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Chapter 4

Tremor-specific neuronal oscillation pattern in dorsal subthalamic nucleus of parkinsonian patients

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ABSTRACT

Background
Subthalamic nucleus (STN) deep brain stimulation effectively improves parkinsonian symptoms. It is hypothesized that distinct functional territories with different neurophysiologic activity within the STN relate to different symptoms.

Objective
Aim of the study was to identify distinctive characteristics of STN neuronal activity related to tremor by directly comparing tremor sides to no-tremor sides. In addition, we studied the spatial pattern of frequency distributions within the STN in more detail.

Methods
We analyzed intraoperative STN single/multi-unit recordings from 33 tremor sides and 23 no-tremor sides. STN tracks were normalized to a length of 1 and subdivided into eight successive layers. The power spectral density was split into six frequency bands: theta (3-8 Hz), alpha (9-12 Hz), lower beta (13-20 Hz), upper beta (21-30 Hz), lower gamma (31-59 Hz), and upper gamma (60-100 Hz).

Results
Tremor sides presented predominant theta frequency oscillations in the most dorsal layers of the STN, while in no-tremor sides beta frequencies predominated. Oscillatory activity was stronger in the dorsal STN than in the ventral, and this pattern was specific for frequencies in the theta, alpha, and beta bands, but not in the gamma bands.

Conclusions
Our study supports the hypothesis that the presence of tremor is associated with a distinctive neuronal oscillations pattern. In particular, we demonstrate the specificity of the association of theta frequencies in the dorsal STN with tremor. Identification of symptom-specific characteristics of intraoperative micro-recordings in the STN may lead to refinement of targeting for each patient, tailored to the specific clinical presentation.
INTRODUCTION

The benefits and side-effects of subthalamic nucleus (STN) deep brain stimulation (DBS) for Parkinson’s disease (PD) are well documented. 1 Anatomical and neurophysiological data suggest that the dorso-lateral portion of the STN has sensory-motor functions, while the ventro-medial portion is involved in affective and limbic functions, which could explain the incidence of cognitive and behavioral side-effects after STN DBS. 2,3 It is hypothesized that symptom-specific topography within STN is determined by distinct neuronal oscillatory activity, with beta-frequency oscillations (13-30 Hz) correlating to bradykinesia and rigidity. 4-8 Theta-frequency oscillations in the basal ganglia (3-8 Hz) have been associated with both parkinsonian tremor and Essential Tremor (ET). 9 However, direct comparison of STN neuronal oscillatory activity between PD patients with and those without tremor, or between body sides with and those without tremor, has been rarely performed, and only in small case series. 10-12 In order to substantiate existing evidence and to demonstrate the specificity of the association of theta frequencies with tremor, direct comparison of a large number of tremor and no-tremor sides is needed. In the present study, we therefore defined the spatial pattern of neuronal oscillatory frequency distributions within the STN from microelectrode recordings of 33 tremor sides and 23 no-tremor sides in 34 PD patients. We hypothesize that the pattern of frequency distribution across successive STN layers would be different between tremor and no-tremor sides.

METHODS AND MATERIALS

All PD patients who underwent STN DBS from March 2003 (when we started using microelectrode recordings (MER)) until June 2007 were considered (Fig. 3, Table 1).

Surgical procedure and MER

We used frame-based three-dimensional MRI and Leksell Surgiplan® software and stereotactic frame (Elekta Instrument AB, Stockholm, Sweden) for targeting. Standard STN coordinates were: 12 mm lateral, 2 mm posterior, and 4 mm below the midcommissural point. Visual adjustments were made based on T2-weighted MRI. Paths were planned with 15-20° anterior angulation to the intercommissural line, 20-30° lateral angulation from midline, avoiding sulci, vascular structures, and lateral ventricles. Surgery and MER were performed bilaterally with patients being awake and without sedatives. Dopamine agonists, when used, were gradually reduced and then stopped 3 days before surgery, while Levodopa was stopped at least 12 hours before surgery (“off-medication”), according to a standard protocol. Single/multi-unit MER were performed using Medtronic microelectrodes 291 (10 µm² exposure; impedance ~1.1MΩ at 220Hz), which capture spikes within ~100-200 µm (1-3 neurons) and background activity. 13 Depending on pre-operative...
MRI, three to five MER needles were placed in an array with central, lateral, medial, anterior, and posterior cannulas, 2 mm apart. Starting from a remote position, electrodes were advanced simultaneously in 0.5 mm-steps until substantia nigra activity was recognizable or activity significantly decreased in all channels. Signals were amplified by 10,000 (Leadpoint, Medtronic), analog bandpass filtered between 500-5,000Hz (-3 dB; 12 dB/oct) and sampled at 24 kHz with a 16-bit A/D converter. Clinical testing was performed at several sites. The definitive DBS electrode was implanted at the site with the best therapeutic window.

**Data analysis**
Starting 6 mm above target, all recordings from the trajectory with the best clinical effect – i.e. the trajectory that was chosen for permanent stimulation – were retrospectively analyzed. Only trajectories with STN activity for 4 mm or longer were included. The final position of all DBS electrodes was checked by co-registration of post-operative CT with pre-operative MRI.

Recordings were visually inspected off-line. Only the longest, artifacts-free, stable sections (minimally 3 seconds long) were analyzed.

The motor part of the Unified Parkinson’s Disease Rating Scale performed off-medication before surgery was used to evaluate parkinsonian symptoms. Each STN was defined as ‘no-tremor side’ when the contralateral sub-score for tremor (items 20 + 21) was 0, and ‘tremor side’, when it was larger than 0. The investigator who performed analyses was blinded to clinical information.

**Determination of STN borders**
To determine STN borders, we analyzed the root mean square (RMS) of the signal. At each recorded site s, we calculated the RMS(s) of the signal x(s) as:

\[ \text{RMS}(s) = \frac{\sum_{i=1}^{n} |x_i(s)|}{n} \]

where \( n \) is the total number of recorded samples at each site and \( i \) is the \( i \)th sample. Since factors like electrode properties or tissue impedance may influence RMS, we calculated a normalized RMS (NRMS) for inter-trajectory comparison. NRMS was defined as

\[ \text{NRMS}(s) = \frac{\text{RMS}(s) - \text{RMS}_{\text{all sites}}}{\text{RMS}(s) + \text{RMS}_{\text{all sites}}} \]

where \( \text{RMS}_{\text{all sites}} \) is the average of RMS(s) across all sites of the chosen trajectory. Thus, NRMS had a value between -1 and 1. STN MER activity is characterized by an increase of background noise amplitude and an increase of neuronal firing: recordings were defined to be inside the STN if NRMS(s) >0 and outside the STN if NRMS(s) <0 (Fig.1). All raw recordings were also visually inspected to verify this attribution.
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Figure 1. Representative spectral activity from a tremor side (left) and a no-tremor side (right). Top: delineation of subthalamic nucleus MER activity based on normalized RMS. Bottom: distribution of power spectral density in 6 frequency bands across subsequent sites (same data as top panels).

Subdivision in layers
In order to average recordings from corresponding STN layers across tracks with variable length, we normalized the portion of the electrode track between entering and leaving the STN to a length of 1.0. We subsequently subdivided it into 8 equal bins, representing successive adjacent layers going from the dorsal to the ventral border (Fig.2).

Spectral analysis
After recorded MER signals are high-pass filtered, the resulting traces contain high-frequency spike activity, which is modulated at low frequency. We extracted low frequency information from the signal envelope by means of full-wave rectification.\textsuperscript{12,15}

After artifacts removal and mean subtraction, power spectral density was obtained by computing the power spectrum of the full-wave rectified signal. For each site, the complete signal was divided into non-overlapping disjoint segments of 32768 samples (segment length of 1.3653 seconds).\textsuperscript{16} This resulted in a frequency resolution of 0.7324 Hz. Spectral analysis
of this signals was performed with MATLAB 7.6 (The Mathworks Inc) using Neurospec scripts.17
MER activity was assumed to be a realization of stationary zero mean time series. Discrete Fourier transform and parameters derived from it were used for spectral analysis. The power spectral density was averaged across corresponding layers of all the trajectories. Power spectral density was split up into six frequency bands: theta (3-8 Hz), alpha (9-12 Hz), lower beta (13-20 Hz), upper beta (21-30 Hz), lower gamma (31-59 Hz), and upper gamma (60-100 Hz).18,19 Frequencies below 3 Hz were not included, to exclude pulsation or movement artifacts. To compare trajectories, we normalized the absolute power in each frequency band at each site by dividing it by the average absolute total power (3-100 Hz) of all the sites outside the STN in the same trajectory. Recorded signals were on average 8 seconds long, with a minimum of 3 seconds (7.6% of the signals), which always resulted in 2 or more segments per single site.

Figure 2. Method for clustering corresponding layers of different subthalamic nuclei (STN). The portion of the electrode track between entering and leaving the STN was normalized to a length of 1.0, and subsequently subdivided into 8 equal bins.
Statistical analysis
We conducted a multilevel linear mixed-effects model analysis. As some patients contributed two sides and data from the same patient might not be independent, “side” and “patient” were used as contextual variables, by allowing a random intercept. The presence or absence of tremor defined two groups. Layers (8 levels) and frequency (6 levels) were used as repeated variables and were considered ordinal variables. We analyzed the main fixed effects of tremor, frequency and layer and their interaction (tremor*frequency, tremor*bin and frequency*bin). A second level interaction (tremor*frequency*bin) was included to test the hypothesis that the pattern of frequency distribution would be different across the layers between tremor and no-tremor sides. The model was estimated with Restricted Maximum Likelihood. For statistical analysis, we used PASW statistics 18 software (former SPSS).

RESULTS

Fifty-five PD patients were eligible for the study. Due to MER unavailability, prior pallidotomy, too short trajectory or suboptimal electrode position, 54 nuclei were excluded from analysis (Fig.3). Thus, 56 STN trajectories from 34 patients were analyzed (22 bilateral and 12 unilateral). Thirty-three sides were classified as ‘tremor sides’ (average tremor score 3.2) and 23 as ‘no-tremor sides’. Patients’ characteristics are described in Table 1.

Table 1. Clinical characteristics of the patients included in the study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>34</td>
</tr>
<tr>
<td>Gender (women/men)</td>
<td>10/24</td>
</tr>
<tr>
<td>Age at STN surgery (years)</td>
<td>57.3±10.4</td>
</tr>
<tr>
<td>Disease duration at STN surgery (years)</td>
<td>10.7 ± 3.8</td>
</tr>
<tr>
<td>Levodopa-equivalent daily dosage (mg)</td>
<td>1085.7 ± 429.2</td>
</tr>
<tr>
<td>UPDRS motor score off medication</td>
<td>39.8 ± 11.7</td>
</tr>
<tr>
<td>Number of sides</td>
<td>56</td>
</tr>
<tr>
<td>Sides with tremor/no-tremor</td>
<td>33/23</td>
</tr>
</tbody>
</table>

* Formula for Levodopa-equivalent daily dosage: regular levodopa dose + slow release levodopa × 0.75 + bromocriptine × 10 + apomorphine × 10 + ropinirole × 20 + pergolide × 100 + pramipexole × 100 + [regular levodopa dose + (slow release levodopa × 0.75)] × 0.2 if taking entacapone. STN = Subthalamic nucleus; UPDRS = Unified Parkinson’s disease rating scale.
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Figure 3. Flow diagram of patient inclusion. MER = Microelectrode recordings. * For 2 patients the other side was included, for 4 patients the other side was discarded due to one of the above mentioned reasons.

As spectral estimation might be less accurate in short recordings, we have performed sensitivity analysis to explore if the presence of short recordings (< 4 sec) could affect the results. The number of trajectories with short recordings was equally distributed among tremor and no-tremor sides (42% and 39% respectively). We compared the power of each frequency band in each layer between trajectories containing short recordings and trajectories with no short recordings and found no significant difference in any value (t-test). Moreover we have applied the main statistical model only to the group of trajectories with no short segments (N=33, 19 tremor sides, 14 no-tremor sides) and obtained similar results (significant effect of layer, significant effect of frequency, significant interaction layer*frequency and significant interaction tremor*frequency). The second level interaction (tremor *layer*frequency) did not reach a significance level (p = 0.056), most likely due to the smaller number of trajectories analyzed. Therefore, we assume that the inclusion of trajectories with short recordings did not significantly affect the results.

There was a significant main effect of the layer (F = 64.565; p < 0.001). Overall power in the top layers appeared higher than in the bottom layers (Fig.4a). There was also a significant main effect of the frequency band analyzed (F 81.444; p < 0.001). Power in the frequency bands 3-8 Hz, 9-12 Hz, 13-20 Hz and 21-30 Hz appeared higher than in the 31-100 Hz band.
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(Fig. 4b). There was a significant interaction effect between the layer and the considered frequency band ($F = 42.264; p < 0.001$). This indicates that the profile of spectral power in the different frequency bands varied according to the layer considered (Fig. 4c).

Indeed, in the upper layers frequencies from 3 to 30 Hz appeared to have a higher power than frequencies from 31 to 100 Hz, while in the lower layers (6 to 8) power was similarly distributed in all frequencies. In other words, only in the dorsal STN theta, alpha, and beta frequencies appeared more represented than gamma frequencies, while in the ventral STN power was lower and equally distributed across all frequency bands. When layers and frequency bands were considered all together, there was no significant difference between tremor and no-tremor sides (main effect of tremor not significant, $p = 0.235$).

The distribution of the overall power across the layers showed a similar pattern in tremor and no-tremor sides (interaction between layer and tremor not significant) (Fig 5 a). However, tremor and no-tremor sides showed a different trend of power distribution across frequencies (significant interaction between frequency and tremor, $F = 6.924; p < 0.05$). This trend was particularly evident for theta and beta frequency bands (Fig. 5b). Moreover, when...
calculated as a function of layer, the trend of power distribution across the frequency bands was significantly different between tremor and no-tremor sides (interaction between layer, frequency, and tremor significant, F = 4.172, p < 0.05). This was reflected by a stronger theta component in the top layers in the tremor sides than in the no-tremor sides and a stronger beta component in the no-tremor sides as compared to the tremor sides, while there was no difference in power distribution between tremor and no-tremor sides in the bottom layers and in the gamma frequencies (Fig.6).

**Figure 5.** a) Distribution of normalized power spectral density across the various layers in the tremor (solid lines) and no-tremor sides (dashed lines). b) Distribution of normalized power spectral density across the various frequency bands in the tremor (solid lines) and no-tremor sides (dashed lines).
Figure 6. a) Normalized power spectral density (PSD) in each of the eight layers in the tremor (green lines) and no-tremor sides (blue lines). b) Distribution of normalized power spectral density as a function of depth in the Tremor and No-tremor sides.
DISCUSSION

The present study shows by means of direct comparison of tremor sides and no-tremor sides, that the prevalence of theta-frequency oscillations in the most dorsal STN layers is specific for the nuclei contralateral to tremor, while in the absence of tremor beta frequencies (and especially lower beta frequencies) predominate. Moreover, we confirmed that in PD patients, off-medication and at rest, oscillatory activity in the dorsal STN is stronger than in the ventral part. This pattern is specific for theta, alpha, and beta frequencies, but not for gamma.

In the present study we analyzed a large number of STN recordings and used particular care in the methodology to reduce any possible bias. Our findings thus provide robust evidence to support current theories on the neurophysiological activity of the STN in parkinsonian patients.

Methodology

To maximize the proportion of optimal STN recordings, we analyzed only the tracks with the best clinical effect and with confirmed correct position. Short trajectories, which possibly did not follow STN major axis, were excluded. In earlier studies, data were either described per patient, or pooled by averaging recordings based on their absolute distance from the borders.20-22 Because STN sizes differ,23 although the anatomo-functional organization within the nucleus is similar, we pooled recordings from different STNs by correcting for the actual size of the nuclei. This novel method allowed us to more accurately study the pattern of frequency distribution in adjoining areas. We analyzed intraoperative MER, which capture spikes from 1-3 neurons, superimposed on background activity.13 In our recordings, LFPs low frequencies were filtered out before analysis. Thus, the low frequencies analyzed after rectification of the filtered signal reflect only the low-frequency modulation of high-frequency neuronal activity.12,24 Although the functional meaning of this signal is not equivalent to that of LFPs, information gathered from single/multi-unit recordings, might be considered as representative for the oscillatory activity in the basal ganglia-cortical circuitry.25

Dorso-ventral pattern

Animal and human studies suggest that the sensorimotor circuit is located in the dorso-lateral STN.2,3,20 Beta-frequency oscillations are considered important in the pathophysiology of PD, since they are prominent in the cortico-basal-ganglia circuitry in parkinsonian patients and inversely correlate with the motor improvement produced by dopaminergic treatment and STN DBS.4,18,26-28 Both in single unit recordings and in LFPs, beta activity appears more represented in dorsal STN.5,29 Our findings on single/multi-unit recordings confirm the functional difference between dorsal and ventral STN, showing a dorso-ventral power
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gradient in theta, alpha, and beta frequencies, thus supporting targeting of the dorso-lateral STN to improve parkinsonian motor symptoms. Gamma-frequency band oscillations did not show this dorso-ventral pattern. Gamma activity is enhanced by dopaminergic treatment and movement. 18,20,27,28 Our patients were off-medication and at rest: this could explain a low and evenly distributed gamma power. Moreover, gamma power distribution was similar in tremor and no-tremor sides. Lower-gamma activity (35-55 Hz) might be associated with tremor, but its power is modulated by tremor amplitude.21,30 As we averaged recordings of tremor sides irrespective of fluctuations in tremor intensity, we may have missed a variation in gamma power.

Oscillation pattern associated with tremor

We applied the analysis of spectral power as a function of layers to directly compare tremor sides and no-tremor sides. Contralateral to tremor, theta band power appeared predominant in top layers. On the opposite, in the absence of tremor, beta band power was higher. These differences would be missed if analysis was performed irrespective of layers. The theta-frequency range (3-8 Hz) equals that of parkinsonian tremor. Since we did not perform spike discrimination, we cannot exclude mechanical artifact due to tremor of the head or of the electrode. However, the strong theta component in the top layers is unlikely due to a transmission artifact from the trembling limbs, because it was selectively present only in some of the layers and not in others. Tremor is supposed to originate from pathological oscillatory activity in the cortico-thalamic-basal-ganglia circuitry. 31,32 LFPs recorded in the STN of patients with tremor present peaks at the tremor frequency and are coherent with EMG at the tremor frequency. 33-36 The presence of single neurons oscillating at tremor frequency in the dorsal STN has been demonstrated in studies on PD patients with tremor. 12,20,21,37,38 However, a direct comparison between tremor sides and no-tremor sides has only been performed in a small number of patients. In one study comparing four patients with tremor and two with no tremor, 10 and similarly in another one comparing seven tremor sides with four no-tremor sides, 11 single neurons oscillating at the tremor frequency were found only in the presence of tremor. In another study, no significant correlation was found between the oscillatory characteristics of single STN neurons and clinical sub-scores for tremor, rigidity and bradykinesia. 12

Alpha and beta frequency oscillations in STN neurons are characteristic of PD and not found in Essential Tremor, whereas theta frequency oscillations are present in both groups.9 These findings suggest that theta oscillations are related to tremor, irrespective of the underlying pathology. Our data strongly support an association between the presence of tremor and neuronal oscillatory activity at the theta-frequency. In addition, we show that this activity is predominant in the top layers of the STN and that this pattern is not found in the absence of tremor.
Beta band power was, in our study, more pronounced in no-tremor than in tremor sides. This is in agreement with earlier studies, which demonstrated that in patients with predominant tremor only a minority of neurons oscillate or are coherent at beta frequencies $^{21,38,39}$ and that power in the beta-frequency band correlates to bradykinesia and rigidity rather than tremor. $^{4-8}$

Biochemical and clinical studies suggest that parkinsonian tremor has a different pathophysiologic mechanism than other symptoms.$^{40-43}$ Also, the effect of dopaminergic drugs is usually lower on tremor, while thalamic stereotactic surgery controls tremor but does not improve other parkinsonian symptoms.$^{44}$ Our findings support the hypothesis that different parkinsonian symptoms originate in segregated circuits.$^{2,45}$

The analysis of neuronal oscillatory activity could help identifying tremor-associated cell-clusters in each patient. Further studies are needed to clarify whether a specific frequency profile in the dorsal STN is involved in tremor generation or whether it results from proprioceptive inputs prompted by tremor. However, it appears that tremor improvement can be obtained by stimulating the STN at the level of tremor-cells $^{20}$ or at the sites where LFP-EMG coherence at tremor frequency is detected.$^{33}$ Thus, intraoperative identification of tremor-related cell-clusters by means of MER could ultimately help refining STN targeting.

Our study supports the hypothesis that the presence of tremor is associated with a distinctive neuronal oscillations pattern. In particular, we demonstrate the specificity of the association of theta frequencies in the dorsal STN with tremor. Further studies are needed to replicate these results, possibly with a prospective design. Identification of symptom-specific characteristics of intraoperative MER may lead to further refinement of STN targeting, tailored to the specific clinical presentation.

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