Deep brain stimulation of the subthalamic nucleus reestablishes neuronal information transmission in the 6-OHDA rat model of parkinsonism

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Dorval AD, Grill WM. Deep brain stimulation of the subthalamic nucleus reestablishes neuronal information transmission in the 6-OHDA rat model of parkinsonism. J Neurophysiol 111: 1949–1959, 2014. First published February 19, 2014; doi:10.1152/jn.00713.2013.—Pathophysiological activity of basal ganglia neurons accompanies the motor symptoms of Parkinson’s disease. High-frequency (>90 Hz) deep brain stimulation (DBS) reduces parkinsonian symptoms, but the mechanisms remain unclear. We hypothesize that parkinsonism-associated electrophysiological changes constitute an increase in neuronal firing pattern disorder and a concomitant decrease in information transmission through the ventral basal ganglia, and that effective DBS alleviates symptoms by decreasing neuronal disorder while simultaneously increasing information transfer through the same regions. We tested these hypotheses in the freely behaving, 6-hydroxydopamine-lesioned rat model of hemiparkinsonism. Following the onset of parkinsonism, mean neuronal firing rates were unchanged, despite a significant increase in firing pattern disorder (i.e., neuronal entropy), in both the globus pallidus and substantia nigra pars reticulata. This increase in neuronal entropy was reversed by symptom-alleviating DBS. Whereas increases in signal entropy are most commonly indicative of similar increases in information transmission, directed information through both regions was substantially reduced (>70%) following the onset of parkinsonism. Again, this decrease in information transmission was partially reversed by DBS. Together, these results suggest that the parkinsonian basal ganglia are rife with entropic activity and incapable of functional information transmission. Furthermore, they indicate that symptom-alleviating DBS works by lowering the entropic noise floor, enabling more information-rich signal propagation. In this view, the symptoms of parkinsonism may be more a default mode, normally overridden by healthy basal ganglia information. When that information is abolished by parkinsonian pathophysiology, hypokinetic symptoms emerge.

basal ganglia; Parkinson’s disease; subthalamic nucleus; high-frequency stimulation

Similarities between symptom relief from DBS and ablative lesion kindled the view that the two therapies produce similar changes in neural firing rates in downstream structures (Benabid et al. 1998; Benazzouz et al. 2000; Beurrier et al. 2001; Boraud et al. 1996). This hypothesis posits that both DBS and ablative lesion alleviate hypokinetic PD symptoms by reducing GABAergic drive to thalamus, thereby disinhibiting the thalamocortical motor loop. However, recent studies have reported that parkinsonism-alleviating DBS does not reduce GABAergic drive to thalamus, but rather may increase that inhibitory drive and thereby reduce thalamic firing rates (Anderson et al. 2003; Hashimoto et al. 2003; Hershey et al. 2003; Jech et al. 2001; Windels et al. 2000).

In addition to changes in firing rate, altered patterns of neural activity appear to contribute to parkinsonian symptoms. Neurons in the parkinsonian brain exhibit irregular spike trains that include more bursts (Bergman et al. 1994; Magnin et al. 2000; Tang et al. 2005; Wichmann and Soares 2006) and oscillations (Brown et al. 2004; Raz et al. 2000). Therapeutic DBS regularizes neuronal activity (Bar-Gad et al. 2004; Degos et al. 2005; Dorval et al. 2008; Hashimoto et al. 2003; McIntyre et al. 2004) and reduces bursts (Anderson et al. 2003; Grill et al. 2004; Tai et al. 2012) and coherent oscillations (McConnell et al. 2012; Meissner et al. 2005). Irregularity, bursts, and oscillations all contribute to the firing pattern entropy that bounds the maximum information that a neuronal firing pattern can convey. Changes in activity patterns associated with parkinsonism may increase firing pattern entropy from the surprisingly low levels in the healthy condition (Darbin et al. 2006).

In a nonhuman primate model of parkinsonism, we found that symptom-alleviating DBS decreased entropy, whereas behaviorally ineffective DBS increased entropy (Dorval et al. 2008), but we did not quantify changes between the healthy and parkinsonian state. Furthermore, computational studies have proposed that regularized firing patterns in basal ganglia allow thalamic neurons to relay motor commands with higher fidelity (Dorval et al. 2009, 2010; Guo et al. 2008; Rubin and Terman 2004; So et al. 2012), suggesting that low entropy is imperative for effective information transmission.

In this study we quantified information transmission in the 6-hydroxydopamine (6-OHDA)-lesioned rat model of PD in the healthy and parkinsonian states, and with DBS. We found that parkinsonism increased firing pattern entropy in globus pallidus (GP; the homolog of the external segment of globus pallidus in primates) and substantia nigra pars reticulata (SNr; the principal basal ganglia output structure in rodents) and that
these increases were partially reversed by symptom-alleviating DBS. However, increases in entropy were overshadowed by large reductions in information transmission following lesion, and information transmission was partially restored by DBS.

METHODS

All surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee of Duke University and complied with U.S. Public Health Service policy on care and use of laboratory animals.

Animal Procedures

Young adult Long-Evans rats (250–350 g) of both sexes were divided randomly into two groups, left and right, based on which brain hemisphere was to receive implanted stimulating and recording electrode arrays and 6-OHDA injection to lesion the dopaminergic neurons in substantia nigra pars compacta (SNc). The overall timeline of each animal was as follows: surgical implantation, followed by 1 wk of recovery; 3–5 control recording sessions, each at least 2 days apart; neurotoxin injection, followed by 1 wk of recovery; 3–12 experimental recording sessions, each at least 2 days apart; and formalin perfusion and brain removal.

Surgical implantation. Rats were anesthetized with 2–3% isoflurane and positioned in a stereotaxic frame on a heating pad to maintain body temperature. The surgical site was shaved, washed with alcohol and povidone-iodine, and opened to the skull. Four stainless steel bone screws were inserted through small burr holes in the skull to anchor the brain implants and the acrylic cap. Stainless steel wires had been welded to at least two of the bone screws to serve as ground contacts for the electrode arrays. The following craniotomies were made over the same hemisphere, anatomically positioned with respect to bregma (Paxinos and Watson 2008). A 26-gauge stainless steel cannula was implanted into the medial forebrain bundle: 2.0 mm posterior and 2.0 mm lateral, to a depth of 6.0 mm. A four-channel stimulating electrode array (2 × 2 grid, 10 kΩ, platinum-iridium electrodes on 400-μm spacing; Microprobes, Gaithersburg MD) was implanted into the STN: 3.6 mm posterior and 2.6 mm lateral, to a depth of 7.8 mm. These arrays were constructed such that the medial electrodes were 0.2 mm longer than the lateral electrodes, enabling all four tips to reside within the STN. Eight-channel recording microelectrode arrays (MEAs; 2 × 4 grid, ~1 μM, stainless steel electrodes on 400-μm spacing; Microprobes) were implanted into the GP via a craniotomy running posterolaterally from 0.8 mm posterior and 2.6 mm lateral to 2.3 mm posterior and 4.0 mm lateral, to a depth of 7.0 mm, and into the SNr via a craniotomy running anterolaterally from 5.7 mm posterior and 1.7 mm lateral to 5.3 mm posterior and 2.9 mm lateral, to a depth of 7.7 mm. The ground contacts of all arrays and bone screws were wrapped together, and all of the hardware was encased in dental acrylic. The skin was sutured around the electrode connectors and swabbed with antibiotic gel. The animal was returned to its home cage.

Neurotoxin injection. After 2–3 wk of control recordings (see below), rats were anesthetized with 2–3% isoflurane and returned to the stereotaxic frame. A 36-gauge needle was inserted through the cannula over the medial forebrain bundle to a depth of 8.0 mm below the cranial surface. The needle was retracted by 0.5 mm, and 8.0 μg of 6-OHDA dissolved in 4.0 μl of saline were injected over a 5-min interval. After another 5 min wait, the needle was withdrawn at a speed of ~2 mm/min. Rats were returned to their home cage for at least 1 wk before recordings began.

Recording sessions. Paw-use asymmetry was quantified with a cylinder task (Chang et al. 2008; Choi-Lundberg et al. 1998). Animals were briefly anesthetized with 2–3% isoflurane. A four-channel tether was connected to the stimulating array, and a pair of eight-channel head stages with associated tethers were connected to the recording MEAs. The stimulating tether connected to an isolated stimulator (FHC, Bowdoin ME) that was turned off except in experimental sessions (see below). The head stage tethers were connected to a Multichannel Acquisition Processor (MAP; Plexon, Dallas, TX). The animals were placed in a 25-cm-diameter vertical cylinder within a dark chamber. An infrared camera beneath the cylinder monitored the rat behavior through the transparent floor of the cylinder. Several minutes after the animal awoke from the anesthesia, a 30-min recording session began. The video and electrophysiological activity were recorded simultaneously. At the end of the 30-min session, the animal was removed from the cylinder and briefly anesthetized with 2–3% isoflurane. The tethers and head stages were disconnected, and the animal was returned to its home cage.

Stimulation. The stimulator was connected to a digital-to-analog board (National Instruments, Austin, TX) running custom software written in LabView on a standard computer. The stimulation was 100 μs per phase symmetrical biphasic pulses at 100 Hz. Large-amplitude stimulation induced dyskinesias, manifesting as stereotyped rearing up and over onto the dorsum or dystonic twitching to one side with a contralateral head direction. Stimulation amplitude was established for each animal as the lesser of the value just below the threshold to elicit dyskinesias or 400 μA, large relative to some studies but with low-impedance electrodes maintaining safe current densities. Bipolar stimulation was delivered with the medial-ventral contacts as the anode and the lateral-dorsal contacts as the cathode. To establish a parkinsonian baseline following 6-OHDA injection, animals underwent one to three experimental behavioral sessions of 30 min each. Subsequently, each session began with 10 min of no stimulation, followed by 15 min with stimulation on, followed by 5 min with stimulation off. In all other regards, the experimental sessions were identical to the control sessions.

Histology. The experiment ended after 12 experimental recording sessions or when the acrylic head cap became dislodged from the skull. Animals with at least three experimental recording sessions (11/16) were included in subsequent analysis. Histology was used to assess dopaminergic cell loss in most of those animals (9/11). Animals were deeply anesthetized with pentobarbital sodium and transcardially perfused with 10% formalin. The brain was removed and postfixed for 1–7 days, blocked, and mounted. Sections through SNc were stained for tyrosine hydroxylase and counterstained with cresyl violet.

Data Analysis

Of the 16 animals in the study, behavioral analysis was performed on all 11 surviving through 3 experimental recording sessions. Of those 11, 6 animals experienced at least 3 recording sessions with DBS; the other 5 animals were evaluated only for the non-DBS conditions.

Behavior. When in the 25-cm-diameter vertical cylinder, the rats frequently reared up and touched the outside walls with their forepaws. A data analyst blinded to the side of 6-OHDA injection determined the times that each paw was in contact with the cylinder wall. For all trials and all conditions, the duration of paw touches was summed for the forepaw ipsilateral (Tipsi) and contralateral (Tcontra) to the 6-OHDA injection. An ipsilateral paw preference (range 100%) was calculated as 100 times the ratio of the difference between the ipsilateral and contralateral durations, divided by their sum; i.e., ipsilateral paw preference = 100 × (Tipsi − Tcontra)/(Tipsi + Tcontra).

Neural recordings. Unit activity was band-pass filtered from 150 Hz to 6 kHz and sampled at 40 kHz on all 16 channels. Channel thresholds on the Plexon MAP system were set manually during each experimental session to record 1.2-ms snippets around every threshold crossing. Subsequently, units were isolated on each channel in Offline Sorter (Plexon) via principal component analysis and the valley seek algorithm, with manual supervision (e.g., requiring the presence of a
Table 1. Number of units

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<th>Ctrl</th>
<th>6-OHDA</th>
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<td>242</td>
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<tr>
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<td>203</td>
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<td>SNr -&gt; SNr</td>
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Values are total numbers of isolated neuronal units and neural pairs analyzed from 8 animals in control (Ctrl), 6-hydroxydopamine (6-OHDA)-lesioned, and 6-OHDA-lesioned + deep brain stimulation (DBS) conditions. GP, globus pallidus; SNr, substantia nigra pars reticulata.

Refractory period, and firing rates between 0.333 and 250 Hz). For sessions including stimulation, the large DBS artifacts were removed from the data before the principal components were identified. Epochs during DBS included an ~1-ms time window following each stimulation pulse in which no unit activity could be recorded because of the DBS artifact. A 1.0-ms time window, repeating at 100 Hz, was also blanked out of all the non-DBS epochs to keep all comparisons equal. Note that this 100 ms/s (10%) blanking implies that reported firing rates in the control and 6-OHDA conditions may be as low as ~90% of their actual value. Spike times for each unit were exported to GNU-Octave for analysis.

**Spiketime analysis.** From the spike times in each condition, firing rates were calculated via standard methods. Firing pattern entropy was calculated from the distribution of interspike intervals (ISIs) (Rieke et al. 1993; Strong et al. 1998), but binned logarithmically (Dorval 2008; Dorval et al. 2008), to distinguish events occurring at different time scales, e.g., bursting from nonbursting (Selinger et al. 2007). To minimize biases in the entropy estimates introduced by changes in data set size, exactly 10 min of data were analyzed from each recording in the control, 6-OHDA, and 6-OHDA + DBS conditions. Where transient conditions are analyzed separately (e.g., Fig. 5), exactly 3 min of data were analyzed in the DBS-onset and DBS-offset cases. Briefly, ISI probability distributions were generated by rounding ISIs into bins of logarithmic time divided into 10 bins per decade, i.e., 10 bins between 1 and 10 ms, 10 bins between 10 and 100 ms, and so on. Two-dimensional ISI probability distributions were generated similarly from all pairs of consecutive ISIs: P(ISI\(_i\), ISI\(_j\)) was found as the number of times ISI\(_j\) followed ISI\(_i\) divided by the total number of ISIs. Maximum likelihood estimates of the entropy per spike were calculated as \(H_{\text{unit}} = -\sum_i \sum_j P(\text{ISI}\_i, \text{ISI}\_j) \log_2 P(\text{ISI}\_i, \text{ISI}\_j)\), where the inner sum is over the final ISI (\(a\)), and the outer sum is over the preceding ISI (\(b\)). For low-firing rate neurons, higher dimensional ISI distributions yielded biased entropy estimates, and thus analysis was restricted to the two-dimensional paired ISI case.

**Neuron-pair analysis.** Cross-correlations between all simultaneously recorded spike trains were calculated via standard methods. Briefly, spike times were converted into trains of delta functions and convolved with a Gaussian kernel having a standard deviation width of 5.0 ms. Each signal was then cross-correlated with all other signals built from simultaneously recorded spike trains normalized as a percentage against the autocorrelation of each signal, i.e., the value of the autocorrelation at time 0 was 100%. There were no appreciable oscillations in the cross-correlations, except in the DBS cases where the DBS frequency was strongly represented. Thus we report only the maximum value of the cross-correlation function.

Information was calculated from the entropies of the logarithmically binned interval distributions (Dorval 2011). Directed information between each pair of neurons was calculated from the paired entropy (\(H_{\text{pair}}\)) and single-unit entropy (\(H_{\text{unit}}\)). The paired entropy is not temporally symmetric and depends on the cross-spike interval (CSI; the time delay between one spike of the input neuron and the next spike of the output neuron): \(H_{\text{pair}} = -\sum_i \sum_j P(\text{ISI}\_i, \text{ISI}\_j) \log_2 \frac{P(\text{ISI}\_i, \text{ISI}\_j, \text{CSI})}{P(\text{ISI}\_i, \text{ISI}\_j)}\). Here, the inner sum is over the final ISI (\(a\)) in the output neuron, the middle sum is over the preceding ISI (\(b\)) in the output neuron, and the outer sum is over the CSI (\(c\)) from the input to the output neuron. Although higher dimensional ISI distributions could have been incorporated, the definition used is matched to the unit entropy measurements from above, and this matching ensures that the CSI could not raise the pattern entropy, consistent with information theory (Shannon and Weaver 1949).

The raw directed information was defined as the information about the input neuron spike train present in the output neuron spike train, \(I_{\text{raw}} = H_{\text{unit}} - H_{\text{pair}}\), and bounded the information that an ideal observer could gather about the input neuron by watching the output neuron. However, given finite data sets, inclusion of the input neuron spike times must decrease the calculated entropy (\(H_{\text{pair}} < H_{\text{unit}}\)), even if the two neurons are completely independent, e.g., in different animals. To compensate for this bias, the raw information was compared against a shuffled information (\(I_{\text{shuf}}\)) calculated from the shuffled entropy (\(H_{\text{shuf}}\)), found in the same way as the paired entropy but with the CSIs shuffled to remove any true dependence between the two neurons without modifying their underlying entropy estimates.

![Behavioral effects of parkinsonism and deep brain stimulation (DBS).](http://jn.physiology.org/)

Fig. 1. Behavioral effects of parkinsonism and deep brain stimulation (DBS). A: paw preference in a vertical column exploration task in control (n = 11), 6-hydroxydopamine (6-OHDA)-exposed (n = 11), and 6-OHDA-exposed with DBS (+DBS; n = 6) conditions. Positive values indicate a preference for the paw ipsilateral to 6-OHDA injection. Data are confidence intervals of the mean: boxes indicate the middle 50% and error bars span 90%. Horizontal brackets indicate statistical significance (\(p < 0.01\)). B: individual behavior of the 6 animals treated with DBS. Four rats (top; different triangles) exhibited a shift in paw preference of \(>10\%\) following 6-OHDA injection. These animals, an average shift of \(\sim 25\%\) following 6-OHDA injection. Histology analysis verified a lesion of substantia nigra pars compacta (SNc) in 3 of these 4 animals; histology was not available for the animal represented by upright triangles (↑). Two rats (bottom; different symbols) did not exhibit a shift in paw preference of \(>10\%\) following 6-OHDA injection. Histology confirmed the presence of a lesion in one animal (lesion, ◆) and the absence of a lesion in the other (no lesion, ◆). These data are presented merely for completeness, because these animals were included in the summary results in A; they reduced the average 6-OHDA effect and increased the length of error bars for both the 6-OHDA and +DBS conditions. C: tyrosine hydroxylase-stained slice from the animal represented with inverted triangles (▼) in B. Dopaminergic cells, stained in the left SNc (arrow), are absent in the right SNc.
The reported directed information values are the excess information in the raw estimates not found in the shuffled versions: $I_{\text{dir}} = I_{\text{raw}} - I_{\text{shuf}}$.

The ISIs and CSIs were apportioned into distributions with 10 bins per decade, and the entropies were calculated from the two-dimensional ISI pairings, for all reported summary values and statistical comparisons. Results were qualitatively similar over the range of 5 to 20 bins per decade and when using the entropy estimate found by extrapolating the number of relevant ISIs to infinity (Dorval 2008; Strong et al. 1998). However, for illustrative purposes, some figures in this manuscript show distributions divided into something other than 10 bins per decade. In particular, Figs. 2 and 4 show an overdiscretized 20 bins per decade on the (unused) isolated ISI distributions but the correct 10 bins per decade on the paired ISI distributions, and Fig. 6 shows the correct 10 bins per decade for the ISIs but only 3 bins total for the CSIs.

Statistical analysis. Bootstrapping was used to estimate confidence intervals on the electrophysiological measurements. In each case, the population was resampled with replacement to yield a new population of equivalent size. This process was repeated 50,000 times, and the statistic was calculated for each population. The distribution intervals of these statistics are presented as the confidence intervals. Where noted, the bootstrapped significance value separating two populations was found as the product of the integrals of the potentially overlapping tails of the distribution intervals. Comparisons highlighted in gray denote statistical significance ($P < 0.05$, with Bonferroni correction for 3 comparisons, i.e., $P < 0.016667$) on this bootstrapped confidence interval test.

In addition, one-way analyses of variance (ANOVA) were performed on all reported measures, and the results of each ANOVA are discussed where appropriate. Where ANOVA revealed an effect of condition ($P < 0.05$), paired $t$-tests were used to identify pairwise changes. Comparisons highlighted in black denote statistical significance ($P < 0.05$) on the paired $t$-test. All comparisons denoted in black (ANOVA and $t$-test, $P < 0.05$) also met the criteria for gray (bootstrap, $P < 0.016667$).

RESULTS

Of 16 animals that were implanted, 11 yielded sufficient data in control and parkinsonian conditions, and 6 of those 11 yielded sufficient data during DBS. Behavioral and histological analyses were conducted to determine which animals exhibited hemiparkinsonian symptoms and which had a confirmed dopaminergic lesion in SNc. Data from animals lacking either parkinsonian symptoms or a dopaminergic lesion were not included in the electrophysiological results: one animal not receiving DBS was excluded for having no SNc lesion; one animal receiving DBS was excluded for having no 6-OHDA-induced behavioral effect; and one animal receiving DBS was excluded for having neither a 6-OHDA-induced behavioral effect nor a SNc lesion. The total numbers of isolated neuronal units and neuron pairs analyzed from the remaining eight animals are listed in Table 1.

Evaluation of Parkinsonism

Symptoms of hemiparkinsonism were quantified with a vertical column exploration task that evaluated the relative use of forepaws ipsilateral and contralateral to the 6-OHDA injection. Control sessions conducted after surgical implantation of

![Fig. 2. Changes in activity of example single neurons in globus pallidus (GP; A) and substantia nigra pars reticulata (SNr; B) following 6-OHDA lesion. Left, rastergrams of spike times from a typical 10-s period, before and after 6-OHDA lesion, illustrate that spike bursts and long pauses are more common in the parkinsonian condition. Right, normalized histograms of single interspike intervals (ISIs; top) and ISI pairs (bottom) from the same units; contrast the narrow distributions of regular ISIs in the control with the broad distributions of irregular ISIs following 6-OHDA lesion. ISI$_a$ and ISI$_b$ represent the final ISI and the preceding ISI, respectively.](http://jn.physiology.org/doi/abs/10.1152/jn.00713.2013)
the three electrode arrays and cannula but before 6-OHDA injection did not reveal asymmetry in forepaw use (Fig. 1A). Experimental sessions were conducted at least 1 wk after 6-OHDA injection, and some included 100-Hz DBS of the STN. Injection of 6-OHDA produced a preference for exploration with the forepaw ipsilateral to injection, and the ipsilateral preference was decreased by DBS in the population of six animals with at least three DBS recording sessions (Fig. 1A). Of those animals, four showed a strong ipsilateral paw preference that was completely reversed by DBS (Fig. 1B, top). The other two animals did not show a preference following 6-OHDA administration. However, DBS did affect behavior in these two animals, driving ipsilateral paw preference in one rat, but contralateral paw preference in the other (Fig. 1B, bottom).

Histological assessment was conducted on seven of nine animals with symptoms of PD following 6-OHDA injection; six of seven had a confirmed dopaminergic lesion in SNc (Fig. 1C). Histological assessment was also performed on the two animals without 6-OHDA-induced symptoms (i.e., Fig. 1B, bottom); the one with the DBS-induced ipsilateral paw preference had a near complete lesion; the one with the DBS-induced contralateral paw preference had no detectable lesion. The subsequent results quantify the activity of multiple single neurons in the remaining eight animals, four of which received DBS that was confirmed to be effective in reversing paw-use asymmetry.

**Single-Unit Activity**

Action potentials were recorded extracellularly from single neurons in GP and SNr during 30-min trials and isolated offline (Table 1). Probability distributions of single and consecutive ISIs showed a similar effect of 6-OHDA lesion on activity in GP and SNr (Fig. 2). Units recorded before 6-OHDA injection tended to have narrow ISI distributions, indicative of a well-defined mode frequency with minimal variation. In contrast, units recorded after injection had broader ISI distributions, indicative of irregular firing patterns.

The average firing rate of each unit was calculated during 20-s intervals following onset of 100-Hz DBS and normalized to the average firing rate during the 2-min preceding DBS onset (Fig. 3). On a unit-by-unit basis, and averaged across the population, firing rate in both GP and SNr increased rapidly at the onset of DBS, but the majority of the increase in firing rate waned after 1–3 min. Since transient effects are less relevant to the enduring behavioral responses, we focused on neural activity after the first 3 min of DBS, except where otherwise noted (e.g., Fig. 5).

Probability distributions of single and consecutive ISIs in GP and SNr (Fig. 4) revealed both decreases (A) and increases (B) in firing rate. However, the more notable change was the periodicity that manifested in the ISI distributions as high-probability modes at the subharmonics of the DBS frequency. In some cases, these modes narrowed the ISI distribution by reducing the tail probabilities, decreasing firing pattern entropy from the 6-OHDA condition, e.g., Fig. 4B, in which DBS was associated with fewer short (<2 ms) and long (>100 ms) ISIs. In other cases, however, these modes were coupled with a broadening of the ISI distribution by elevating the tail probabilities, and yielded unchanged or even increased firing pattern entropy from the 6-OHDA condition, e.g., Fig. 4A, in which DBS was associated with more short (<2 ms) and long (>100 ms) ISIs.

Firing rates and firing pattern entropy were calculated for all neurons in GP and SNr in the control, 6-OHDA lesioned, and stable DBS conditions (Table 1); the 3 min immediately following DBS onset and offset were considered separately. Condition contributed to firing rate in SNr (ANOVA, P < 0.05), and modest changes in GP firing rates were evident with bootstrapped statistics (Fig. 5A). Neither GP nor SNr exhibited a significant difference in firing rate between the control and 6-OHDA conditions, whereas DBS (and DBS onset) caused modest increases in firing rate.

Firing pattern entropy depended on condition in GP and SNr (ANOVA, P < 0.05). In both regions, 6-OHDA lesion increased firing pattern entropy, whereas therapeutic DBS reversed the increase, and no difference was observed between control and DBS conditions (Fig. 5B). Firing pattern entropy was reduced further in the 3 min following DBS onset, and even more so in the 3 min following DBS offset. Entropy values were calculated from the paired ISI distributions as explained in METHODS, but single ISIs and ISI triplets yielded qualitatively similar results.

**Paired-Unit Activity**

CSI distributions were calculated from the spike times of simultaneously recorded units. This process is illustrated for two pairs of units: one pair recorded in the healthy condition and one pair recorded after 6-OHDA lesion, with and without DBS (Fig. 6A). Three ISI distributions are shown for one neuron in SNr (unit a), conditioned on the time since a spike in

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**Fig. 3. Changes in firing rate produced by 100-Hz DBS of the subthalamic nucleus (STN).** A: example unit in SNr from a 6-OHDA-lesioned rat. Rastergram spanning 2 min before through 3 min after DBS onset (top) illustrates that the irregular activity before DBS is replaced by higher frequency firing that gradually decreases. Histogram of the firing rate of the same unit averaged over 20-s intervals (means ± SE; bottom), where each 1-s bin was treated as a unique data point for the SE calculation, shows the transient and sustained changes induced by DBS. B: firing rate histograms from all recorded units in GP (top) and SNr (bottom), normalized to their average rates for the 2 min before DBS onset. Graphs show means ± SE of all normalized histograms. Error bars for the baseline value (mean = 1.0) represent the standard deviation of the normalized firing rate in the 2 min before DBS onset.
Fig. 4. Changes in activity of single example neurons in GP (A) and SNr (B) from one 6-OHDA-lesioned animal during DBS. Left, rastergrams of spike times from a typical 10-s period with and without DBS. Periodic firing imposed by DBS is not easily visualized at this scale. Right, normalized histograms of single ISIs (top) and ISI pairs (bottom) from the same units; contrast the unimodal distributions of the 6-OHDA condition with the multimodal distributions of the DBS condition. DBS drives neural firing at subharmonics of the 100-Hz stimulation frequency, i.e., 10 ms, 20 ms, 30 ms, etc.

another neuron in GP (unit b), the cross-spike interval. Figure 6A, top, shows the ISI distribution of unit a, given that unit b fired a short (S) time ago, less than 18 ms before the end of the ISI; Fig. 6A, bottom, shows the ISI distribution of unit a, given that unit b fired a long (L) time ago, at least 56 ms before the end of the ISI; and Fig. 6A, middle, shows the ISI distribution of unit a, given that unit b last fired a medium (M) time ago, 18–56 ms before the end of the ISI. Vertical dashed lines divide the ISIs of unit a into common neural rhythms: ISIs at alpha frequencies or slower (α), beta frequencies (β), gamma frequencies (γ), or faster (χ).

The integral of the ISI distribution within each of these four frequency bands was calculated and compared across the three CSI conditions (Fig. 6B). For example, the first three bars of the graph for the control condition (Fig. 6B, left) show that whenever unit a fired a very fast ISI (χ), there was a 44% chance that unit b fired in the preceding 18 ms, a 39% chance that unit b fired between 18 and 56 ms ago, and a 17% chance that unit b had not fired in the past 56 ms. Thus spikes by unit a carried some information about the spikes in unit b. Alternatively, if unit a had been completely independent of unit b, the three ISI distributions would look approximately the same, and all of the bars at the bottom would rise to ~33%, as they do for ISIs from unit a in the beta range (β). Horizontal dashed lines depict probability at 33 ± 7%, providing a visual illustration of whether spikes from unit a carried information about spikes from unit b. Bars outside the 33 ± 7% range refute that ISI is independent of CSI (binomial statistics, P < 0.03) for the least number of ISIs observed.

From the example pair of neurons recorded in the control condition (Fig. 6B, left), a χ-, γ-, or β-rhythm ISI in unit a indicated that unit b had probably fired in the last 56 ms, and an α-rhythm ISI strongly indicated that unit b had probably not fired in the past 56 ms. Interestingly, a β-rhythm ISI provided negligible information about unit b. Data from the other example pair of neurons recorded following 6-OHDA lesion revealed that the ISIs of unit a provided very little information about unit b, illustrated by all bars rising into the 33 ± 7% range (Fig. 6B, middle). During DBS, however, unit a provided substantial information about unit b when the ISIs were in the χ-, γ-, or α-rhythm range (Fig. 6B, right). As in the control condition, β-rhythm ISIs did not provide substantial information about unit b in any condition.

Converting the percentages to probabilities and using the information equations in METHODS yielded a measure of the information that unit a carries about unit b spikes, or how much information in unit b is passed to unit a. The procedure that we used to calculate information was identical to this example, except we operated with unit a distributions of paired ISIs (c.f., isolated ISIs), divided CSIs into the same bin widths as ISIs (10 bins per decade; c.f., 3 bins of S, M, and L), and calculated joint probabilities over all ISI bins (10 bins per decade; c.f., 4 bins of α, β, γ, and χ).

Directed information per spike was calculated for all simultaneously recorded pairs and averaged by pair-coupling iden-
DBS-induced entropy reduction was more pronounced in both the onset and reversed the 6-OHDA lesion-induced entropy increases in both regions. The P exhibited a large increase relative to 6-OHDA. 6-OHDA conditions were not significant. In both regions, the onset condition DBS relative to control in both conditions. Rate changes between control and 53). These increased correlations were not corrected by A (Fig. 8). Vertical brackets highlight significant changes from the 6-OHDA condition. A: firing rates depended on condition in SNr (ANOVA, *P < 0.05) but not GP. However, bootstrapped comparisons (gray brackets) show firing rates were elevated in DBS relative to control in both conditions. Rate changes between control and 6-OHDA conditions were not significant. In both regions, the onset condition exhibited a large increase relative to 6-OHDA. B: firing pattern entropy changed as a function of condition in both regions (ANOVA, *P < 0.05). DBS reversed the 6-OHDA lesion-induced entropy increases in both regions. The DBS-induced entropy reduction was more pronounced in both the onset and offset periods. Ctrl, control.

In summary, correlations between unit firing times on short time scales were increased by parkinsonism, but were further increased by DBS. Conversely, directed information and the information rate were dramatically reduced by parkinsonism and partially restored by DBS. In particular, in the pairing most likely to represent basal ganglia output (SNr to SNr), the information rate decreased from 12.7 ± 1.2 to 1.6 ± 0.3 bits/s (mean ± SE) between the control and 6-OHDA conditions, respectively, whereas DBS increased this information rate in the parkinsonian animal from 1.6 ± 0.3 to 8.7 ± 1.9 bits/s.

**DISCUSSION**

Many of the motor symptoms of PD are driven by changes in neural activity in the basal ganglia. Although the death of dopaminergic neurons in SNc precipitates these changes, clinical intervention (in the form of dopaminergic medication, surgical lesion, or DBS) can alleviate the cardinal motor symptoms of parkinsonism: rigidity, tremor, and bradykinesia. In the present work, we show that lesions of the SNc decreased information transmission in and between GP and SNr, and that symptom-alleviating DBS increased information transmission back toward the levels observed prior to SNc lesion. However, the neural elements mediating the neural and behavioral effects of DBS are not clear and may include STN neurons, surrounding axon tracts, and presynaptic axons projecting into STN (Johnson and McIntyre 2008; McIntyre et al. 2004). The ability of neuronal information to separate healthy from pathological states may contribute to biomarkers to help diagnose conditions and constrain therapeutic interventions in PD, as well as in other neurological disorders, e.g., epilepsy (Trevelyan et al. 2013).

The present data provide evidence that parkinsonian symptoms arise when neurons in the ventral basal ganglia fail to deliver information to thalamic relay neurons. Highly entropic neural signals can convey excess information if, and only if, the receiving neuron can interpret the message from the transmitting cell. Neurons in the parkinsonian basal ganglia transmit signals that other basal ganglia neurons (and likely thalamic neurons) cannot adequately interpret. Behaviorally effective DBS partially restores information transmission through these networks. In support of this view, firing patterns recorded from basal ganglia efferents during DBS are partially entrained to the stimulation pulses (Bar-Gad et al. 2004; Cleary et al. 2013) and restore thalamic relay neuron information transmission in a computational model (Guo et al. 2008). Furthermore, novel temporal patterns of stimulation (Brocker et al. 2013) may restore information rates fully toward healthy levels and thereby improve symptom relief.

Neural firing rates were rightly the focus of early studies that led to the model that bradykinesia in PD results from over...
inhibition of the thalamus by the basal ganglia (Bergman et al. 1994; Molnar et al. 2005). However, that model does not account for results showing that DBS may increase the firing rate of neurons that inhibit thalamus (Hashimoto et al. 2003), thereby decreasing the firing rate of neurons in thalamus (Anderson et al. 2003), yet still alleviate bradykinesia. Collectively, these results are interpreted as parkinsonian symptoms being generated by changes in neural activity (Wichmann and DeLong 2003), where changes encompass a wide variety of characteristics, including excessive oscillations (Brown et al. 2004; Raz et al. 2000) and increased bursting (Bergman et al. 1994; Magnin et al. 2000; Soares et al. 2004; Tang et al. 2005). This view is supported by observations that behaviorally effective DBS decreases oscillations (Brown et al. 2004; McConnell et al. 2012; Meissner et al. 2005), bursting (Anderson et al. 2003; Grill et al. 2004; Tai et al. 2012), and other irregular unit activity (Bar-Gad et al. 2004; Degos et al. 2005; Hashimoto et al. 2003). Our results continue this line of work by quantifying changes in neuronal activity with firing pattern entropy and show that changes in neural firing patterns precipitate a large decrease in transmitted information that is partially restored by behaviorally effective DBS.

An important difference between the DBS-induced changes in neuronal activity in the present and previous studies is the accounting for onset dynamics. The majority of single-unit studies of DBS focus on changes in the first 1–3 min following DBS onset, despite the fact that optimal relief from parkinsonian symptoms other than tremor does not occur until 2–5 min after DBS onset (Lopiano et al. 2003; Temperli et al. 2003). Although we observed more pronounced early changes in firing rate and firing patterns (Figs. 3 and 5), these immediate changes may not be the most relevant to long-term effects on symptoms. Our findings on the transient effects of DBS may help to explain discrepancies in the literature on whether DBS increases (Dorval et al. 2008; Hashimoto et al. 2003), decreases (Benazzouz et al. 2000; Burbaud et al. 1994), or drives no net change (Bosch et al. 2011; Moran et al. 2011; Shi et al. 2006) in neuronal firing rates, which are also affected by the tripartite effects of DBS (McConnell et al. 2012).

Neuronal Entropy in the Parkinsonian Basal Ganglia

Distinct from firing rates, firing pattern entropy increases in both GP and SNr during the transition from healthy to parkinsonism. This increase was presumed in our prior work in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) primate (Dorval et al. 2008), but the present data show that this is indeed the case. As spike times phase lock at the fundamental and subharmonics of the stimulation frequency (Garcia et al. 2005; Hashimoto et al. 2003; Reese et al. 2011), DBS reduces firing pattern entropy from the elevated level present in parkinsonism.

The DBS-induced reduction in entropy may be magnified in successive downstream regions. The present study shows that behaviorally effective DBS produced a small entropy reduction in GP (excluding the DBS-onset interval) and a larger reduc-
all couplings (I/H9004) the average change in these pairs only. DBS increased directed information in the directed information was calculated in both conditions. Bar graphs depict bars) in each condition. /H11021 1.5 bits/spike; open 0.5 bits/spike; solid bars) and high-information pairs (significant changes between conditions. mean: median, 25–75% boxes, and 5–95% bars. Horizontal brackets highlight depict the mean; box plots represent bootstrapped confidence intervals on the relative to 6-OHDA, but not back to the levels of the control condition. Bars decrease in information transmission that was partially restored by DBS. Bars depict the average change in these pairs only. DBS increased directed information in all couplings (ΔI > 0; *P < 0.01).

Neural Information Through the Parkinsonian Basal Ganglia

We distinguished between these hypotheses by recording simultaneously from multiple pairs of neurons in GP and SNr following 6-OHDA lesion without and with DBS. A: directed information was substantially reduced in the 6-OHDA condition, relative to control. Directed information was increased in the DBS condition, relative to 6-OHDA, but not back to the levels of the control condition. Bars depict the mean; box plots represent bootstrapped confidence intervals on the mean: median, 25–75% boxes, and 5–95% bars. Horizontal brackets highlight significant changes between conditions. B: percentage of low-information (<0.5 bits/spike; solid bars) and high-information pairs (>1.5 bits/spike; open bars) in each condition. C: for all pairs recorded both with and without DBS, the directed information was calculated in both conditions. Bar graphs depict the average change in these pairs only. DBS increased directed information in all couplings (ΔI > 0; *P < 0.01).

The findings that parkinsonian states are associated with high levels of entropy in the basal ganglia is consistent with work by others (Cruz et al. 2009; Mallet et al. 2008). However, subsequent studies examining the STN-to-GPe pathway report information transmission increases in the parkinsonian state (Cruz et al. 2011) and decreases in the presence of STN DBS (Rosenbaum et al. 2013). Along with not examining STN activity, there are several other important differences between these prior studies and the present study. First, the neural recordings in those studies were conducted in anesthetized rats or computational models, as opposed to the awake and behaving rats of the present study. Second, some of those studies calculate entropy and information capacity from mathematical models fit to the firing rates, autocorrelations, and cross-correlations; these linear measures do not consider any nonlinear interactions contributing to information capacity and transmission. Third, directed information was calculated from the firing rate in 1–30 bins of 5 ms each, compared with the ISI time series binned logarithmically (Dorval 2011), to distinguish events occurring at different time scales. We have compared these approaches, and the ISI time series approach yields less biased estimates with substantially fewer data (Dorval 2008), an important consideration for the prior in vivo studies that examined trials lasting less than 2 min. Information entropy either masks or disrupts information transmission.

The reduced information transmission in PD is large in both absolute and relative terms and is independent of any changes in firing rate. Information per spike as calculated from logarithmically binned ISI distributions is independent of firing rate (Dorval 2008, 2011), and information per spike (Fig. 7) and information per time (Fig. 8) were similarly decreased following 6-OHDA lesion relative to control. In particular, the average information rate between all pairs of neurons in both nuclei decreased by ~80% from the healthy to parkinsonian conditions. This reduction suggests that despite an increase in thalamic inhibition, the more salient interpretation is that parkinsonian basal ganglia have ceased to convey meaningful information to thalamus.

We distinguished between these hypotheses by recording simultaneously from multiple pairs of neurons in GP and SNr following 6-OHDA lesion without and with DBS. A: directed information was substantially reduced in the 6-OHDA condition, relative to control. Directed information was increased in the DBS condition, relative to 6-OHDA, but not back to the levels of the control condition. Bars depict the mean; box plots represent bootstrapped confidence intervals on the mean: median, 25–75% boxes, and 5–95% bars. Horizontal brackets highlight significant changes between conditions.

Fig. 7. Summary of information per spike from all simultaneously recorded pairs in GP and SNr following 6-OHDA lesion without and with DBS. A: directed information was substantially reduced in the 6-OHDA condition, relative to control. Directed information was increased in the DBS condition, relative to 6-OHDA, but not back to the levels of the control condition. Bars depict the mean; box plots represent bootstrapped confidence intervals on the mean: median, 25–75% boxes, and 5–95% bars. Horizontal brackets highlight significant changes between conditions. B: percentage of low-information (<0.5 bits/spike; solid bars) and high-information pairs (>1.5 bits/spike; open bars) in each condition. C: for all pairs recorded both with and without DBS, the directed information was calculated in both conditions. Bar graphs depict the average change in these pairs only. DBS increased directed information in all couplings (ΔI > 0; *P < 0.01).

Fig. 8. Summary of correlation and information rate data from all simultaneously recorded pairs in GP and SNr following 6-OHDA lesion without and with DBS. A: average peak %cross-correlation of spike trains convolved with a 5-ms-wide Gaussian kernel. In the GP-to-SNr pairings in particular, 6-OHDA drove a correlation increase that was exacerbated by DBS. B: average information rate for the same pairs. In all couplings, 6-OHDA drove a large decrease in information transmission that was partially restored by DBS. Bars depict the mean; box plots represent bootstrapped confidence intervals on the mean: median, 25–75% boxes, and 5–95% bars. Horizontal brackets highlight significant changes between conditions.
transmission can change dramatically in 2 min, e.g., in the DBS onset period (Figs. 3 and 5). With the careful analysis of long trials in the present work, we show that information transmission is decreased in the parkinsonian state relative to healthy control and is increased by symptom-alleviating DBS.

It may be that much of the information in the healthy state is unnecessary or underutilized. As dopaminergic neurons in SNc degenerate, the information rate in these regions slowly decreases (e.g., from ~12 bits/s). When the average information rate drops below some symptom threshold (e.g., ~5 bits/s), symptoms emerge. Therapies may work if they can restore the information rate to back above that threshold. However, it is worth noting that the behavioral correlate of this neural information is not completely understood. Recent work in the nonhuman primate demonstrated that pallidal information about motor activity may be decreased by symptom-alleviating DBS (Agnesi et al. 2013). To understand fully these relationships, we should record motor activity simultaneously with multiple neuronal channels in progressive models of parkinsonism, responding to both DBS and other therapies (e.g., L-DOPA).

Conclusion

Modern circuit models of PD electrophysiology include changes in neuronal firing patterns that underlie the development of parkinsonian motor symptoms. Regardless, the firing rate framework is too pervasive to be readily dismissed, and the notion that symptoms arise from pathological inhibition remains central to most discussions of parkinsonian pathophysiology. We proposed amending this framework such that firing rates are replaced with measures of neuronal information. A dramatic reduction in transmitted information through efferent basal ganglia regions occurred coincident with the development of parkinsonism in the 6-OHDA rat model. Therapeutic DBS partially restored information transmission and alleviated symptoms. Thus symptom severity is determined by information transmission through efferent basal ganglia neurons.

REFERENCES


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