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Prior pallidotomy reduces and modifies neuronal activity in the subthalamic nucleus of Parkinson's disease patients

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Abstract

Parkinson's disease (PD) patients with prior radio-frequency lesions in the internal segment of the globus pallidus (GPi, pallidotomy), whose symptoms have deteriorated, may be candidates for further invasive treatment such as subthalamic deep brain stimulation (STN DBS). Six patients with prior pallidotomy (five unilaterally; one bilaterally) underwent bilateral STN DBS. The microelectrode recordings (MERs, used intraoperatively for STN verification), ipsilateral and contralateral to pallidotomy, and MERs from 11 matched PD patients who underwent bilateral STN DBS without prior pallidotomy were compared. For each trajectory, average, variance and mean successive difference (MSD, a measure of irregularity) of the root mean square (RMS) of the STN MER were calculated. The RMS in trajectories ipsilateral to pallidotomy showed significant reduction of the mean average and MSD of STN activity when compared with trajectories from patients without prior pallidotomy. The RMS parameters contralateral to pallidotomy tend to lie between those ipsilateral to pallidotomy and those without prior pallidotomy. The average STN power spectral density of oscillatory activity was notably lower ipsilateral to pallidotomy than contralateral, or without prior pallidotomy. The finding that pallidotomy reduces STN activity and changes firing characteristics, in conjunction with the effectiveness of STN DBS despite prior pallidotomy, calls for reappraisal and modification of the current model of the basal ganglia (BG) cortical network. It highlights the critical role of direct projections from the BG to brain-stem structures and suggests a possible GPi–STN reciprocal positive-feedback mechanism.

Introduction

Current models of the basal ganglia (BG), based on the classical studies of Albin *et al*. (1989) and DeLong (1990), propose a closed-loop BG–thalamo-cortical network that facilitates movement via feed-forward direct and indirect striato-pallidal pathways. These models propose that movement is controlled by modulating inhibitory globus pallidus (GPi) and substantia nigra, pars reticulata (SNr) projections to the thalamus and explain the pathophysiology of Parkinson's disease (PD) by GPi/SNr over-activity. Accordingly, excessive inhibition of the thalamo-cortical network results in the hypokinetic motor deficits typical of PD. The subthalamic nucleus (STN), which lies upstream of the GPi/SNr and is noted to have an abnormal increase in activity in Parkinsonian monkeys (Wichmann *et al*., 1994), drives the GPi/SNr by excitation via the indirect pathway. The models are consistent with many experimental findings, e.g. inactivation of the GPi (Lozano *et al*., 1995) or STN (Bergman *et al*., 1990) alleviates PD symptoms, but are incongruous with others. For example thalamotomy does not lead to PD-like symptoms, and in addition to alleviating hypokinetic disorders, GPi and STN inactivation alleviate hyperkinetic disorders as well (Obeso *et al*., 2000).

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Surgical treatment of PD includes posteroventral pallidotomy (Svennilson *et al*., 1960; Laitinen *et al*., 1992) and deep brain stimulation (DBS) of the GPi and the STN. STN DBS has proven to be a beneficial and accepted treatment of advanced PD (Limousin *et al*., 1995; Starr, 2002; Machado *et al*., 2006) and is also effective on patients who have had a prior pallidotomy, but whose symptoms have deteriorated. Kleiner-Fisman *et al*. (2004) found STN DBS post pallidotomy to be no less efficacious than STN DBS in patients without prior pallidotomy, whereas Ondo *et al*. (2006) found less motor improvement in patients with prior pallidotomy (dyskinesia scores, however, showed no less of an improvement).

In order to implant a stimulating macroelectrode for STN DBS, microelectrode recording (MER) is used to verify localization of the STN physiologically (Israel & Burchiel, 2004; Gross *et al*., 2006). The effects of pallidotomy on STN activity have been studied, but require further elucidation due to apparent discrepancies. For unilateral pallidotomy, Mogilner *et al*. (2002) found that STN single-cell activity demonstrated a lower mean firing frequency on the side ipsilateral to prior pallidotomy compared with the contralateral side. In contrast, electrophysiological studies of the STN conducted by Kleiner-Fisman *et al*. (2004) found no significant differences.

In the present study we tested whether STN activity ipsilateral to prior pallidotomy is different in comparison with STN activity in patients who had no pallidotomy as well as contralateral to prior pallidotomy. We analysed the raw multi-unit MERs in order to avoid

possible errors and biases induced by spike detection and sorting (Lewicki, 1998; Joshua *et al*., 2007). Insight into the effects of prior pallidotomy on STN activity could improve our understanding of the pathophysiology of PD, the mechanisms of its surgical treatments, and general functioning of the BG. In addition, it can aid the neurosurgeon and neurophysiologist in correctly localizing the STN target in post-pallidotomy patients, which might have slightly different physiological characteristics.

Methods

Patients

Thirty-three trajectories were analysed from 17 patients. In 16 patients, we analysed two trajectories per patient – one in each hemisphere. In the remaining (no-pallidotomy) patient, one trajectory was excluded due to STN length shorter than 2 mm. It is presumed that the short length indicates that the trajectory skimmed the edge of the STN and did not pass through the center. This is a potential variable of surgery and the reason that MER is performed. As there is the possibility that these recordings are not representative of STN activity they were excluded *a priori*. All the other trajectories had STN length > 3 mm. Five patients had unilateral pallidotomies, one had a bilateral pallidotomy and 11 had no pallidotomy. This resulted in seven trajectories with an ipsilateral pallidotomy, five with a contralateral pallidotomy and 21 without (due to the excluded trajectory – as explained above). These trajectories will be called ipsilateral, contralateral and no-pallidotomy trajectories, respectively. Patient details and clinical histories are given in Table 1. Pallidotomies were verified on the preoperative magnetic resonance image (MRI; Fig. 1). The position of the implanted STN DBS electrodes was verified on postoperative computerized tomography (CT), fused with the preoperative MRI (Framelink 4, Medtronic, Minneapolis, MN, USA).

This study was authorized and approved by the Institutional Review Board of Hadassah University Hospital in accordance with the Declaration of Helsinki (Ref. code: 2334). All patients met accepted selection criteria for STN DBS and signed informed consent for surgery with MER. Surgery was performed using the CRW stereotactic frame (Radionics, Burlington, MA, USA). STN target coordinates were chosen as a composite of anterior commissure – posterior commissure-based location and MRI using Framelink 4 software (Medtronic). No sedative was used and all patients were awake during surgery. The patient's level of awareness was continuously assessed clinically, and when drowsy the patient was stimulated by conversation with one of the surgical team and awoken. Data were obtained off dopaminergic medications (> 12 h after last medication) and during periods of rest, but with no objective measure of tremor or the other PD signs. One of the patients (patient E from Fig. 5) had many tremor episodes. We repeated the root mean square (RMS) analysis of this patient after removing the sections with clear oscillations, but this procedure did not affect the population RMS parameters analysed.

In this study a single trajectory using one or two microelectrodes was made starting from 10 mm above the calculated target. When two microelectrodes were used, the second was advanced in parallel (2 mm anterior) to the central (aimed at the calculated target) electrode track. Further trajectories were only made if the results of microrecording or macrostimulation were suboptimal in the first. In 27 of the 33 trajectories analysed for this study (82%), either only one microelectrode was used or there was no STN recorded on the other microelectrode. For the six remaining trajectories the

TABLE 1. Patient details

Pallidotomy	Age (years)	Gender	Disease duration (years)	Predominant motor symptoms		Motor UPDRS before STN DBS		Time elapsed since pallidotomy (years)	LEDD* at time of STN DBS operation
				Before pallidotomy	After pallidotomy	On	Off		
None	59	M	12	–	–	NA	NA	–	975
None	45	M	13	–	–	NA	NA	–	975
None	53	M	24	–	–	47	91	–	2461
None	43	M	10	–	–	40	60	–	249
None	51	F	8	–	–	NA	NA	–	750
None	57	M	11	–	–	25	43	–	2195
None	69	M	8	–	–	18	46	–	1370
None	59	M	7	–	–	7	22	–	1463
None	57	M	7	–	–	9	38	–	1490
None	65	M	13	–	–	27	60	–	370
None	66	F	5	–	–	NA	33	–	750
(Mean)	(56.7)		(10.7)	–	–	(25)	(49)	–	(1207)
Pallidotomy									
Left	59	M	14	Rigidity, bradykinesia	Rigidity, bradykinesia	23	49	8	1800
Right	65	F	35	Tremor (L), freezing	Tremor	19	44	8	830
Right	56	F	20	Tremor (L)	Tremor (R), dysarthria†	26	59	8	620
Right	66	F	14	Bradykinesia, freezing, dysarthria, tremor	Freezing†	57	77	9	1546
Left	54	M	20	Tremor (R)	Tremor (L)†	9	34	12	333
Bilateral	72	F	32	Tremor (R)	Tremor†	40	52	12	1450
(Mean)	(62.0)		(22.5)	–	–	(29)	(53)	(9.5)	(1096)

*LEDD, Levodopa equivalent daily dose as calculated by Deuschl *et al.* (2006); L/R, left and right limbs, respectively (specified when noted in medical diagnosis). †Considerable clinical improvement following pallidotomy was reported. NA, not available; STN DBS, subthalamic nucleus deep brain stimulation; UPDRS, Unified Parkinson's Disease Rating Scale.

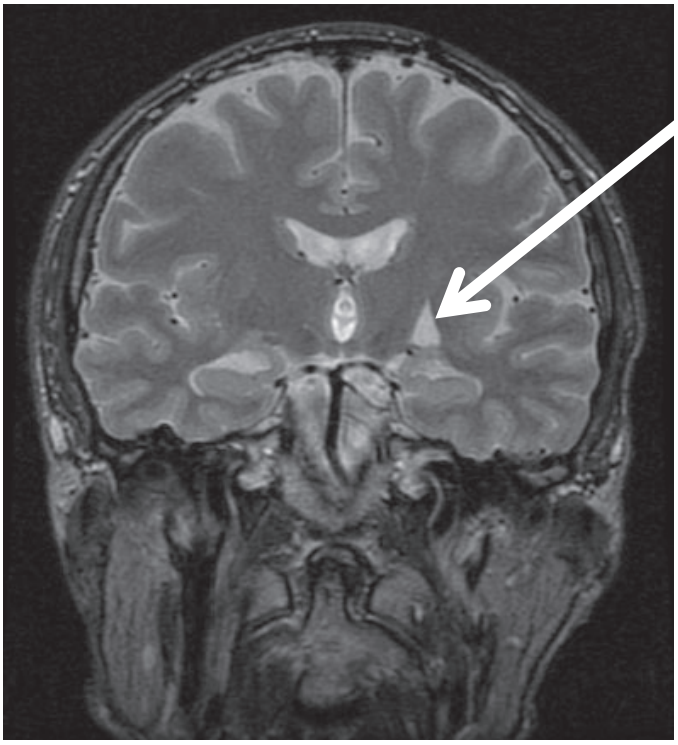

FIG. 1. Pallidotomy verification by MRI. Representative coronal T2-weighted image showing a left-sided pallidotomy (white arrow).

microelectrode recording with the greater STN recording span was chosen for analysis (i.e. a single trajectory per hemisphere). In all but one (no-pallidotomy) trajectory, the greater STN span and the recording analysed was from the central (aimed at the calculated target) electrode track.

Data acquisition and analysis

Data acquisition was conducted with the MicroGuide system (AlphaOmega Engineering, Nazareth, Israel). Neurophysiological activity was recorded via polyamide-coated tungsten microelectrodes (Frederick Haer; impedance = 0.48 ± 0.17 MΩ; measured at 1 kHz, at the beginning of each trajectory). The recorded signal was amplified 10 000-fold and band-passed between 250 and 6000 Hz using a hardware four-pole Butterworth filter. The signal was sampled at 24 or 48 kHz, by use of a 12-bit A/D converter, using a ±5 V input range (i.e. ~0.25 μV amplitude resolution). The electrodes were advanced in small discrete steps, from 10 mm above, towards the estimated center of the STN. Step size (ranging from 500 down to 50 μm in our recordings) was controlled by the neurophysiologist in order to achieve optimal unit recording and identification of upper and lower borders of the STN. Typically, shorter steps were used when the electrode was advanced closer to the presumed location of the STN. Following a 2-s signal stabilization period after electrode movement cessation, multi-unit segments were recorded for 5–60 s. Nevertheless, in many cases the human operating-room conditions have resulted in non-stable recording (e.g. due to further movement of brain tissue in relation to the electrode tip or due to neuronal injury). The data segments were therefore analysed offline for stability (Gourevitch & Eggermont, 2007) by visual inspection of one of the authors (A.Z.). When instability was observed, the longest stable section was selected from the recording, discarding the rest. All recordings chosen for further analysis were longer than 1 s (duration mean ± SD, 9.87 ± 8.97 s). All the statistics presented in this article use mean mean ± SD notation.

STN raw activity measure

The RMS estimate of the raw multi-unit activity recorded by the microelectrode at each electrode depth was used as a measure for evaluating STN activity. The RMS estimate is defined as follows:

$$\mathrm{RMS}(\vec{\mathrm{X}}) = \sqrt{\frac{\sum_{i=1}^{n}(\mathrm{X}_i - \mu)^2}{n-1}} \quad (1)$$

where $\vec{\mathrm{X}}$ is the vector of the sampled analog signal with mean μ, X_i is each sample and n is the number of samples. As our signal is band-pass filtered, the DC component (μ) is negligible and might exist only because of minor differences between the amplification/filtering system and the sampling/acquisition system. The RMS about the mean (standard deviation) is therefore an unbiased and optimal estimator of the RMS of the neuronal activity (Moran *et al*., 2006). RMS values are susceptible to electrode properties and other external factors (e.g. amplifier gain). Hence, the RMS requires normalization in order to allow intertrajectory comparison. The RMS for each trajectory was normalized by the average activity from 10 mm estimated distance to (center of STN) target (EDT), until entry into the STN, creating a normalized RMS (NRMS; Fig. 2A–D). Once the electrode enters the STN, the NRMS increases dramatically. This is used as a marker for the point of entry into the STN (Moran *et al*., 2006). The point of exit was deducted in a likewise, but reverse, manner – a decrease in the NRMS and return to the normalized baseline. The NRMS values between the point of entry and the point of exit of the STN comprise the STN recordings and were used for comparison between trajectories.

The average and variance of the STN NRMS were calculated for the ipsilateral, contralateral and no-pallidotomy trajectories (see above). In comparison with what seemed a smoother NRMS in the STN of ipsilateral trajectories (Fig. 2B and D), the STN NRMSs of the no-pallidotomy (and to a lesser degree the contralateral) trajectories (Fig. 2A and C) were more irregular, containing large jumps between consecutive steps in a trajectory. In order to quantify this lack of smoothness, we used the mean successive difference (MSD) as described by Cranz & Becker (1921) and von Neumann *et al*. (1941). The MSD is defined as the average absolute difference of the NRMS between two consecutive steps in the STN of a trajectory:

$$\mathrm{MSD} = \frac{\sum_{i=Q+1}^{P}\left|\mathrm{NRMS}_i - \mathrm{NRMS}_{i-1}\right|}{P-Q} \quad (2)$$

where P and Q are the entry and exit point indexes of the STN, respectively. In order to demonstrate the MSD measure, the NRMS of a no-pallidotomy trajectory (Fig. 3A) was redrawn with the STN activity reordered in an ascending and descending manner (Fig. 3B). This was done by sorting the top and bottom halves separately and then combining the two. For example, if the STN comprised 30 RMS measurements, the first 15 were arranged in ascending order and the next 15 in descending order. The irregularity of the original STN NRMS is seen exclusively in the MSD values (0.50 vs. 0.07), because the average and variance are measures that are not dependent on sequence and therefore remain unchanged in the re-ordered figure.

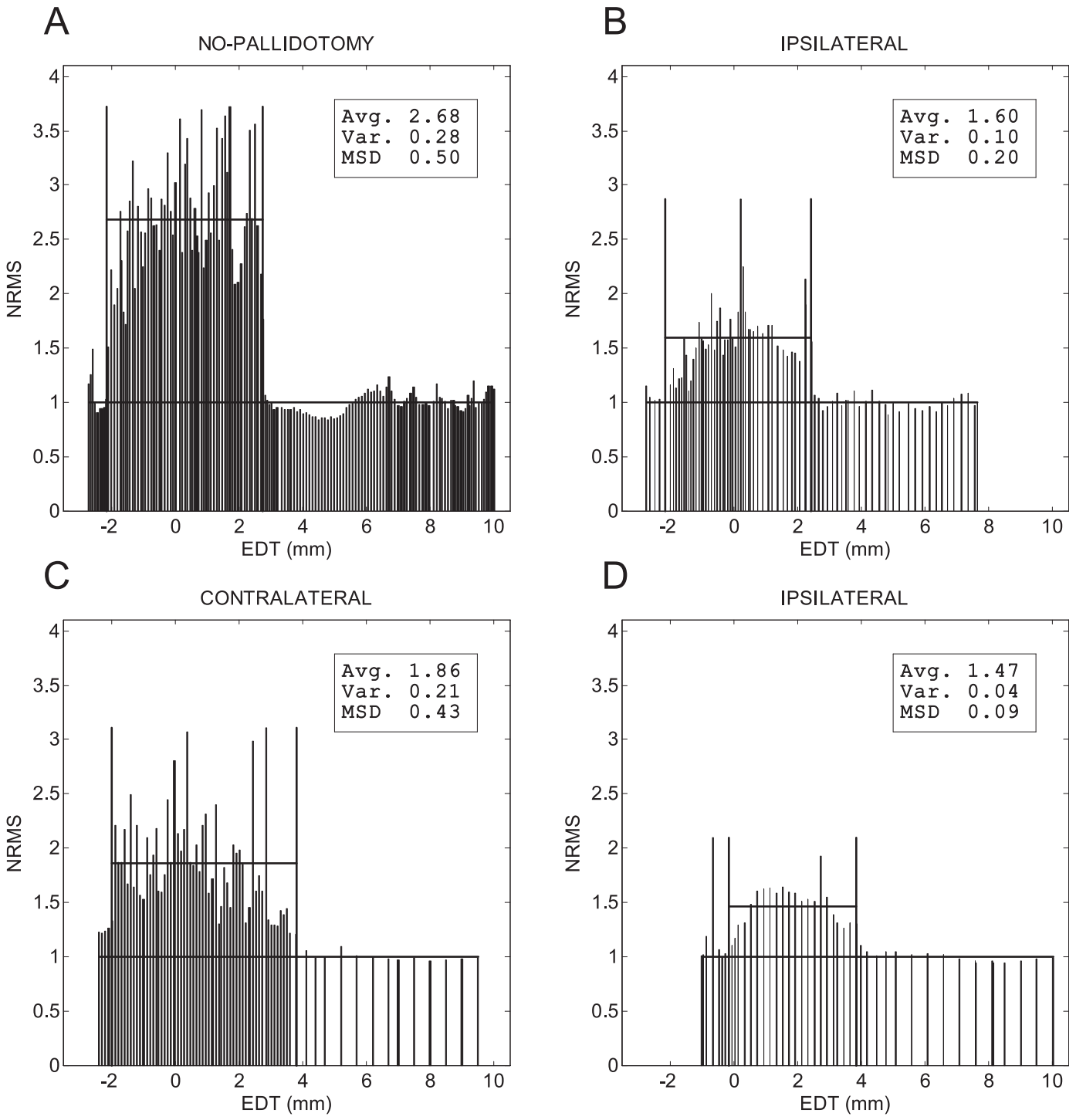

FIG. 2. The NRMS plot of a trajectory. Each solid line represents the NRMS calculated at discrete steps of estimated distance to target (EDT) in the surgical trajectory. The vertical dashed, horizontal dashed and horizontal dotted lines indicate the STN borders, the normalized baseline and the mean STN NRMS, respectively. (A) A trajectory with no prior pallidotomy. (B) A trajectory ipsilateral to prior pallidotomy. (C) A trajectory contralateral to pallidotomy (D) The trajectory ipsilateral to pallidotomy of the same patient as in C.

Statistical analysis

In all statistical mean comparisons a one-way analysis of variance (ANOVA) was used across the three trajectory groups (ipsilateral, contralateral and no-pallidotomy) with statistical significance declared at $P = 0.05$. When ANOVA detected significance, a *post hoc* multiple comparison test using the Bonferroni correction was performed. The mean of the STN NRMS average, variance and MSD were compared as well as average length of the STN. Significance of step size of electrode depth in the STN of the different groups was negated by statistical analysis.

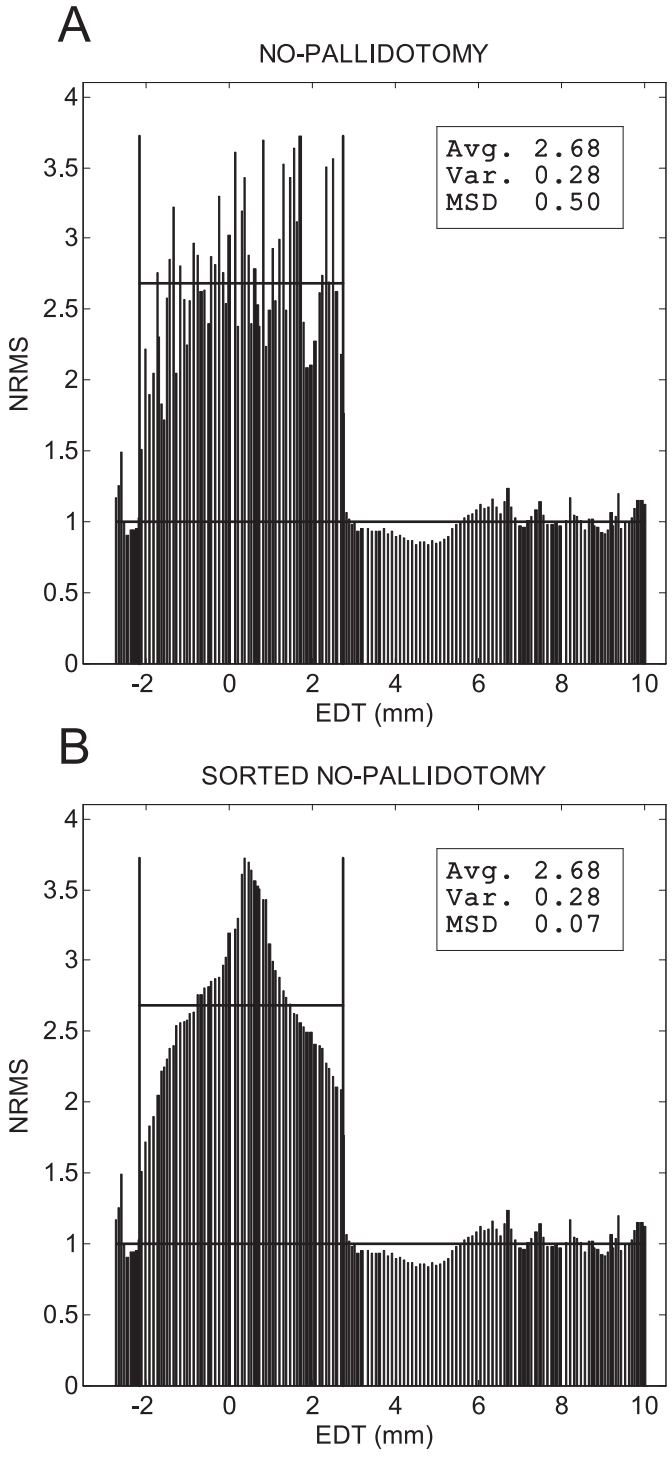

FIG. 3. MSD irregularity measure demonstrated. (A) A no-pallidotomy trajectory (same as in Fig. 2A). (B) The same trajectory, but with the STN NRMS reordered in an ascending and descending manner. The smoothness of the RMS in the reordered STN is demonstrated by the low MSD value compared with that in A, while the mean and variance are unchanged. Convention and borders as in Fig. 2.

Spectral analysis

For spectral analysis, the raw analog signal was rectified by the 'absolute' operator and the mean subtracted. This procedure is required in order to expose the frequency band of interest (below 60 Hz) as the original analog data are filtered at 250–6000 Hz (see section on data acquisition and analysis). The average power spectral density (PSD) was calculated in each STN using Welch's method with a 1-s Hamming window and zero-padding, resulting in a spectral resolution of 0.33 Hz. For each recording, the PSD was normalized by the baseline activity between 55 and 95 Hz. The average PSD for ipsilateral, contralateral and no-pallidotomy trajectories was calculated.

Software

Data analysis was carried out on custom software using MATLAB V7.1 (Mathworks, Natick, MA, usa). The software used in this article can be found online (http://basalganglia.huji.ac.il/links.htm)

Results

Statistically significant differences were found between the ipsilateral and no-pallidotomy STN trajectories when comparing the averages of the NRMS average (Fig. 4A) and MSD (Fig. 4C). Although the STN RMS variance showed similar changes (Fig. 4B), the results were not statistically significant. The mean average, variance and MSD of the contralateral trajectories were between the ipsilateral and no-pallidotomy trajectory values without statistical significance in either direction. The lack of significance in contralateral trajectory comparisons is attributed both to the small differences (as opposed to

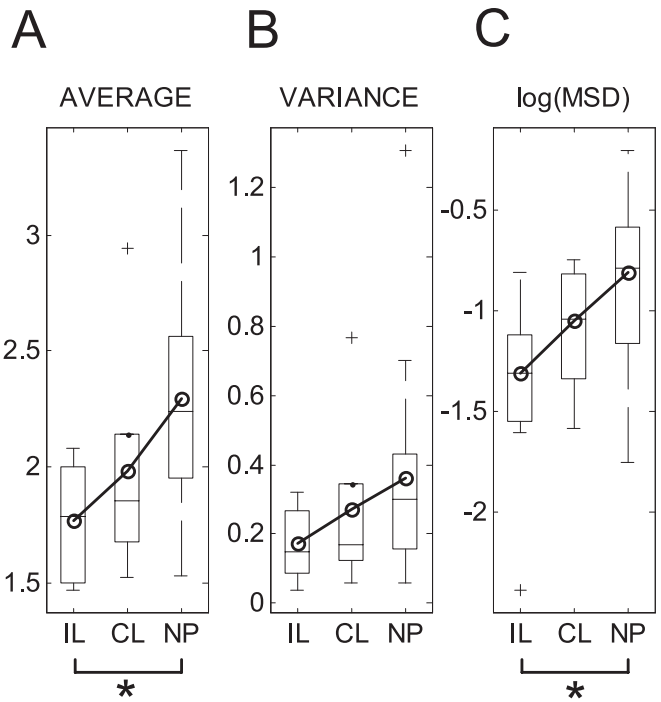

FIG. 4. Inter-trajectory comparison. A box plot comparison of STN (A) NRMS average, (B) NRMS variance and (C) NRMS MSD (after the logarithmic transformation) across trajectories. Horizontal lines represent the lower quartile, median and upper quartile values. Whiskers show the extent of the rest of the data (maximum whisker length was set at 1.5 units of interquartile range) and crosses represent outliers. The average values (circle markers) are joined by dashed lines. Differences in STN mean average and MSD between trajectories ipsilateral (IL) to pallidotomy and those with no-pallidotomy (NP) were significant (*$P < 0.05$). Contralateral (CL) trajectory values were systematically between the other two.

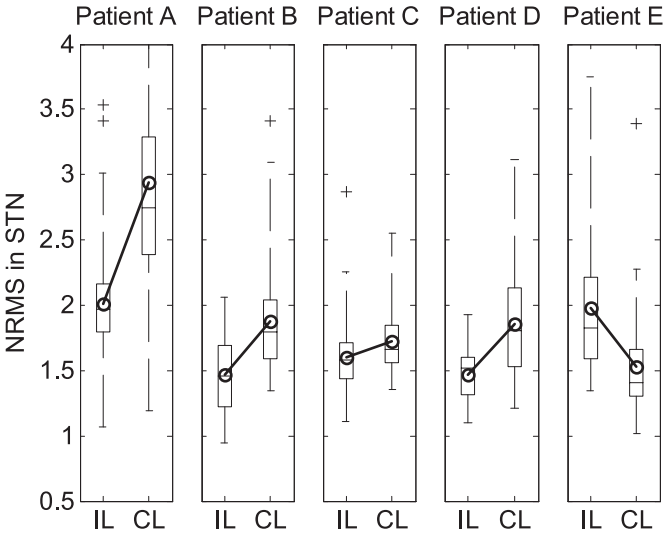

FIG. 5. Comparison of ipsilateral vs. contralateral STN NRMS in unilateral pallidotomy patients. Individual box plot comparisons of five patients with unilateral pallidotomy. Horizontal lines represent the lower quartile, median and upper quartile NRMS values. Whiskers show the extent of the rest of the data (maximum whisker length was set at 3 units of interquartile range) and crosses represent outliers. Average values (circle markers) are joined by dashed lines. For four of the five patients the mean and median NRMS in STN were lower ipsilateral (IL) to pallidotomy when compared with the contralateral (CL) side.

ipsilateral vs. no-pallidotomy) and to the small sample size. There was no significant difference in mean STN length between the three trajectories. Below we provide the full details of these findings.

Average normalized RMS

For each patient we calculated the average value of the STN NRMS. The mean of the NRMS average was significantly larger in the no-pallidotomy trajectory recordings (2.30 ± 0.48) than in the ipsilateral recordings: (1.77 ± 0.24; $P < 0.05$). The contralateral mean of the NRMS average was between the two (1.98 ± 0.50) with no statistical significance in either direction. Figure 4A depicts the population statistics of the average NRMS.

Variance of normalized RMS

There were no statistically significant differences seen in the variance of the NRMS, but the trend across trajectories was the same as that seen in the average NRMS (Fig. 4B), i.e. the mean of the NRMS variance tends to be larger in the no-pallidotomy trajectory recordings (0.36 ± 0.27) than in the ipsilateral recordings (0.17 ± 0.09). The contralateral mean NRMS variance was between the two (0.27 ± 0.25).

Irregularity of normalized RMS

The irregularity of the NRMS was estimated by the MSD (see Methods). The logarithm (log) transformation was applied to the MSD in order to improve homogeneity of variance in the ANOVA analysis. The mean MSD was significantly larger in the no-pallidotomy trajectory recordings (0.44 ± 0.17) than in the ipsilateral recordings (0.27 ± 0.10; $P < 0.05$). The contralateral mean MSD (0.35 ± 0.97) was between the two with no significant difference in either direction. Figure 4C depicts the population statistics of the irregularity of the NRMS. The fact that MSD showed significant differences ($P < 0.05$) despite the non-significant differences in variance ($P = 0.30$) emphasizes that STN activity in no-pallidotomy trajectories is characterized by significant irregularity rather than by a larger span of RMS values.

Ipsilateral vs. contralateral STN activity in cases with unilateral pallidotomy

The patients with unilateral pallidotomy provide an opportunity to study ipsilateral vs. contralateral trajectories in the same subject. This is particularly attractive due to considerations of control and symmetry. A box plot comparison of the normalized STN activity (NRMS) indicates that the average and median NRMS on the contralateral side were increased in four out of the five patients when compared with the ipsilateral side (Fig. 5). Although differences in the mean average NRMS were not statistically significant (probably due to small sample size), a trend of reduced activity ipsilateral to pallidotomy was evident. Interestingly, it was noted that patient E seemed to have considerably lower (tremor-dominant) Unified Parkinson's Disease Rating Scale (UPDRS) scores and levodopa-equivalent medications when compared with the other patients. This clinical observation may explain the difference between patient E and the other patients, and should be tested in future studies of these patients.

Spectral analysis

Periodic oscillations are evident in the average PSD of no-pallidotomy trajectories at 9 and 15 Hz (Fig. 6C), contralateral trajectories at 5 and 14 Hz (Fig. 6B) and ipsilateral trajectories at 13 Hz (Fig. 6A). The average PSD of oscillatory activity is notably lower in the ipsilateral trajectories than in contralateral and no-pallidotomy trajectories (Fig. 6A–C). In order to assess the relationship between RMS and PSD, the RMS measurements of each STN were divided into two: high RMS (with values above the trajectory mean) and low RMS (with values below the trajectory mean). For each group the PSD was calculated and averaged across trajectories (Fig. 6D–F). Our results show that RMS is correlated with PSD in the oscillatory bands – increased RMS seems to be coupled with higher PSD. These results are in line with previous findings of coupling between increased firing rate (FR) and oscillatory activity in the GPi (Bergman *et al*., 1994) and in the STN (Levy *et al*., 2000). However, no significant differences in the RMS–PSD correlation were observed between the ipsilateral, contralateral and the no-pallidotomy groups.

Step size

It was noticed that the average step size of electrode depth in the ipsilateral trajectories (105.24 ± 37.22 μm) was larger than that of the no-pallidotomy trajectories (83.30 ± 15.73 μm); this, however, was not statistically significant. The question nonetheless arose as to whether this could bias the MSD measurement. However, reducing step size could reduce, but not increase, the average MSD, as smaller steps on an NRMS gradient would cause smaller difference values. Our findings were opposite (no-pallidotomy trajectories with a smaller average step size had a larger MSD than ipsilateral trajectories), indicating that not only were the differences not a result of step size bias, but if the average step size were the same, the MSD differences would be even more robust.

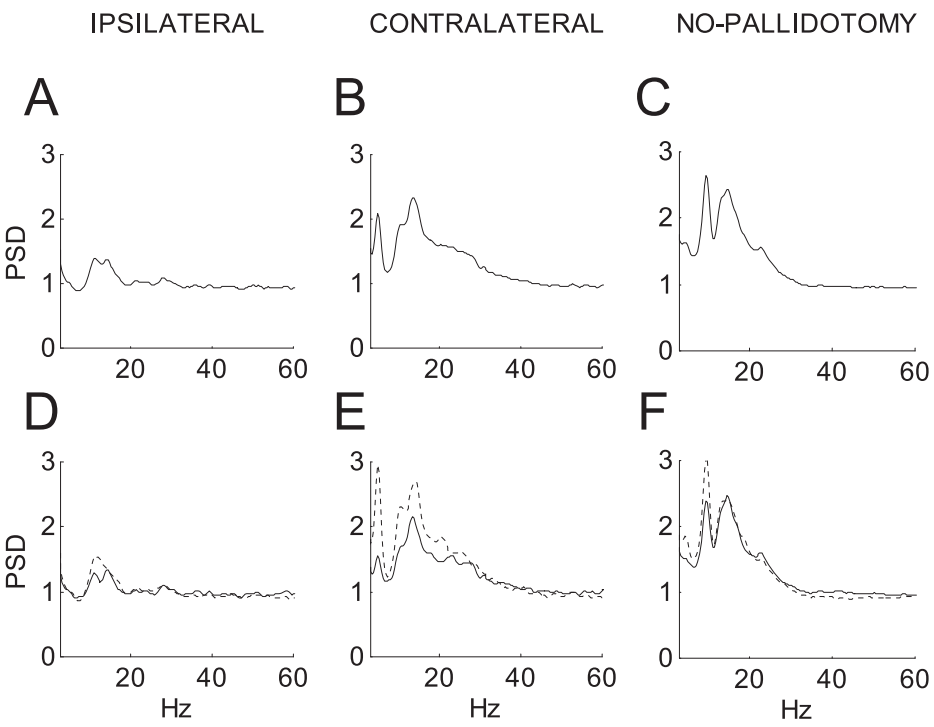

FIG. 6. Average power spectral density (PSD). The average PSD of oscillatory activity is notably lower in the ipsilateral trajectories (A) when compared with the contralateral (B) and no-pallidotomy (C) trajectories. The RMS measurements of each STN were divided into two – RMS values below trajectory mean and RMS values above trajectory mean. Solid and dashed lines (D–F) represent the average PSD of below-average and above-average RMS measurements, respectively.

Discussion

This study has two main findings: the STN ipsilateral to pallidotomy has (i) altered RMS characteristics (increased regularity of successive measures) and (ii) reduced activity (lower RMS average). In extracellular recording, spike size is a function of the size and geometrical structures of the cells, the density of the excitable channels of the cells, the cell environment (other cells and extracellular medium) as well as the electrode properties and the distance between the electrode and the cells (Gold *et al*., 2006). We have assumed that the many factors influencing the spike waveform average to a common value (central limit theory) and therefore our multi-unit RMS measure represents the mean firing rate, cell density or both. We consequently refer to the RMS as a general measure of population activity.

Our first finding of reduced MSD in the ipsilateral trajectories indicates that the STN RMS ipsilateral to pallidotomy is less volatile and more constant. In contrast, a high irregularity in the STN population activity such as that observed in the no-pallidotomy trajectories (high MSD) could suggest high STN synchronization. Population synchrony in the STN could induce diverse states of both higher and lower RMS activity causing a large MSD, whereas fully asynchronous activity would average to a more homogeneous RMS with low MSD. This is in line with the reduced oscillatory activity observed in the PSD of ipsilateral trajectories (Fig. 6). Our observation of lower MSD and PSD of oscillatory activity ipsilateral to pallidotomy could suggest that pallidotomy reduces STN synchrony. STN synchrony has been documented as a core feature in the pathophysiology of PD (Wichmann *et al*., 1994; Levy *et al*., 2000; Brown *et al*., 2001; Priori *et al*., 2004; Fogelson *et al*., 2005). In order to verify this, STN synchrony with and without pallidotomy would need to be analysed and compared. However, the present limitations on the duration and quality of recording in human patients do not enable this study on our data.

The findings of altered STN RMS characteristics and reduced STN activity ipsilateral to pallidotomy could be the result of two separate phenomena or outcomes of the same underlying dynamic. Coupling between oscillatory activity and increased FR has been described in the GPi (Bergman *et al*., 1994) and in the STN (Levy *et al*., 2000). In addition, neural simulations also indicated that average neuronal population activity and synchrony are tightly coupled (Chawla *et al*., 1999), and de la Rocha *et al*. (2007) showed that correlation between neural spike trains increases with FR. Hence, if the reduced MSD is a result of a reduction of synchrony as was proposed above, it could be suggested that the two findings come in conjunction and share a common pathway and mechanism. We suggest two possible explanations for reduced STN activity and synchronization ipsilateral to pallidotomy.

Pallidotomy possibly causes retrograde degeneration of projections to the Gpi

A recently described major input to the GPi is the globus pallidus, external segment (GPe). In order for retrograde degeneration of the GPe to be considered as a factor affecting STN activity, it would need to be shown that there exists a population of GPe cells that projects both to the GPi and to the STN. Based on a recent single-axon tracing of the GPe in primates (Sato *et al*., 2000) fewer than one-third of GPe axons branch to innervate both GPi and STN. Regardless, it seems improbable for retrograde degeneration of the Gpe–STN projection to be the physiological explanation behind our findings as it is predicted that this should lead to an increase in STN activity and not a decrease as we found. Another major input to the GPi is the STN. Retrograde degeneration of the massive STN projection to the GPi resulting in the death of a large portion of this glutaminergic cell population would reduce overall STN activity and hence multi-unit RMS measurements. Mogilner *et al*. (2002) found reduced STN cell density ipsilateral to

pallidotomy when compared with the contralateral STN; however, this was not statistically significant. Hence, further studies are required to validate the STN retrograde degeneration hypothesis.

It is possible that pallidotomy reduces STN activity by interrupting a GPi–STN reciprocal positive-feedback loop, for example via a (polysynaptic) excitatory GPi–STN pathway

This explanation would require modification of the classical direct and indirect pathways models of the BG cortical network, as within the context of these models the GPi is considered to be downstream from the STN. Via the entire BG–thalamo-cortical closed loop, reduced GPi activity would facilitate thalamo-cortical network activity. If we were to assume that the principal (projection) neurons of the cortex are the sole targets of thalamic axons, then this would lead to a net increase of STN activity and not a decrease as was found. This is based on excitatory glutaminergic projections from the cortex to the striatum, and γ-amino butyric acid (GABA)ergic projection from the striatum to the GPe and from the GPe to the STN (the classically described indirect pathway). The effects of the hyper-direct cortical–STN pathway would lead to the same result. If, on the other hand, the thalamo-cortical projections were to activate a great number of GABAergic interneurons, then increased thalamo-cortical activity could effectively reduce the activity of principal neurons of the cortex as described recently by Paz *et al*. (2007). This would result in decreased activity in the STN as was found in this study. As the STN closes this loop by projecting back to the GPi, a possible (long loop) reciprocal positive-feedback mechanism can be suggested.

An alternative (short loop) GPi–STN reciprocal positive-feedback mechanism can be suggested based on GPi to STN projections (Parent & Parent, 2004) and significant rebound properties of STN neurons. Post-inhibitory rebound firing has been described in the dorsolateral thalamus of the zebra finch (Luo & Perkel, 1999) and burst firing in STN neurons may be driven by rebound depolarization by pallidal fibers (Hallworth & Bevan, 2005). Pallidotomy can possibly reduce STN burst activity by breaking a long or short GPi–STN reciprocal positive-feedback loop (or both).

Our results show that the STN ipsilateral to prior pallidotomy has reduced RMS activity when compared with the STN of PD patients with no-pallidotomy, and tends (without significance) to have reduced RMS activity when compared with the STN contralateral to prior pallidotomy. The findings of Mogilner *et al*. (2002) of a lower mean STN single-cell FR on the side ipsilateral to prior pallidotomy compared with the contralateral side are in line with our findings. In contrast, Kleiner-Fisman *et al*. (2004) found no differences when comparing STN neuronal FR ipsilateral to prior pallidotomy vs. contralateral and also when comparing with a previously measured (Levy *et al*., 2000) no prior pallidotomy FR. The discrepancy between our results and those of Kleiner-Fisman *et al*. (2004) can possibly be explained by the following differences between the studies: (i) Kleiner-Fisman *et al*. (2004) compared neuronal FR whereas in our study the multi-unit RMS activity was compared; and (ii) different patient groups were used. Of particular note, the mean time from pallidotomy to STN DBS and mean disease duration at time of STN DBS in the Kleiner-Fisman *et al*. (2004) study (41.6 months from pallidotomy and 12 years mean disease duration) are considerably shorter than those in our study (114 months and 22.5 years, respectively).

The reduced motor improvement of STN DBS post pallidotomy found by Ondo *et al*. (2006) in conjunction with our results and those of Mogilner *et al*. (2002) of reduced STN activity ipsilateral to pallidotomy could suggest that clinical improvement of STN DBS is correlated with STN over-activity or synchrony. It could be explained accordingly that STN DBS will be more effective on a 'more pathological' STN. This would also explain why the post pallidotomy patients in Kleiner-Fisman *et al*.'s (2004) study, which showed no reduction in STN activity ipsilateral to pallidotomy, also demonstrated comparable motor improvement and efficaciousness of STN DBS. It would seem plausible to suggest that pallidotomy reduces STN activity in a once-off (set-back) manner, but does not break the continual increase associated with disease progression. Hence, pallidotomy has a palliative effect, and several years later, further treatment (such as STN DBS) may be required.

The fact that STN DBS is efficacious post pallidotomy (Kleiner-Fisman *et al*., 2004; Ondo *et al*., 2006) in conjunction with the classical BG models would compel the notion that at least part of the effects of STN DBS are mediated via the SNr. Nevertheless, prior GPi lesion (pallidotomy) should cause reduced effectiveness of STN DBS. The fact that Kleiner-Fisman *et al*. (2004) found no difference in motor improvement, and Ondo *et al*. (2006) found no difference in dyskinesia reduction with/without pallidotomy suggest that DBS antidromic activation of the cortex (Li *et al*., 2007) or direct projections from the STN to brain-stem structures could play a more critical role than previously thought. Many recent reports suggest a significant role for the upper brainstem and the pedunculopontine nucleus (PPN) in particular in BG physiology and PD pathology (Kojima *et al*., 1997; Aziz *et al*., 1998; Pahapill & Lozano, 2000; Nandi *et al*., 2002).

Increased STN and GPi/SNr activity is a hallmark of PD (Wichmann & DeLong, 1996) and it is well known that STN inactivation reduces GPi activity (Wichmann *et al*., 1994). This together with the second potential explanation of our results introduced above (that pallidotomy reduces STN activity by interrupting a GPi–STN positive-feedback loop) could shed light on our understanding of over-activity and synchrony within the BG. Excessive positive feedback could aggravate GPi–STN over-activity and cause increased GPi–STN synchronization such as that observed by Brown *et al*. (2001). This is because volatility and excessive oscillatory activity are innate in a positive-feedback loop (and would be characterized by high MSD). Breaking this loop by pallidotomy could account for the reduced activity and lower STN irregularity seen ipsilateral to pallidotomy in this study.

In conclusion, we suggest that pallidotomy sets PD symptoms back by partially breaking a GPi–STN positive-feedback loop. This is in agreement with our results of reduced STN RMS activity and reduced MSD post pallidotomy. In this case, part of the therapeutic effect of pallidotomy could come from reduced activity and synchrony in the STN. We propose that with disease progression, STN over-activity and synchrony continue to deteriorate, and that bilateral STN DBS further aids in alleviating PD symptoms by directly targeting the STN. STN DBS is efficacious even post pallidotomy either via back-propagation to the cortex or STN direct projections to brain-stem structures such as the PPN and not exclusively through the GPi/SNr.

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Abbreviations

BG, basal ganglia; CT, computerized tomography; DBS, deep brain stimulation; EDT, estimated distance to target; FR, firing rate; GPe, globus pallidus, external segment; GPi, globus pallidus, internal segment; MER, microelectrode recording; MRI, magnetic resonance imaging; MSD, mean successive difference; NRMS, normalized root mean square; PD, Parkinson's disease; PPN, pedunculopontine nucleus; RMS, root mean square; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus.

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