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Disruption of the blood-brain barrier in parkinson's disease: curse or route to a cure?

Heyne Lee[1]; Ilse S. Pienaar[1]

[1]Centre for Neuroinflammation and Neurodegeneration, Division of Brain Sciences, Department of Medicine, Imperial College London, London, W12 ONN, United Kingdom

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1. ABSTRACT

The vertebrate blood-brain barrier (BBB) is critical for ensuring the maintenance of brain homeostasis, whilst protecting the brain against toxic insults. Various pathological events disrupt BBB integrity, holding several important clinical implications. In instances where the normal mechanisms controlling passage of substances into the brain are compromised, these could sensitize or even worsen endogenous pathological conditions. Recognition has grown recently that patients diagnosed with Parkinson's disease (PD) present with concurrent medical problems, including cerebrovascular lesions. However, cerebrovascular disturbances may also result from PD-related disease processes; the pathological mechanisms which could entail interaction between environment-derived and genetic factors. The current review addresses the accumulation of studies aimed at better understanding the series of processes affecting the neurovascular unit in human Parkinsonism, due in part to the BBB presenting as a formidable opponent in the effective delivery of therapeutics that have shown promise as therapeutic strategies for treating aspects of PD when tested *in vitro*.

2. INTRODUCTION

Parkinson's disease (PD), the second most common neurodegenerative disorder, is typified in part by motor disturbances, including tremor, rigidity and bradykinesia. A key neuropathological signature of PD pathology comprises of the loss of ventral tier mesencephalic neuromelanin-containing dopaminergic neurons. Recently, evidence has emerged that PD pathology is not restricted to the dopaminergic system, but progressively also affects the noradrenergic, serotonergic and cholinergic systems, locating to the locus coeruleus, raphe nucleus and pedunculopontine nucleus, respectively (1-3). Degeneration of such structures was shown to associate with the onset and progression of non-motor symptoms, including disruptions of the autonomic nervous system, mood, arousal, cognition and sleep architecture (4). These key neuropathological hallmarks are accompanied by intracytoplasmic inclusion bodies, termed Lewy bodies (LBs) and dystrophic Lewy neurites (LN) in surviving neurons (5). In addition, peripheral abnormalities, i.e. olfactory deficit or constipation, feature predominantly in

PD patients in the early course of the disease, particularly before the appearance of any pathological motor signs (6).

Despite several decades of intense research efforts, the etiological factors of PD remain unknown. However, exposure to environmental toxins has been strongly implicated in the onset and progression of the disease. Potential environmental triggers of PD may include the widely used herbicide (7), exposure to pesticides in well-water, participating in farming activities and rural living (8-10).

Evidence is emerging that such environmental toxins may access the brain in instances where a breakdown of the vascular barrier exists. Brain neurons are normally protected against the adverse effects of environmentally-derived noxious blood-borne chemicals by means of the blood–brain barrier (BBB), a barrier system comprising of specialized proteins locating to the inside of blood vessels in the brain. However, under circumstances of a leaky BBB this can have deleterious effects on perivascular astrocytes and neurons, which may contribute to slow neurodegenerative changes (11).

A striking illustration of the association between Parkinsonism and environmental toxin exposure is given by a case study published by Langston and Ballard (12) on the effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a compound with a similar chemical structure than paraquat on humans. Albeit tragic (humans exposed to the toxin developed severe, acute PD), the discovery resulted in MPTP administration becoming one of the most frequently used toxins for generating toxin-based animal models of PD. Due to its high lipophilicity, MPTP is capable of being transported across the BBB, after which it is converted to its toxic byproduct, 1-methyl-4-phenylpyridinium (MPP+). This conversion occurs in astrocytes via the enzyme monoamine oxidase (MAO). Once converted, MPP+ is released into the extracellular milieu by means of the plasma membrane transporter, organic cation transporter (*OCT*)-3 (13). MPP+ subsequently enters dopaminergic neurons by specific DA transporters, resulting in cell death and the concomitant release of oxidative stress.

This review article aims to present current knowledge of how PD pathology may compromise BBB architecture and function, and evidence to conversely suggest that BBB breakdown could underlie the development and even onset of PD symptoms, whilst also discussing novel therapeutic approaches designed to address such deficits.

3. OVERVIEW OF BBB COMPOSITION

The cellular and molecular characteristics of the cerebral blood-carrying vessels, overviewed in the current section and illustrated in Figure 1, make up essential parameters for experimental characterization of BBB. The differentiated BBB consists of a complex system of barriers comprising of highly specialized endothelial cells (ECs). The EC barrier is characterized by the presence of high-capacity carriers/transporters, paucity of pinocytotic vesicles and lack of fenestrations. An additional second line of defence consists of pericytes, basal lamina substance, astrocytes and microglia (14). The functional role of these constituents for maintaining BBB integrity remains largely undetermined. However, aquaporin 4 (AQP4), expressed at the end-feet of astrocytes, is known to play a crucial role in BBB integrity, whilst fulfilling a role in the onset of cerebral edema (15).

The overall permeability of the BBB is regulated by the ECs and their junctional complexes, consisting of adherens junctions (AJs) and tight junctions (TJs). A critical element of the AJs is the vascular endothelial (VE)-cadherin/β-catenin complex. On the other hand, TJs consist of the integral membrane proteins claudin, occludin, and junction adhesion molecules and a number of accessory cytoplasmic proteins including zonula occludens-1 (ZO-1). Together these are responsible for maintaining cell polarity, whilst controlling paracellular permeability across the lateral intercellular space. This “barrier” property of TJs allows the BBB to exert control, by preventing solutes and water from freely crossing the paracellular pathway (16). Glucose transporter protein 1 (GLUT-1), which mediates glucose transfer across the BBB, together with the junctional proteins are concomitantly downregulated *in situ*ations in which breakdown of the BBB occurs (17).

Both TJs and AJs are composed of transmembrane adhesive proteins and cytoplasmic plaque proteins, which are linked to the actin cytoskeleton of the ECs. Hence, cerebral EC cytoskeleton-associated proteins such as caldesmon appear to be critical for maintaining endothelial integrity (18).

4. A DISRUPTED BBB MAY BE A CRITICAL FEATURE OF PD PATHOLOGY

To date, BBB disruption in PD patients and in animal models of the disease involving dopaminergic neurodegeneration has been minimally evaluated, despite mounting evidence for its involvement in progressive PD pathology. Oxidative stress, resulting in protein, lipid and DNA oxidation, to culminate in cell death, has been implicated as harmful to BBB components whilst having been identified as a putative molecular contributor to PD pathogenesis. In this regard, increased BBB permeability was demonstrated *in vitro* following brain ECs’ exposure to xenobiotics, promoting reactive oxygen species (ROS) generation. The effect was dose-dependent, whilst a pre-incubation step with the antioxidants superoxide dismutases and catalase blocked excessive ROS production (19). Further evidence that ROS contributes to BBB dysfunction was given by Gaillard and colleagues (20) who by exposing EC’s to the ROS generator lipopolysachcharide, observed EC dysfunction. Similarly, the defect was prevented with anti-oxidant treatment. Moreover, metalloproteinase-9 (MMP-9) activity, involved in degrading the basal lamina was increased by oxidative stress (21), whilst antioxidant treatment of brain ECs prevented MMP-9-induced TJ disassembly (22). BBB ECs may be rendered more vulnerable to oxidative stress due to having increased

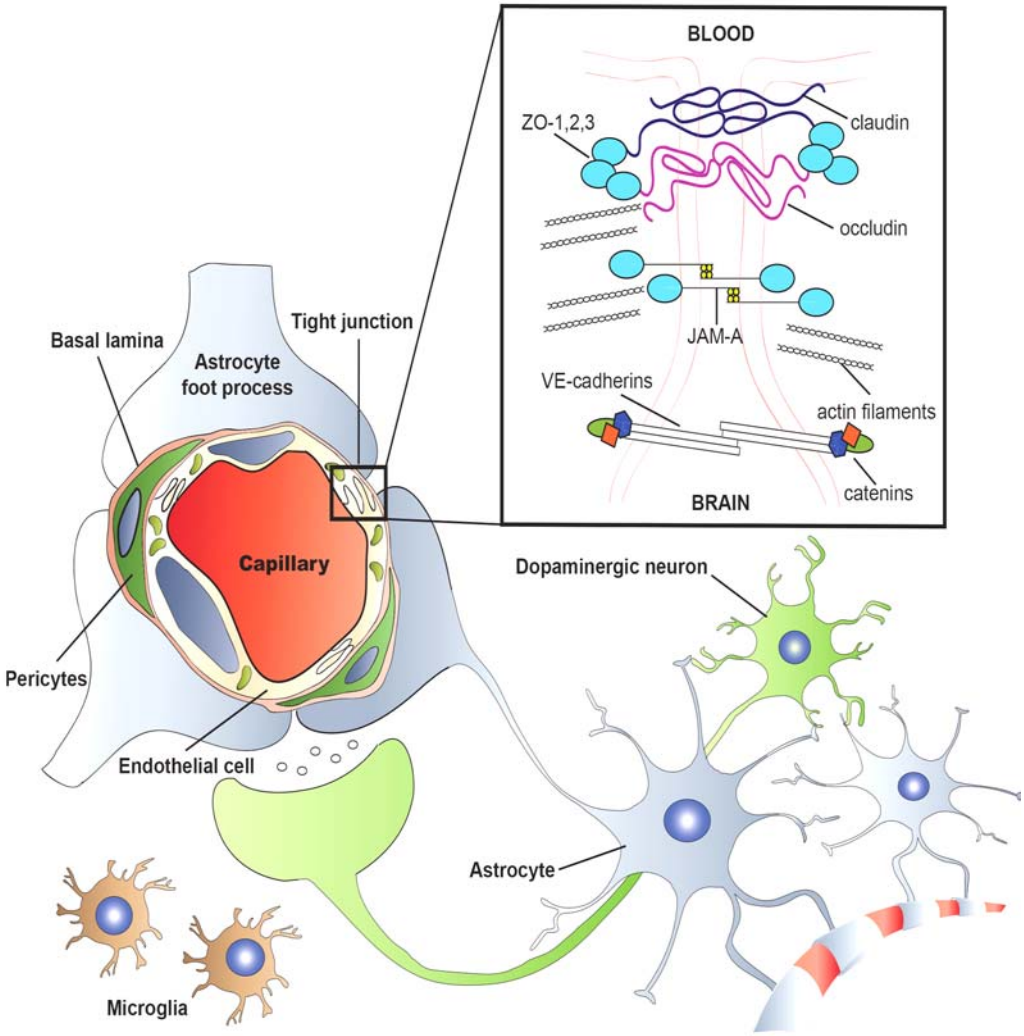

Figure 1. (A) Schematic of an intact neurovascular unit characterised by normal TJ proteins connecting the ECs of the capillaries that irrigate the brain parenchyma. Together these components, along with the basal lamina, pericytes and astrocytic end-feet comprise the neurovascular unit. Several PD-related pathological events could impact on the BBB, which in turn could result in neuronal death or –damage. These include: (1) Diminished expression of BBB transporters such as for glutamate (GLUT-1, expressed in capillaries and astrocytes) and amino acids such as L-type amino acid transporter 1 (LAT1) could diminish glutamate and amino acid substrate supply from the circulating blood into the brain, (2) thickening and collagen accumulation in the capillary basement membrane, (3) disrupted contact, and hence miscommunication between the pericytes, lining the abluminal side of endothelial cells in the perivascular space, and brain endothelial cells, to the detriment of brain microcirculation, (4) inflammatory-mediated processes, including brain microglial activation could potentiate damage to BBB components. The precise sequential order of these steps to result in cell death remains unclear. Moreover, whether and the extent to which PD-related pathological processes, such as accumulation of toxic protein, and bioenergetics, mitochondrial deregulation or oxidative stress that affect neurons, are involved in BBB disruption also, eventuating in cell death, remains to be ascertained. Note the presence of receptors to allow for brain influx of nutrients such as insulin, leptin and iron (transferrin) and growth factors such as VEGF, critical for survival or self-renewal and BDNF that promotes differentiation, migration and proliferation. The insert (B) shows the presence of TJ between adjacent ECs. These are formed by an intricate complex of transmembrane proteins (junctional adhesion molecule-1, occludin, and claudins) along with cytoplasmic accessory proteins (zonula occludens-1 and -2, cingulin, AF-6, and 7H6). Interaction and communication between astrocytes, pericytes and ECs maintain TJ protein integrity. Disrupted expression or localization of TJ proteins could increase CNS vascular permeability, resulting in pathological leakage of blood components into the surrounding brain parenchyma.

mitochondrial content compared to ECs of the peripheral vasculature (23). On the other hand, dopamine could result in oxidative stress and mitochondrial dysfunction (24), potentially impacting on BBB integrity. The proposal that oxidative stress and neuroinflammation, both playing critical contributory roles in PD pathology, may also be

involved in BBB dysfunction raises the possibility that BBB disruption may form a pathological feature of the disease.

Various studies have suggested that a relationship exists between cerebral ischemic lesions and PD-related motor symptoms (25, 26). However, despite finding a correlation between pathology of vessels supplying the brain and PD severity in patients, a study by Rektor and others (27) concluded that patients did not present with any clinical manifestation of cerebrovascular disease, referring to such lesions as "silent" (27). Song and colleagues (28) conducted a longitudinal 2-year follow-up to the study results reported by Rektor and others (27) to evaluate whether or not silent cerebral ischemic lesions impact on the progression and severity of PD. However, although reporting a statistically significant correlation between atherosclerotic risk factors and the development of ischemic lesions, no evidence was found to support an association between silent cerebral ischemic lesions and PD-related motor severity. However, this result may, at least partly, be explained by the fact that Song and others (28) evaluated the impact of silent cerebral ischemic lesion patients, categorised only as grade 1 and 2 on PD-related motor feature severity, whereas Rektor and colleagues' study (27) cohort consisted of high-grade silent cerebral ischemic lesions. Moreover, the cohort studied by Song and others (28) can also be deemed small, demanding a larger cohort of patients in order to conclusively enlighten on whether a correlation exists between PD motor severity and the extent of vascular lesions.

On the other hand, several studies have reported detecting angiogenic activity in PD-affected regions, including the basal ganglia (29, 30). For instance, Faucheux and others (31) revealed an increased number of endothelial cells in the substantia nigra (SN) of PD patients. Moreover, Barcia and colleagues (32) observed increased numbers of blood vessels in close proximity to degenerating DA neurons in the SN of non-human primates, which correlated with increased expression of the pro-angiogenic molecule Vascular Endothelial Growth Factor (VEGF).

Data from another study indicated that expression of integrins reflect the level of angiogenesis in patients and in a reliable toxin-induced animal model of PD (33). Moreover, by also using an animal model of PD, an association was revealed between BBB leakage/dysfunction in the SN and striatum and β3 integrin. Hence, the release of cytokines might activate microglia, with activated microglia associating with human Parkinsonism, which appear to not only be pro-inflammatory, but pro-angiogenic also (34). Alternatively, angiogenesis may compromise BBB integrity in PD-affected brains, which in turn contributes to ongoing neuroinflammation by allowing peripheral molecules and immune cells access to brain parenchyma.

To evaluate whether changes in the vascular system might constitute a contributing factor for the death of nigral dopaminergic neurons in PD, Rite and colleagues (35) intranigrally injected VEGF, a potent inducer of BBB permeability, into the unihemispheric SN of rats. In these animals, BBB leakage, visualized as immunoglobulin G extravasation and immunohistochemical detection of albumin in the brain parenchyma, occurred rapidly following the injection. The observed BBB breakdown was accompanied by lost integrity of nigral dopaminergic neurons. The authors further revealed through use of TUNEL histochemistry that BBB breakdown could induce apoptotic death of nigral dopaminergic neurons. This observation ties in with previous reports which revealed that BBB disruption would allow blood-residing molecules, such as thrombin, a multifunctional serine protease responsible for blood coagulation, to leak into the brain parenchyma (36). This could exert toxic, neurodegenerating effects on nigral dopaminergic neurons, creating an intense inflammatory reaction.

5. THE BBB AS AN OBSTACLE FOR EFFECTIVE DRUG DELIVERY TO PD PATIENTS

Currently, no disease modifying treatment is available for PD or for any of the other synucleopathies. A major obstacle in drug development is the unmet need to deliver clinically useful therapeutic compounds to the brains of PD patients due to the BBB's poor permeability to drugs (37).

P-glycoprotein (P-gp), also known as ABCB1, is an ATP dependent, 170 kDa transmembrane glycoprotein expressed on the luminal side of the brain capillary ECs composing the BBB. From this position, P-gp actively transports P-gp substrates back into the blood, thereby restricting such compounds from penetrating the brain parenchyma (38). This ability results in P-gp avoiding or at least reducing adverse effects on the CNS by peripherally acting drugs, whilst reducing pharmacoresistance associated with other drugs (39). The role of P-gp, to serve as a molecular efflux-pump to deliver drugs to the brain, has been deemed especially profound at the level of the BBB due to the lipophilic nature that make up the BBB capillary membrane.

Kortekaas and colleagues (40) provided the first evidence in support of BBB dysfunction in PD patients by performing a positron emission tomography (PET) imaging study; a study technique which measures the concentration in tissue of biomarker, labeled with short-lived positron emitting isotopes. (R)-[^{11}C]verapamil (verapamil labeled with ^{11}C) is currently the most commonly used P-gp PET radiotracer. The group used PET for measuring brain uptake of [^{11}C]-verapamil, which is ordinarily extruded from the brain by means of P-gp. The results showed that levels of [^{11}C]-verapamil are significantly upregulated in the midbrain of PD patients compared to healthy controls. In other work, Bartels and others (41) showed that P-gp function was decreased in the frontal lobes of advanced PD patients compared to healthy controls. However, in a different study the same group detected no difference between controls and patients diagnosed with early PD (42). Such studies suggest that the decrease in P-gp function seen in advanced PD may result from the disease process itself or alternatively, from long-term treatments

received by PD patients.

The associated prevalence of functional polymorphisms of the *MDR1* gene, encoding for P-gp with susceptibility for PD was explored in several separate studies. Lee and others (43) detected three single *MDR1* nucleotide polymorphisms in PD patients of Chinese descent, absent in control patients. It was also revealed that PD patients exposed to pesticides were five times more likely to carry the 2425T allele of *MDTR1*, presumed to result in reduced pump function (44). However, although these findings support the hypothesis that BBB disruption forms a clinical characteristic of the disease, Furuno and colleagues (45) found no significant association between expression levels of three common *MDTR1* polymorphisms and PD. Yet, the group did report observing that the frequency of the 3435T/T genotype, associated with decreased P-glycoprotein expression and function, was highest in the early-onset PD, second-highest in late-onset PD and lowest in the control group. Moreover, the MDR1 exon 21 and exon 26 polymorphisms were in significant linkage disequilibrium (45). Taken together, the results suggest that *MDR1* and other drug transporters remain plausible candidates as PD risk genes.

In other work, Chen and co-workers (46) revealed decreased expression of the TJ proteins ZO-1 and occludin in the striatum, with this decrease that was seen to associate with disruption of the BBB in an MPTP-induced mouse model of PD. Moreover, the study reported that changes in protein levels as well as increased BBB permeability resulting in vessel leakage was prevented by administering caffeine.

Although the extent of the impact left on surrounding neurons by a compromised BBB remains to be fully ascertained, one proposed mechanism entails the invasion of peripheral factors such as immunoglobulins and environmental toxins into the brain. This could instigate release of pro-inflammatory cytokines that could potentially trigger profound changes in gene expression in ATP-binding cassette (ABC) drug efflux transporter proteins, a protein category that regulates chemical trafficking of endogenous and exogenous substances across biological membranes. Evidence was given that cellular stress deriving from microglia and brain capillary ECs could affect ABC drug efflux transporters and thus BBB function (47).

The pathophysiology of L-DOPA-induced dyskinesia, following this dopaminergic precursor's long-term usage, entails large and transient elevations in brain extracellular levels of DA (48, 49), resulting in abnormal involuntary movements (dyskinesia). Functional and structural alterations of brain microvasculature have been deemed a potentially critical contributor to the onset and progression of L-DOPA-induced dyskinesia. In this regard, L-DOPA reaches the brain parenchyma via the blood through endothelial transport mechanisms (50), hence it's been postulated that disruptions of the brain endothelium or its barrier properties could influence the kinetics of L-DOPA entry into the brain. Seminal work by the group of Angela Cenci at Lund University provided convincing evidence that L-DOPA administration induces endothelial proliferation and overall angiogenesis in the basal ganglia using both a rat model of PD (51, 52). Moreover, in search of putative mechanisms that could mediate the observed L-DOPA-induced angiogenic response, the same group reported that the expression levels of VEGF, regarded as an important inducer of angiogenesis, was upregulated in striatal tissue from both L-DOPA-treated dyskinetic rats and in PD patients with a clinical history of dyskinesia (30). Very recently, this group made use of rats that had been lesioned unilaterally with the parkinsonian agent, 6-hydroxydopamine (6-OHDA) to show that permeability of an already compromised BBB in PD may in fact increase, following L-DOPA administration (53). The study served to strengthen previous observations made in PD patients, where cerebral blood flow and metabolism were shown to be increased in the basal ganglia following L-DOPA administration, particularly in dyskinetic PD patients (54). The authors postulated that the angiogenic response to L-DOPA in the DA-denervated brain might depend on stimulation of D_1-class receptors.

6. DRUG DELIVERY SYSTEMS DEVELOPED FOR PENETRATING THE BBB OF PARKINSONIAN BRAINS

As set out above, the BBB provides a formidable obstacle in the effective delivery, and hence, treatment of common neurological disorders such as PD, Alzheimer's disease and Multiple Sclerosis. In light of findings that expression and function of membrane drug transporters such as P-gp may be altered in PD (40, 41), several studies have explored pharmacological strategies aimed at stimulating P-gp expression and function as a promising neuroprotective strategy. For instance, use of antagonists against the σ_2 receptor was proposed for reducing transcription of *MDR1* (55). Moreover, P-gp function stimulating properties have been assigned to a variety of prescription drugs, as well as grapefruit juice and St. John's wort (56). Pharmacological exploitation of the endogenous factors identified as stimulatory for P-gp expression, such as progesterone and HSP90 (57) represent further opportunities for molecular targeting and correcting this BBB dysfunction in Parkinsonism to improve drug delivery.

Nanoparticles, being solid colloidal matrix-like polymeric particles or lipids, ranging in size 10-1000 nanometers (58) offer a novel drug delivery system capable of reaching the affected lesion sites, and providing good bioavailability. In particular, due to their relatively large surface area, nanoparticles are capable of binding, absorbing and transporting compounds such as drugs, probes and proteins. Nanobiotechnology offer an opportunity to bypass the limited permeability of the BBB, which make it possible for only lipophilic molecules or those of molecular weight <600 Da to successfully traverse the BBB. Moreover, it offers a non-invasive delivery device over those of other methods for bypassing the BBB, such as direct intracranial- or intraventricular injection, which, apart from a significantly higher risk of infection,

also associate with high surgical expense and less loading capacity within the brain then the non-invasive alternative (59). Drugs are commonly conjugated to a nanoparticle by incorporation during nanoparticle production or by surface adsorption onto a preformed nanoparticle (60). A drug conjugated to a nanoparticle receives enhanced protection against degradation and phagocytosis by the reticulo-endothelial system, whilst also offering more sustained and slower release (58).

7. CONCLUSIONS

Here we discussed the growing body of evidence in favour of the suggestion that underlying disruption of neurovascular mechanisms may precede, accelerate, or contribute to chronic disease processes in neurodegenerative disorders such as PD. Moreover, a more thorough understanding of the mechanisms by which BBB disruption occurs might lead to the development of new approaches for the treatment of PD. Hence, although not attempting to provide a comprehensive overview of this rapidly developing field of research, attention was devoted to technologies for delivering small and large molecules across the ECs composing the BBB to the brains of PD-affected patients. Whilst the challenge to fully describe the extent and nature of BBB disruption in PD-affected brains remain numerous and substantial, the current review highlighted the potential diagnostic and therapeutic implications for exploring aspects of BBB dysfunction in both patients and in relevant animal models of the disease. This includes growing consensus that high-throughput genomic and proteomic discovery platforms as well as advances in neuroimaging may be critical experimental tools by which to discover novel BBB transporters and junctional barrier proteins to exploit in the development of therapeutic endeavors aimed at either restoring or overcoming the BBB deficit seen in PD.

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Abbreviations: Adherens junctions, AJs; ATP-binding cassette, ABC; Aquaporin 4, AQP4; Blood-brain barrier, BBB; Deep brain stimulation, DBS; Endothelial cells, ECs; Glucose transporter protein 1, GLUT-1; L-type amino acid transporter 1, LAT1; Lewy bodies, LBs; Lewy neuritis, LNs; Magnetic resonance imaging, MRI; 1-methyl-4-phenylpyridinium, MPP+; Metalloproteinase-9, MMP-9; 1-

methyl-4-phenyl-1,2,3,6-tetrahydropyridine, MPTP; Monoamine oxidase, MAO; Reactive oxygen species, ROS; Silent information regulator 1, SIRT1; 6-hydroxydopamine, 6-OHDA; Substantia nigra, SN; Subthalamic nucleus, STN; Tight junctions, TJs; Vascular endothelial growth factor, VEGF

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Send correspondence to: Ilse S. Pienaar, Centre for Neuroinflammation and Neurodegeneration, Division of Brain Sciences, Department of Medicine, Imperial College London, London, W12 ONN, United Kingdom, Tel.: 0044 207 594 6665, Fax: 0044 207 594 6548, E-mail: i.pienaar@imperial.ac.uk