Increased Gamma Oscillatory Activity in the Subthalamic Nucleus During Tremor in Parkinson’s Disease Patients

M. Weinberger, W. D. Hutchison, A. M. Lozano, M. Hodaie, and J. O. Dostrovsky

INTRODUCTION

Parkinson’s disease (PD) is a movement disorder of basal ganglia-origin. A severe loss of midbrain dopaminergic neurons is the pathological hallmark of PD (Hornykiewicz 1966). Despite significant advances in our understanding of the anatomy and cellular physiology of the basal ganglia, the casual link between the pathology and the symptoms remain obscure. Tremor at rest is a well-recognized cardinal symptom of PD affecting ~70% of PD patients. It is classically defined as the 4- to 6-Hz tremor with or without postural/kinetic tremor. Unlike rigidity and akinesia, tremor does not necessarily get worse with disease progression, and the severity of tremor does not correlate with dopamine deficiency in the striatum (Deuschl et al. 2000, 2001). Postmortem and imaging studies suggest that the pathophysiology of rest tremor may be distinct from that of rigidity and akinesia (Jellinger 1999; Pavese et al. 2006). However, the mechanisms underlying parkinsonian tremor remain to be elucidated.

Most recently, hypotheses regarding the pathophysiology of PD have emphasized dynamic changes in the basal ganglia network. Changes in firing patterns and in particular oscillatory activity, in addition to mean firing rates, are considered critical in PD pathophysiology (see reviews by Bevan et al. 2002; Brown 2003, 2006; Hammond et al. 2007). Studies in monkeys with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism suggest that one of the consequences of loss of dopaminergic inputs to the basal ganglia is increased oscillatory firing and synchronization in basal ganglia nuclei, i.e., the subthalamic nucleus (STN) and globus pallidus (Bergman et al. 1994; Heimer et al. 2006; Nini et al. 1995; Raz et al. 2000). Oscillatory neuronal firing at the frequency of tremor and the beta frequency band (15–30 Hz) in these structures has also been observed in PD patients undergoing functional neurosurgery and presumed to be related to the pathophysiological changes (Hurtado et al. 1999; Hutchison et al. 1997, 1998; Levy et al. 2000, 2002b; Magnin et al. 2000; Weinberger et al. 2006).

Beta oscillatory activity in the basal ganglia has been previously associated with the pathology that gives rise to tremor in PD (Levy et al. 2000). However, more recent studies in PD patients failed to find a clear positive correlation between tremor severity and the degree of beta oscillations (Amirnovin et al. 2004; Kuhn et al. 2006; Silberstein et al. 2003; Wang et al. 2005; Weinberger et al. 2006). Instead there is growing evidence suggesting that beta oscillations play an anti-kinetic role and contribute to bradykinesia and rigidity in PD (Brown 2003; Chen et al. 2007; Kuhn et al. 2006, 2008; Ray et al. 2008). On the other hand, oscillatory firing in the tremor frequency range is frequently coherent with the tremor of a particular limb although uncorrelated oscillations are commonplace as well (Hurtado et al. 1999, 2005; Lemstra et al. 1999; Raz et al. 2000). It was originally suggested by Alberts et al. (1969) that PD tremor is generated by segregated parallel networks, each involving a different limb. This idea was supported by studies showing that different limbs oscillate independently of each other (Ben-Pazi et al. 2001; Hurtado et al. 2000; Raethjen et al. 2000). A recent study has further confirmed this hypothesis by showing that tremor-related activity in the globus pallidus can be coherent with one tremulous limb but not with the other (Hurtado et al. 2005).

In spectral analysis terms, tremor is considered a nonstationary phenomenon because it comes and goes over time (Hurtado et al. 2000).
et al. 2005). Therefore assessments of the interactions between basal ganglia oscillatory activities and tremor should be obtained by analyzing these signals over time. One means of studying changes in the pattern of local neuronal synchrony is through a frequency-based analysis of local field potential (LFP) signals. The LFP is believed to reflect synchronized dendritic currents in a group of neurons. Tremor-related oscillations, although common in single-unit recordings (Hutchison et al. 1997; Levy et al. 2000, 2002a,b) are not a consistent or strong feature in LFP signals, probably due to the variable phase relationships between neurons oscillating at tremor frequencies (Hurtado et al. 1999, 2005; Levy et al. 2000). Instead oscillatory field potential activity in PD patients off medications is particularly prominent in the beta band (Brown et al. 2001; Kuhn et al. 2005; Levy et al. 2002a; Weinerberger et al. 2006) and, to a lesser extent, in the gamma band (35–90 Hz) (Fogelson et al. 2005; Pogosyan et al. 2006; Trottenberg et al. 2006). In contrast to the beta activity, gamma band oscillations in the basal ganglia have been hypothesized to play a prokinetic role and to contribute to movement generation (Brown 2003; Brown and Williams 2005), as these oscillations are increased during movement and by treatment with dopaminergic medications, in tandem with clinical improvement (Alonso-Frech et al. 2006; Androuldakis et al. 2007; Cassidy et al. 2002; Williams et al. 2002). However, their possible involvement in PD tremor has not been reported.

The aim of this study was to examine the relationship among tremor, beta, and gamma frequency oscillatory activity in the STN and how these change with time and in relation to variations in tremor intensity to gain a better understanding of the role of STN and oscillatory activity in mediating parkinsonian tremor. As part of these studies, we examined the coherence of STN neurons displaying oscillatory firing at the tremor frequencies with the simultaneously recorded limb tremor and how the coherence varied over time. In addition we examined and compared the spatial distribution of these neurons and beta oscillatory neurons along the dorsoventral STN axis and their relation to the simultaneously recorded LFP.

**METHODS**

**Patients**

We studied nine patients with advanced PD who exhibited intermittent periods of resting tremor during stereotactic surgery for the implantation of deep brain stimulating electrodes in the STN. The group consisted of two women and seven men, who at the time of operation had a mean age of 59.5 yr (range: 55–67) and a mean duration of PD of 10.7 ± 3.7 (mean ± SD) years. All patients were assessed preoperatively using the Unified Parkinson’s Disease Rating Scale (UPDRS) (Fahn et al. 1987) before and after an acute levodopa challenge (Moro et al. 2002). During surgery, the patients were awake and off dopaminergic medications for ≥12 h from the last oral dose of antiparkinsonian medications. Demographic details of the patients are given in Table 1. The studies were performed with approval of the University Health Network Ethical Review Board, University of Toronto. Patients gave written and informed consent before surgery.

**Recordings**

Neuronal data were recorded simultaneously with signals from surface EMG electrodes and triaxial accelerometers (with summated x-y-z signals) to monitor muscle activity and limb movement. In all cases, the tremor was contralateral to the side of the recordings. Neuronal activity and LFPs were recorded simultaneously from each of two independently driven microelectrodes (~25 μm tip length, axes 600 μm apart, ~0.2-MΩ impedance at 1,000 Hz) (Levy et al. 2007) during the electrophysiological mapping procedure used to obtain physiological data for localizing the target for the deep brain stimulation electrode placement. The localization procedure of the STN using microelectrode recording was previously described in detail (Hutchison et al. 1998). Briefly, the dorsal border of STN was noted by an increase in background activity and high-frequency neuronal discharge. As the electrodes were advanced past the ventral border of STN, the background noise decreased until they reached the substantia nigra pars reticulata, which was identified by higher-frequency, more regular and lower-amplitude discharges compared with STN. All recordings were amplified 5,000–10,000 times, filtered at 10–5,000 Hz (analog Butterworth filters: high-pass, 1 pole; low-pass, 2 poles) using two Guideline System GS3000 (Axon Instruments, Foster City, CA) amplifiers. The monopolar recordings from both microelectrodes shared a common ground consisting of the stainless steel guide tube and frame attachments to the head. The amplifier ground was also connected to the frame. The signals were digitized at 10 kHz with a CED 1401 [Cambridge Electronic Design (CED), Cambridge, UK] interface.

**Data analysis**

Neuronal discharges were discriminated using spike sorting algorithms in Spike2 (CED). Spike times, unfiltered LFP and accelerometer/EMG data were imported into MATLAB (version 7, The MathWorks, Natick, MA) for further analysis. Recordings from 13 sides in nine patients were analyzed. In four patients, both right and left STN were analyzed; only left STN was analyzed in three patients and right STN in two patients. Only periods without voluntary movements or artifacts were analyzed. Recording depths were realigned to the top of STN in each track, where 0 is the dorsal border of STN and negative values are ventral to this border.

**Table 1. Patient details**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age and Gender</th>
<th>Disease Duration</th>
<th>Motor UPDRS On/Off Drugs Pre-Op</th>
<th>No. of Cells Sampled</th>
<th>Number of Neurons with Tremor/Beta Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>11</td>
<td>11/28</td>
<td>7</td>
<td>1/–</td>
</tr>
<tr>
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<td>59 F</td>
<td>14</td>
<td>10/52.5</td>
<td>16</td>
<td>5/1</td>
</tr>
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<td>19/60.5</td>
<td>13</td>
<td>1/3*</td>
</tr>
<tr>
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<td>6/33</td>
<td>14</td>
<td>1/5</td>
</tr>
<tr>
<td>6</td>
<td>55 F</td>
<td>10</td>
<td>18/41</td>
<td>4</td>
<td>2/–</td>
</tr>
<tr>
<td>7</td>
<td>67 M</td>
<td>6</td>
<td>14/33</td>
<td>11</td>
<td>–/–</td>
</tr>
<tr>
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<td>55 M</td>
<td>14</td>
<td>4.5/39</td>
<td>8</td>
<td>1/3*</td>
</tr>
<tr>
<td>9</td>
<td>63 M</td>
<td>8</td>
<td>17/42</td>
<td>9</td>
<td>4/–</td>
</tr>
</tbody>
</table>

Age is in years. UPDRS, United Parkinson’s Disease Rating Scale. *Including the neuron with both tremor and beta activity.
Tremor amplitude was quantified off-line by calculating the root mean square (RMS) value of the sampled accelerometer signal. For frequency domain analysis, we used the discrete Fourier transform and its derivations calculated according to Halliday et al. (1995). After signals were downsampled to 1 kHz, spectra of LFP power were estimated by dividing the waveform signal into a number of sections of equal duration of 1.024 s (1.024 data points, 512-point overlap), each section was windowed (Hamming window), and the magnitudes of the 1.024 discrete Fourier transform of each section were squared and averaged to form the power spectrum, yielding a frequency resolution of 0.97 Hz. The power was transformed to a logarithmic scale and shown in decibels (dB). Because the estimated power spectrum has a distribution analogous to a $\chi^2$ distribution, the 95% confidence intervals were given on the basis of the $\chi^2$ distribution (Jarvis and Mitra 2001), whereas degrees of freedom values are based on the number of windowed sections.

Spectral analysis of spike trains was performed using the Fourier transform (Halliday et al. 1995) and significant oscillations were detected using shuffling of spike trains (Rivlin-Etzion et al. 2006). Interspike interval (ISI) shuffling generates a new spike train by using the time differences between adjacent spikes (1st-order ISIs). Thus the spectrum of the new spike train is determined solely by the first-order ISIs of the original spike train, whereas higher-order effects (i.e., the time difference between spikes that are separated by 1 spike or more) are abolished by the shuffling process. Comparing the original spectrum to the new one enables one to detect patterns such as oscillations that are generated by higher-order ISIs. To obtain an accurate and less-noisy estimate, we repeated the shuffling process 100 times and averaged the results. Subtraction of the new spectrum from the original spectrum resulted in a corrected spectrum, in which peaks were considered significant when they exceeded the upper 95% confidence limit. The confidence limit was estimated from the corrected spectrum based on the $\chi^2$ distribution as described in the preceding text. Because the variance for the corrected spectrum is the same for all frequencies, the confidence interval depends solely on the degrees of freedom and the term is a constant that does not depend on the frequency (Rivlin-Etzion et al. 2006).

Coherence (Halliday et al. 1995; Rosenberg et al. 1989) was used to estimate the relationship between simultaneously recorded data. The coherence function provides a frequency domain bounded measure of association, taking on values between 0 and 1, with 0 indicating no relationship. Coherence can be estimated by direct substitution of the appropriate spectra as: $|f_{xx}f_{yy}|/f_{xx}^2f_{yy}^2$, with 95% confidence level of $1 - (0.05)^{1/2}$, where $L$ is the number of disjoint sections.

We used the continuous wavelet transform to analyze changes in LFP power in the time-frequency domain (Wang et al. 2005). The wavelet function is convolved with the observed data at each time point and across a range of scales, allowing the identification of specific frequency components over time. In the present study, we used the Morlet wavelet function where a sinusoidal oscillation ($e^{i\omega t} = \cos(\omega t) + i \sin(\omega t)$) is modulated by a Gaussian bell curve ($e^{-\tau^2}$) to give a brief oscillation localized in both time and frequency. The absolute value of the transform is then squared to give a continuous time-frequency representation of the power content of the signal (Torrence and Compo 1998). In addition, wavelet-based coherence was calculated to characterize the temporal relationship between neuronal firing and the simultaneously recorded LFP and tremor. The classical coherence definition was modified using the smoothed Morlet wavelets to yield time-frequency coherence, defined as the ratio of the wavelet cross spectrum to the product of the wavelet autospectra of the two signals (Gurley et al. 2003). Areas of significant coherence were detected with 95% confidence level of $1 - (0.05)^{1/2}$, where $df$ is the number of degrees of freedom required to detect coherence values $>0.1$. The percent time during the overall record when the two signals were significantly coherent with each other for a given frequency band was calculated.

RESULTS

Incidences of neurons with tremor and/or beta oscillatory firing is higher in the dorsal STN

A total of 102 neurons was recorded from the STN during periods of relatively constant tremor amplitude. The mean duration of recordings was 34.6 ± 20.5 (SD) s (range: 14–129 s). Of these 102 neurons, 15 neurons exhibited significant oscillatory firing at a frequency in the range of parkinsonian tremor (tremor-frequency activity) [mean oscillation frequency: 4.1 ± 0.6 (SD) Hz]. In addition, 12 neurons exhibited significant oscillatory firing in the beta frequency range (mean frequency: 22.1 ± 5 Hz). Two of these neurons had both beta and tremor-frequency activity. Examples of power spectra of neurons firing at tremor and/or beta frequencies are shown in Figs. 1A and 2. There was no significant difference between the mean firing rate of neurons with tremor-frequency activity and neurons with beta activity (55.7 ± 30 and 67.8 ± 27 Hz, respectively, $t$-test); however, the oscillatory neurons ($n = 25$) had a significantly higher mean firing rate than...
nonoscillatory neurons ($n = 77$; medians: 60.5 and 36 Hz, respectively, $P = 0.007$, Mann-Whitney rank-sum test). The distribution of the observed neurons among patients is indicated in Table 1.

The majority (13/15) of the neurons with tremor-frequency activity was localized in the dorsal 2.5 mm of STN. This distribution was similar to that of the beta oscillatory neurons. The mean location within the STN (where 0 indicates the dorsal border) of neurons with tremor-frequency activity was not significantly different from that of neurons with beta activity $[-1.3 \pm 1.0$ and $-1.7 \pm 1.0$ (SD) mm, respectively, $t$-test]. Figure 1B shows the distributions of the oscillatory and nonoscillatory neurons within the STN at successive 0.5-mm intervals.

Neurons with tremor-frequency oscillations are coherent with the LFP at the beta frequencies

Seven of the 15 neurons (46.6%) with tremor-frequency oscillatory firing showed significant coherence with the simultaneously recorded LFP in the tremor frequency range (Fig. 2, A and B). Interestingly however, 13 tremor-frequency neurons (86.6%) showed significant coherence with the LFP in the beta frequency range. Eight of these neurons were coherent with the LFPs recorded from both microelectrodes (including the 2 neurons with both tremor and beta activity; Fig. 2). The electrophysiological characteristics of the neurons with tremor-frequency activity are summarized in Table 2. It is important to note that the values of coherence, at the beta frequencies, between the tremor-frequency neurons and the LFP were significantly lower than the values of coherence between the beta oscillatory neurons and the LFP (median: 0.17 and 0.35, respectively, $P \leq 0.001$, Mann-Whitney rank-sum test, excluding neurons with both tremor and beta activity).

Of the 12 neurons with beta oscillations, 11 (92%) showed significant coherence with the LFP, and 10 of these neurons were coherent with the LFPs from both microelectrodes. In contrast, only 19 of the 77 nonoscillatory neurons (25%) showed coherent firing with the LFP at the beta frequencies. These neurons might exhibit weak beta oscillatory firing that failed to reach significance but nonetheless had significant coherence when compared with the LFP.

Coherence between neuronal firing and LFP varies over time

To further characterize the coherence between neurons and the simultaneously recorded LFP, we used wavelet-based coherence, which allowed us to estimate the percentage of time during which the two signals were significantly related. Examples of temporal coherence between tremor/beta oscillatory neurons and the simultaneously recorded LFP are shown in Fig. 3, A and B, respectively. During these periods, tremor amplitude was relatively constant. Only 8 of the 15 tremor-frequency neurons displayed periods of significant coherence with the LFP recorded from one or both microelectrodes at the

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**Figure 2.** A—C: examples of neurons with tremor-frequency activity (top) and their coherence with the local field potentials (LFPs) recorded from the 2 microelectrodes (middle) and with the electromyogram (EMG) recorded from the tremulous joint (bottom). These neurons are represented in Table 2 as A, 3; B, 14; and C, 1.
tremor frequencies. On average, this coherence lasted for 68 ± 23% (mean ± SD) of the time. In addition, 14 tremor-frequency neurons were coherent with the LFP at the beta frequencies for an average of 50 ± 26% of the time. It is important to consider that although more neurons displayed coherence with the LFP at the beta frequencies, there was no significant difference between the durations of coherence in the two frequency bands (P = 0.13, t-test). Beta oscillatory neurons were coherent with the LFP for 77 ± 33% of the time, and only one neuron was not coherent with the LFP. At the beta frequencies, beta oscillatory neurons were coherent with the LFP for a significantly longer duration relative to tremor-frequency neurons (P = 0.03, t-test). The data are summarized in Fig. 3C. Note that neurons displaying only beta oscillatory activity were not coherent with the LFP at the tremor frequencies.

**Neurons with tremor-frequency oscillations are not constantly correlated with tremor**

Of the 15 neurons with tremor-frequency oscillations, only 7 were coherent with the simultaneously recorded tremor (EMG and/or accelerometer; Table 2, Fig. 2). However, the frequency of oscillations of 12 of the 15 neurons was similar to the frequency of tremor, ranging between 4 and 4.9 Hz. In the remaining three neurons, the frequency of oscillations was 3 Hz and was lower than the frequency of tremor (~4.8 Hz). Nevertheless, the activity of one of these neurons was coherent with the EMG at 4.8 Hz (see Fig. 2C), suggesting that some of its activity was nevertheless related to the tremor.

Because the relationship between tremor-frequency neuronal activity in the STN and tremor is most likely a nonstationary process, we examined this relationship over periods of relatively constant tremor amplitude. Consistent with our conventional coherence analyses, only 7 of the 15 tremor-frequency neurons were significantly related to the tremor over time. These neurons were coherent with the tremor for 86 ± 23% (mean ± SD) of the time. Figure 4A shows an example of temporal coherence between a tremor-frequency neuron and the simultaneously recorded tremor.

Six tremor-frequency neurons were recorded during periods of altering tremor amplitude. Three of these neurons were oscillating significantly only during periods of stronger tremor, whereas two neurons were oscillating significantly only when tremor ceased. One neuron was oscillating during both periods of tremor and nontremor. Figure 4D shows an example of the activity of a tremor-frequency neuron over time. This neuron exhibited significant tremor-frequency oscillations only during episodes of simultaneous limb tremor (although, in this case, there was no significant coherence with the recorded tremor). The tremor-frequency activity could be observed together with significant beta oscillations.

**LFP oscillatory activity in the 35- to 55-Hz gamma frequency band is increased during tremor**

LFP signals during periods with altering tremor amplitude were recorded from 58 sites within the STN [mean recording duration: 91.1 ± 69.7 (SD) s, range: 34–436 s]. Interestingly, in 49 of the 58 sites (85%), we observed an increase in the LFP power in the low gamma-frequency range (35–55 Hz) during periods of stronger tremor compared with periods of weak or no tremor. Examples of increased LFP gamma power with increased tremor amplitude are shown in Figs. 5 and 6. The majority of these sites (40/49) were located in the dorsal 3.0 mm of the STN. In contrast, seven of the 9 sites where the LFP gamma power did not increase during periods of stronger tremor were in the ventral 3.0 mm. The distributions of sites where gamma activity increased and sites with no increase were significantly different (mean depths: −2.0 ± 1.4 vs. −3.3 ± 1.5 mm, respectively, P = 0.013, t-test). The increase in the LFP power in the low gamma-frequency range was observed in each of the nine patients (Fig. 7A).

In 37 of the 58 sites, neuronal spiking activity was recorded simultaneously with the LFP. The mean firing rate of these neurons was significantly higher during periods of stronger tremor amplitude relative to periods of weak/no tremor (48.7 ± 27 and 38.3 ± 23.9 Hz, respectively, P ≤ 0.001, paired t-test).

The LFP power over the gamma frequencies was then averaged across patients for the periods of weak tremor (mean tremor RMS = 0.14) and periods of stronger tremor (mean tremor RMS = 1.12). On average, the gamma power was significantly higher during the periods of stronger tremor (median power: 0.014 and 0.026 for weak and stronger tremor, respectively; n = 58,

<table>
<thead>
<tr>
<th>Cell</th>
<th>Location within STN, mm</th>
<th>Oscillation Frequency, Hz</th>
<th>Mean Firing Rate, Hz</th>
<th>Coherence with LFP at Tremor Frequencies</th>
<th>Coherence with LFP at Beta Frequencies</th>
<th>Coherence with EMG/accel</th>
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<td>EMG</td>
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</table>

STN, Subthalamic nucleus; LFP, local field potential; EMG, electromyogram. *Neurons with both tremor and beta activity.
Figure 3. A: wavelet-based coherence showing 58 s of temporal relationship between a tremor-frequency neuron (3 in Table 2) and the simultaneously recorded LFP. Gray boundaries indicate areas of 95% significance level. B: wavelet-based coherence showing 65 s of temporal relationship between beta oscillatory neuron and the simultaneously recorded LFP. Accelerometer traces in A and B indicate a relatively constant tremor state in patients 2 and 4, respectively. C: bar graph showing the mean duration (±SE) of significant coherence with the LFP for tremor and beta oscillatory neurons. Note that each group contains only the neurons that showed significant coherence with the LFP. Asterisk, *P = 0.03 (t-test).

Altered balance between beta and gamma rhythms during periods of stronger tremor

An increase in the LFP power in the tremor frequencies was also observed; however, we cannot rule out the possibility that this increase is due to tremor-movement related artifacts.

In addition to the increased power of individual LFPs, the coherence between two LFPs in the low gamma frequency range was also increased during periods of stronger tremor (see Fig. 7C).
Figs. 5 and 6: B3 and C3). This was observed in 17 (65%) of the 26 sites where the two LFPs were recorded simultaneously. In comparison, the coherence between LFPs in the beta frequency range was increased in 46% of the cases (12/26 sites). When averaging across sites for the periods of weak tremor (mean RMS = 0.13) and stronger tremor (mean RMS = 1.12), we observed that the gamma coherence was significantly increased with increasing tremor (medians: 0.01 and 0.23 for weaker and stronger tremor, respectively; n = 26, P ≤ 0.001, Wilcoxon signed rank test). On the other hand, beta coherence did not change significantly (means: 0.38 and 0.33; P = 0.28, paired t-test). Figure 8A shows the relative changes in beta and gamma coherence that were calculated by normalizing the coherence values during periods of strong tremor to the values that were measured during weak tremor. On average, the coherence in the beta frequencies decreased only by 0.04 ± 0.2, whereas the coherence in the gamma frequencies increased by 0.14 ± 0.2 (mean ± SD). In addition, the ratio of the coherence value in the beta frequencies and the coherence value in the gamma frequencies was significantly lower during periods of strong tremor versus weak tremor (medians: 3.95 and 1.28; P = 0.003, Wilcoxon signed rank test; Fig. 8B).

To provide further support for the relative increase in gamma coherence compared with beta coherence, we used temporal coherence to calculate the percentage of time during which the simultaneously recorded LFPs were coherent with each other. We found that during the periods of strong tremor, gamma coherence significantly increased in duration and lasted for 58.7 ± 33.5% of the time (mean ± SD) compared with 34.5 ± 32.8% during periods of weaker tremor (P ≤ 0.001, paired t-test). The duration of beta coherence, on the other hand, did not alter significantly (83.5 ± 34.2 and
DISCUSSION

This study provides data documenting the relationship of STN neuronal and LFP oscillatory activity to rest tremor in PD. This is the first study to show that STN neurons oscillating at PD tremor frequencies are not only located in the same region as beta oscillatory neurons (in dorsal STN) but are also coherent with the simultaneously recorded LFPs in the beta frequency band, and that the oscillatory LFP activity in the low-gamma frequency band is enhanced during periods when patients exhibit tremor at rest.

Tremor-frequency neuronal activity: relation to tremor

In the present study, we found that in tremulous PD patients, nearly 15% of STN neurons exhibit significant oscillations at the tremor frequency and that in 80% of the cases, the frequency of oscillations was similar to the frequency of limb tremor suggesting that this oscillatory activity might be tremor-related. However, significant coherence between tremor-frequency oscillation and limb tremor was only observed in 47% of the cases (Table 2). Tremor characteristically varies in location and timing, and practical limitations preclude simultaneous monitoring of all somatic muscles that may be tremulous. Also in our study, in many cases, accelerometer signals were used to determine coherence with firing and may not detect low-intensity tremors. This may account at least in some of the cases for the lack of correlation between the STN tremor-frequency neuronal activity and the tremor recorded in the sampled muscles. Our observation is consistent with previous reports showing that neuronal oscillations in the globus pallidus are not always coherent with the tremulous limb (Hurtado et al. 1999; Lemstra et al. 1999; Raz et al. 2000), although their oscillation frequency is similar to the frequency of the limb tremor (Hutchison et al. 1997). Several studies of multilimb EMG recordings in PD patients also indicated that tremor in different limbs is largely uncorrelated (Ben-Pazi et al. 2001; Hurtado et al. 2000; Raethjen et al. 2000). These studies, together with the fact that only half of the tremor-frequency neurons fire coherently with the LFP, support the earlier hypothesis that independent oscillatory circuits may underlie parkinsonian tremor in different extremities (Alberts et al. 1969). This idea was further strengthened by Hurtado et al. (2005), who showed that tremor-related activity in the globus pallidus can be coherent with one tremulous limb but not with the other (Hurtado et al. 2005).

Interestingly, we found that oscillatory activity in single cells, as well as their coherence with limb tremor, occurs intermittently and, in some cases, independently from the fluctuations in tremor amplitude. This finding suggests that tremor oscillatory circuits are not only independent but that their oscillatory activity and synchrony with tremor can fluctuate over time. These findings are similar to those of Hurtado...
et al. (1999, 2005), who reported transient synchronization between limb tremor and neuronal oscillations in globus pallidus of PD patients.

Tremor-frequency neuronal activity: relation to beta oscillations

We have demonstrated that the vast majority (~87%) of the tremor-frequency neurons are located in the dorsal part of STN where neurons firing with beta oscillatory rhythms are observed. This observation is consistent with an earlier report that 84% of the tremor-related neurons are located in the dorsolateral STN (Rodriguez-Oroz et al. 2001) and with our previous study showing that most of the beta oscillatory neurons are located in the dorsal portion of STN (Weinberger et al. 2006) where also the beta LFP oscillatory activity is maximal (Kuhn et al. 2005; Weinberger et al. 2006). Anatomical studies indicate that the dorsolateral region receives inputs from the primary motor cortex (Monakov et al. 1978; Nambu et al. 1996), whereas inputs from the premotor and supplementary motor areas project mainly to the medial region of the STN (Nambu et al. 1997). The dorsolateral STN contains neurons responsive to passive and active movements in both monkey and human (Abosch et al. 2002; DeLong et al. 1985; Rodriguez-Oroz et al. 2001). In contrast, neurons located in the ventral and medial region of the STN have relatively weak or no sensorimotor responses and are reciprocally connected with the associative and limbic cortical regions (Maurice et al. 1998; Parent and Hazrati 1995). It is therefore not surprising that neurons with tremor-related activity are clustered in the dorsolateral STN, which is most likely to be the STN region involved in mediating the cardinal motor features of Parkinson’s disease. It has been shown that microstimulation in the dorsolateral STN can induce tremor arrest with a short latency of <200 ms (Rodriguez-Oroz et al. 2001) and that local block of this region by lidocaine or muscimol microinjections reduces tremor (Levy et al. 2001). Our results provide further support for the association of this region with tremor and as a clinically effective target for deep brain stimulation to alleviate PD tremor (Herzog et al. 2004).

Interestingly, apart from the similar distribution of tremor frequency and beta oscillatory neurons within the STN, our results demonstrate that although only half of the tremor-frequency neurons oscillate coherently with the LFP at the tremor frequencies, the majority fire in coherence with the LFP at the beta frequencies. This is surprising because only 2/25 showed both tremor and beta oscillatory activity and also in light of the growing evidence for the lack of relationship between beta oscillations and tremor (Amirnovin et al. 2004; Kuhn et al. 2006; Ray et al. 2008; Silberstein et al. 2003; Wang et al. 2005; Weinberger et al. 2006). Instead beta oscillations have been suggested to contribute to bradykinesia and rigidity (Brown 2003; Chen et al. 2007; Kuhn et al. 2006, 2008; Ray et al. 2008). The low incidence of neurons with both tremor and beta oscillation could indicate the existence of two separate but interdigitated populations of neurons with different func-
tions and connectivity within the same region. However, the fact that tremor-activity neurons are coherent with LFP beta oscillatory activity suggests the opposite—i.e., that most if not all of the neurons in the dorsal region receive oscillatory inputs in both frequencies. The tremor-frequency neurons might therefore exhibit weak beta oscillations that failed to reach significance. Coherence analysis is much more sensitive and reveals activity that may not reach significance in the power spectrum.

In light of these results, we hypothesize that those neurons in STN that are likely to oscillate at tremor frequencies are also those prone to oscillate at beta frequencies. In other words, the same STN neuron that oscillates at tremor frequencies during tremulous behavior can oscillate at beta frequencies during akinetic/rigid behavior. When examining the distribution of the neurons among our tremulous group of patients, it can be observed that tremor oscillatory activity was detected in most patients (7 of 9), whereas beta oscillatory neurons were only observed in four patients (see Table 1). It is possible that in tremulous patients neurons in the dorsal STN are more likely to oscillate at the tremor frequencies because cortical oscillations at these same frequencies are also increased, and this region receives direct projections from the corresponding cortical regions (Hellwig et al. 2000). In a recent study (Weinberger et al. 2006), we found that 28% of the neurons in STN display significant beta oscillatory activity. On the other hand, in this study, which included a different population of patients, only 12% of the neurons were oscillating at beta frequencies. However, overall 24.5% of the neurons were oscillatory at either tremor or beta frequencies. These neurons are perhaps those that, in the dopamine depleted state, become more sensitive to rhythmic cortical inputs (Bevan et al. 2007; Magill et al. 2001; Paz et al. 2005; Sharott et al. 2005). Supporting this hypothesis and consistent with previous finding by Levy et al. (2000), there was no significant difference between the mean firing rate of neurons with tremor activity and neurons with beta activity. These neurons fire at a faster rate relative to the nonoscillatory neurons (see also Levy et al. 2000; Weinberger et al. 2006). The higher mean firing rates of the oscillatory neurons is perhaps due to a greater efficacy of the excitatory cortico-subthalamic inputs on those neurons following dopamine depletion (Bevan et al. 2007; Magill et al. 2001). The chronic loss of dopamine in the STN (in addition to its loss in the striatum) might further promote the capability of the cortex to drive rhythmic STN activity through actions both on the excitability of STN neurons and on the activity-dependent plasticity at synapses in the STN (Bevan et al. 2006).

Low gamma LFP oscillatory activity: relation to tremor

Increased LFP power in the low gamma frequency range (35–55 Hz) was observed during periods of stronger tremor. We have shown that the increase in gamma activity during tremor is most likely to occur at sites in the dorsal STN, suggesting it is related to sensorimotor processing. It is yet to be elucidated, however, whether this increase is associated with the mechanism of generation of resting tremor in PD patients or is simply a result of increased motor drive to, or output from, STN during tremor. In addition to the increased low gamma power at individual sites within STN, we also observed increased coherence between nearby sites, suggesting that the low gamma LFP oscillations are distributed and synchronized over at least a few millimeters within the STN. It has been previously demonstrated that subthalamic gamma LFP oscillations recorded from PD patients during rest are greater within the upper STN and bordering zona incerta, where they...
are consistently synchronized to neuronal discharge and might therefore reflect synchronized population activity of local neurons (Trottenberg et al. 2006).

In our study, the mean firing rate of local neurons was significantly higher during stronger tremor, suggesting that the elevated gamma LFP activity is associated with changes in STN neuronal firing rates during tremor. Indeed it has been recently shown that elevations of STN gamma LFP activity do not only influence the relative timing of spikes in spike trains (Trottenberg et al. 2006) but also have a major effect on information carrying capacity (Pogosyan et al. 2006). It has been argued that STN gamma oscillations can increase the information that can be transmitted and decoded by neurons downstream of STN (Foffani and Priori 2007). Thus exaggerated increase in overall information flow in downstream neurons may indirectly contribute to the generation of rest tremor in PD.

We found that the ratio of the beta to gamma coherence was significantly lower during stronger tremor, suggesting that alteration of the balance between beta and gamma oscillations in the STN is related to tremor and/or its magnitude. STN beta oscillations are prominent in PD patients withdrawn from dopaminergic therapy (Brown et al. 2001; Levy et al. 2002a; Priori et al. 2004; Williams et al. 2002) and are thought to play an anti-kinetic role (Brown 2003; Kuhn et al. 2004). Increasing evidence suggests that beta oscillations contribute to bradykinesia rather than to tremor in PD (Chen et al. 2007; Kuhn et al. 2006, 2008; Weinberger et al. 2006). In contrast, STN oscillations in the gamma range are increased following dopaminergic therapy and during movement and have been related to specific coding of movement related parameters (Androulidakis et al. 2007; Brown 2003; Cassidy et al. 2002; Williams et al. 2002). Negative correlation between beta and gamma oscillations in STN has been shown to occur after treatment with dopaminergic medications (Alonso-Frech et al. 2006; Fogelson et al. 2005), providing further evidence for the reciprocal interactions between these two functionally different oscillatory patterns. It has been argued that the balance between beta and gamma oscillations determines the effects of abnormal high-frequency synchronization on tremor.

![Relative coherence change](image1)

![Beta/gamma ratio](image2)

![Temporal coherence](image3)

Fig. 8. A: the relative changes (±SE) in beta and gamma coherences between episodes of weak and stronger tremor. The coherence values during episodes of strong tremor (mean RMS = 1.12) were normalized to the values that were measured during weak tremor (mean RMS = 0.13). On average, beta coherence decreased only slightly (by 0.04 ± 0.2, mean ± SD), whereas gamma coherence increased significantly (by 0.14 ± 0.2, mean ± SD). *P ≤ 0.001 (Wilcoxon signed-rank test). B: Box plots of the mean ratio of the beta to gamma coherence during episodes of weak and stronger tremor (medians: 3.96 and 1.28, respectively; P = 0.003, Wilcoxon signed-rank test). C: Bar graph showing the mean duration (±SE) of significant coherence between the 2 LFPs in the beta and gamma frequencies during periods of weak and stronger tremor. *P ≤ 0.001 (paired t-test). D: Accelerometer trace showing 52 s of intermittent resting tremor of the left leg in patient 3 (top) with a wavelet-based coherence between the 2 simultaneously recorded LFPs (bottom). Significant coherence is indicated by the gray boundaries.
suggesting that resting tremor in PD patients is associated with increase in the power of gamma oscillatory activity in STN. Furthermore, during tremor there is an increase of these neurons is frequently coherent with the beta oscillatory activity are found and moreover that the firing of dorsal STN in the same region where neurons firing with oscillatory activity in the tremor range are usually located in the cortex (Hammond et al. 2003). As mentioned in the preceding text, in the dopamine-thalamocortical projections on the motor areas of the cortex (Brown 2003). Our results may therefore suggest that altered balance between these two modes of activity may also be related to resting tremor in PD. It is important to mention that the baseline levels of gamma LFP in our study may be relatively low as the recordings were done during off medication state. The elevated gamma levels together with the relatively high beta activity that (did not decrease during tremor episodes) may characterize the STN state during resting tremor in PD.

It is important to note, however, that the gamma oscillatory activity we observed was in the low gamma range (35–55 Hz), and we did not see activity at the higher range of gamma frequencies (≈90 Hz) usually observed in STN following levodopa treatment and with movement. In fact, parkinsonian rest tremor is known to be suppressed with onset of voluntary movement of the same limb. Thus the low-beta activity observed during tremor might not necessarily be movement-related. Parkinsonian rest tremor is diagnosed only if its amplitude increases during mental stress (Deuschl et al. 1998). Therefore an alternative hypothesis is that subthalamic low-gamma oscillations are related to mental processes that lead to fluctuations in STN state during tremor. Gamma-band synchrony in the 40- to 60-Hz range has been implicated in intense mental activity and in various cognitive functions such as memory and attention (Fitzgibbon et al. 2004; Kaiser and Lutzenberger 2005; Kessler et al. 2000; Tallon-Baudry et al. 1998; Tiitinen et al. 1993). Attention-related gamma power modulations were reported in humans especially over the frontal and central cortices (Tiitinen et al. 1993; Ward 2003). The cerebral cortex is known to influence the activity of the STN directly via monosynaptic projections (Kitai and Deniau 1981). As mentioned in the preceding text, in the dopamine-depleted state, there is an increased coupling between cortex and STN activities (Magill et al. 2001) perhaps due to a greater efficacy of the excitatory synaptic inputs (Bevan et al. 2007). It has been previously suggested that basal ganglia beta- and gamma-band oscillatory activity may arise in the cortex (Hammond et al. 2007). Thus the low-gamma synchrony observed in STN during tremor might reflect an enhanced response to rhythmic cortical input during periods when there is increased low-gamma cortical activity.

In conclusion, this study has shown that STN cells with oscillatory activity in the tremor range are usually located in the dorsal STN in the same region where neurons firing with beta oscillatory activity are found and moreover that the firing of these neurons is frequently coherent with the beta oscillations of the LFP. Furthermore, during tremor there is an increase in the power of gamma oscillatory activity in STN suggesting that resting tremor in PD patients is associated with an altered balance between beta and gamma oscillations.

Acknowledgments

We thank all the patients who participated in this study and thanks to N. Mahant for writing some of the custom software used in the analysis.

Grants

This work was supported by the Canadian Institutes of Health Research Grant MOP-42505 to J. O. Dostrovsky.

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