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Deep-brain stimulation associates with improved microvascular integrity in the subthalamic nucleus in Parkinson's disease

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ABSTRACT

Deep brain stimulation (DBS) of the subthalamic nucleus (STN) has become an accepted treatment for motor symptoms in a subset of Parkinson's disease (PD) patients. The mechanisms why DBS is effective are incompletely understood, but previous studies show that DBS targeted in brain structures other than the STN may modify the microvasculature. However, this has not been studied in PD subjects who have received STN-DBS. Here we investigated the extent and nature of microvascular changes in post-mortem STN samples from STN-DBS PD patients, compared to aged controls and PD patients who had not been treated with STN-DBS. We used immunohistochemical and immunofluorescent methods to assess serial STN-containing brain sections from PD and STN-DBS PD cases, compared to similar age controls using specific antibodies to detect capillaries, an adherens junction and tight junction-associated proteins as well as activated microglia. Cellular features in stained sections were quantified by confocal fluorescence microscopy and stereological methods in conjunction with *in vitro* imaging tools. We found significant upregulation of microvessel endothelial cell thickness, length and density but lowered activated microglia density and striking upregulation of all analysed adherens junction and tight junction-associated proteins in STN-DBS PD patients compared to non-DBS PD patients and controls. Moreover, in STN-DBS PD samples, expression of an angiogenic factor, vascular endothelial growth factor (VEGF), was significantly upregulated compared to the other groups. Our findings suggest that overexpressed VEGF and downregulation of inflammatory processes may be critical mechanisms underlying the DBS-induced microvascular changes.

Introduction

In the brains of Parkinson's disease (PD) patients, the degeneration of neurons in the substantia nigra pars compacta (SNpc) results in loss of dopamine (DA) content in the caudate nucleus and putamen, triggering downstream changes in the activity of the basal ganglia output pathways. These changes include increased activity of glutamatergic pathways originating from the subthalamic nucleus (STN) segment of the basal ganglia's indirect pathway that projects to the internal segment of the globus pallidus (GPi) and substantia nigra reticulata (SNr), resulting in PD motor symptoms (Bergman et al., 1990; Smith et al., 1998). Moreover, a hyperactive STN–SNpc pathway ensues, potentially reinforcing neurodegeneration of the SNpc dopaminergic neurons via glutamate-mediated excitotoxicity (Miller and DeLong, 1987; Bergman et al., 1994).

Such insights resulted in the development of deep brain stimulation (DBS) of the STN (STN-DBS), with studies showing a marked reduction in motor fluctuations and the disappearance of drug-induced dyskinesias in STN-DBS PD patients (Limousin et al., 1998). These patients also exhibited improved axial functions (Ngoga et al., 2014) and slower PD progression (Tagliati et al., 2010). This is in accordance with other studies (e.g. Temel et al., 2006), demonstrating that STN-DBS protected nigral neuronal loss in the 6-hydroxydopamine (6-OHDA) rat model of PD. Similarly, Wallace et al. (2007) observed that STN-DBS applied both before and after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration to primates prevented further loss of SNpc dopaminergic neurons compared to sham-surgery controls.

Although STN-DBS as a therapeutic option has existed for 25 years, the mechanisms responsible for symptom improvement remain largely

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unknown. Recently, a role for the microvasculature in alleviating Parkinsonian syndrome following STN-DBS has been proposed (Nagai et al., 2012; Hill et al., 2013). This includes that DBS may enhance neurogenesis (Segi-Nishida et al., 2008; Warner-Schmidt et al., 2008). Related to this, Vedam-Mai et al. (2014) recently revealed that in PD patients, DBS stimulation induced neural stem cell proliferation, indicative of cellular plasticity, at both a local level and more distally, compared to normal and untreated PD brains. A further possibility is that DBS induces angiogenesis, possibly by upregulating the neurotrophic factors glial-derived neurotrophic factor (GDNF) and vascular endothelial growth factor (VEGF) (Glickstein et al., 2001; Hellsten et al., 2004; Wang et al., 2007; Lindvall and Wahlberg, 2008).

Blood vessel alterations have been implicated in the pathogenesis of several neurodegenerative disorders, including Alzheimer's disease (AD), PD, multiple sclerosis and amyotrophic lateral sclerosis (Lee and Pienaar, 2014). Here we used immunohistochemical and stereological methods to assess markers of the brain microvasculature including tight junction (TJ) complexes and adhesion molecules associated with the blood–brain barrier (BBB) (Ballabh et al., 2004; Abbott et al., 2010) within post-mortem STNs of STN-DBS PD patients, compared to non-stimulated PD and neurologically-intact controls. We provide the first evidence that STN-DBS result in significant microvascular changes, whilst identifying putative protein mechanisms to explain the observed angiogenic benefits.

Materials and methods

Subjects

Post-mortem human brain samples (non-stimulated PD, n = 7; PD STN-DBS, n = 5 and non-PD controls, n = 7) were provided by the Parkinson's UK Brain Bank at Imperial College London (http://www.parkinsons.org.uk/research/parkinsons_brain_bank.aspx) and the Banner Sun Health Research Institute Brain and Body Donation Program.

(BBDP, https://www.brainandbodydonationprogram.org/), Sun City, Arizona, USA. A summary of the demographic and clinical characteristics of the control, PD and PD STN-DBS subjects included in this study is presented in Table 1. Tissue was collected with informed consent by the donors via a prospective donor scheme according to the local Ethics Committee approval. Neuropathological examination was carried out on each case by an experienced neuropathologist.

PD cases (either those that had received STN-DBS or non-stimulated PD cases) had a clinical history of PD and were also selected based on the absence of dementia and the presence of at least 2 of the cardinal clinical signs of PD, as well as histological evidence of Lewy bodies/alpha-synuclein deposition, pigmented neuronal loss in the SNpc and being Levodopa responsive. In all STN-DBS PD cases the DBS electrode was chronically implanted and it was more than a year between placement and death.

Normal control subjects were chosen based on the pathology reports that classified the cases as being within the normal range for their age and not presenting with any signs of neuropathology or histological abnormalities. This included the absence of atrophic changes in the mesencephalic regions, no evidence of plaques or vascular amyloid and the lack of detection of neurofibrillary tangles or excessive glial staining following an immunoperoxidase tau stain. Moreover, in the normal controls, there was no indications of cortical or nigral Lewy body formation nor of infarcts, with the small arteries that appeared void of sclerotic changes. Unless specified, the cause of death was bronchopneumonia.

Moreover, in none of the three case groupings were cases included for any of the three study groups if the neuropathological report mentioned that evidence was found of ischemia, indicative of a stroke-like episode. In addition, cases were excluded where mention was found in the clinical notes that a patient had received chronic treatment with drugs known to potentially influence the circulation, including anti-hypertensives, anti-inflammatories or steroids.

Table 1
Clinical and demographic characteristics of all cases included. Results are shown as the mean ± S.E.M.

	Control (n = 7)	PD non-stimulated (n = 7)	PD STN-DBS (n = 5)
Sex ratio (M/F)	4:3	4:3	2:3
Age of PD symptom onset (years)	N/A	62 ± 4.32	74 ± 5.15
Age at death (years)	77 ± 12.13	76 ± 6.84	80 ± 1.17
Disease duration	N/A	15 ± 6.28	24 ± 4.37
PMI (h)	10 ± 1.13	10 ± 1.5	6 ± 1.5
Braak staging	N/A	II; III; IV	IV; III; IV

Histopathological staining and STN delineation

STN-containing formalin-fixed, paraffin-embedded blocks from PD, control and STN-DBS PD cases were serially cut at 6 μm using a microtome (Microm International, Waldorf, Germany) and mounted onto SuperFrost™ slides (Thermo Fisher Scientific, Runcorn, UK). Standard haematoxylin and eosin (H&E) and luxol fast blue (LFB) staining, the latter for detecting myelin sheaths, were used for general morphological analysis, to accurately identify the STN and for revealing the electrode tract in the STN-DBS PD patient cohort.

The STN boundary was delineated on H&E and LFB stained sections as a discrete, compact structure located just medial to the peduncular portion of the internal capsule and superiolateral to the SN. The extent of the nucleus was marked on the slides with a permanent marker pen, used for overlaying to define the nucleus on the serial immunostained sections (Fig. 1A).

For single-antigen immunohistochemistry, tissue sections were dewaxed with xylene, rehydrated with a series of graded ethanols (EtOH), before washing well in distilled water. Sections were then incubated in 3% (w/w) hydrogen peroxide (H_2O_2, Sigma-Aldrich, Poole, UK) in PBS for 20 min at room temperature (RT) to block endogenous peroxidases. Antigenic epitopes were 'unmasked' to reduce nonspecific background staining and increase antibody labelling. For antigen retrieval, the sections were immersed in 10 mM sodium citrate buffer (pH 6.0) and heated in a steam cooker for 20 min. After heating, the sections were washed and cooled to RT under running tap water and rinsed in Tris-buffered saline (TBS). For blocking non-specific binding sites, the sections were incubated for 1 h at RT in 10% normal horse serum (NHS, Vector Laboratories, Peterborough, UK), diluted in PBS.

Each STN set of serial sections (n = 32) was immunostained for vascular endothelial marker, glucose transporter isoform 1 (GLUT-1, 1:150, Millipore, Watford, UK, #400060) (Lax et al., 2012) and β subunit of the major histocompatibility complex (MHC)-II receptor (HLA-DP/DQ/DR), serving as a microglia marker (1:200, DAKO Cytomation, Glostrup, Denmark, #CR3/43). The primary antibody was applied to the sections overnight at 4 °C. The following day, after washing in PBS, sections were incubated for 2 h at RT in horse anti-mouse secondary antibody (1:200, Vector Laboratories) for visualising the microglia and horse anti-rabbit (1:200, Vector Laboratories) for identifying microvessels. This was followed by incubation in avidin–biotin complex (ABC) elite complex (Vector Laboratories) for 30 min at RT. Immunoreactivity was visualised with the chromogen 3,3′-diaminobenzidine (DAB, Vector Laboratories), applied for 10 min at RT. Sections were then dehydrated through graded EtOH, cleared in 2 changes of xylene, before being mounted in DPX (Sigma-Aldrich) and applying glass coverslips. For each staining protocol, a negative control was included, by omitting the antibody (images not shown).