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More Than Meets the Eye - Myelinated Axons Crowd the Subthalamic Nucleus

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Abstract

High frequency deep brain stimulation (DBS) of the subthalamic nucleus (STN) is a successful treatment for patients with advanced Parkinson's disease (PD). While its exact mechanism of action is unknown, it is currently believed that the beneficial effects of the stimulation are mediated either by alleviating pathological basal ganglia output patterns of activity, or by activation of axons of passage which arise from the cerebral cortex and other sources.

In this study, we show that the anatomical composition of the primate STN provides a substrate through which DBS may elicit widespread changes in brain activity via stimulation of fibers of passage. Using quantitative high-resolution electron microscopy, we found that the primate STN is traversed by numerous myelinated axons which occupy as much as 45% of its sensorimotor territory, and 36% of its associative region. In comparison, myelinated axons occupy only 27% of the surface areas of the sensorimotor and associative regions of the internal segment of the globus pallidus (GPi), another target for therapeutic DBS in PD. We also noted that myelinated axons in the STN have, on average, a larger diameter than those in GPi, which may render them more susceptible to electrical stimulation.

Because axons are more excitable than other neuronal elements, our findings support the hypothesis that STN DBS, even when carried out entirely within the confines of the nucleus, mediates some of its effects by activating myelinated axons of passage.

Keywords

deep brain stimulation; Parkinson's disease; fibers of passage; globus pallidus; antidromic

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Introduction

High frequency stimulation of the subthalamic nucleus (STN) alleviates parkinsonism in patients ¹⁻³ and in MPTP-treated monkeys ⁴. Modeling studies and experimental data have suggested that the stimulation elicits different effects on cell bodies and axons, depending on the stimulation parameters and the electrode placement relative to the stimulated tissue element(s) ^{5, 6}. According to these studies, stimulation with the parameters used in current clinical practice is very likely to predominately activate axons ⁷⁻⁹. Although DBS at the dorsal surface of the STN may exert many of its therapeutic benefits via activation of nearby pallido-thalamic fibers ^{10, 11}, the specific neural elements affected by stimulation within the core of the STN are less well defined ^{12, 13}. In the present study, we approached this question with an electron microscopic analysis of the distribution and relative prevalence of myelinated axons within the monkey STN, and found that the primate STN contains a much larger density of myelinated axons than the internal pallidal segment (GPi), another DBS target in the basal ganglia. This crowding of myelinated axons may contribute significantly to the effectiveness of STN DBS for the treatment of Parkinson's disease (PD) and related disorders. Preliminary results of this study have been presented in abstract form ¹⁴.

Methods

Animals and tissue preparation

We used brain tissue from three adult (3-8 years old) rhesus monkeys (*Macaca mulatta*, 4-15kg; 1 male, 2 females). All experiments were performed in accordance with the National Institutes of Health's "Guide for the Care and Use of Laboratory Animals" ¹⁵, and were approved by the Institutional Health and Biosafety and Animal Care and Use Committees of Emory University. The monkeys were deeply anesthetized with an overdose of pentobarbital (30mg/kg, i.v.) and transcardially perfused with cold, oxygenated Ringer's solution. Following this, the animals were perfused with 2 liters of a fixative – either 4% paraformaldehyde + 0.1% glutaraldehyde in phosphate buffer (PB; 0.1M, pH 7.4) or 2% paraformaldehyde + 3.75% acrolein in PB. After fixation, the brains were cut into 10 mm thick blocks in the frontal plane, removed from the skull, and immersed in fixative (2% or 4% paraformaldehyde in PB, 0.1M, pH 7.4) overnight at 4 °C. Both fixative recipes and post-fixation procedures provided adequate and comparable ultrastructural preservation of the tissue suitable for electron microscopic observations performed in this study.

Processing of brain sections for electron microscopy

Sixty µm-thick vibratome sections were collected in cold phosphate-buffered saline (PBS; 0.01M, pH 7.4). Sections containing the STN and the GPi were selected and treated with sodium borohydride (1% in PBS) for 20 min. They were later soaked in a cryoprotectant solution (PB; 0.05 M, pH 7.4, containing 25% sucrose and 10% glycerol) for 20 min before being frozen at – 80 °C for 20 min, thawed, and placed in a graded series of cryoprotectant (100, 70, 50 and 30% in PBS), and then washed in PBS.

After completion of the cryoprotectant protocol, the sections were transferred to PB (0.1M, pH 7.4) for 10 min and subjected to 1% osmium tetroxide for 20 min. Following subsequent rinses with PB, the sections were dehydrated through solutions containing an increasing gradient of ethanol (50, 70, 90 and 100%). The 70% ethanol solution also contained 1% uranyl acetate to increase tissue contrast under the electron microscope. The sections were then immersed in propylene oxide before being embedded in epoxy resin (Durcupan, ACM; Fluka, Buchs, Switzerland) overnight. Finally, the sections were mounted on microscope slides, dabbed with epoxy resin, coverslipped with oil-coated coverslips and placed in a 60 °C oven for 48 h. After removing the slides from the oven, the coverslips were taken off the

slides. Blocks of tissue from the sensorimotor and associative regions of the GPi and STN^{16, 17}, were cut out from the slides and glued on top of resin blocks with cyanoacrylate glue. The resulting blocks were cut into 60-nm ultrathin sections with an ultramicrotome (Ultracut T2; Leica, Nussloch, Germany) and collected on single-slot Pioloform-coated copper grids. The sections were then stained with lead citrate for 5 min and viewed under an electron microscope (JEM-1011, JEOL, Peabody, MA).

Data Collection and Analysis

Digital micrographs of the neuropil in randomly selected sensorimotor and associative regions of the STN and GPi, as defined in previous anatomical and functional studies^{16, 17}, were taken at 25,000x under the electron microscope. Each image covered a surface area of 39.53 μm^2 . This material was used to quantify the surface area occupied by myelinated axons, and their average cross-sectional diameters.

To do so, the borders of each myelinated axon (including the myelin) seen within a micrograph were traced using NIH's ImageJ software (Fig. 1 A), and the surface area occupied by myelinated axons calculated. Twenty five micrographs from each of the functional regions of both nuclei were analyzed in every animal. The measured surface areas of all myelinated axons were summed and expressed as the proportion of the total analyzed STN or GPi surface areas in each animal, which allowed us to calculate the average percentage surface areas occupied by myelinated axons across animals. Statistical differences between regions were assessed using Student's t-test (SigmaPlot 12.0, Systat Software Inc.). To determine whether any differences in the percent surface area covered by myelinated axons between the STN and GPi was due to differences in the density or sizes of myelinated axons, we counted the number of myelinated axons per area analyzed for each region, and measured the diameters of the myelinated axons (excluding the myelin) in the STN and GPi using NIH's ImageJ software (Fig. 1 B) as the shortest diameter passing through the center of mass. The latter analysis is based on data from 200 myelinated axons per nucleus (100 each from the sensorimotor and associative territory, respectively), per animal. The diameters of myelinated axons were first compared between functional territories, and then pooled to calculate the average diameters for the GPi or STN.

In order to assess whether myelinated axons were clustered in bundles in the STN, we counted the number of myelinated axons in each micrograph. The number of myelinated axons per micrograph was plotted (bin size = 5 myelinated axons) and the normality of the distribution of these measurements was determined using the Shapiro-Wilk test (SigmaPlot 12.0, Systat Software Inc.). Clustering of axons in bundles would be expected to lead to non-normal patterns of distribution.

Results

We found that myelinated axons occupy $40.1 \pm 1.1\%$ (mean $\pm$ SEM) of the surface of the STN (Fig. 2 A), with no significant differences between the sensorimotor ($44.7 \pm 3.9\%$) and associative ($35.6 \pm 4.6\%$) regions. A similar analysis revealed that only $27.0 \pm 1.5\%$ of the surface of the GPi (sensorimotor: $26.9 \pm 3.3\%$ and associative: $27.2 \pm 1.3\%$) is occupied by myelinated axons (Fig. 2 A). The proportion of the surface area covered by myelinated axons in the STN (sensorimotor and associative regions combined) was found to be significantly larger than that in the GPi ($P=0.002$), while the number of myelinated axons in STN and GPi was similar in all sampled regions (Fig. 2 B), suggesting that individual myelinated axons in the STN have larger diameters than those in GPi. In support of this hypothesis, we found that the sizes of myelinated axons in the STN were more broadly distributed than those in the GPi, with a slight propensity for axons of larger diameters in the STN (Fig. 3).

In order to determine if myelinated axons in the STN are aggregated into bundles or homogeneously distributed across the neuropil, we counted the number of myelinated axons in each micrograph taken in the sensorimotor and associative regions of the STN. We found that myelinated axons are normally distributed in the STN (Shapiro-Wilk: $W=0.913$, $P=0.374$), with an average of approximately 20 myelinated axons per micrograph (Fig. 4), in both functional regions of the STN.

Discussion

The results of this study demonstrate that almost half of the sensorimotor and associative STN surface area is occupied by myelinated axons that have a broad size distribution. Although the origin and projection targets of these axons are unknown, they provide a substrate through which deep brain stimulation of the STN could influence widespread areas of the central nervous system. We also found no evidence for clustering of axons into bundles.

We noticed that the thickness of myelinated axons within the STN varied substantially. Throughout the nervous system, long-distance connections use large caliber axons, while shorter connections engage small-diameter axons. The range of axon diameters in the STN may therefore represent a spectrum of short-, medium-, or long-distance fibers traveling through, or terminating in, the STN. A detailed characterization of the source, destination and topographical distribution of these axons across the different functional regions of the STN is warranted. Although speculative, it is reasonable to suggest that the corticosubthalamic system gives rise to a significant contingent of these fibers¹⁸⁻²⁰. Small caliber GABAergic axons from the external segment of the globus pallidus, which represents the main origin of inputs to the STN, should also be considered as a major source of axons in the STN. Additional inputs known to terminate in the STN (i.e., those arising from the thalamus, pedunculopontine nucleus, substantia nigra, raphe, locus coeruleus, or the tectum) or fibers that travel through the nucleus¹⁸ could also account for part of this myelinated axons crowd within the primate STN.

The prominence of axons in the STN may have implications for the effects of deep brain stimulation (DBS) in this area. DBS strongly affects the activity of fibers close to the DBS electrode. If the axons in the STN arise from motor and non-motor cortical areas, stimulation of these fibers may result in antidromic activation of diverse cortical regions^{21, 22}. Fibers could be stimulated regardless of their actual termination or origin in the STN, suggesting that the effectiveness of STN stimulation in movement disorders and other neurologic conditions^{1, 2, 23-25}, as well as the side effects of STN-DBS²⁶⁻²⁹ may have less to do with the specific connections and functions of the STN itself, than with the electrode location relative to fiber tracts that are nearby or traverse it.

This study also emphasizes important structural differences between the STN and GPi: the STN, which contains a dense neuronal population^{30, 31}, is tightly compacted with myelinated axons; while the GPi harbors a much smaller and less tightly compacted population of neurons^{30, 32} and myelinated axons. However, despite these significant differences in terms of neuronal cell bodies and myelinated axons' density between the GPi and STN, DBS in either of these nuclei produces comparable benefits in PD patients^{26, 33, 34}, albeit with slightly different side-effect profiles²⁶⁻²⁹. Therefore, our data add to the growing body of evidence that the anti-parkinsonian effects of DBS in STN or GPi may be mediated through different cellular mechanisms²².

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Authors' Roles

A.M. and Y.S. designed the study. A.M. performed the experiments and analyzed the data. T.W. provided expert guidance throughout the project. A.M., T.W. and Y.S. wrote the manuscript.

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References

1. Benabid AL, Pollak P, Gross C, et al. Acute and long-term effects of subthalamic nucleus stimulation in Parkinson's disease. *Stereotact Funct Neurosurg.* 1994; 62(1-4):76–84. [PubMed: 7631092]
2. Limousin P, Pollak P, Benazzouz A, et al. Bilateral subthalamic nucleus stimulation for severe Parkinson's disease. *Mov Disord.* 1995; 10(5):672–674. [PubMed: 8552123]
3. Limousin P, Pollak P, Benazzouz A, et al. Effect of parkinsonian signs and symptoms of bilateral subthalamic nucleus stimulation. *Lancet.* 1995; 345(8942):91–95. [PubMed: 7815888]
4. Benazzouz A, Gross C, Feger J, Boraud T, Bioulac B. Reversal of rigidity and improvement in motor performance by subthalamic high-frequency stimulation in MPTP-treated monkeys. *Eur J Neurosci.* 1993; 5(4):382–389. [PubMed: 8261116]
5. Ranck JB Jr. Which elements are excited in electrical stimulation of mammalian central nervous system: a review. *Brain Res.* 1975; 98(3):417–440. [PubMed: 1102064]
6. Kringelbach ML, Jenkinson N, Owen SL, Aziz TZ. Translational principles of deep brain stimulation. *Nat Rev Neurosci.* 2007; 8(8):623–635. [PubMed: 17637800]
7. Holsheimer J, Demeulemeester H, Nuttin B, de Sutter P. Identification of the target neuronal elements in electrical deep brain stimulation. *Eur J Neurosci.* 2000; 12(12):4573–4577. [PubMed: 11122371]
8. Nowak LG, Bullier J. Axons, but not cell bodies, are activated by electrical stimulation in cortical gray matter. II. Evidence from selective inactivation of cell bodies and axon initial segments. *Exp Brain Res.* 1998; 118(4):489–500. [PubMed: 9504844]
9. Kringelbach ML, Green AL, Owen SL, Schweder PM, Aziz TZ. Sing the mind electric - principles of deep brain stimulation. *Eur J Neurosci.* 2010; 32(7):1070–1079. [PubMed: 21039946]
10. Stefani A, Fedele E, Pierantozzi M, et al. Reduced GABA Content in the Motor Thalamus during Effective Deep Brain Stimulation of the Subthalamic Nucleus. *Front Syst Neurosci.* 2011; 5:17. [PubMed: 21519387]
11. Stefani A, Fedele E, Galati S, et al. Deep brain stimulation in Parkinson's disease patients: biochemical evidence. *J Neural Transm Suppl.* 2006; (70):401–408. [PubMed: 17017559]
12. Kuncel AM, Grill WM. Selection of stimulus parameters for deep brain stimulation. *Clin Neurophysiol.* 2004; 115(11):2431–2441. [PubMed: 15465430]
13. Deniau JM, Degos B, Bosch C, Maurice N. Deep brain stimulation mechanisms: beyond the concept of local functional inhibition. *Eur J Neurosci.* 2010; 32(7):1080–1091. [PubMed: 21039947]
14. Mathai, A.; Smith, Y. Myelinated axons crowd the subthalamic nucleus – Could this underlie the effects of deep brain stimulation?. *Southeast Medical Scientist Symposium*; Atlanta, GA. 2011.
15. Garber, JC.; Barbee, RW.; Bielitzki, JT., et al. *Guide for the Care and Use of Laboratory Animals.* 8. Washington, D.C: The National Academies Press; 2010.
16. Shink E, Bevan MD, Bolam JP, Smith Y. The subthalamic nucleus and the external pallidum: two tightly interconnected structures that control the output of the basal ganglia in the monkey. *Neuroscience.* 1996; 73(2):335–357. [PubMed: 8783253]

17. Mathai A, Smith Y. The corticostriatal and corticosubthalamic pathways: two entries, one target. So what? *Front Syst Neurosci.* 2011; 5:64. [PubMed: 21866224]
18. Haynes WI, Haber SN. The organization of prefrontal-subthalamic inputs in primates provides an anatomical substrate for both functional specificity and integration: implications for Basal Ganglia models and deep brain stimulation. *J Neurosci.* 2013; 33(11):4804–4814. [PubMed: 23486951]
19. Monakow KH, Akert K, Kunzle H. Projections of the precentral motor cortex and other cortical areas of the frontal lobe to the subthalamic nucleus in the monkey. *Exp Brain Res.* 1978; 33(3-4): 395–403. [PubMed: 83239]
20. Nambu A, Takada M, Inase M, Tokuno H. Dual somatotopical representations in the primate subthalamic nucleus: evidence for ordered but reversed body-map transformations from the primary motor cortex and the supplementary motor area. *J Neurosci.* 1996; 16(8):2671–2683. [PubMed: 8786443]
21. Li S, Arbuthnott GW, Jutras MJ, Goldberg JA, Jaeger D. Resonant antidromic cortical circuit activation as a consequence of high-frequency subthalamic deep-brain stimulation. *J Neurophysiol.* 2007; 98(6):3525–3537. [PubMed: 17928554]
22. Devergnas A, Wichmann T. Cortical potentials evoked by deep brain stimulation in the subthalamic area. *Front Syst Neurosci.* 2011; 5:30. [PubMed: 21625611]
23. Ostrem JL, Racine CA, Glass GA, et al. Subthalamic nucleus deep brain stimulation in primary cervical dystonia. *Neurology.* 2011; 76(10):870–878. [PubMed: 21383323]
24. Pahapill PA, O'Connell B. Long-term follow-up study of chronic deep brain stimulation of the subthalamic nucleus for cervical dystonia. *Neuromodulation.* 2010; 13(1):26–30. [PubMed: 21992761]
25. Mallet L, Polosan M, Jaafari N, et al. Subthalamic nucleus stimulation in severe obsessivecompulsive disorder. *N Engl J Med.* 2008; 359(20):2121–2134. [PubMed: 19005196]
26. Bronstein JM, Tagliati M, Alterman RL, et al. Deep brain stimulation for Parkinson disease: an expert consensus and review of key issues. *Arch Neurol.* 2011; 68(2):165. [PubMed: 20937936]
27. Weaver FM, Follett KA, Stern M, et al. Randomized trial of deep brain stimulation for Parkinson disease: thirty-six-month outcomes. *Neurology.* 2012; 79(1):55–65. [PubMed: 22722632]
28. Zahodne LB, Okun MS, Foote KD, et al. Greater improvement in quality of life following unilateral deep brain stimulation surgery in the globus pallidus as compared to the subthalamic nucleus. *J Neurol.* 2009; 256(8):1321–1329. [PubMed: 19363633]
29. Hariz MI, Rehncrona S, Quinn NP, Speelman JD, Wensing C. Multicenter study on deep brain stimulation in Parkinson's disease: an independent assessment of reported adverse events at 4 years. *Mov Disord.* 2008; 23(3):416–421. [PubMed: 18067188]
30. Oorschot DE. Total number of neurons in the neostriatal, pallidal, subthalamic, and substantia nigral nuclei of the rat basal ganglia: a stereological study using the cavalieri and optical disector methods. *J Comp Neurol.* 1996; 366(4):580–599. [PubMed: 8833111]
31. Lange H, Thorner G, Hopf A. Morphometric-statistical structure analysis of human striatum, pallidum and nucleus subthalamicus. III. Nucleus subthalamicus. *J Hirnforsch.* 1976; 17(1):31–41. [PubMed: 965719]
32. Thorner G, Lange H, Hopf A. Morphometrical-statistical structure analysis of human striatum, pallidum and subthalamic nucleus. II. Globus pallidus. *J Hirnforsch.* 1975; 16(5):401–413. [PubMed: 1214059]
33. Follett KA, Torres-Russotto D. Deep brain stimulation of globus pallidus interna, subthalamic nucleus, and pedunculopontine nucleus for Parkinson's disease: which target? *Parkinsonism Relat Disord.* 2012; 18(Suppl 1):S165–167. [PubMed: 22166422]
34. Odekerken VJ, van Laar T, Staal MJ, et al. Subthalamic nucleus versus globus pallidus bilateral deep brain stimulation for advanced Parkinson's disease (NSTAPS study): a randomised controlled trial. *Lancet Neurol.* 2013; 12(1):37–44. [PubMed: 23168021]

Figure 1.

Electron micrographs of the ultrastructural composition of the monkey STN (A) and GPi (B) neuropil. The black line with double ended arrows in panel A illustrates an example of the measurement used to assess the cross-sectional diameter of individual myelinated axons, defined as the shortest diameter passing through its center. The scale bar in B corresponds to 1 μm and applies to both portions of the figure.

Figure 2.

(A) Percentage surface area occupied by myelinated axons in the STN and GPi. The percent surface area occupied by myelinated axons in the STN is significantly higher than in the GPi (**, $P=0.002$). (B) Number of myelinated axons per 100 μm^2 of surface area in GPi and STN. The columns represent means $\pm$ SEM across 3 monkeys, i.e. a total of 1976.5 μm^2 surface area/nucleus/animal. No significant difference was found between the data from STN and GPi.

Figure 3.
Distribution of the diameters of myelinated axons in the STN and GPi (based on measurements of 200 myelinated axons/nucleus/animal in 3 monkeys).

Figure 4.

Distribution of the number of myelinated axons per micrographs of the STN neuropil. Myelinated axons are homogenously distributed in the STN. Data expressed as means $\pm$ SEM across 3 monkeys, based on the analysis of 50 micrographs/nucleus/animal (each micrograph contributed $39.53 \mu\text{m}^2$, total surface area: $1976.5 \mu\text{m}^2/\text{nucleus}/\text{animal}$).