The Threshold Theory for Parkinson's Disease

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Parkinson's disease (PD) is recognized by the accumulation of α-synuclein within neurons. In contrast to the current ascending theory where α-synuclein would propagate from neuron to neuron, we now propose the threshold theory for PD based on evidence of parallel degeneration of both central nervous system (CNS) and peripheral nervous system (PNS) in PD. The functional threshold is lower for the emergence of early symptoms before the classical motor symptoms of PD. This is due to the larger functional reserve of the midbrain dopamine and integrated basal ganglia motor systems to control movement. This threshold theory better accounts for the current neurobiology of PD symptom progression compared to the hypothesis that the disease ascends from the PNS to the CNS as proposed by Braak's hypothesis.

Neuronal Dysfunction in PD

Parkinson’s disease (PD) is a progressive neurodegenerative disease that affects millions of people around the world. The prominent motor symptoms in PD, which include tremors, rigidity, and bradykinesia, are due to the degeneration of dopaminergic neurons in the substantia nigra [1], and occur when the threshold of approximately 30% dopamine synapses and levels remain in the caudate-putamen (corresponding to a 70% loss of dopaminergic synapses) [2,3]. Nonmotor symptoms, such as constipation, cardiac arrhythmias, and sleep disorders, are nowadays also recognized as being part of the disease and are due to dysfunction of peripheral neurons and brain stem nuclei [4]. Cognitive deficits are also observed in the disease and represent a nonmotor symptom that appears in late PD stages [5].

The cause for the neuronal degeneration in PD is still not clear but the neuronal accumulation of α-synuclein is a common denominator in the disease. Mutations and multiplications in the α-synuclein gene cause autosomal dominant PD [6], implying that the increased expression of α-synuclein in neurons is enough to promote disease. Additionally, α-synuclein is the major component of Lewy bodies present in genetic and sporadic PD [7]. The toxicity caused by α-synuclein is thought to rely on its accumulation and oligomerization and to precede the formation of large α-synuclein aggregates or fibrils [8]. In this context, increased neuronal levels of α-synuclein interfere with many intracellular functions, in particular vesicular homeostasis and microtubular transport systems [9,10]. Lipids interact with α-synuclein through its amphipathic N-terminal region [11] and its accumulation leads to dysfunction of vesicular systems, such as mitochondria, lysosomes, autophagy, endoplasmic reticulum, early endosomes, and synaptic vesicles [9,12,13]. The interaction of α-synuclein with tubulins, kinesin-containing complexes, and dynein-containing complexes [14], and the fact that its accumulation causes the blockage of anterograde and retrograde motor proteins along axons [15], further indicate that intracellular vesicular pathways become dysfunctional after increases of α-synuclein levels.

Despite these findings, and based mainly on intracerebral injections of preformed α-synuclein fibrils in mice, PD has been recently been proposed to be a prion-like disease [16,17]. We will
An Alternative to the Anatomical α-Synuclein Spreading Theory

Neuropathology of PD and the Lack of Consensus on Lewy Bodies Ascension

It has been hypothesized that neuronal damage in PD occurs in a spreading and ascending pattern from peripheral nerves to the brainstem and midbrain, before finally reaching higher cortical structures [18]. Even though the sequential appearance and enrichment of Lewy bodies from lower to upper brain regions has been contested [19], the findings that α-synuclein can be released from cells [20] and that some neurons inside midbrain transplants develop Lewy bodies [21–23], which is not seen in all transplant cases and methods [24,25], has been offered by many to support the belief that PD is a form of prion disease [16,17,26].

Postmortem analysis of incidental and symptomatic PD suggested that the appearance of α-synuclein-positive Lewy bodies occurs in a progressive and anatomical way [18]. According to this hypothesis, Lewy bodies appear first at the nuclei of the glossopharyngeal and vagal nerves and also in the olfactory nucleus. While few inclusions were found in the olfactory nucleus in this report, Lewy pathology in the brain stem was more obvious [18], suggesting that there was an ascending pattern towards midbrain, temporal, and finally neocortex.

On the contrary, retrospective clinicopathologic studies have suggested that up to 47% of cases do not follow an ascending progression of Lewy bodies [27,28]. For example, around 7% of patients from a UK-based brain bank did not show α-synuclein pathology in the dorsal motor nucleus of the vagus, while the substantia nigra was largely affected in these cases [27]. Accumulation of α-synuclein was also observed in spinal cords of typical PD patients together with dorsal motor nucleus of the vagus (some cases even exhibited α-synuclein accumulation in the spinal cord without accumulation in the dorsal nucleus of the vagus) [27], defying the idea that α-synuclein accumulates at peripheral neurons in close proximity to environmental gateways. In another study, the presence of accumulated α-synuclein in PD patients in the spinal cord was found to be even more prevalent than that observed in the vagus nerve and gastrointestinal tract. Furthermore, this accumulation showed a rostrocaudal (descending) distribution [29], instead of the suggested caudorostral (ascending) gradient [18]. Further supporting a more neutral and parallel distribution of α-synuclein pathology is the finding that Lewy bodies are less prevalent in the colon compared to the stomach [30] but that constipation is the most common peripheral symptom in PD (occurring in almost 50% of patients) [31]. Finally, another study identified robust Lewy pathology in the dorsal vagal nucleus but degeneration of substantia nigra in the absence of Lewy bodies [32,33], further challenging the obligatory ascending spreading of α-synuclein to promote disease.

Limited Functional Reserve of Neurons Involved in Early Nonmotor Symptoms

Moreover, instead of the physical spreading of aggregated α-synuclein, in vivo image analysis, revealed that both symptomatic and at-risk subjects showed only a partial match to the putative ascending Lewy body pathology [34]. This indicates that the selective vulnerability of different regions to accumulated α-synuclein could account for the progression of PD. Supporting this view, nigral dopaminergic neurons are a highly sensitive group of neurons [15,35–42], and death of these neurons are a prominent feature of PD.

It is now recognized that gastrointestinal symptoms may in part be due to local α-synuclein accumulation. Enteric neuronal dysfunction appears before onset of motor symptoms [43,44].
Gastrointestinal problems are generally observed in other neurological dysfunctions and therefore not specific to PD [45]. Likewise, cardiac autonomic dysfunction also appears before obvious motor symptoms in PD patients, and also in α-synuclein model transgenic mice [46–48]. Finally, the appearance of REM sleep disorder a decade before motor symptoms [49] together with the accumulation of α-synuclein in the reticular activating system (RAS) at the brainstem [50], points also to widespread neuronal dysfunction in PD.

The mechanisms underlying these early brainstem-RAS, peripheral, and autonomic nervous system mediated symptoms is not understood, as these neurons are not considered as sensitive as midbrain dopaminergic neurons [51] (Figure 1). In fact, in contrast to midbrain dopaminergic neurons, brainstem and peripheral neurons are more resilient to insults. For instance, neurons in the vagal and hypoglossal nuclei showed little or no degeneration detected by Nissl and True blue staining 28 days postaxotomy, depending on the distance of the axotomy relative to the nuclei [52,53]. Degeneration of neurons in the dorsal motor nucleus was observed.

**Figure 1. Increased Sensitivity of Dopaminergic Midbrain Neurons Compared to those Present at Brainstem and Peripheral Systems.** On the left, the significant decline of dopaminergic synapses in the putamen was measured by PET-CFT in MPTP-treated monkeys [1–3,63,64,129,130], and confirmed in PD patients by metabolic labeling and immunohistochemical assays [1,129,130]. The decline of dopaminergic synapses in the putamen is pronounced and the measured threshold for parkinsonian motor symptoms occurs when only 30% of them remain intact [129]. On the right, we depict a hypothetical curve of observed brainstem-RAS, PNS, ANS, and ENS cellular pathologies, including α-synuclein – and the dysfunction it causes. This hypothesis is supported by a collection of studies showing heterogeneous presence of aggregated α-synuclein in the lower nervous system (brainstem to enteric neurons) of PD patients [131,132] as well as in transgenic α-synuclein mice models [47,48]. In essence, the threshold for the brainstem and peripheral nonmotor symptoms to become apparent is lower than that for motor symptoms (on the left). Therefore, the presence of 70% loss of dopamine synapses in the high functional caudate-putamen system produces symptoms later than the comparable 20–30% presence of pathologies in the brainstem-RAS, peripheral, autonomic, and enteric nervous systems. These differences in functional reserves explain the progression of the disease symptoms observed. In agreement, several of the nonmotor symptoms, such as constipation and REM sleep disorder, are observed 10 and even 20 years before the onset of motor symptoms in PD patients [49,133,134]. In addition, transgenic α-synuclein mice have robust dysfunction of enteric motility with no obvious death of enteric neurons and no overt aggregation of α-synuclein [47]. Abbreviations: ANS, autonomic nervous system; ENS, enteric nervous system; PNS, peripheral nervous system; RAS, reticular activating system.
mainly after a prolonged period following vagotomy [54,55]. In addition, different studies have shown significant neuronal regeneration of neurons in the dorsal motor nucleus after vagotomy [56,57]. Similarly, although age-related neuronal loss has been reported, not all evidence suggests an extensive death of enteric neurons during ageing [58]. (In agreement, no obvious death of enteric neurons is observed in PD [30] but rather accumulation of α-synuclein in these cells along with altered or reduced activity [59,60]). One possibility here is that the functional network of enteric neurons is much less developed than the brain dopaminergic neuron circuitry used for movement initiation and control, suggesting that enteric neurons would have less functional reserve than dopaminergic neurons (Figure 2). In agreement with this proposal, humans have extensive interconnected midbrain, striatal, pallidal, thalamic, and cortical nuclei [61,62] providing vast compensatory mechanisms and redundancy to allow initiation of movement (Figure 2). This is in agreement with the appearance of motor symptoms in PD only after approximately 70% of nigral dopaminergic neurons are dead [2,3,63–65] (Figure 1). In other words, parallel cellular pathologies in PD occurring at similar rates would give the first symptoms in the peripheral nervous system, compared to midbrain dopaminergic circuitry, due to an earlier

Low functional threshold systems
Brainstem-RAS, peripheral, autonomic, and enteric nervous system

High functional threshold systems
Caudate-putamen and midbrain integrated motor system

Key:
- DA
- SHT
- Glu
- GABA

CNS

Brainstem-RAS

Peripheral nervous system
Sympathetic
Parasympathetic

Enteric neurons

Cortex

Medium spiny neurons

Globus pallidum SNpr

Thalamus

Motor cortex

DRN

SNpc

STN

Figure 2. Differences in Neuronal Circuitry Relevant to PD. The emergence of nonmotor symptoms and progression of PD is explained by the threshold theory, where pathophysiological changes are translated into symptoms based on functional reserve of each system affected at similar times and degrees throughout the body. On the left, a simplified scheme of neuronal connections from the CNS to brainstem, peripheral, autonomic, and enteric nervous systems showing the lack of redundant connections to support their functions, to which we ascribe a low functional threshold to the emergence of nonmotor symptoms. The broken arrow represents a combination of neuronal transmission coming from peripheral, sympathetic, and enteric systems but also humoral signals that can influence brainstem-RAS and CNS from the periphery [136]. In the right panel, the intricate midbrain circuitry responsible for initiating movements depicts the different types of inputs to the striatal medium spiny neurons, that can compensate for deficits of inputs from the substantia nigra pars compacta that occur in parkinsonism of PD. The arrow from medium spiny neurons to SNpc represents the innervation of striatal neurons to the dendrites of SNpc dopaminergic neurons located in the SNpr [136]. Abbreviations: SHT, serotonin; ACh, acetylcholine; CNS, central nervous system; DA, dopamine; DRN, dorsal raphe nucleus; GABA, γ-aminobutyric acid; Glu, glutamate; NE, norepinephrine; Ob, olfactory bulb; RAS, reticular activating system; SNpc, substantia nigra pars compacta; SNpr, substantia nigra pars reticulata; STN, subthalamic nucleus.
functional threshold in the autonomic nervous system. This threshold function explains the progression of early symptoms in PD.

Evidence against the idea that Propagation of PD Pathology Depends on Transynaptic Spread of α-Synuclein

Even though cognitive deficits in late-stage PD would be somewhat consistent with the ascending spreading of α-synuclein [63], the preferential accumulation of α-synuclein in the cerebral cortex in Dementia with Lewy bodies (DLB) without initial prominent pathology in the substantia nigra [67] also contests the idea of the indispensable ascending spreading of α-synuclein for promoting pathology. Although PD and DLB classically represent distinct disease classifications, they share a main feature, which is the prominent intraneuronal accumulation of α-synuclein [68,69]. In addition, autonomic dysfunction is observed not only in PD and DLB, but also for instance in Alzheimer disease, multiple sclerosis, and psychiatric diseases [70], which are not associated with the idea of ascending pathology from enteric neurons. These findings suggest that PD is a general disorder that affects an array of different neurons, including nigral, cortical, enteric, and cardiac neurons. Thus, even if α-synuclein can ascend through neuronal synapses when recombinant α-synuclein viruses are injected in peripheral neuronal tracts, or rotenone is infused in the stomach [71,72], the wide neuronal expression of α-synuclein [73,74] argues against the requirement of protein ascension to promote propagation of the disease. Furthermore, even though injection of recombinant α-synuclein viruses in the vagus nerve in the neck of mice led to certain levels of α-synuclein in the dorsal motor nucleus of the vagus (and in higher brain structures), α-synuclein reached the substantia nigra pars reticulata, and not the pars compacta [72], suggesting that the anatomic path speculated to facilitate the ascending spreading of α-synuclein (enteric neurons→vagus→substantia nigra pars compacta→cortex) may not be sound. Finally, knockout of α-synuclein did not counteract the retrograde synaptic transfer of α-synuclein to higher brain structures when recombinant α-synuclein viruses were injected in the vagus nerve [75], challenging the idea of PD as a prion-like disease. Instead it supports the idea that α-synuclein, as a small synaptic protein, may move across synapses under certain conditions.

Mice injected with systemic MPTP and rotenone [76,77] and those engineered to overexpress α-synuclein in neurons [47,48,78] develop gastrointestinal dysfunction prior to motor ones. It has been proposed that the vagus nerve can propagate α-synuclein and inflammatory signals [79,80]. A study analyzing a 20 year cohort of Danish patients that had different degrees of vagotomy, found that full truncal vagotomy was associated with a slight decreased risk of developing PD when compared to patients that had superselective vagotomy [81]; however this finding was contested in another study following this same cohort over a longer (35 year) period [82]. Taken together, these findings indicate that the idea of performing vagotomy to prevent the progression of PD disease may be premature.

PD as a Systemic Cellular Neurological Disease

In summary, we believe that all evidence available from genetic, cellular, and functional data support a view of PD as a global systemic disease. According to this view, disease mechanisms simultaneously influence different neurons in the CNS and PNS. The death and dysfunction of neurons in a particular area depend on their individual vulnerability but the appearance of symptoms is a result of the degree to which damaged neurons are functionally connected to and modulated by other neuronal groups. Therefore, the hypothesized ascending progression theory of disease might in fact represent a combination of differences in vulnerability and functional reserve of affected neurons. We propose the notion of ‘functional threshold theory’ as opposed to Braak’s α-synuclein ‘ascending anatomic theory’.

For example, in this functional threshold theory, a small remaining percentage of dopaminergic synapses and neurons are enough to allow normal movement initiation (Figure 1). In agreement
with this reasoning, a relatively small number of surviving implanted dopamine neurons, less or equal to 10% of normal dopaminergic neurons, are sufficient to bring the patient above this threshold and allow movement initiation [63]. Secondly, according to the functional threshold theory, the functionally more sensitive autonomic nervous system will show earlier signs of dysfunction than the midbrain. Below, we further discuss gaps in the α-synuclein spreading theory by considering recent investigations of α-synuclein cell biology and neurobiology that better explain the sequence of symptoms observed in PD and Lewy body disease.

**α-Synuclein Properties and Aggregates in the Brain**

Cells can release α-synuclein in a passive manner and also through exosomes [20,83,84], and it can translocate under experimental conditions to adjacent cells in coculture experiments [85]. However, based on the fact that the amount of released α-synuclein relative to total intracellular levels is slight [84], as well as the ability of α-synuclein to be incorporated by glial cells [86,87], it is possible that small amounts of released α-synuclein may activate glial cells which in turn cause further neuronal accumulation of α-synuclein and, ultimately, toxicity in PD. In agreement, immunoreactive astrocytes containing α-synuclein inclusions are present in PD brains [88] and inflammation parallels the accumulation of α-synuclein in central neurons after intragastric administration of rotenone to mice [71], and after peripheral infection by HSN1 influenza virus [79]. Moreover, the large number of postencephalitic cases of PD [89] and the recent evidence of neuroinflammatory mechanisms involved in PD [90,91] indicate that the spreading of α-synuclein is not required to explain the pathogenesis or symptoms of the disease, but inflammation may be involved.

In such a framework, the idea of ascending anatomic spreading PD may be an oversimplification of events, one that is only supported by models using artificially high loads of aggregated or preformed α-synuclein fibrils [92–97], extracts of brains with α-synucleinopathy [94,98–100] or insoluble brain fractions of mice with α-synuclein pathology [93,101]. Furthermore, not every study using these approaches confirmed a significant spread of α-synuclein when injections were performed in nontransgenic mice [102,103]. Even considering that some degree of spreading occurs when injecting mice with high amounts of aggregated α-synuclein, it does not follow that this must be the pathological mechanism occurring in the brains of PD patients. In agreement with the uncertain spread of α-synuclein in more relevant pathophysiological conditions, healthy grafts of human midbrain transplants do not necessarily show signs of pathology associated with α-synuclein [24,25]. Moreover, no spreading of pathology to substantia nigra is observed when α-synuclein is specifically overexpressed in discrete mouse brain areas, such as cortex, hippocampus, striatum, and even ventral substantia nigra pars compacta [104]. This rules out long-distance, across synapse, and cell-to-cell α-synuclein propagation in *in vivo* models that do not use injected α-synucleinopathy homogenates or preformed fibrillated α-synuclein.

In support of the ascending propagation theory, injecting high loads of preformed α-synuclein fibrils into the brain parenchyma and olfactory bulb can lead to accumulation of α-synuclein within neurons and promote neuronal death [92,105–107]. However, in these experiments, the artificially loaded α-synuclein outside neurons and glia may actually cause inflammatory responses that reduce neuronal function and survival for long periods after the initial or parallel retrograde transport of the protein aggregates. In support of such damaging effects of the inflammatory response, are the findings that α-synuclein aggregates are: (i) incorporated by glial cells [108,109] and cause profound inflammation with astrogliosis, and (ii) do not cause distant spread from the point of brain injection when using nontransgenic mice [97,103,109,110]. Injection of α-synuclein devoid of its amyloidogenic core and thus unable to aggregate by itself also promotes profound neuroinflammation and α-synuclein pathology [97]. In addition, the proinflammatory stimulant LPS leads to α-synuclein aggregation and death of dopaminergic
neurons in the substantia nigra [111]. Finally, traumatic brain injuries, known to cause inflammation, increase the levels of α-synuclein and the risk of developing PD [112–114].

α-Synuclein and Midbrain Grafts

The idea that PD could be caused by the spreading of α-synuclein is also supported by misinterpretations of findings of α-synuclein inclusions in human midbrain grafts in some studies [21,23]. Embryonic midbrain transplants have been shown to improve symptoms in patients subjected to optimal procedures, wherein grafts remain healthy and viable for more than one decade [21,22,24,25]. Nevertheless, some studies question the benefit of such grafts, based on poor clinical improvement or the appearance of Lewy bodies in transplanted neurons [21,22,115–117]. We disagree with both these findings and conclusions. We argue that the findings could be explained by many other variables, such as intracellular biology, as well as transplantation, cell preparation, and surgical methods [63]. Graft survival, inflammatory responses, and the formation of Lewy bodies in human fetal embryonic transplants are dependent on the technique used to isolate and to implant the fetal dopaminergic neurons in the host [63,118]. While transplants performed with dissociated neurons are perfectly healthy after 14 years [24,25], those done with cellular aggregates show grafts that contain neurons surrounded extensively by microglial activation [21,116]. In addition, while transplantations of dissociated neurons have no or very rare α-synuclein inclusions after 14 years [24,25], transplantations done with small cellular aggregate suspension or small tissue pieces have approximately 2–12% inclusions after 12–24 years [21–23].

Furthermore, a recent study reported the postmortem examination of an embryonic dopaminergic transplant in the putamen 24 years following surgery [22]. Not only was there a dense and near to normal dopaminergic innervation by the graft in the putamen, but the clinical benefits were remarkable even after the reintroduction of L-Dopa after a decade spent drug-free post-transplantation [22]. Since these clear benefits, the authors observed that 12% of grafted neurons developed Lewy bodies. They claim that these findings support the spread of pathology from host neurons to transplants [22]. However, there is no evidence for this interpretation. First, due to the caveats we have already mentioned, it is not clear if the Lewy bodies they observe in the graft are due to spread of α-synuclein from host neurons or if they are actually a result of the reactive inflammation to the graft or even a result of the established inflammation process in the host brain [37,63,118]. Second, the presence of 5–12% of Lewy bodies in the grafts does not seem an excessive number of inclusions after 16–24 years of functionally efficient transplantation [21]. On the contrary, it indicates that the relatively small amounts of Lewy bodies found in these studies might be related to intracellular events, or inflammation and glial recruitment due to the increased immunogenicity of blood vessels present in solid tissue pieces grafts [63,118].

Overall, when done properly, transplantation of embryonic dopaminergic neurons can be highly effective, devoid of α-synuclein inclusions (abrogating the idea of prion-like propagation of α-synuclein from host to graft) and beneficial to PD patients. Importantly, these findings also question the idea of using active and passive α-synuclein immunization to prevent the suggested α-synuclein propagation to treat PD patients [119]. Even for Alzheimer disease, where the pathology is largely extracellular [120], both active and passive Aβ immunization in patients caused meningoencephalitis and cerebral vasogenic edema [121–123]. Therefore, pursuing the immunization therapeutic avenue may even pose serious risks to PD patients.

Concluding Remarks

The chronic intracellular processes that promote the accumulation of endogenous α-synuclein, such as increased expression and decreased degradation [124,125], and the subsequent deleterious role of accumulated α-synuclein [9] can by themselves account for most, if not all, of the Lewy-body pathology observed in PD and related disorders. Nonetheless, it is...
possible that slight release of α-synuclein to the extracellular space could initiate inflammatory damage due to an increase of proinflammatory cytokines and glial reactivity [15,37,126–128]. In relatively late stages of pathobiology, α-synuclein (in all different conformations) and other molecules from dying neurons now present in the extracellular space could be endocytosed and hence further increase the intracellular load of adjacent neurons with prior compromised proteolytic and metabolic pathways, exacerbating the burden of accumulated intracellular α-synuclein and increasing the proteinopathy in PD (see Outstanding Questions). Overall, we hypothesize that one of the main processes of the disease is the locally accumulated α-synuclein within neurons. While artificial animal and cellular models of excessive toxic protein transfer can be created, no real pathophysiological evidence exists in patients that verify anatomical spreading of α-synuclein to support the notion that PD is primarily a prion-like disease. Instead, the functional threshold theory proposed here accurately explains the progression of disease, where the symptoms only begin when the functional reserve of neurons (and their connecting brain regions) is unable to allow for network compensation. Consequently, early symptoms of PD reflect loss of function in the least compensated systems, such as the GI tract, olfactory system, and brain stem, rather than the spread of α-synuclein from the peripheral to central nervous system.

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