Pallidal stimulation suppresses pathological dysrhythmia in the parkinsonian motor cortex

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Historically, pathological changes in tonic firing rate at critical nodes of the cortico-basal ganglia (BG) network were thought to underlie parkinsonism (Albin et al. 1989; DeLong 1990). This hypothesis is challenged by other studies suggesting instead the importance of increased burst firing, excessive oscillatory and synchronous activity in the beta-frequency range, and a breakdown of functional segregation in the cortico-BG network (Filion and Tremblay 1991; Raz et al. 2000; Goldberg et al. 2002, 2004; Hutchison et al. 1994, 2004; Wichmann et al. 1999; Soares et al. 2004). Excessive beta-oscillatory activity is hypothesized to induce an antikinetic state, which inhibits voluntary movement in downstream BG-recipient motor centers [e.g., primary motor cortex (M1) (Marsden et al. 2001b; Brown et al. 2004b)]. Therefore, M1 activity is believed to play a critical role in the expression of PD symptoms.

Early studies hypothesized that DBS works by inhibiting neuronal activity in the vicinity of the stimulating electrode, analogous to a reversible pallidotomy (Benazzouz et al. 1995; Boraud et al. 1996; Dostrovsky et al. 2000). It was suggested that this inhibition of BG output reduces the excessive inhibitory tone impinging on the motor cortex.

We have recently reported that therapeutic GPI-DBS in parkinsonian nonhuman primates (NHPs) induces a complex combination of changes in activity in the pallidum (McCairn and Turner 2009). At the single unit level, this includes increases or decreases in tonic firing rate, plus phasic and dynamic entrainment by the stimulation pulse. Across the population, however, we observed only a negligible reduction in mean firing rate (−6.9 sp/s) and no change in burstiness. In contrast, beta oscillations and synchrony were reduced dramatically. Similar observations have been made by others during high-frequency microstimulation of the GPI (Bar-Gad et al. 2004; Erez et al. 2009; Chiken and Nambu 2013) or the STN (Meissner et al. 2005; Moran et al. 2011).

These findings suggest that suppression of system-wide pathological oscillations and synchrony are important components of the mechanism of action of GPI-DBS. To test that hypothesis, we studied the effects of GPI-DBS on the activity of multiple single units in the M1 of macaque monkeys that were rendered parkinsonian using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).
Antidromic-driven high-frequency resonance in cortex has been postulated to play a key role in the therapeutic mechanism of STN-DBS (Li et al. 2007; Dejean et al. 2009; Gradinaru et al. 2009; Li et al. 2012) and perhaps GPI-DBS as well (Johnson et al. 2009). Our experimental methods allowed us to assay directly the prevalence of antidromic-like driving and thus the potential involvement of high-frequency resonance of M1 during therapeutic GPI-DBS.

**METHODS**

Two mature female (3 and 4 kg) cynomolgus macaques (Macaca fascicularis) were used for this study (monkey C and monkey E). All aspects of animal use were approved by the University of California, San Francisco Animal Care and Use Committee and were in accord with the Guide for the Care and Use of Laboratory Animals (National Research Council 1996).

*Surgery.* After the animal was acclimatized to the recording environment, the animal underwent aseptic surgery under isoflurane anesthesia, following ketamine induction (10 mg/kg im). A cylindrical titanium recording chamber (19-mm inner diameter) was implanted over a craniotomy to allow access to the right GP and M1 via a coronal approach (Szabo and Cowan 1984) (35° from vertical; Horsley-Clark anterior: 15 mm, lateral: 13 mm, depth: 18 mm) using an Alpha Omega four channel microdrive. The chamber and head fixation hardware were fixed to the skull using bone screws and methylmethacrylate cement. Prophylactic antibiotics and analgesics were administered postsurgically.

*MPTP administration.* Experimental parkinsonism was induced using the overlesioned hemi-parkinsonian model (Oiwa et al. 2003; McCairn and Turner 2009). Animals received initial infusions of MPTP via the right intracarotid artery (0.55 mg/kg), followed by supplemental systemic doses (0.4 mg/kg iv, 0.35–0.75 mg/kg im) over the course of several weeks, until a stable, moderate to severe parkinsonism was induced. Symptom severity was rated regularly according to a scale developed by Schneider et al. (2003).

*Indwelling GPI-DBS macroelectrode.* Standard microelectrode mapping was used to identify the chamber coordinates for DBS electrode placement, which allowed targeting of the GPI: A custom DBS electrode was assembled from two Teflon-coated Pt-Ir microwires (50-μm diameter) glued inside a 5-mm length of 28-gauge stainless steel cannula (Turner and DeLong 2000; McCairn and Turner 2009). The cut ends of the microwires extended below the cannula tip by 1.0 and 1.5 mm. Insulation was stripped from ~0.5 mm of the distal ends of each microwire (surface areas: ~0.08 mm², impedance: 5–10 kΩ at 1 kHz, charge density: 100 μC·cm⁻²·phase⁻¹). The electrode assembly was implanted transdurally, via the chronic recording chamber using a protective guide cannula (26 gauge) and stylog mounted in the microdrive. Upon reaching the target location for implantation, the guide tube and stylos 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 (which was subsequently sealed with cyanoacrylate glue) and soldered to a head-mounted connector.

*Clinical testing.* To measure the therapeutic effects of GPI-DBS, transient changes in posture and rigidity were measured at the elbow joint using a servo-controlled torque motor (Acrotech, Pittsburgh, PA). 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° around the elbow’s neutral position at a cycle rate of 1 Hz, with reactive torque sampled from the motor at a rate of 1 KHz (see Fig. 2Bi). Significant changes in the work required to move the elbow were detected by converting the torque transients per sinusoid into total “work” required expressed as Newton meters per degree (McCairn and Turner 2009). These data were then tested for significance by constructing histograms of the mean work on- and off-stimulation, with confidence limits set at P = 0.01 (see Fig. 2Bi).

*Data acquisition and artifact subtraction.* The extracellular activity of multiple single neurons in M1 was sampled using a four-electrode microdrive and glass-insulated tungsten microelectrodes (0.5–1.0 MΩ 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 to 6 kHz; First order Butterworth) and saved to disk either as continuous data or as 38 sample-long snippets of the continuous data stream.

During periods of GPI-DBS, online signal processing (Tucker Davis Technologies) was used to eliminate the large stimulation-induced electrical artifacts in the neuronal recordings (see Fig. 2, Ai–Aii). This was accomplished through use of a low gain headstage and preamplifier and 16-bit A/D conversion to prevent artifact-induced saturation of the analog electronics, synchronization of stimulus delivery, analog-to-digital conversion, and artifact subtraction using the same microsecond-accurate clock, thereby eliminating shock-to-shock variation in the shape of the artifact caused by temporal jitter, and subtraction of an artifact “template” from the data stream at the time of every shock. The template 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). This artifact subtraction algorithm was applied in real-time during simultaneous data acquisition across four channels 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. Offline analyses, using custom Matlab scripts, 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. Additional quality testing of spike waveforms during stimulation was achieved through constructing peristimulus sweeps of the recorded signal aligned to each stimulus pulse. The mean waveform shape was analyzed in 2-ms epochs pre- and poststimulus delivery to determine that action potentials were of a consistent size and shape with those recorded outside the stimulus period (see Fig. 2Bii). Interspike interval (ISI) histograms were then constructed to determine if there were significant increases or decreases in activity equal to the stimulation rate. Further details on the efficacy of the artifact subtraction methods can be found in McCairn and Turner (2009).

*Recording and stimulation protocol.* The animal was seated in a primate chair and placed in a sound attenuating booth. The primary motor cortex was identified before experimental recording sessions using sensory examination and intracortical microstimulation (5–50 μA, 20 biphasic pulses, 200 μs each phase, 300 Hz; Fig. 1B). During recording experiments, up to four microelectrodes were lowered transdurally into the primary motor cortex via the implanted recording chamber. As soon as the action potentials of one or more single neurons were 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 to the GPI using an 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. Data were then collected during an initial 1-min off-stimulation ("baseline" or "control") period, followed by multiple 30-s blocks of stimulation (DBSstim) each separated by >30 s of no stimulation (DBSoff). Stimulation currents ranged from 200 to 1,000 μA (mean current 400 μA, SD = 120 μA). All of the recordings were obtained during rigidity testing. During recording, the animal’s left (contralateral) arm was secured in...
evidence that single unit isolation was corrupted by the appearance of voltage transients caused by GPi-DBS. Following sorting, each neuron’s autocorrelation function was inspected to determine that each unit displayed a clear refractory period. This precluded the possibility that the identified unit was corrupted by shock artifacts.

The primary tests for long-duration effects of DBS on a neuron’s firing were performed on a histogram constructed around DBS on [i.e., a peri-DBS histogram (PDBSH), bin size = 1 s]. Significant responses to GPi-DBS were initially determined via 99% confidence interval threshold crossings. The latency of any significant response was taken as the first bin that crossed the 99% confidence interval relative to the start of DBS. To calculate population-scale mean firing rate changes during GPi-DBS, the data were normalized by subtracting the mean of the 30-s prestimulation data just before DBS on to give a measure of neural activity around a baseline of zero. To assess the stability of any rate changes across the stimulation period, the peristimulus histogram was divided into equal epochs covering the pre-, actual, and post-stimulation period, each consisting of 10 s. The data for each stimulation period (epochs 4–6) were compared with control periods (epochs 1–3 and poststimulation periods (epochs 7–9) using multiparameter ANOVA with Tukey’s honestly significant difference error correction (see Fig. 3).

DBS-induced changes to the pattern of firing were investigated by building ISI histograms and testing the coefficient of variation (CV). The different experimental states (DBS on and DBS off) were concatenated, and only sessions with >1,000 spikes were used for further analysis. The data from each neuron were then pooled and differences in mean CV between the experimental states were determined using Student’s t-test.

Burst discharges were detected using the Legendy surprise method (Legendy and Salcman 1985), which is described in detail by Wichmann and Soares (2006). Bursts were defined as groups of three or more spikes whose ISIs were unusually short compared with other ISIs of a spike train. The parameters for analysis used a surprise threshold of 5, which equates to an alpha <0.001 that the candidate burst would occur as part of a Poisson-distributed sequence of spikes. Spike trains were concatenated across all off- and on-stimulation periods. The concatenated control and stimulation trains were analyzed separately. Three fundamental measures for burst analysis were used: 1) the number of identified bursts in the 30 s before stimulation was compared with the number of bursts in the 30 s of stimulation; 2) the fraction of time within a spike train spent in bursts; and 3) the fraction of total spikes contained within bursts. A change in a single neuron’s propensity to fire in bursts between different experimental states was tested using a Wilcoxon rank-sum test. 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 (McCairn and Turner 2009; Pasqueau and Turner 2011). Separate averages were computed across the onset of bursts detected in control and stimulation trains. The mean preburst firing rate (500 ms immediately before burst onset) was subtracted from each burst-triggered average to aid comparison between stimulation conditions. Significant differences between the two states were tested for by performing a t-test across the concatenated pre- and post-DBS matrices, which gave a P value for each analyzed bin. Comparing burst-triggered averages provided a way to detect potential effects of DBS on the general structure of bursts without extracting an unreasonably large number of burst parameters.

Oscillations in neuronal activity were detected using methods adapted from previous studies (McCairn and Turner 2009; Pasqueau and Turner 2011). To detect oscillations, a discrete Fourier transform was applied to nonoverlapping 1,024-point segments of the spike delta function (1-kHz resolution) and 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. Peaks in the normalized spectra between 3 and 200 Hz were tested for significance relative to the SD of the normalized spectra in the 340- to

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**Fig. 1.** Histology and intracortical mapping. A: cresyl violet stained slide, showing a coronal section through the electrode implanted hemisphere, which highlights the gliosis mark produced by placement of the stimulating electrode (black arrow). Aii: tyrosine hydroxylase was depleted throughout the dorsal caudate (Cd) and putamen (Pu) but preserved in the limbic regions of the ventral caudate (black arrow). GPi, globus pallidus internus; GPe, globus pallidus externus. B: chamber schematic showing the areas of the chamber sampled for primary motor cortex (M1) recording and microexcitability sites at which intracortical microstimulation elicited movement of specific body parts (filled shapes). OF, orofacial.
500-Hz “control” range. Frequencies up to 200 Hz were included to detect the fundamental spectral correlates of phasic responses to stimulation. 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 3–160 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). Note that we did not perform shuffled normalization of the computed spectra (i.e., following Rivlin-Etzion et al. 2006), consistent with our previous study of M1 activity in parkinsonism (Pasquereau and Turner 2011). Thus, in the current study, spectral peaks could reflect either rhythmic oscillations in firing rate or the rhythmic generation of individual spikes. A full justification for this approach is provided in Pasquereau and Turner (2011).

Functional connectivity and the presence of correlated activity between pairs of neurons in the spectral domain were determined using coherence analysis. Coherence was calculated by dividing the cross spectral density of the two cells being analyzed by the square root of the product of the autospectra of each signal (1-ms resolution, maximum lag = 1 s, smoothed with a \( \sigma = 3 \) ms Gaussian).

**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- \( \mu \)m sections, mounted, and stained with cresyl violet (Fig. 1Ai). To verify depletion of dopaminergic terminals in the striatum, selected sections were stained using tyrosine hydroxylase (TH) immunohistochemistry. These sections were incubated for 24 h with a mouse anti-TH monoclonal antibody, followed by 1 h of incubation with a biotinylated horse anti-mouse antibody (Chemicon International, Temecula, CA). Sections were then incubated with streptavidin for 1 h and revealed using DAB-Vector SK-400 (Vector Laboratories, Burlingame, CA; Fig. 1Ai). The locations of stimulating electrodes within the GPi were clearly visible based on the locations of defects left in the histologic sections by the electrode shaft.

**RESULTS**

**Clinical status and reconstruction of the stimulation site.** After MPTP administration, both animals showed moderate to severe parkinsonian signs bilaterally. Daily symptom ratings using the Schneider scale for parkinsonism (Schneider et al. 2003) were 37 and 38 for the two animals, compared with a mean of 8 before MPTP treatment. Observable symptoms included decreased spontaneous movement, limb rigidity, bradykinesia, action tremor, and stooped posture. Both animals had very low rates of spontaneous movement in their home cages (mean 9.0 movements/min), compared with the >50 movements/min rate typical for neurologically intact macaques.

Postmortem histological examination confirmed that the stimulating electrode targeted the GPi in both animals, an example of which is shown in Fig. 1Ai. In the figure, gliosis caused by the macroelectrode assembly is visible; the tip of the stimulating probe and actual site of stimulation (indicated by an arrow) is located in the dorsomedial regions of the GPi, adjacent to the internal capsule. The animals also showed widespread depletion of tyrosine hydroxylase, the rate-limiting enzyme for conversion of tyrosine to dopamine (Fig. 1Ai). Preserved fibers in the example shown here (indicated by arrows) can be seen at the level of the ventral caudate (Fig. 1Ai), a common feature observed with MPTP toxicity (Jan et al. 2003).

GPI-DBS was associated with a reduction in muscular rigidity, which persisted for \( >10 \) s following the cessation of GPI-DBS (Fig. 2Bi). GPI-DBS was also associated with an increase in transient postural shifts; these changes in body posture were readily detectable in the torque traces as the animal moved against the arm that was fixed in the splint of the torque motor (Fig. 2Bii). The behavioral results with respect to muscular rigidity and postural transients have been published previously, and a more detailed description of the experimental findings can be found in McCain and Turner (2009).

**Electrophysiological data and responses to GPI-DBS.** A total of 132 cells (monkey C ( \( n = 78 \) ) and monkey E ( \( n = 54 \) )) were sampled from the arm area of M1 during GPI-DBS. All of the cells were recorded while a torque motor moved the contralateral elbow through 20° sinusoidal displacements. Of the 132 cells sampled, 36% (monkey C ( \( n = 22 \) ) and monkey E ( \( n = 25 \) ), total \( n = 47 \) ) showed a significant change in mean firing rate during GPI-DBS. The changes in firing rate manifested as either increases in activity [27%, \( n = 35 \), monkey C ( \( n = 17 \) ) and monkey E ( \( n = 18 \) ); Fig. 3, A and C] or decreases [9%, \( n = 12 \), monkey C ( \( n = 5 \) ) and monkey E ( \( n = 7 \) ); Fig. 3, B and D]]. Increases in firing were more common than decreases (\( \chi^2 = 9.4; P < 0.01 \)). There was, however, no significant difference in the latency of firing rate changes for the two response types. The mean onset latency was 2.7 s (SD ± 2.9) for increases in firing rate and 2.0 s (SD ± 3.4; \( P = 0.4, \) t-test) for decreased firing rates. Across the population, GPI-DBS had no net effect on the population mean firing rate (Fig. 3E). The population mean firing rate was slightly elevated during the stimulation period (+0.46 ± 0.17 sp/s), but this increase was nonsignificant (\( P = 0.05, \) ANOVA).

**Minimal antidromic-like driving in M1 during GPI-DBS.** Previous studies, using GPI-DBS in MPTP monkeys (Johnson et al. 2009), have suggested that stimulation of the output nucleus of the BG leads to an antidromic activation motor cortices, specifically M1. We examined our recordings for evidence of short-latency antidromic-like activation of M1 by searching for driving of a neuron’s action potentials at a fixed latency following individual stimuli delivered to the Gpi. Our ability to record continuously throughout the peristimulus interval, unimpeded by shock artifacts, allowed us to determine the prevalence of short-latency driving of M1 during GPI-DBS and thereby assess the idea that antidromic induction of high-frequency resonance in the cortex plays a critical role in the therapeutic mechanism of DBS (Li et al. 2007; Dejean et al. 2009; Johnson et al. 2009; Li et al. 2012).

Among the 132 cells in the database, short fixed-latency driving was found in only one neuron (<1% of all neurons studied). For this one neuron, GPI stimulation at 400 \( \mu \)A (150 Hz) induced action potentials at a constant short latency (2.0 ± 0.4 ms) in response to a small fraction of the stimuli delivered (fraction of stimuli that yielded an antidromic spike = 6.4%). The temporally locked activity was present for short periods (mean 0.18 ± 0.04 s) separated by long periods of silence (5.3 ± 14.3 s). The mean firing rate during the periods of antidromic-like driving was 101.3 ± 15.6 Hz. The low fraction of cells showing any indication of antidromic-like driving runs contrary to the idea that antidromic-induced cortical resonance is a primary component of therapeutic GPI-DBS.

**GPI-DBS did not suppress burst firing.** Although one therapeutic mechanism hypothesized for GPI-DBS is suppression...
of burst firing (Shi et al. 2006; Vitek 2002), our previous work found no evidence for a decrease in burst firing in the pallidum during GPI-DBS (McCairn and Turner 2009). Similarly, we found here that few cells (11%, \( n = 11005 \)) showed a change in the prevalence of burst firing between the two states and those cells working shows no evidence of shock artifacts while action potentials and recording noise are preserved. (Note the different voltage scales in Aii and Aiii.) Action potentials that were completely obscured by artifacts in the unsubtracted data stream were easily detected in the processed data stream. Aiv: peristimulus sweeps (\( n = 479 \)) of the recorded signal (top trace) demonstrate that it was possible to record and discriminate action potentials across the entire peristimulus period, including times close to stimulus pulse delivery (time zero). The bottom traces show statistical tests of the efficacy of the artifact rejection. The mean waveforms of spikes discriminated during DBS (thick black lines; ±SE indicated by light gray shading) were required to fall within the 95% confidence interval for all spikes discriminate during nonstimulation periods (dark gray shading). The test was conducted separately for four 2-ms epochs, defined by the vertical dashed lines. Bi: clinical testing using the torque motor. The figure shows one unprocessed record of constant angular displacement (gray trace) ±20° at 1 Hz and corresponding torque trace (black trace) off- and on-stimulation. Bii: measure of elbow rigidity calculated as cycle-by-cycle work derived from the integrated resistive torque (i.e., “work”) required to move the elbow joint through a ±20° cycle at 1 Hz from one recording session that consisted of ten 30-s long periods of stimulation. Note the large reduction of work during application of GPI-DBS and the persistence of the effect after stimulation has stopped. Biii: postural transients (*) increased in frequency during GPI-DBS. This particular example shows raw torque (black traces) for 7 successive blocks of GPI-DBS (gray shading) sampled from monkey C.

Fig. 2. Stimulation artifact rejection and behavioral response to deep brain stimulation (DBS). Ai: neuronal signal from M1 acquired just before and during GPI-DBS, but with artifact subtraction disabled. Note the presence of large voltage transients during DBS (black) preventing discrimination of single unit spikes. Aii: expanded view of a short time period during DBS showing the large voltage transients that occurred time locked to the delivery of the stimulation pulse (vertical dashed lines; 150-Hz, 1-mA, 200-μs pulse width). Aiii: 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. (Note the different voltage scales in Aii and Aiii.) Action potentials that were completely obscured by artifacts in the unsubtracted data stream were easily detected in the processed data stream. Aiv: peristimulus sweeps (\( n = 4500 \)) of the recorded signal (top trace) demonstrate that it was possible to record and discriminate action potentials across the entire peristimulus period, including times close to stimulus pulse delivery (time zero). The bottom traces show statistical tests of the efficacy of the artifact rejection. The mean waveforms of spikes discriminated during DBS (thick black lines; ±SE indicated by light gray shading) were required to fall within the 95% confidence interval for all spikes discriminate during nonstimulation periods (dark gray shading). The test was conducted separately for four 2-ms epochs, defined by the vertical dashed lines. Bi: clinical testing using the torque motor. The figure shows one unprocessed record of constant angular displacement (gray trace) ±20° at 1 Hz and corresponding torque trace (black trace) off- and on-stimulation. Bii: measure of elbow rigidity calculated as cycle-by-cycle work derived from the integrated resistive torque (i.e., “work”) required to move the elbow joint through a ±20° cycle at 1 Hz from one recording session that consisted of ten 30-s long periods of stimulation. Note the large reduction of work during application of GPI-DBS and the persistence of the effect after stimulation has stopped. Biii: postural transients (*) increased in frequency during GPI-DBS. This particular example shows raw torque (black traces) for 7 successive blocks of GPI-DBS (gray shading) sampled from monkey C.
corresponds to the start of an identified burst (DBSoff burst/s median = 12, DBSon burst/s median = 0; P = 0.0003, Wilcoxon rank sum). Figure 4Aii shows an example of a cell that increased its propensity to fire in bursts following the onset of stimulation. DBSoff burst/s median = 0, DBSon burst/s median = 5; P = 0.0006, Wilcoxon rank sum). The low number of cells with modified burst firing between the two states and roughly equal propensity to decrease and increase burstiness led to no significant change in measures of burst firing across the population (P > 0.2, paired t-test; Fig. 4, Bi and Bii). Similarly, the population-averaged burst structure was indistinguishable on- and off-stimulation (Fig. 4Biii).

CV of ISIs also showed no difference between the off- and on-stimulation conditions (DBSoff CV = 1.29, SD ± 0.5 vs. DBSon CV = 1.3, SD ± 0.7; P = 0.7, paired t-test; Fig. 4Biv). Therefore, although GPi-DBS affected the prevalence and structure of bursts in some individual neurons, these effects yielded no net change in the amount or pattern of burst firing or regularity of firing when averaged across the population.
**GPi-DBS suppressed beta oscillatory activity.** A subset of M1 neurons (20%, \( n = 27 \)) displayed significant rhythmic firing in the beta (12–30 Hz)-frequency band (Fig. 5). The mean oscillation frequency for these cells was 17.25 ± 4.0 Hz. Figure 5A shows an example of an M1 neuron’s autocorrelation function off- and on-stimulation. In the off-stimulation condition (black), the autocorrelation function shows substantial peaks and valleys indicative of oscillatory firing in the beta-frequency range. This motif transitioned to a flatter profile in the on-stimulation condition (Fig. 5A, gray trace). Spectral analysis of this neuron’s spike train showed a marked peak at 14.8 Hz in the off-stimulation condition that was attenuated significantly during GPi-DBS. Consistent with the observation that short-latency entrainment and high-frequency resonance were rare in our data, the reduction in beta oscillations was achieved without the appearance of peaks at the frequency of stimulation (150 Hz) or its lower harmonics (Fig. 5B). GPi-DBS reduced oscillatory power in all beta-rhythmic cells but one, causing a significant reduction in the overall prevalence of oscillatory activity (\( \chi^2 = 27.01; P < 0.0001; \) Fig. 5C). In contrast, the prevalence of high-frequency oscillations did not differ significantly between DBSon and DBSoff conditions (i.e., the gamma band and oscillations between gamma and 200 Hz).

GPi-DBS also reduced oscillations that were coherent (i.e., synchronized) between neuron pairs in M1. A total of 207 neuronal pairs were sampled simultaneously from different electrodes. Coherent activity <30 Hz was found in 18% of the recorded pairs. Figure 6A shows an example in which, before stimulation, the firing of a pair of neurons was highly synchronized at 15.6 Hz (\( R^2 = 0.15; \) Fig. 6A, black trace). It can be seen that GPi-DBS completely abolished this synchronized oscillation (Fig. 6A, gray trace). In agreement with this example, stimulation reduced the overall prevalence of synchronized firing across the population of cell pairs studied (Fig. 6B; \( \chi^2 = 28.13; P < 0.0001 \)). In summary, GPi-DBS reduced the prevalence of pathological low-frequency oscillations (<30 Hz), both in the firing of individual neurons and in the synchronized firing of neuronal pairs.

**DISCUSSION**

Our results demonstrate that GPi-DBS induces both increases and decreases in the firing rates of individual neurons within M1, with excitation being the predominant response among the population of cells that were directly affected by stimulation. However, population-scale mean firing rates were only marginally affected between experimental states (DBSoff vs. DBSon). GPi-DBS also had a minimal impact on burst statistics and firing variability measured at the population level. The most obvious neurophysiological correlate of reduced parkinsonism was a suppression of rhythmic firing and synchronous activity in the beta (12–30 Hz) range. Antidromic-driven activity and associated high-frequency resonant activity were uncommon in this study. It is, therefore, unlikely that high-frequency resonance in the cortex (e.g., at the 150-Hz stimulation frequency or harmonics) played an important role as a therapeutic mechanism during GPi-DBS.

**Potential confounds and limitations.** Although we used low-impedance macroelectrodes and clinically relevant currents and frequencies, the experimental electrodes did not replicate the exact geometry of clinical electrodes. Additionally, the pulse width used in this study was 0.2 ms (vs. <0.1 ms used clinically). Another limitation is that we were unable to confidently reconstruct the laminar position or the efferent targets of the M1 neurons studied. Recent work has shown that subpopulations within M1 are affected differently by the parkinsonian state (Pasquereau and Turner 2011). It is also important to recognize that the present results, collected while an animal’s arm was being moved passively via a torque motor, may have limited relevance for other behavioral contexts [e.g.,
We observed two primary effects of GPi-DBS on the animal’s behavior: a reduction in limb rigidity and an increase in the frequency of postural transients. The latter presumably reflected movements executed by the animal to achieve a more comfortable resting position. A potential confound is that the observed suppression of beta activity may be secondary to the increased frequency of movement and not a direct effect of stimulation. It should be pointed out, however, that although GPi-DBS often increased the frequency of spontaneous movement, these movements appear sporadically. The suppression of low-frequency oscillatory activity was still present in the absence of any detectable movement by the animal. A detailed analysis and discussion of the clinical effects of GPi-DBS can be found in McCain and Turner (2009).

Modulation of rate firing. Supporting evidence for GPi-DBS-induced modulation of the motor cortexes of PD patients comes from a number of studies. These reports have relied on noninvasive PET and MRI measures, and although clear changes have been observed in pre- and supplementary motor areas, there are as yet no conclusive reports of gross changes in M1 activity from GPi-DBS (Davis et al. 1997; Limousin et al. 1997; Devoval et al. 2002; Fukuda et al. 2002; Valalik et al. 2009). We report both increases and decreases in the firing rate of individual cells during GPi-DBS, with no net change in the population mean firing rates. This observation would appear to support the previous imaging studies.

Can current understanding of the network effects of DBS explain our observations? The local effects of GPi-DBS on firing rates within the pallidum appear to be mixed, with some neurons showing dramatic reductions in firing rate (Benazzouz et al. 1995; Borau et al. 1996; Dostrovsky et al. 2000; Wu et al. 2001; McCain and Turner 2009; Bar-Gad et al. 2004; Erez et al. 2009; Liu et al. 2012; Chiken and Nambu 2013) and others showing increases (McCain and Turner 2009; Bar-Gad et al. 2004; Erez et al. 2009; Liu et al. 2012; Chiken and Nambu 2013). These observations may explain our observation of a mixture of increases and decreases in M1 firing rates, by way of DBS-induced disinhibition and inhibition (respectively) of GPi-receiving thalamo-cortical projections (Montgomery 2006; Anderson et al. 2003).

Direct electrophysiological support for pallidal stimulation modulating M1 has recently been demonstrated in NHPs (Johnson et al. 2009). GPi-DBS was found to reduce the mean firing rates of cortical neurons, often in a phasic stimulus-locked manner. The latency of inhibition was on the order of ~3 ms following delivery of the stimulation pulse; the short-latency timing of these responses appears to rule out mediation via the known multisynaptic pathways from pallidum to cortex (Anderson and Turner 1991; Nambu et al. 1988; Buford et al. 1996; Inase et al. 1996). Methodological differences (artifact rejection techniques) and differences in the stimulating probe most likely explain the difference between this and our study. The macroelectrode used in the previous study was significantly larger than the one used here and may have been more effective at exciting fibers in the internal capsule. It remains unclear, however, how stimulation in the GPi is able to elicit short-latency inhibition, not excitation as would be predicted (Li et al. 2007; Dejean et al. 2009; Gradinaru et al. 2009; Li et al. 2012).

Fig. 5. Firing pattern and spectral properties in M1 during stimulation. A: exemplar autocorrelation function and its change during GPi-DBS. In the DBSoff condition (black trace), the cell tended to spike at regular intervals, revealed as significant peaks and valleys in its autocorrelation function, within the beta-frequency range. During stimulation (gray trace), the cell’s autocorrelation function flattened and lost the characteristic peaks and valleys observed off-stimulation. B: power spectra for the same exemplar data used in A under DBSoff and DBSon conditions. A prominent spectral peak at 14.8 Hz (*) was abolished during stimulation. Dashed horizontal line: threshold for significance. C: fraction of cells with one or more significant spectral peak under DBSoff and DBSon conditions (black and gray bins, respectively). Note that the prevalence of cells showing oscillatory activity in the beta-frequency range was reduced significantly during stimulation (*).
Claims of direct activation of the cortex following GPI-DBS have also recently been published from studies in dystonic patients (Tisch et al. 2008; Bhanpuri et al. 2014). These studies used EEG recording with relatively slow rates of stimulation (~9–10 Hz). It was reported that any changes in EEG amplitude were detectable at ~25 ms after pulse delivery, which is close to the latencies expected for orthodromic pallido-thalamo-cortical transmission (Herrojo et al. 2014). As our study did not use EEG or LFP measurement in M1, it is difficult to make a direct comparison with the results reported here.

**Antidromic-driven high-frequency resonance in M1 was minimal.** Recent work using DBS in the STN of rodents has suggested that antidromic modulation of the cortex (and induction of “high-frequency resonance”), through activation of fibers in the internal capsule, may underlie the mechanism of STN-DBS (Li et al. 2007; Dejean et al. 2009; Li et al. 2012; Gradinaru et al. 2009). The short-latency inhibition of M1 during GPI-DBS reported by Johnson et al. (2009) may also be seen as consistent with a high-frequency resonance mechanism for DBS.

Our artifact subtraction method makes it possible to identify short-latency responses (0–3 ms) in close spatial proximity to the stimulating electrode (McCain and Turner 2009; McCain et al. 2013). Using this technique, we found only one M1 cell (out of 132 cells studied) that responded at a short fixed latency as would be expected of antidromic driving. The precise pathway responsible for this effect is a matter of speculation. Observations that high-frequency microstimulation of the GPi is not transmitted reliably to M1 (Rivlin-Etzion et al. 2008) makes an orthodromic pathway (i.e., GPi→thalamus→cortex) unlikely. It appears more likely that GPI-DBS in our animals spread to the internal capsule where it excited a small number of corticofugal fibers. Alternatively, there is some evidence for a sparse but direct cortical innervation of the pallidum (Naito and Kita 1994; Smith and Wichmann 2014). The low fidelity of driving observed here (spikes elicited by ~6% of shocks) is consistent with a previous study of antidromic activation of cortical neurons during high-frequency stimulation (Iremonger et al. 2006). The paucity of stimulation driven high-frequency resonance in our recordings, combined with pronounced and persistent therapeutic effects of GPI-DBS, is inconsistent with the idea that antidromic-driven resonance in cortex is a primary therapeutic mechanism of action for GPI-DBS.

**GPI-DBS 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 corresponds with our observations from the GP of MPTP-treated NHPs (McCain and Turner 2009) and is supported by the recent work of Hahn and colleagues (Hahn et al. 2008). This finding brings into question the concept that GPI-DBS reduces parkinsonism by suppressing burst firing (Vitek 2002; Shi et al. 2006). The concept that DBS does not alleviate parkinsonism through abolition of burst firing may, however, only apply to GPI-DBS. Recent work in rodents has suggested that the activation of the fibers surrounding the STN and subsequent antidromic activation of cortical structures can lead to destabilization of synchronized bursting activity within M1 (Li et al. 2012).

**GPI-DBS suppressed beta-oscillatory activity.** Parkinsonian symptoms have been associated with the presence of synchronized beta oscillations in the BG-thalamo-cortical network both in animal models (Goldberg et al. 2004; Mallet et al. 2008; McCain and Turner 2009; Pasquereau and Turner 2011) and in human patients (Hutchison et al. 2004; Kuhn et al. 2004, 2009; Brown and Williams 2005; Hammond et al. 2007; Eusebio et al. 2011). It is important to note, however, that some parkinsonian signs may appear before excessive beta firing emerges (Leblois et al. 2007; Mallet et al. 2005), and the assumption that increased beta oscillations play a causal role in parkinsonism has been questioned (Montgomery 2012). We demonstrated previously that, at the level of the GP, the most consistent features associated with DBS-induced reductions in parkinsonism were reductions in oscillations and coherent oscillations between pairs of pallidal neurons (McCain and Turner 2009).

Similarly, in the present study, we found a subset of M1 neurons that showed beta-oscillatory activity and pairs of simultaneously recorded neurons whose firing rates were modulated coherently at beta frequencies. These presumed pathological oscillations were reduced in strength or eliminated completely by DBS. Similar reductions in beta activity have been observed in the STN in NHPs (Meissner et al. 2005; Moran et al. 2012) and have been inferred from studies in PD patients (Marsden et al. 2001a; Brown et al. 2004a; Hammond et al. 2007; Eusebio et al. 2011). Our results suggest that the oscillation-suppressive effects of DBS extend beyond the local
neuronal circuits that are directly modulated by the stimulation pulse.

The mechanism for suppression of oscillatory activity in M1 is most likely dependent on the specific nucleus being stimulated. In the case of the STN, it appears that much of the suppressive effect in M1 is achieved through “sporadic” antidromic activation of the cortex via the internal capsule (Li et al. 2007; Dejean et al. 2009; Gradinaru et al. 2009; Li et al. 2012). Data from the present study suggest that it is sufficient to suppress oscillatory activity in the output nucleus of the BG (i.e., the GPi) without significantly activating the fibers of the internal capsule to cause a reduction in M1 oscillatory activity.

Therapeutic GPI-DBS also suppressed beta-range coherence. The role that coherence/synchrony plays in the pathophysiology of parkinsonism has yet to be elucidated. It has been suggested that the increase in coherence/synchrony may reflect a breakdown in the functional segregation of discrete cortico-BG neural circuits (Filion and Tremblay 1991; Raz et al. 2000; Goldberg et al. 2002, 2004; Hutchison et al. 2004; Kuhn et al. 2004; Brown and Williams 2005; Bergman et al. 1998). Presumably, as with beta oscillations, the mechanism that mediates a DBS-induced reduction in synchrony depends on the nucleus being stimulated. In the case of GPI-DBS, it is probable that a reduction in synchrony local to the site of stimulation in the GPi (McCairn and Turner 2009) is then communicated to downstream circuits including M1.

Conclusion. In summary, the effects of GPI-DBS on M1 activity closely resembled those reported for the pallidum. GPI-DBS caused significant reductions in the prevalence of oscillatory and synchronous activity in single and pairs of neurons but no gross alteration in population-level measures of baseline firing rate or burst firing. These results reinforce the concept that reductions of pathological oscillations and synchrony play a major role in the therapeutic mechanism of GPI-DBS.

The observations and conclusions presented here, however, should be placed in the context of the established clinical effects of GPI-DBS, which are known to evolve over multiple time scales from minutes, hours, days, and months (Temperli et al. 2008). The changes to the prevalence of oscillatory and synchronous activity reported here were observed at short time intervals relative to the typical duration of clinical DBS treatment. Therefore, our results may not reflect the totality of the therapeutic mechanisms of GPI-DBS and further studies are needed to test for more long-term effects.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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