Review

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Impaired intracellular trafficking defines early Parkinson's disease

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Parkinson's disease (PD) is an insidious and incurable neurodegenerative disease, and represents a significant cost to individuals, carers, and ageing societies. It is defined at post-mortem by the loss of dopamine neurons in the substantia nigra together with the presence of Lewy bodies and Lewy neurites. We examine here the role of α-synuclein and other cellular transport proteins implicated in PD and how their aberrant activity may be compounded by the unique anatomy of the dopaminergic neuron. This review uses multiple lines of evidence from genetic studies, human tissue, induced pluripotent stem cells, and refined animal models to argue that prodromal PD can be defined as a disease of impaired intracellular trafficking. Dysfunction of the dopaminergic synapse heralds trafficking impairment.

Early PD: a traffic jam

PD is a common neurodegenerative disease characterised by insidious deterioration of motor control, often associated with mood, sleep, and cognitive disturbances [1]. Over 1% of all people over the age of 65 suffer from PD [2]. Similarly to other neurodegenerative diseases, age is a key risk factor and by 2030, an estimated 9 million people worldwide will be living with PD [3]. PD carries a significant economic cost, including direct and indirect health care costs, and lost productivity [4], estimated annually at £500 million per year in the UK, and $6 billion in the USA [5,6]. PD is pathologically characterised by the loss of midbrain dopamine (DA; see Glossary) neurons, and the development of Lewy bodies and Lewy neurites that are predominantly composed of the protein α-synuclein [7].

The aim of this review is to integrate lines of evidence from human tissue, human iPSCs, refined animal models, and genetic studies that have suggested early PD pathogenesis is likely to be a consequence of, and be defined by, impaired intracellular trafficking. We discuss how dysfunction of key intracellular trafficking proteins, together with the large demand on the intracellular trafficking system that the massive axonal arbor of midbrain DA neurons impose, underlie the pathogenesis of PD.

A complex road-map: the DA neuron

The motor manifestations of PD are largely due to degeneration of DA neurons in the substantia nigra pars compacta (SNc), and to a lesser extent the ventral tegmental area (VTA) and other midbrain regions. These nigral neurons project via the mesostriatal pathway to the striatum (caudate-putamen). In the striatum, the axons of DA neurons branch extensively giving rise to a dense lattice that provides non-selective DA innervation of the principal

Glossary

α-Synuclein: a presynaptically enriched protein that acts in conjunction with SNARE proteins to regulate neurotransmitter release. It is the major component of two pathological hallmarks of PD: proteinaceous aggregates termed Lewy bodies and Lewy neurites. α-Synuclein is encoded by the gene *SNCA*.
Dopamine (DA): a neurotransmitter derived from the amino acid tyrosine. It is released by DA neurons of the midbrain onto medium spiny neurons in the striatum to help regulate movement.
Glucocerebrosidase (GBA): an enzyme that cleaves glucocerebroside, an intermediate protein in glycolipid metabolism. It is encoded by the gene *GBA*. Patients who suffer from Gaucher's disease, a lysosomal storage disease, are homozygous for mutations in *GBA*. A subset of patients suffering Gaucher's disease exhibit parkinsonian symptoms. Patients who are *GBA* mutation heterozygotes have an increased risk of developing PD.
iPSCs: mutipotential cells derived from differentiated adult cells that can be manipulated to produce different cell types including neurons.
Leucine rich repeat kinase 2 (LRRK2): a large multidomain protein with GTPase and kinase domains that has multiple intracellular roles, including regulating autophagy. It is encoded by the gene *LRRK2*. Mutations in *LRRK2* cause familial PD, and polymorphisms in *LRRK2* confer risk of sporadic PD.
Mesostriatal pathway: the neural pathway that connects the midbrain to the striatum. DA-producing neurons whose cell bodies and dendrites reside in the midbrain, and in particular the substantia nigra, make synaptic connections within the striatum.
SNARE (soluble *N*-ethylmaleimide–sensitive factor attachment protein receptor) proteins: form complexes which mediate docking of neurotransmitter-containing vesicles to the presynaptic membrane. Important SNARE proteins include synaptobrevin, syntaxin, and SNAP-25.
Substantia nigra: literally 'black substance', a region of the midbrain where pigmented neuromelanin-containing dopaminergic neurons are located. It is divided into two main parts: the pars compacta (SNc) and pars reticulata. Loss of neurons from the SNc is one the pathological hallmarks of PD. Lewy bodies and neurites are present in some of the remaining neurons in most cases of PD.
Tau: a microtubule-associated protein that is present in the axons of neurons, and assists and helps to regulates axonal transport. Tau is encoded by the gene *MAPT*.

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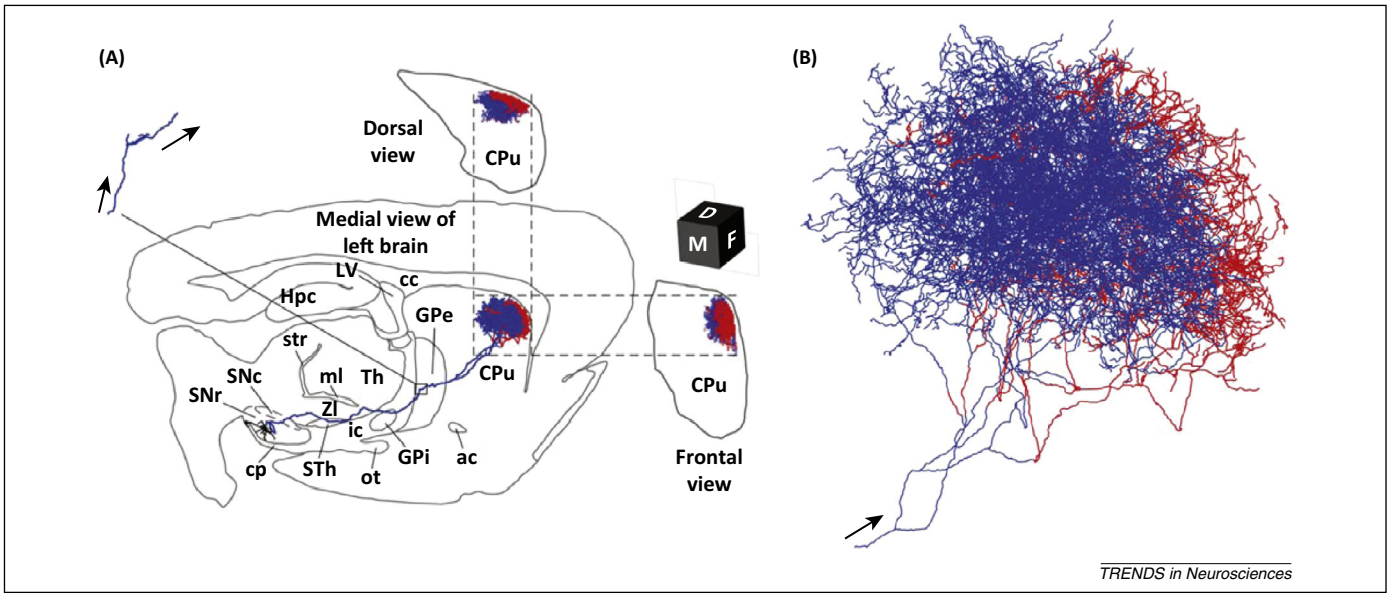

Figure 1. Complex axonal arborisation of midbrain dopaminergic neurons. **(A)** Medial, dorsal, and frontal reconstructions of the axonal projections of midbrain dopaminergic neurons generated using a GFP protein targeting neuronal membranes. Red (striosome) and blue (matrix) lines indicate the striatal compartments in which axonal fibres are located. **(B)** Striatal arborisation of a typical midbrain dopaminergic neuron projected onto the parasagittal plane. Figures adapted from Matsuda *et al.* [13] with permission from the Society for Neuroscience. Abbreviations: ac, anterior commissure; cc, corpus callosum; cp, cerebral peduncle; CPu, caudate-putamen (neostriatum); GPe, external segment of the globus pallidus; GPi, globus pallidus interna; Hpc, hippocampus; ic, internal capsule; LV, lateral ventricle; ml, medial lemniscus; ot, optic tract; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STh, subthalamic nucleus; str, superior thalamic radiation; Th, thalamus; Zl, zona incerta.

efferents of the striatum, the medium spiny neurons (Figure 1) [8,9]. Neuropathological studies have clearly demonstrated that the key aspects that define neuronal susceptibility in PD are the axonal length, axonal calibre, and the degree of myelination [10]. For example, cortical motor neurons that have relatively long processes but high-calibre axons that are heavily myelinated are relatively protected in PD, whereas most of the subcortical nuclei (chiefly the SNc, but also the magnocellular nuclei of the basal forebrain, hypothalamic tuberomamillary nucleus) have thin axons, are lightly myelinated, and develop Lewy pathology.

A close examination of the neuroanatomical and physiological characteristics of SNc DA neurons helps us to understand why these neurons are most severely affected in PD. Quantitative anatomical data lead to an estimate that each SNc DA neuron in the rat gives rise to 100 000–250 000 synapses at the level of the striatum [11]. The extraordinary number of synapses formed by each DA neuron is put into context by considering other neuronal types in the rat: striatal spiny neurons form ~300 synapses, striatal inhibitory interneurons give rise to ~5 000 synapses, and on average there are ~10 000 synapses per cortical neuron [12]. An elegant study in the rat has corroborated these findings by labelling individual DA neurons in the SNc in their entirety, allowing the immense nature of their axonal arbors to be visualised and quantified (Figure 1) [13]. The average total length of the axon of a rat SNc DA neuron was estimated at ~50 cm and the average volume occupied by the axonal arbor at about ~0.5 mm^3. Extrapolation of these findings to the human brain, the striatal volume of which is about 300-fold greater than in the rat, but with only ~30-fold more SNc neurons to provide the DA innervation, indicates that an individual human DA neuron provides 10-fold more innervation than does a rat DA neuron. This suggests that each human SNc DA neuron gives rise to between 1 and 2.5 million synapses in the striatum, with a total axonal length in excess of 4 m [11]. Trafficking of vesicles and organelles, including synaptic vesicles, mitochondria, and ribosomes, throughout the cell is dependent on transport along microtubules (reviewed by Hancock [14]). The requirement for cellular trafficking machinery in human SNc DA neurons is far in excess of other neuron types, and means that any impairment of cellular trafficking preferentially affects SNc DA neurons. Cellular trafficking places energy demands on the cell because the predominant motor proteins, kinesin and dynein, are powered by hydrolysis of ATP; one molecule of ATP is required for 8 nm of cellular travel [15]. In addition, computational analysis [16] reveals that such a large and complex axonal arborisation incurs a disproportionately high energy-cost for action potential propagation and recovery of membrane potential.

Other factors collude to increase the vulnerability of SNc DA neurons. SNc DA neurons compared to adjacent VTA neurons have elevated somatodendritic calcium entry associated with intrinsic pacemaking currents [17], while their axons use different complements of calcium channels with evidence for differential calcium handling [66]. Some studies also describe DA and its metabolites as neurotoxic, and there is also the potential for deleterious DA modification of α-synuclein [18].

In summary, the massive highly-tortuous axonal arborisation and large synapse number of vulnerable human SNc DA neurons imposes a disproportionately large burden on the machinery for cellular transport and synaptic release, and may explain why these neurons are preferentially susceptible in PD, despite the wider distribution of key pathogenic proteins such as α-synuclein.