<td>26.70 (2.15)</td>
</tr>
<tr>
<td>(n=57)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPi</td>
<td>6.82 ( .80 )</td>
<td>7.01 ( .80 )</td>
<td>22.16 (2.94)</td>
</tr>
<tr>
<td>(n=28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7.49 ( .49 )</td>
<td>7.57 ( .49 )</td>
<td>24.43 (1.82)</td>
</tr>
<tr>
<td>(n=85)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>