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Neuronal vulnerability, pathogenesis and Parkinson's disease

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Abstract

Although there have been significant advances, pathogenesis in Parkinson's disease (PD) is still poorly understood. Potential clues about pathogenesis that have not been systematically pursued are suggested by the restricted pattern of neuronal pathology in the disease. In addition to dopaminergic neurons in the substantia nigra pars compacta (SNc), a significant number of other central and peripheral neuronal populations exhibit Lewy pathology (LP), phenotypic dysregulation or frank degeneration in PD patients. Drawing on this literature, there appears to be a small number of risk factors contributing to vulnerability. These include autonomous activity, broad action potentials, low intrinsic calcium buffering capacity, poorly myelinated long highly branched axons and terminal fields, and use of a monoamine neurotransmitter, often with the catecholamine-derived neuromelanin pigment. Of these phenotypic traits, only the physiological ones appear to provide a reachable therapeutic target at present.

1. Introduction

While the loss of substantia nigra (SN) dopaminergic (DA) neurons in PD is responsible for its core motor symptoms [1], a variety of other neurons exhibit signs of pathology in post-mortem analysis. Indeed, on the basis of symptoms and autopsy results, James Parkinson reasoned that the disease was caused by a primary lesion in the medulla, stating "From the impediment to speech, the difficulty in mastication and swallowing, the inability to retain, or freely to eject the saliva, may with propriety be inferred an extension of the morbid change upwards through the medulla spinalis to the medulla oblongata, necessarily impairing the powers of the several nerves derived from that portion into which the morbid change may have reached" [2].

Fritz Jakob Lewy identified intracellular protein aggregates in the dorsal motor nucleus of the vagus (DMV), a region within the medulla oblongata, in the brains of PD patients [3, 4]. These aggregates became known as Lewy bodies (LB) and Lewy neurites (LN). The discovery that alpha-synuclein (aSYN) is a major component of LP has enabled the use of immunocytochemical approaches to characterize these aggregates, which are now often labeled together as Lewy pathology (LP). With this assay, pathologists and the anatomist Heiko Braak have mapped LP in postmortem samples from PD patients [5].

These studies provide important insights, but several caveats should be noted. First, the relationship between LP and neuronal dysfunction and death is uncertain. In fact, the existence of LP in a particular cell type could reflect a successful response to proteostatic stress and not a loss of function [6, 7]. Second, LP is found in other synucleinopathies that have an uncertain relationship to PD; particularly patients diagnosed with dementia with Lewy bodies (DLB) and incidental Lewy body disease (iLBD), the latter of which is widely suspected by anatomists to be an earlier stage of PD or DLB. Patients diagnosed with

multiple system atrophy (MSA) also show abnormal aSYN label, although the cell types and their distribution pattern, including a virtual lack of peripheral and enteric nervous system pathology, differ from PD, DLB, and iLBD [8]. Pathogenesis in these diseases undoubtedly differs from that in PD, at least in some key respects and it appears that aSYN aggregation is reachable by multiple paths. This uncertainty also makes it difficult to draw firm conclusions about the relationship between aSYN aggregation in a particular group of neurons and PD [9], as patients with PD could also be suffering to varying extents from other diseases that have such sequelae.

In contrast to LP maps, cell loss maps in postmortem PD samples using modern stereological approaches have been rare. This is unfortunate because it would allow for a more definitive correlation to be established between symptoms and disease targets. What is known will be reviewed below.

2. Vulnerable neuronal populations in PD

A. Peripheral nervous system

The peripheral nervous system can be divided into three parts: sensory, motor and autonomic. Peripheral sensory and motor neurons do not display LP or signs of degeneration in PD patients (with a possible exception of the sensory lingual nerve, mentioned below). In contrast, there are signs of pathology in the autonomic nervous system (ANS). There are sympathetic and parasympathetic divisions, each of which has preganglionic and postganglionic components.

Preganglionic sympathetic neurons arising from the ventral spinal cord as a rule release acetylcholine and postganglionic sympathetic neurons release norepinephrine (NE). The postganglionic sympathetic neurons that innervate sweat glands and release acetylcholine had been considered to be an exception in that they release acetylcholine: however, recent work demonstrates that a subset of these neurons can express tyrosine hydroxylase (TH) and the CNS vesicular monoamine transporter (VMAT2) in human and so are suggested to co-release NE [10].

Preganglionic parasympathetic neurons arise from neurons in the brainstem nuclei, including the DMV and neurons in the sacral spinal cord. These neurons are cholinergic. Postganglionic parasympathetic neurons are typically located in ganglia close to or embedded in the target organ. These neurons are largely cholinergic as well, but some postganglionic parasympathetic neurons express proteins associated with NE release as well as proteins associated with acetylcholine release [10]. These neurons are found in the heart, skin, and salivary glands.

In addition to the sympathetic and parasympathetic neuron of the ANS, there are intrinsic neurons found in target organs that provide local regulation of function. The largest component of this intrinsic division of the ANS is the enteric nervous system (ENS), which is embedded in the gastrointestinal tract. ENS neurons can be divided into myenteric (Auerbach's) or submucosal (Meissner's) plexuses. The myenteric plexuses are located between the inner and outer layers of the muscular layer (muscularis externa), and the submucosal plexuses in the adjacent submucosa. ENS neurons have a wide range of transmitter phenotypes, including cholinergic, serotonergic (most of the serotonin in the body is found in the gut [11]), noradrenergic and dopaminergic types [12].

Enteric neurons—The ENS displays LP in PD patients and the original characterization of PD noted severe constipation in many PD patients [2]. It is now widely thought that a loss of DA neurons in PD myenteric plexus underlies constipation [13]. Enteric LBs were first

identified in neurons of the esophagus or colon of 2 of 22 Parkinson's patients, both of whom also had dysphagia; 2 of 8 patients with a primary diagnosis of difficulty in swallowing (achalasia) also exhibited LBs in degenerating ganglionic cells of the esophageal myenteric plexus [14], and it is possible that the achalasia patients had PD. LP in the enteric neurons of the alimentary tract of a PD patient was confirmed by Cote and colleagues [15]. Wakabayashi and colleagues reported LP in both the myenteric and submucosal LP of all (of 7) PD patients studied, but also in 8 of 24 control age-matched subjects [16]. A follow-up study on 3 PD patients from that group reported that while in the sympathetic system nearly all LBs were found in catecholamine cells, that in the alimentary tract, LBs were mostly found in non-dopaminergic VIP secreting neurons of the myenteric plexus without accompanying loss of those neurons [17]. That conclusion has however been challenged in part by recent work on colonic biopsies from PD patients using phosphorylated alpha-synuclein and TH immunolabel [18]. In that study, 60% of neurons of the myenteric plexus with LP are TH+, and most of those are also immunolabeled for dopamine-beta hydroxylase and are thus NE neurons. It is possible that the 40% of LP neurons that apparently lacked TH had low levels of expression. There are also reports of LP in innervation and regions proximal to stomach [16, 19].

Most of the early reports found a similar distribution and number of TH+ cells in the myenteric and submucosal plexuses in PD and controls, which was interpreted as evidence against an overt death of catecholamine neurons. This view has been challenged by Chandar Singaram and colleagues who used a DA-specific monoclonal antibody to demonstrate that the number of DA neurons in the plexus was decreased by over 90% in 9 of 11 PD patients [20]. There was further a very striking loss of DA neurites in PD enteric tissue, and levels of DA measured in the nerve ending regions of the muscularis externa were decreased by 70% (note that DA is also present in NE neurons). There appear to be a large fraction of DA enteric neurons in human [12, 21].

These studies show that ENS LP is a frequent, but not necessary concomitant of PD. They also suggest that DA ENS neurons display LP and can be lost in PD. However, it is not clear whether other transmitter phenotypes, like serotonergic neurons, display LP and are lost in PD. Note further that although gastrointestinal dysfunction is common in PD patients [22], it is also common among aged individuals without any sign of parkinsonism [23].

Sympathetic and parasympathetic neurons—The principal divisions of the ANS are also reported to be affected in PD patients. Orthostatic hypotension occurs in a significant fraction of late stage PD patients. This symptom has been attributed to a drop in cardiac NA innervation [22, 24]. However, a more generalized sympathetic denervation that impairs vasogenic reflexes that maintain blood pressure could also be a factor [24]. In four PD patients, there was a profound loss of both TH immunolabel and neurofilament immunolabel compared to controls [25]. This report was corroborated by another small study of PD patients [26]. While the loss of TH might reflect in part a loss of phenotypic dysregulation, the extensive loss of neurofilament suggests that neurons were lost in these patients. This conclusion is consistent with a decreased number of unmyelinated axons in cardiac sympathetic nerve of PD patients [27]. A recent study found that in four of five PD patients, all classes of postganglionic sympathetic NE neuron that innervate the heart, including the left right ventricles, the right atrium and the sinus node, were reduced; this apparent neuronal loss was found in all PD patients that exhibited orthostatic hypotension [28]. Three of these patients showed LP and axonal beading in NA neurons. Loss of cardiac sympathetic NA innervation is seen in some iLBD patients but not others [26].

In a number of PD and DLB patients, LP is present in the peripheral vagal nerve and ganglia [29, 30]. These axons originate in the DMV. There are reports of strong LP in the

DMV preganglionic parasympathetic neurons of nearly all PD patients studied (see below). The DMV projects to many areas, including the gastrointestinal tract.

A subset of parasympathetic neurons in the intrinsic cardiac ganglia also appear to be at risk in PD [28]. These parasympathetic neurons are classically considered to be cholinergic, but a subset in human are reported to express VMAT2, suggesting they might co-release NE [10]. The parasympathetic inferior salivatory nucleus (IXth cranial nerve), which innervates the parotid gland, also features LP in PD [30, 31], but neuronal number even in control patients remains unknown (Kelly del Tredici, personal communication).

Salivary glands—Reduced salivary secretion (sialopenia; dry mouth) can accompany PD. The submandibular gland (the major source of saliva) is innervated by parasympathetic postganglionic neurons of the submandibular ganglion and by sympathetic postganglionic neurons of the superior cervical ganglion. In PD patients, LP has been seen in both in the submandibular gland and the superior cervical ganglion [30]. Inferior to the submandibular gland within the perviascular stroma, one of two parasympathetic lingual nerves, which are not considered parasympathetic but rather have sensory functions, showed LP. Within the superior cervical ganglion, LP was present in all cases, including the cell bodies of the large postganglionic neurons of PD patients. We are not aware of reports of neuronal loss there.

Skin—The unmyelinated neurites of the skin are NE releasing sympathetic postganglionic neurons that innervate blood vessels and other structures including sweat glands, which as above were previously thought to release only ACh but are now thought to also release NE in human [10]. Djaldetti and colleagues have found a marked denervation of all autonomic neurites in skin [32], work supported by analyses of skin biopsies from PD patients [22, 33].

Adrenal gland—Although they are not considered neurons, chromaffin cells of the adrenal medulla were the first cells shown to secrete catecholamines (hence the term adrenaline). den Hartog Jager found that PD patients display a large inclusion body in these cells, which he called “adrenal bodies” [34]. LP was subsequently described to be present in adrenal neurons [35]. However, the later work indicated that adrenal chromaffin cells themselves did not show LP and the adrenal bodies described by den Jager were not immunoreactive for αSYN [35]. In contrast, postganglionic sympathetic neurons innervating the adrenal gland exhibited LP in about ~30% of PD patients [35], although the TH innervation of the adrenal appeared to be normal.

Summary—LP is found in several types of peripheral neuron in PD. The only neurons that are known to be lost in PD are NE neurons innervating the heart and skin and DA neurons of the ENS: the loss of these neurons might be responsible for orthostatic hypotension, sweating (hyperhydosis) and constipation that commonly accompany PD.

B. Central Nervous System

The vast majority of the neurons lost or displaying signs of pathology in early and mid-state PD patients are found in the brainstem. LP and cell loss has been reported in the region of the DMV, the medullary reticular formation, the raphe nuclei (RN), the locus ceruleus (LC), the pedunculopontine nuclei (PPN), the substantia nigra pars compacta (SNc) and, to a lesser extent, the ventral tegmental area (VTA) and retrorubral area (RRA). The evidence for the involvement of these nuclei will be discussed in turn.

SNc and LC neurons that have high levels of neuromelanin (NM) pigment appear to be selectively targeted. Preganglionic neurons of the DMV do not contain neuromelanin, but some neurons in regions neighboring the DMV contain NM, and these are sometimes found

to express LP [31]. NM is a derivative of oxidized catecholamine that reacts with lipids and proteins, and acts as a reservoir that avidly traps iron, metals and other toxic substances; NM is sequestered and accumulated throughout life within autophagic organelles in neuronal cell bodies [36], apparently as a neuroprotective response. Although most obvious in the SNc and LC, NM is found in other central catecholamine neurons [37] and by more sensitive chemical methods in additional non-catecholaminergic neurons [38, 39]. NM autophagic organelles are essentially identical to lipofuscin (LF) organelles, a different pigment derived from oxidized lipids and proteins that accumulate throughout life within these organelles, save for the catechol-derived pigments and substances that accrue to them [40]. Hence, the presence of NM indicates a history of cytosolic oxyradical formation from catecholamines, while the presence of LF indicates a history of reactive lipid derivatives. Current research suggests that neurons cannot degrade NM, and that loss of NM depends first on cell death, which produces extraneuronal NM deposits followed by hydrogen peroxide-mediated degradation following phagocytosis by microglia [41]: remarkably, this was theorized on the basis of neuropathological observations by Foix and Nicolesco (1925). The presence of extraneuronal NM remnants and disappearance of NM neurons therefore indicates that the neurons have been lost.

DMV—As mentioned, James Parkinson theorized that damage to the medulla caused the disease. The caudal medulla oblongata includes a number of nuclei implicated in PD including some that exhibit NM and LF pigmented areas [37, 42]. The DMV was one of the first nuclei found by Fritz Lewy to express LB [3]. The NM neurons in this region do not appear to be parasympathetic preganglionic fibers that project via the vagus nerve. Axons entering the vagus nerve from the DMV can have strong LP [43], while LP within the DMV has been reported in essentially all PD patients examined [31], although other reports find exceptions [44, 45]. Additional neurons in this region have some variable degree of LP [31].

Neuronal counts demonstrate that DMV neurons are lost in PD [46], but there are differences in the literature regarding which neurons are lost, probably due to differences in stages of the disease. Classical studies report NM containing neurons in the DMV region were lost in PD brains, including the original report [47], which states that “The pigmented cells of the dorsal vagal nucleus also degenerated, often with vacuolation, by contrast with the non-pigmented cells in this nucleus which remained healthy.” While this was subsequently reported in a number of other studies [48], Eadie himself did not confirm this and reported that motoneurons were lost, consistent with a later study [46]. Saper and colleagues note that some of the most heavily NM pigmented neurons survive the disease [49]. Braak and collaborators [42] suggest that the preganglionic parasympathetic projection neurons are first to degenerate, and that loss of the NM neurons may occur later (Kelly del Tredici, personal communication).

New work suggests that some DMV neurons, while cholinergic, also express tyrosine hydroxylase and aromatic acid decarboxylase, the enzymes that produce dopamine, and thus may express cytosolic catecholamines but not release them due to a lack of vesicular monoamine transporter [50]. There is also a local A2 catecholamine group in this area, and it is not clear which other DMV neurons may express catecholamines, and whether these are projection neurons.

The brainstem reticular formation also includes several nuclei in which LP is observed in PD patients [31]. While neuronal death has not been reported per se, the depigmentation of this area in some patients again indicates that some catecholaminergic neurons must be lost.

SNc, VTA, RRF—The loss of DA neurons in the SNc is the best documented sequela of PD. In 1894, Blocq and Marinesco reported a study of a patient with unilateral parkinsonism

who had a tuberculoma that damaged the SNc [51]. The loss of NM in the SNc of PD patients was reported early in the 20th century [4] and confirmed by many subsequent studies [47, 48, 52]. The loss of NM is seen in all PD patients [53], but not all patients with parkinsonism [54]. LP is nearly always found in the NM neurons in the SNc, particularly in the posterolateral regions [31]. LB has been reported in the neighboring VTA and RRF neurons as well [53]. DA neuronal loss in these two regions is variable [55] but has been estimated to be as much as 50% [39].

Unlike the situation with the LC, neuronal loss in the SNc appears to be specific to PD. A comparative study reported a profound (~80%) loss of NM SNc neurons in PD patients, but only a modest 7% loss in AD patients [56]. The most heavily NM pigmented neurons that are lost in the SNc of PD patients are suggested to be those with lower levels of VMAT2 and with decreased vesicular accumulation of DA [57] as well as lower levels of calbindin [58, 59]. Note that while NM neurons are particularly targeted [39], some of the most highly pigmented neurons are spared in PD patients [49, 60].

LC—The neighboring NE neurons in the LC have long been known to be lost in PD [47, 52]. LP is observed in the larger projection NM containing LC neurons and is absent from non-NM cells in the complex [53], confirming selective damage to NE neurons. Virtually all PD patients appear to have a substantial loss of LC neurons, with a mean neuronal loss of 83% in later stages of the disease [56]. There is also an average 68% loss of these neurons in Alzheimer's disease (AD) [56] but the extent of LC loss is more variable in AD patients than PD patients.

RN—Neurons in the RN are responsible for serotoninergic innervation of the CNS. In iLBD and PD, LP is most apparent in medium-sized neurons of the caudal RN [31]. The ventral rostral raphe region around the medial lemniscus also exhibits LB in PD [53]. RN neurons not only display LB but are lost in PD, with a reduction of over half these neurons in the median raphe, and a somewhat smaller fractional loss reported in the raphes obscurus [53, 61].

PPN—The PPN is rostral to the LC and includes a mixture of cholinergic, glutamatergic and GABAergic neurons; the cholinergic and glutamatergic neurons are projection neurons with an array of targets in the mesencephalon and diencephalon [62]. The PPN was reported to exhibit LP by Braak's group in some cases [5, 42], and LBs and neuronal loss were reported by Jellinger [63]. Death of PPN neurons in PD appears to be highly variable. The nucleus itself was smaller in 4 of 6 PD brains examined by Hirsch and colleagues as defined by NAPDH diaphorase and ACh esterase label [64]; they also estimated that about half of the neurons were lost in that subgroup of patients. As in many other regions, loss appears not to be specific to PD as PPN neurons also were dramatically less abundant (~80% reduction) in the brains of individuals with progressive supranuclear palsy (PSP) (Hirsch et al., 1987). Jellinger [63] attempted to address the specificity issue by comparing the nuclei in patients with PD, PSP, and AD using cresyl violet stain. He found nearly the same neuronal loss in PSP and PD (~50–60%) and less loss in AD (~30%). A recent study found a 40% drop on average in the number of neurons displaying cholinergic markers in the PPN of PD patients and the magnitude of this reduction was positively correlated with symptom severity (Rinne et al., 2008). Halliday and collaborators also have reported a loss of substance P immunoreactivity in the PPN and neighboring structures in PD brains [61]. However, in neither of these studies is it clear whether this reflects cell loss or phenotypic dysregulation [65].

NBM—The nucleus basalis of Meynert (NBM) is a prominent site of LP in PD patients. This cholinergic basal forebrain structure supplies the majority of cholinergic input to the

cerebral cortex. Neurons in this region, along with the DMV, were first reported to exhibit LP by Lewy [3]. Indeed, the NBM is nearly always found to display LP in PD. The specificity of this loss is less clear as the NBM also figures prominently in AD [66]. This issue was addressed by a comparative study [67] that found a loss of about half of the NBM neurons measured by cresyl violet label in 11 PD patients, which was significantly greater than age-matched control subjects. Not all of these subjects displayed AD-like symptoms, suggesting that there was a symptomatic threshold. There was no correlation between PD symptom severity and NBM loss in this study. The most extensive comparative study of PD and AD brains found similar NBM neuronal losses, also as labeled by cresyl violet, on average (~40%), but there was a great deal of variability [56]. Thus, LP and neuronal loss in the NBM appears to be a common feature of PD and AD.

Olfactory neurons—There are several other regions of the brain rostral to the mesencephalon that have been reported to exhibit signs of pathology in PD. One of these is the olfactory system. In the olfactory bulb, mitral cells exhibit LP [68] but this is also seen in AD [69]. The anterior olfactory nucleus also shows LP in iLBD [31]. The existence and targeting of neuronal loss in the olfactory system is controversial [70, 71]. This issue is complicated by the neurogenesis in this region [72].

Additional areas—There are several other regions that have been reported to have either neuronal loss or LP in PD, many in single studies. These include the hypothalamus [73] and the intralaminar nuclei of the thalamus [74]. The specificity of these changes is unclear. In the case of the thalamic nuclei, the loss is also seen in PSP [74]. In apparent later stages of PD, LP is also scattered throughout the cerebral cortex, amygdala and hippocampus [75, 76]. Retinal DA also has been reported to decline [77], but LP was not identified.

Summary

There are obviously major gaps in the description of neuronal pathology and loss seen in PD patients, particularly insofar as how frank cell loss contrasts with phenotypic dysregulation. Nevertheless, it is clear that the pathology in PD is distributed across both the CNS and PNS. There is well-documented neuronal loss of NM+ catecholamine neurons of the SNc and LC in nearly all PD patients, and of DMV neurons in most patients. Loss of VTA, RRF, PPN, RN and BNM neurons in PD appears common but quite variable. Only the loss in SNc is specific to PD, although this is in part a circular argument due to reliance on SN-dependent motor dysfunction to diagnose the disease, and there are diseases with parkinsonian symptoms that do not exhibit SNc death [1].

3. Determinants of vulnerability

The obvious question is what links this seemingly diverse set of neurons. There are several possible phenotypic traits that have been proposed to underlie vulnerability. All are consistent with age being the single largest risk factor in PD.

- **Lewy pathology.** Immunolabel for aSYN aggregates is widely used as a marker for PD-related pathology, and with the caveat that it is also associated with several disorders that variably overlap with PD, it is remarkable that nearly all neurons that experience cell death in PD also display LP. The converse, however, does not appear to be true, as various peripheral and central neurons are found to express LP without current evidence for eventual neuronal death. It may be that LP results from a neuroprotective response to stabilize and detoxify otherwise toxic forms of aSYN [6], as is thought to occur for the intracellular accumulation of toxic cysotolic protein aggregates [78]. It is nevertheless possible that LP, even if it indicates a

protective stress response, may have deleterious effects on intraneuronal function [65], such as axonal organelle transport.

- **A common reactive neurotransmitter.** SNc, LC, RN, enteric DA neurons, and sympathetic postganglionic neurons each synthesize a monoamine neurotransmitter, and high levels of cytosolic monoamines are reactive and hypothesized to underlie selective neuronal death under several conditions [79–83]. Additional targeted neurons including some vulnerable parasympathetic neurons of the DMV appear to possess and/or secrete monoamines.

  Obviously, DA SN and, NE LC are the most similar in this regard and both exhibit NM deposition in humans. These two populations display the greatest loss in PD. VTA and RRF DA neurons are less pigmented and less vulnerable, although still substantially more than other neurons often included in lists of targeted neurons; the lower level of neuronal loss than in the SNc has been hypothesized to stem from lower levels of cytosolic catecholamines [81], which is consistent with the lower NM [36], and evidence for lower levels of vesicular accumulation of DA in the highly targeted and more pigmented SNc neurons [57]. Nevertheless, some highly pigmented A2 catecholamine NM neurons of the DMV do not appear to be lost in PD [49], and so NM synthesis per se, which may be another neuroprotective response [36, 40], is not sufficient for neuronal death.

  In the periphery, the loss of NE neurons is variable, with some sympathetic neurons appearing to be lost and others not. Moreover, their loss is not specific to PD, as is also the case for LC neurons. DA neurons in the ENS, which are vulnerable in PD, do not have visible NM, suggesting they may not experience the cytosolic oxidant stress typical of SNc and LC neurons. Two other observations militate against a reactive monoamine transmitter hypothesis in a simple form. One is that there clearly is pathology and loss of neurons that do not use monoamines. Targeted neurons in the PPN and BNM use either acetylcholine or glutamate as neurotransmitters. It must be acknowledged that the loss of neurons in PPN and BNM is not specific to PD, raising the possibility that their loss has a different origin and is not really a reflection of PD per se: it has been recently suggested that loss of cholinergic neurons is a downstream response to previous loss of monoamine neurons [84]. Another observation of relevance is that use of L-DOPA – the precursor for DA – to treat PD does not accelerate the progression of the disease as one might expect if DA was the toxic agent in the disease [1].

- **A long, highly branched axon with multiple release sites.** SNc, LC, RN, PPN and BNM neurons all have unusually long highly branched axons that are unmyelinated or thinly myelinated [27, 85]. This feature is particularly well documented for SNc DA neurons. Single SNc axons terminating in the striatum are highly branched and possess as many as several hundred thousand synaptic release sites [86]. This is an order of magnitude (or more) greater than most neurons that have been carefully studied. Interestingly, these terminals do not appear to have an elevated mitochondrial oxidant stress [87]. However, maintaining a massive terminal field is very likely to create a metabolic and proteostatic burden on the cell body. Mitochondrial trafficking could prove particularly problematic; in fact, mitochondrial density in the somatodendritic region of SNc DA neurons is low [88], possibly reflecting the need to traffic mitochondria to axons. It is worth noting that aSYN is thought to be a presynaptic regulator of synaptic vesicle exocytosis [89–91]; the proteostatic burden it creates could scale with the number of synaptic release sites, and may contribute to mishandling of presynaptic mitochondria [92]. It is unclear whether VTA and RRF DA neurons have as extensive an axonal field as do

SNc DA neurons. Matsuda et al. did not report profound differences in dorsal and ventral striatal terminal fields, which should correspond to SNc and VTA; as a consequence, the differences in vulnerability between these regions would have to be explained by other factors. The LC also forms very long and complex projections. Based upon the distance traveled and terminal field, the axons of DMV neurons are also long and highly branched, and DA enteric neurons are also highly branched.

- **A common physiological phenotype.** An extended discussion of this hypothesis has recently been published [93]. PD is a disease of neurons, not of the liver, kidney or heart. An implication of this fact is that one or more of the features distinguishing neurons from these other cell types must contribute in a seminal way to pathogenesis. A cardinal feature of neurons that separates them from nearly all other cell types is excitability. Neurons use steep electrochemical gradients across their plasma membrane to perform computations on incoming chemical signals from other neurons and to pass the outcome of this computation to other cells. Each step in this process expends energy. Action potentials (or spikes) and synaptic transmission dissipate the ionic gradients for sodium, potassium, calcium and chloride that are maintained by ATP dependent pumps and exchangers. Although all neurons share this basic set of properties, the parameters of spikes and synaptic transmission vary dramatically. The physiological phenotype of neurons ranges from what might be called a ‘wallflower’ or quiescent phenotype to a ‘chatter box’ phenotype that never stops spiking. SNc, LC, RN, BNM, PPN and DMV neurons all fall into the chatterbox phenotype. That is, all of them spike continuously in vivo during the waking state [94–99]. SNc, LC, DMV and PPN neurons are autonomous pacemakers (they spike on their own in the absence of synaptic input). Moreover, each of these neurons have very broad spikes that further dissipate ionic gradients, particularly those for calcium. Although less well studied, many of the neurons in the ANS, particularly those in the ENS, also are spontaneously active and have broad spikes [100–102].

  Calcium entry is energetically expensive because it must be pumped out of the cell against a much steeper electrochemical gradient than any of the other ions. Many neurons ameliorate this burden by expressing specialized calcium binding proteins that effectively buffer calcium and minimize the metabolic cost of pumping it back across the plasma membrane. The expression of calcium binding proteins in the ANS and ENS varies from cell type to cell type [103, 10460]. SNc, LC and DMV neurons express relatively little of these calcium binding proteins [105] (DJS, unpublished results) (RN, PPN, and BNM have not been rigorously characterized in this regard to our knowledge). In contrast, VTA neurons robustly express the calcium binding protein calbindin; most other, relatively PD-resistant autonomous pacemakers in the brain also express calcium binding proteins (e.g., Purkinje neurons, globus pallidus neurons, striatal cholinergic interneurons).

  It is worth noting that autonomous pacemaking neurons spend all of their time at relatively depolarized membrane potentials where NMDA receptors are relieved of their magnesium block, creating another point of sodium and calcium entry during excitatory synaptic transmission; excitotoxicity is perhaps one of the oldest theories of pathogenesis in PD [106, 107]. High cytosolic calcium levels also act to increase cytosolic DA in SNc neurons [81], possibly due to an effect on synthesis, and can lead to aSYN-dependent neuronal death. Thus, while there certainly are gaps in our physiological characterization of PD-vulnerable neurons, at this point it appears that they all are a variant of the chatterbox phenotype: continuous generation of slow, broad spikes and low intrinsic calcium buffering capacity.

This physiological phenotype creates a sustained metabolic burden carried largely by mitochondria. In most neurons, the metabolic burden associated with activity and synaptic transmission is thought to significantly diminish the respiratory reserve of mitochondria [108]. In SNc DA neurons, and others of its kind, this reserve should be even smaller. In fact, there is a measurable increase in the oxidation of mitochondrial thiol proteins when SNc DA neurons are simply pacemaking [109]. Unpublished work by our group (DJS) has revealed that LC and DMV neurons have a similar (albeit smaller) mitochondrial oxidant stress. Mitochondrial dysfunction is widely viewed as a pivotal step in PD pathogenesis [110]. A sustained mitochondrial oxidant stress should in principle lead to the accumulation of mitochondrial DNA (mtDNA) mutations and impaired complex I function seen in the SNc with aging and PD [111]. Genetic mutations, environmental toxins, and proteostatic stress that further challenge mitochondria could synergize with this cell type specific stress to produce a bioenergetic crisis, resulting in impaired protein degradation and ultimately degeneration.

This scenario does not exclude a role for transmitter phenotype or axonal branching in pathogenesis. In fact, these traits, particularly the axonal one, readily fit into this scenario. The translational question is how to devise a therapeutic strategy to stop or slow the progression of PD. Clearly, changing a neuron's transmitter phenotype or axonal branching is not an option. Strategies could be developed to ameliorate the consequences of these traits, but what these might be is not yet obvious. In contrast, there is a way in which the consequences of the chatterbox phenotype on mitochondrial stress could be diminished. One of the ion channels contributing to the basal metabolic stress in SNc DA neurons is the L-type calcium channel. There are FDA approved antagonists (dihydropyridines) of these channels that have an excellent safety record in humans. Moreover, there is epidemiological evidence that sustained use of brain penetrant dihydropyridines reduces the observed risk of PD [112, 113]. This effect is surprising given the relatively low affinity of most dihyrdropyridines for the subtype of L-type calcium channel responsible for most of the calcium entry in SNc DA neurons, i.e., one with a Cav1.3 pore-forming subunit [93, 114].

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