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High functional connectivity of tremor related subthalamic neurons in Parkinson's disease

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ABSTRACT

Objective: Tremor is a core symptom of Parkinson's disease (PD). The subthalamic nucleus (STN) seems to be crucial for tremor pathophysiology considering that deep brain stimulation (DBS) of the STN leads to an effective reduction of Parkinsonian tremor. Here, we investigate the functional connectivity between STN neurons in patients with Parkinsonian tremor.
Methods: STN activity was analyzed in 7 patients with Parkinsonian rest tremor who underwent stereotactic surgery for DBS. Spike activity was registered in different depths of the STN using an array of five microelectrodes. Interneuronal coherence within the STN was analyzed.
Results: Significant interneuronal coherence at the tremor frequency was detected in 78 out of 145 neurons. In contrast, interneuronal coherence in the beta band occurred only in 26 out of 145 neurons. Functional connectivity at the tremor frequency can be characterized by a slowly decaying exponential curve which describes coherence between STN neurons as a function of interneuronal distances between 0 and 4 mm.
Conclusions: Spatially distributed synchronization at the tremor frequency seems to be a key feature of STN pathophysiology in patients with Parkinsonian tremor.
Significance: The findings suggest a subthalamic tremor network which is widely extended and strongly coupled.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder which is characterized by bradykinesia, rigidity, postural instability and tremor. The pathology underlying the motor deficits in PD consists mainly in a loss of dopaminergic neurons in a midbrain nucleus, the substantia nigra pars compacta (SNpc). The SNpc projects to the striatum which can be considered as the main input structure of a collection of subcortical nuclei, the basal ganglia. The dopamine loss in the SNpc leads to a modification of striatal activity which is relayed via a set of feedback and feedforward connections to other basal ganglia nuclei as well as to the thalamus and cerebral cortex. Thus, the loss of dopaminergic neurons in the SNpc gives rise to a profound functional disturbance of extensive cortico-subcortical circuits (Albin et al., 1989; Alexander et al., 1990; DeLong and Wichmann, 2007).

Tremor in PD is thought to be generated by pathological oscillations in these cortico-subcortical circuits (Elble, 1996, 2000). This is supported by recordings from the sensorimotor cortex (Hellwig et al., 2000; Timmermann et al., 2003; Volkmann et al., 1996), the basal ganglia (Amtage et al., 2008; Hurtado et al., 1999; Reck et al., 2009) and the ventrolateral thalamus (Lenz et al., 1994). The subthalamic nucleus (STN) which is part of the basal ganglia seems to play an important role in tremor generation. This has first been observed in an animal model of PD, monkeys injected with the toxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Lesioning the STN in MPTP-treated monkeys relieved a number of Parkinsonian deficits including tremor (Bergman et al., 1990). Based on these findings, deep brain stimulation (DBS) of the STN has been developed as a functional neurosurgical treatment of PD. Today, DBS has become an established method to reduce tremor and other

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Parkinson-related symptoms (Hamani et al., 2006; Krack et al., 1997; Limousin et al., 1995, 1998).

Despite these findings, it is still controversial how the STN contributes to tremor generation. An increase of synchronous oscillatory activity may be important. In MPTP-treated monkeys, STN neurons tend to fire in oscillatory bursts (Wichmann and Soares, 2006; Bergman et al., 1994). Moreover, in the STN of PD patients, recordings of local field potentials reveal prominent oscillations, typically in the beta frequency range between 15 and 30 Hz (Hammond et al., 2007). This beta synchronization is thought to be related to motor impairment in PD, in particular to bradykinesia (Kühn et al., 2004, 2006a, 2006b; Brown, 2003; Weinberger et al., 2006). For instance, the application of dopaminergic drugs leads to an improvement of bradykinesia and to a simultaneous drop in beta synchronization (Doyle et al., 2005; Kühn et al., 2006b).

As to Parkinsonian tremor, beta synchronization seems to be less important. This can be inferred from the analysis of local field potentials in the STN of tremulous PD patients where STN–EMG coherence occurs mainly at the tremor frequency, possibly organized in topographically distributed sub-loops (Liu et al., 2002; Reck et al., 2009). In a recent study, Amtage et al. (2008) highlighted the role of oscillations at the tremor frequency by showing a high amount of coherence between subthalamic spike activity and the tremor EMG.

The question arises whether this high correlation between STN neurons and the tremor EMG is reflected by interneuronal STN connectivity. In this study, we describe the functional connectivity within the STN. We provide evidence for spatially distributed synchronizations at the tremor frequency suggesting the recruitment of an extended network of subthalamic neurons for tremor generation in PD.

2. Methods

2.1. Subjects

Seven patients (6 male, 1 female) were included in this study with a mean age of 59.1 years (range: 40–70 years) and an average duration of PD of 12.4 years (range: 7–27 years) by the time of stereotactic operation. They all met the criteria of the British Brain Bank for idiopathic PD. All patients presented rest tremor ranging from 3.2 to 7.0 Hz of at least one upper limb. Due to unsatisfactory treatment with medication, these patients were selected for stereotactic implantation of electrodes to apply high frequency stimulation in the STN. All patients were preoperatively evaluated by the Unified Parkinson's Disease Rating Scale part III (UPDRS motor score), which had a mean value of 37.4 ± 1.7 of 108 points in the defined "off" state after absence of dopaminergic drugs of at least 12 h. The best "on" state was achieved by a dosage of 200 mg of rapidly effective and soluble levodopa (Madopar LT® 250 mg, Hoffman-La Roche, Grenzach-Wyhlen, Germany). The rating for the "on" condition took place 30 min and 1 h after oral application, yielding a mean of 25.6 ± 1.7 points based on the best score obtained. In all, measurements from 9 STN could be used for offline analysis. The remaining recordings from 5 STN were not evaluated, since tremor was unilateral in 4 patients and suppressed by thalamotomy in one patient. Detailed information on patients can be gathered from Table 1. This study was approved by the local ethical committee.

2.2. Stereotactic procedure

Patients' informed consent for surgery including microelectrode recording and EMG was obtained prior to surgery. Calculation of the target nucleus was performed after fusion of a stereotactic computerized tomography (CT) with a preoperative contrast enhanced magnetic resonance imaging (MP-RAGE 1 mm) using STP4 software (Stryker-Leibinger, Freiburg, Germany). The target coordinates in relation to the mid commissural point (mean values: $x = 12$ mm, $y = -2.5$ mm, $z = -3$ mm) were calculated. Electrode implantation was carried out under local anesthesia without sedation and in a defined "off" condition after >12 h discontinuation of dopaminergic medication.

Microelectrode recording was performed during surgery using the MeKIT® system (inomed GmbH, Teningen, Germany). An array of five microelectrodes was advanced simultaneously in 1 mm steps using a manual MicroDrive® (inomed GmbH, Teningen, Germany). Microelectrode recordings (ISIS MER®, inomed GmbH, Teningen, Germany) were sampled at 25 kHz (filter settings: 250 Hz; 2.5 kHz) and were registered over a period of 30–120 s. Simultaneously, electromyography (EMG) of the contralateral forearm flexors and extensors was recorded using surface electrodes (Kendall Soft-E®, TYCO Healthcare Group LP, Mansfield, MA, USA) and sampled at 2.5 kHz. EMG data were band-pass filtered between 30 Hz and 1 kHz and full-wave rectified. A detailed description of stereotactic surgery and intraoperative recordings is given in Amtage et al. (2008).

2.3. Data post-processing

Each dataset contains five microelectrode recordings and two EMG recordings (wrist flexor and extensor). In order to convert

Table 1
Clinical data at the time of stereotactic operation and STN recording.

Patient	Age [years]	Sex	Disease duration [years]	UPDRS motor score on/off [total 108]	Hoehn & Yahr Score in off	Number of neurons used for analysis within the right or left STN
B	40	M	7	30/37	2	R 23 L 0 (no tremor)
E	69	M	15	29/47	2	R 6 L 7
L	68	M	14	17/33	3	R 20 L 0 (thalamotomy)
M	60	M	7	28/37	2	R 16 L 23
P	70	M	27	22/35	3	R 0 (no tremor) L 12
S	53	M	9	27/36	3	R 0 (no tremor) L 23
W	50	F	8	26/37	2	R 0 (no tremor) L 15

Abbreviations: M, male; F, female; R, right; L, left.

the recorded neuronal activity into point processes that contain only the points in time when neurons fired, a wavelet-based spike-sorting algorithm was applied (Quiroga et al., 2004). As a necessary condition, the spike amplitude had to be at least four times larger than the standard deviation of the entire signal. Subsequently, spikes were grouped into clusters by comparing their shapes. Datasets were accepted for the analysis if rest tremor activity was present. Furthermore, in order to assure sufficient stationarity of the process, spike activity had to exceed 10 spikes per second over a period of at least 30 s. By applying these criteria, data of high quality was obtained. In each dataset, sections which fulfilled the above criteria were selected in such a way that the maximum number of neurons could be considered for analysis.

2.4. Data analysis

For each neuron thus selected and for each EMG, spectral and cross-spectral analysis has been performed. The spectral estimation is based on the so-called periodograms, which is the squared Fourier transform of the signals. For point process, i.e., neuronal spike activity in the STN, the Fourier transform was derived as suggested in Henschel et al. (2008) and Amtage et al. (2008). For time series, i.e., the EMG data, a standard approach was used (Timmer et al., 2000). The neuronal activity as well as the EMG data were tapered using a triangular window to avoid leakage prior to the Fourier transformation (Henschel et al., 2008). The periodograms were smoothed using a triangular kernel with a smoothing width of 1 Hz to obtain estimators for the spectra and cross-spectra.

To quantify both interneuronal interactions within the STN and interactions between the STN activity and the EMG, cross-spectral analysis was performed (Timmer et al., 2000). The normalized cross-spectrum, the so-called spectral coherence, provides the strengths of linear interactions between processes in the frequency domain (Brockwell and Davis, 1991). Coherences at the tremor frequency were rated as significant if the peak in the coherence analysis exceeded the level of significance ($p < 0.01$), and if it deviated from the tremor frequency by less than the peak width at half peak power. The same procedure was applied to the first harmonic of the tremor frequency.

Ordinary cross-spectral analysis does not allow distinguishing direct and indirect interactions. Partial coherence measures the coherence between two processes, correcting for the influence of other processes which are simultaneously observed. This enables separating direct and indirect interactions. Partial coherence is estimated based on the information of all pairwise cross-spectra of the multivariate system (Dahlhaus, 2000). Again, it is possible to apply this technique to time series and point processes (Henschel et al., 2008).

3. Results

Recordings from 42 sites each corresponding to one defined depth within an STN were considered for analysis. By application of the criteria described in Methods, between 2 and 6 neurons per site were selected. In all, 145 neurons were analyzed.

As an example, Fig. 1 illustrates the statistical analyses for one recording in a matrix with frequency spectra and bivariate and partial coherence analyses. All recordings were analyzed as in Fig. 1.

3.1. Bivariate coherence

Bivariate coherence quantifies the interaction strength between two processes. Fig. 2b illustrates that bivariate coherence between STN neurons at the tremor frequency or its first harmonic was found in 78 out of 145 neurons (53.8%). In one particular site, the maximum number of interneuronal coherences per neuron was three. Of the 78 STN neurons with interneuronal coherence, 49 (62.8%) were also coherent with the tremor EMG. For the 67 neurons without interneuronal coherence at the tremor frequency, coherence with the tremor EMG was significantly less frequent (27 out of 67 neurons = 40.3%; Fisher's exact test $p < 0.01$). Combining interneuronal coherence and coherence with the tremor EMG, 105 out of 145 neurons (72.4%) showed coherence at the tremor frequency or its first harmonic (Fig. 2c).

As to coherence in the beta band, a broad frequency range between 15 and 30 Hz must be evaluated. This is in contrast to coherence analysis at the tremor frequency, where one particular frequency is of interest. If coherence is tested for a broad frequency range as the beta band, the significance level has to be adjusted to higher values. This is due to the increased likelihood of a significant coherence if more than one frequency is considered (see Amtage et al. (2008) for details of this argument). Applying this correction to our data, 27 out of 145 neurons (18.6%) showed interneuronal coherence at the beta frequency. Of the 27 STN neurons with interneuronal beta coherence, only 2 (7.4%) were also coherent with the EMG at the beta frequency. For the 118 neurons without interneuronal coherence in the beta frequency range, beta coherence with the EMG was equally rare (8 out of 118 neurons = 6.8%). There was no correlation between the amount of significant beta coherence and the hypokinetic-rigid UPDRS score, i.e., the UPDRS motor score excluding the items for tremor and postural instability.

3.2. Partial coherence

Partial coherence measures the coherence between two processes, correcting for the influence of other processes which are simultaneously observed. In this study, 66 out of 145 neurons were partially coherent with other STN neurons (45.5%). This means that 84.6% of the STN neurons (66 out of 78) with bivariate interneuronal coherence were also partially coherent at the tremor frequency or its first harmonic. Out of the 66 STN neurons with partial interneuronal coherence, 35 (53.0%) were also partially coherent with the tremor EMG.

Concerning the beta frequency range, 23 neurons (15.9%) of all analyzed subthalamic neurons were partially coherent with other STN neurons. This means that 88.5% of the STN neurons (23 out of 26) with bivariate interneuronal coherence in the beta band were also partially coherent. None of the neurons with partial interneuronal coherence at the beta frequency showed partial coherence with the EMG.

3.3. Functional and spatial organization

Fig. 2b displays the spatial arrangement of the 145 STN neurons analyzed here. Neurons with significant interneuronal coherence at the tremor frequency were widely distributed over the whole STN. The highest percentage of coherent neurons was found the middle section of the STN (83.3%), the lowest percentage in its ventral part (25.0%). In Fig. 2c, interneuronal coherences are complemented by coherences between STN neurons and the tremor EMG (Amtage et al., 2008): the dorsal parts of the STN (75.0–91.7%) show higher rates of significant coherences at the tremor frequency than the ventral parts (40.0–68.0%).

For each recording site, the percentage of interneuronal tremor-coherence as a function of the distance between neurons can be determined. Due to the arrangement of the five microelectrodes this can be achieved for four distances (Fig. 3a): approximately 0 mm in the case of neurons recorded by the same microelectrode but separated by spike-sorting, 2, 2.83 and 4 mm. As an example, interneuronal coherences in one STN of patient M are displayed

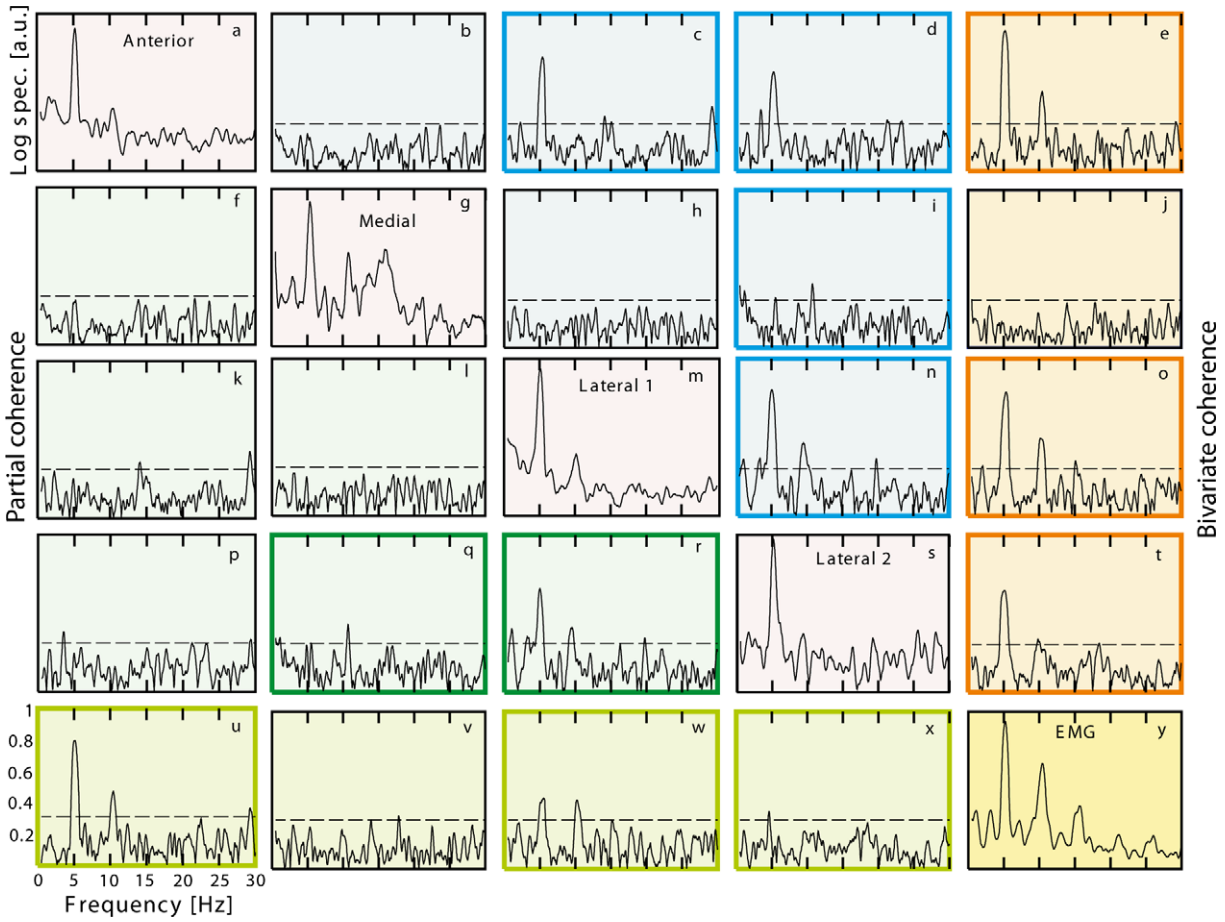

Fig. 1. Illustration of frequency spectra of four subthalamic neurons (red panels, (a) (anterior), 1 g (medial), 1 m (lateral, first neuron) and 1 s (lateral, second neuron)) and tremor surface-EMG (yellow panel, (y)) as well as bivariate and partial coherence analyses for one site of patient M. The bivariate coherence analyses between neurons and tremor EMG are shown in orange panels (e, j, o, t). Bivariate interneuronal coherence spectra (b–d, h, i, n) are highlighted by a blue background. Partial coherences are displayed below the diagonal and are colored in green (f, k, l, p–r for interneuronal partial coherence; (u–x) for partial coherence between STN neurons and tremor EMG). The horizontal dashed line indicates the level of significance ($p < 0.01$). Panels with significant coherences at the tremor frequency and/or its first harmonic are highlighted by a colored frame. Spec. = spectrum; a.u. = arbitrary units.

in Fig. 3b. It is illustrated that significant interneuronal coherence occurs for all the above distances.

The question arises to what extent the amount of interneuronal coherence depends on the distance between neurons. Bivariate coherence was found in 9 out of 15 neuron pairs (60%) which were recorded within the reach of one microelectrode. For neurons 2, 2.83 and 4 mm apart, interneuronal coherence was detected in 24 out of 73 neuron pairs (32.9%), 20 out of 70 neuron pairs (28.6%) and 4 out of 29 neuron pairs (13.8%), respectively. In Fig. 4a, these percentages are plotted against the corresponding interneuronal distances. The data points can be approximated by the exponential function $p(x) = 0.64 * e^{-0.35*x/mm}$, which estimates the probability $p(x)$ of interneuronal tremor-coherence as a function of the interneuronal distance x in mm. A similar function yielding only slightly lower values for $p(x)$ can be obtained using partial coherences (Fig. 4a).

4. Discussion

Our results demonstrate that interneuronal synchronization at the tremor frequency is an outstanding feature of subthalamic neurons in PD patients with tremor. More than 50% of the neurons analyzed here showed significant interneuronal coherence at the tremor frequency. Combining the findings of this study with the results by Amtage et al. (2008), 105 out of 145 neurons (72.4%) showed significant coherence at the tremor frequency or its first harmonic with the EMG or with other STN neurons (Fig. 2c). Significant interneuronal coherence significantly increased the probability of STN–EMG coherence, indicating that neurons tightly involved in the STN tremor network are more likely to contribute to the clinical expression of peripheral tremor.

Interneuronal coherence in the beta band was markedly less frequent (17.9%). This is in line with a recent study by (Steigerwald et al., 2008) in which subthalamic spike activity in PD patients with and without tremor was investigated. It was shown that oscillatory activity in the theta band was much more frequent than in the beta band. Our results seem, however, in contrast to studies on patients with the hypokinetic-rigid type of Parkinson's disease in whom beta synchronization is a prominent feature of STN activity (Kühn et al., 2004, 2006b; Weinberger et al., 2006; Brown, 2003). Since it was the objective of this study to investigate tremor pathophysiology, patients with strong rest tremor were selected while bradykinesia and rigidity were less pronounced. Thus, different study designs may explain why we found relatively low beta synchronization compared to previous work of other groups on PD patients with prominent bradykinesia. Moreover, there might also be methodological differences, since we investigated spike activity, i.e., efferent signals, while the high amount of beta synchronization

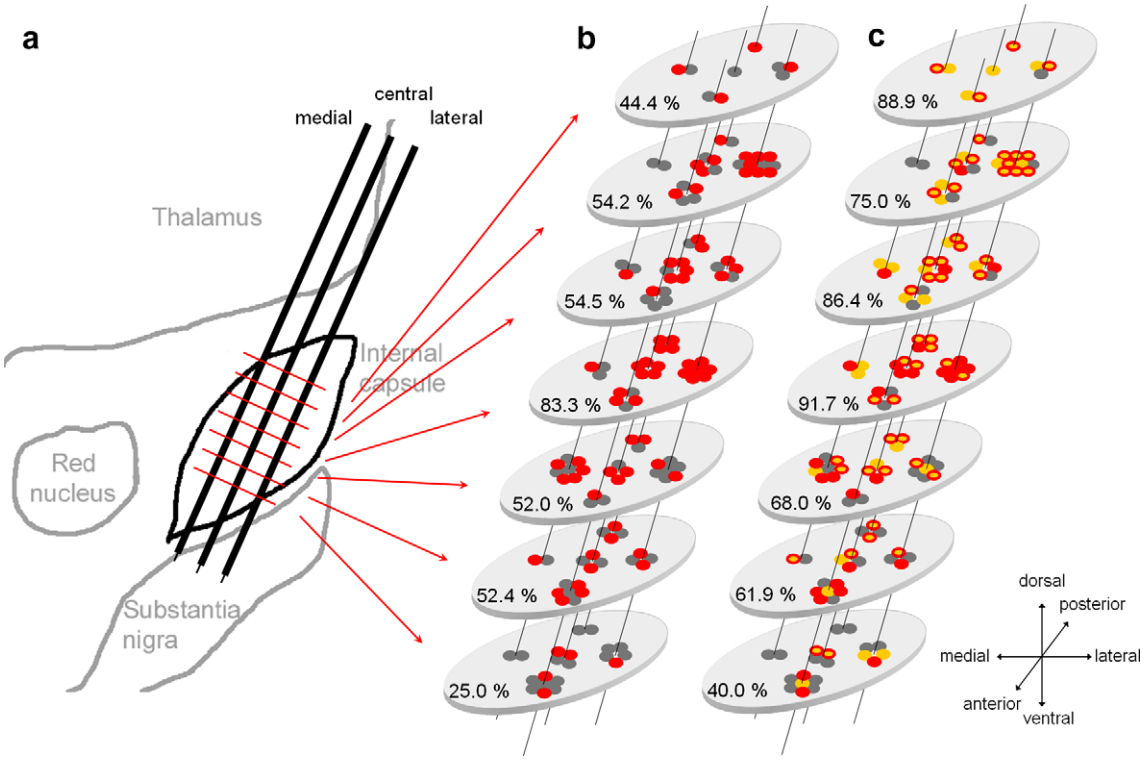

Fig. 2. (a) Schematic frontal section of the right subthalamic nucleus (black) and neighboring structures (grey). The putative positions of the medial, central and lateral microelectrodes are indicated by black lines. Recordings of neuronal STN activity took place in different depths separated by 1 mm (red lines). (b) Schematic illustration of sections through the STN at different recording depths indicated by the red lines in (a). The results for 145 neurons are displayed. Red dots: neurons coherent with other STN neurons at the tremor frequency. Grey dots: neurons not coherent with other STN neurons at the tremor frequency. The numbers on the left indicate the percentage of tremor-coherent neurons per section. (c) Schematic illustration of the same sections through the STN showing in addition significant coherence between tremor EMG and neuronal spike activity. Yellow dots: neurons coherent with the tremor EMG. Red dots: neurons coherent with other STN neurons at the tremor frequency. Yellow dots with red margin: neurons coherent with both the tremor EMG and other STN neurons.

described by Brown and colleagues is based on local field potentials, which correspond mainly to presynaptic input.

The observation that most of the tremor-coherent neuron pairs were also partially coherent indicates that the influence of other simultaneously observed processes was small. In the case of two neighboring neurons recorded by one microelectrode and separated by spike-sorting, partial coherence may be due to direct anatomical coupling. For STN neurons several millimeters apart, direct coupling is less likely, the maximum extent of dendritic trees being around 750 μm (Rafols and Fox, 1976; Yelnik and Percheron, 1979). Instead, significant partial coherence argues for a large number of subthalamic neurons which are implicated in the subthalamic tremor network but which were not observed in our recordings. In other words, the high amount of significant partial coherence found here supports the assumption of an extended subthalamic network of tremor-coherent neurons.

The percentage of tremor-coherent neurons diminishes in the ventral direction although neurons seem to be more densely packed (Yelnik and Percheron, 1979). The decrease of tremor-coherence is consistent with the observation that the ventro-medial STN corresponds to the associative-limbic subdivision of this nucleus (Parent and Hazrati, 1995; Hamani et al., 2004; Aravamuthan et al., 2007). Otherwise, tremor-coherent neurons seem to be widely and isotropically distributed in the sensorimotor subdivision of the STN (Fig. 2c) consistent with its homogeneous anatomical appearance (Yelnik and Percheron, 1979).

Functional connectivity within the STN tremor network can be described by the exponential functions in Fig. 4a. Tremor-coherence may be present over distances of several mm. Using anatomical data, such distances can be paraphrased as numbers of neurons along one axis in three-dimensional space. In the human STN, there are approximately 560,000 neurons in a volume of 240 mm^3 (Hardman et al., 2002). In other words, about 13 neurons can be found along a stretch of 1 mm of STN tissue. Applying this information to the exponential functions of Fig. 4a, it can be inferred that two tremor-coherent STN neurons need not be adjacent, but may be separated by tens of neurons.

The above-mentioned anatomical data combined with the exponential functions of Fig. 4a lead to further conclusions concerning network size and spatial extent. Considering a neuron in the center of the STN, it can be estimated that it is functionally connected to ~135,000 other subthalamic neurons (~24%). A neuron at the dorso-caudal pole of the STN is estimated to be functionally connected to ~56,000 neurons (~10%). Fig. 4b illustrates the number of neurons to which one particular neuron is functionally connected dependent on its position within the STN. In other words, Fig. 4b provides an estimate of the size of the network of tremor related neurons starting out from different locations within the STN. The network thus estimated might seem surprisingly large and widely distributed. Yet, our findings correspond well to the clinical appearance of patients whose motor activity during stereotactic recordings consisted essentially of rest tremor.

There seem to exist multiple central oscillators in Parkinsonian tremor (Lauk et al., 1999; Hurtado et al., 2000; Raethjen et al., 2000; Ben Pazi et al., 2001). In particular, Reck et al. (2009) suggested that there are topographical distributed tremor sub-loops within the STN. In our study we provide evidence for a spatially extended subthalamic tremor network. However, we show also that

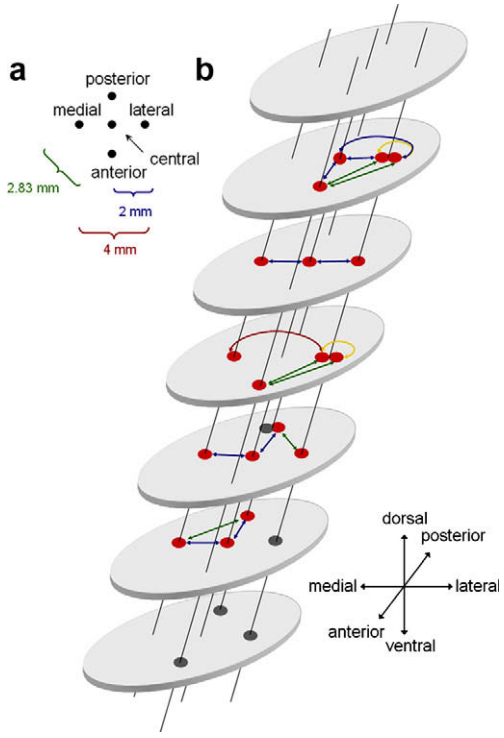

Fig. 3. (a) Spatial arrangement of the tips of the five microelectrodes and the distances between them, as seen from above. (b) Schematic illustration of sections through the STN at different recording depths, analogous to Fig. 2. The results for the left STN of patient M (23 neurons) are displayed. Red dots: neurons coherent with other STN neurons at the tremor frequency. Grey dots: neurons without coherence at the tremor frequency. Yellow arrows represent interneuronal coherence between two neurons recorded within the reach of one microelectrode but separated by spike-sorting. Blue arrows indicate interneuronal coherence between neurons 2 mm apart. Green arrows show interneuronal coherence for neurons 2.83 mm apart and red arrows for neurons 4 mm apart.

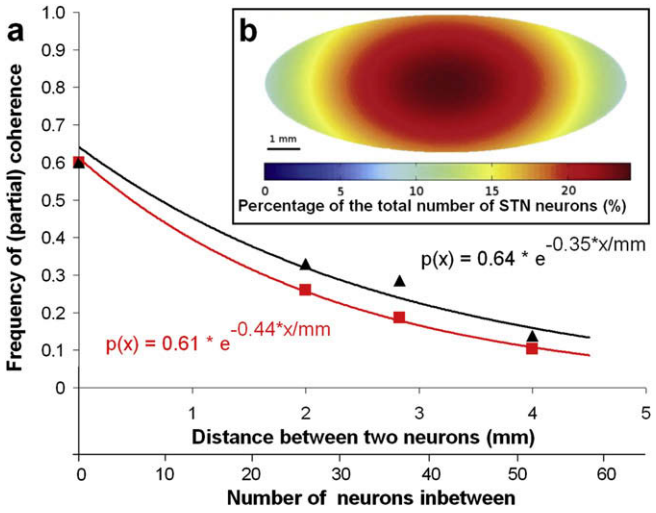

Fig. 4. (a) Interneuronal connectivity as a function of the distance between neurons. The exponential functions estimate the probability $p(x)$ of bivariate or partial coherence at the tremor frequency or its first harmonic at one particular distance x in mm. The mathematical function of bivariate coherence (black) follows the equation: $p(x) = 0.64 * e^{-0.35*x/mm}$ ($R^2 = 0.948$), the equation for partial coherence (red) is: $p(x) = 0.61 * e^{-0.44*x/mm}$ ($R^2 = 0.998$). The distance between two neurons can also be expressed by estimating the number of neurons lying between them (estimation based on anatomical data (Hardman et al., 2002), for details see text) (b) Longitudinal section through the STN schematically displayed as a prolate ellipsoid with the semimajor axis $a = 6.5$ mm and the semiminor axes $b = c = 2.5$ mm. Based on the exponential function for bivariate coherence in (a) the colors code for the percentage of the total number of STN neurons (=560,000) to which a neuron at given position within the STN is functionally connected.

there are neurons with STN–EMG coherence but without interneuronal coherence – and vice versa. This finding is well compatible with the hypothesis of functionally segregated tremor sub-loops within the STN.

The question remains if the present findings imply that the STN is the pacemaker for tremor in Parkinson's disease. It has been questioned whether the basal ganglia are involved in tremor pathogenesis (Rivlin-Etzion et al., 2006). Cerebello-thalamo-cortical circuits may also contribute to the generation of Parkinsonian tremor, since high frequency stimulation or lesioning of a cerebellar relay nucleus in the thalamus, the nucleus ventralis intermedius, can suppress Parkinsonian tremor effectively (Lozano, 2000; Schuurman et al., 2000).

Thus, the present findings do not prove a pacemaker function of the STN. However, subthalamic neurons have indeed the intrinsic property of firing in rhythmic bursts (Beurrier et al., 1999; Bevan and Wilson, 1999). Moreover, oscillatory activity in the STN can be maintained by forming functional loops involving other centers, in particular the cerebral cortex and the external globus pallidus (Plenz and Kital, 1999; Magill et al., 2000). Thus, both the intrinsic properties of subthalamic neurons and the involvement of the STN in cortico-subcortical loops argue for a fundamental contribution of the STN to tremor generation. It might be possible that the cerebello-thalamo-cortical loops and the basal ganglia loops are connected with each other, e.g., via projections from the cerebellum to the striatum (Hoshi et al., 2005). Within these interconnected loops, the STN network may play a crucial role for tremor generation.

Probably, the functional connectivity between STN neurons is shaped dynamically depending on the fluctuations of tremor strength. This issue could not be addressed in the present study, since our patients showed stable tremor during the recordings. However, the dynamics of the subthalamic tremor network as a function of tremor fluctuations is certainly an intriguing subject for future studies.

In conclusion, coherent activity at the tremor frequency or its first harmonic characterizes the neuronal network within the STN. The subthalamic tremor network is widely extended and strongly coupled. Thus, spatially distributed synchronization at the tremor frequency seems to be a key feature of STN pathophysiology in patients with Parkinsonian tremor.

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