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Multiple hit hypotheses for dopamine neuron loss in Parkinson's disease

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Parkinson's disease arises from genetic and possibly neurotoxic causes that produce massive cell death of the neuromelanin-containing dopaminergic neurons of the substantia nigra. Loss of these neurons is essential for the diagnostic parkinsonian features. Although many genetic mutations have been suggested as causes or risk factors for Parkinson's disease, the low penetrance of some mutations and the low disease concordance in relatives suggests that there must be interactions between multiple factors. We suggest that 'multiple hits' that combine toxic stress, for example, from dopamine oxidation or mitochondrial dysfunction, with an inhibition of a neuroprotective response, such as loss of function of parkin or stress-induced autophagic degradation, underlie selective neuronal death. We discuss the properties of substantia nigra dopamine neurons that might make them particular targets of such multiple hits.

Introduction

This issue of TINS celebrates the 50th anniversary of Arvid Carlsson's identification of dopamine (DA) as a neurotransmitter and his prescient comment that, 'it is interesting to note that reserpine, which depletes the dopamine from the corpus striatum, may produce a syndrome very similar to Parkinsonism' [1]. Virtually all subsequent work bears out Carlsson's suggestion that loss of striatal DA causes the diagnostic parkinsonian features of Parkinson's disease (PD) (Box 1).

Although Lewy bodies (LBs) and α-synuclein (α-syn) pathology can be present in many neurons in PD patients (Box 2), extensive (~80%) neuronal death in PD has been reported only for substantia nigra (SN) DA and locus coeruleus (LC) norepinephrine (NE) neurons. The most extensive comparative study between PD and Alzheimer' disease (AD) [2], the most common neurodegenerative disorder, found that for cholinergic neurons of the nucleus basalis, which contain LBs in PD, there was a loss of 37% in PD and 41% in AD. For LC neurons, there was a highly variable loss of 83% in PD and 68% in AD. The major difference between PD and AD was in the SN, where there was a loss of 78% of DA neurons in PD but only 7% in AD. Thus, extensive loss of SN neurons seems specific to PD.

Although the causes of PD remain unknown, even in Huntington's disease (HD), where a single genetic cause was identified over a decade ago, little is known about how the mutation leads to disease. Even though there is consensus among many investigators that age-related neurodegenerative disorders with inclusion bodies result from protein aggregation or defects in intracellular degradation, this remains a conjecture.

There are numerous nuclear genes suggested to cause familial PD, and additional genes suggested to predispose the carrier to PD (Table S1 in the supplementary material online). These seem to fall into several categories:

- Proteins affecting mitochondria (e.g. PINK1, DJ-1, Omi/HtrA2 and POLG).
- Proteins that might be involved in organelle trafficking and vesicular fusion (e.g. α-syn and tau).
- Proteins of macromolecular degradation pathways, such as ubiquitination or ubiquitination-like degradation pathways (e.g. parkin and DJ-1) and lysosomal function (e.g. β-glucocerebrosidase).
- Proteins that modify oxidative stress or antioxidant function [e.g. sepiapterin, DJ-1 and fibroblast growth factor-20 (FGF-20)].

For some of the mutations, the pathogenic role must be subtle because PD penetrance is often low. In the case of the common G2019S *LRRK2* mutation in dominant PD, only 24% of carriers exhibit PD symptoms by 80 years of age [3]. Twins, moreover, usually do not both exhibit PD: in a study of 14 000 pairs of twins aged 50 years and older, 247 individuals were diagnosed with PD and 517 individuals were suspected to have PD, but in only two twin pairs did both members exhibit PD [4]. Studies of incidence in monozygotic and dizygotic twins differ, probably because of differences in experimental design [5,6]. PD within the population at large shows little clear inheritance: in Iceland, 772 PD patients diagnosed over the previous 50 years contained a subgroup of 560 patients who were more related to each other than to the controls [7], but there was no highly penetrant inheritance and the disease often skipped generations.

What is unusual about SN neurons?

Here, we outline features of ventral midbrain DA and SN neurons that might selectively dispose them to stress in PD.

Structure

Neurodegenerative disorders tend to strike projection neurons with long unmyelinated axons. α-Syn is a presynaptic protein, and all neurons with α-syn pathology in PD

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Box 1. Background information on dopamine neuronal loss in Parkinson's disease

Parkinsonism is defined as a combination of tremor at rest, bradykinesia, rigidity, flexed posture, loss of postural reflexes and freezing of gait [12]. Cases of parkinsonism can broadly be divided into those that stem from a loss of striatal DA and those that result from impaired striatal DA receptors. Primary parkinsonism, here called Parkinson's disease (PD), represents ~80% of parkinsonian cases [12] and is the most common neurodegenerative disorder after Alzheimer's disease (AD). As with AD, PD incidence correlates with age, with a greater than 40-fold increase in prevalence between the ages of 55 and 85 [31].

PD diagnosis requires a strong reduction in parkinsonian features after administration of L-DOPA but it cannot be fully confirmed until autopsy, which reveals loss of NM^+ SN neurons and the presence of large ubiquitinated eosinophilic inclusions (i.e. pathological intracellular structures that lack a membrane) known as Lewy bodies (LBs). These are also found in a range of CNS, PNS and enteric neurons but are absent in juvenile 'PD' stemming from parkin mutations [41].

Although various groups of neurons exhibit pathology in PD, the parkinsonism is clearly caused by loss of DA following death of substantia nigra (SN) neurons, as shown by the extensive (~80%) loss of neuromelanin-pigmented (NM^+) DA neurons in the SN with a comparative sparing of the neighboring NM^- ventral tegmental area (VTA) DA neurons [56,57]. Parkinsonian symptoms occur concurrently with loss of striatal DA levels, which can be measured by *in vivo* imaging [58]. The parkinsonian symptoms of PD, moreover, are treated by the DA precursor, L–DOPA, as above. L-DOPA exposure loads more DA into the synaptic vesicles of remaining DAergic terminals to increase the quantal size of DA release [29], compensating for the loss of innervation.

As in Huntington's disease (HD), where motor symptoms are caused by loss of medium spiny neurons but in which there is additional neuronal death associated with non-motor symptoms [59], PD has non-motor symptoms that have not been used as diagnostic criteria. For example, loss of smell is associated with PD, although it is also common in AD [60]. Patients with non-motor features of PD but without loss of striatal DA are generally presumed to suffer from another disorder, such as AD.

In conclusion, despite multiple causes and neuronal targets, PD pathogenesis in nearly all cases converges upstream of LB formation and death of DA SN neurons.

Box 2. A special role for α-syn?

The A30P and A53T α-synuclein (α-syn) mutations were the first genes found to underlie familial PD [61], Of the pathogenic genes suggested and/or elucidated to date, α-syn has received particular attention for several reasons:

- It is a major component of LBs and Lewy neurites [62].
- Overexpression of wild-type α-syn through gene multiplication (also known as the *PARK4* gene for familial PD) triggers PD [63].
- Virus-mediated α-syn overexpression can kill SN DA neurons [64].
- α-Syn knockout mice are resistant to DA neuronal death by MPTP toxicity [52].
- MPTP and 6-hydroxy-DA increase α-syn expression in rodent SN DA neurons [65,66].

PD is now widely considered a 'synucleinopathy' as are 'parkinson-plus' disorders such as multiple system atrophy (MSA) and diffuse Lewy body disease. These diseases also show accumulations of aggregated α-syn.

The physiological role of α-syn might be to control vesicle exocytosis at a late stage before fusion [67]. This might involve alternate binding and dissociation from membrane, shifting between an α–helical conformation in 'lipid rafts' to an unfolded conformation in the cytosol. Provocatively, the A30P PD mutation binds the membrane poorly and might be more subject to aggregation. The dissociation from the synaptic vesicle membrane seems to result from and regulate synaptic vesicle exocytosis [68]. It is by no means clear whether the normal physiological function of α-syn is involved in PD, and it is widely suspected that problems in degradation might initiate a 'toxic gain of function', perhaps through an aggregate.

Braak *et al.* [8] examined immunolabeled α-syn to study aggregation in autopsy of PD patients and also in asymptomatic individuals who might have been en route to developing PD. They suggest a progression of PD through ascending brain regions, beginning with medulla oblongata/pontine tegmentum and olfactory bulb, to locus coeruleus (LC), raphe, then SN, and at late stages to the neocortex.

There is no clear relationship between α-syn expression and neuronal death. Expression is much higher during early human development than at maturity [69], when pathogenesis takes place. In normal adult humans, the α-syn mRNA label is strong in NM^+ SN neurons but is also high in unaffected regions [70]. Moreover, there are no data indicating extensive neuronal loss of cortical neurons in PD despite the presence of LBs. In other words, the presence of α-syn pathology in neurons does not indicate that the neuron will necessarily die.

have long and poorly myelinated or unmyelinated axonal projections [8]. Axonal transport through microtubules is needed to remove damaged mitochondria, to transport vesicles and autophagic vacuoles and to contribute or replace membrane proteins. Appropriate trafficking on microtubules requires mitochondrial ATP production for the molecular motors dynein, kinesin and myosin, and for actin function.

The axonal projections of ventral midbrain DA neurons, entailing SN and ventral tegmental area (VTA) neurons, are particularly impressive. A low number (~300–600 K in human and ~45 K in rat [9]) of these neurons provide massive innervation of the striatum. Each SN neuron might give rise to ~150 000 presynaptic terminals in the striatum [10,11]. SN dopamine neurons also have massive dendrites that extend into the SN reticulata; the SN cell body represents far less than 1% of the total cell volume.

The size and complexity of SN neurons might explain the observation that mutations in several genes related to organelle trafficking seem to predispose individuals to developing PD. Loss of DA axonal terminals seems to precede SN cell body loss in PD [12], and it is tempting to guess that the etiology might stem from problems in the axons or terminals. There are, however, other neurons with long and branched neurites, including DA neurons of the VTA, and so additional attributes are required to explain SN loss.

Function

The SN acts within the basal ganglia loop, which evaluates actions in the environment and signals to the premotor cortex to facilitate or inhibit specific voluntary movement. This system provides 'implicit sequence' or 'habit' learning, that is, learning a skill that eventually does not require conscious effort to recall, for example, running a maze. Once learning is acquired, the basal ganglia might be active only during its initiation or endpoint [13]. This function of SN DA neurons has been most thoroughly studied for reward-based learning [14]; once an animal successfully predicts the outcome of a stimulus or of its own effort, DA neurons seem to be no longer activated and DA is no longer released. That DA release from SN neurons is involved in the initiation of motor tasks and feedback evaluation during behavior is clear from the freezing and postural instability that occur in PD. DA neurons, however, show little or no change in firing before or during

arm or eye movements in primates or spontaneous behavior in rats, but rather increased activity during goal-directed behavior.

Ventral midbrain DA neuronal activity is often described by two patterns. The ongoing tonic firing pattern is slow (mean frequency ~4 Hz) [15] and intrinsic. In mature mice, tonic activity is instigated by Ca^{2+} currents that drive the cell into oscillations between spikes owing to a slowly inactivating L-type Cav1.3 channel that opens in the subthreshold range (~50 mV), and is modulated by Ca^{2+}-activated K^+ channels (SK channels) that produce an afterhyperpolarization that delays return to threshold [16].

Superimposed on the oscillatory tonic activity are phasic bursts of two to six action potentials with a mean frequency of ~20 Hz that require triggering from excitatory inputs to SN dendrites. Bursts are triggered by sensory stimuli and appetitive or conditioned stimuli [14], previously thought to be through the prefrontal cortex and subthalamic nucleus. Recent reports, however, indicate that excitation is primarily from sensory related brainstem nuclei and cholinergic inputs [17].

This characteristic SN activity is related to features that might contribute to PD; several of these require ATP or GTP, and therefore need intact mitochondrial function.

- The Ca^{2+} flux underlying tonic activity is comparatively large, and midbrain DA neurons are unique in having a greater interspike Ca^{2+} current than they do Na^+ current [16]. Together with high Ca^{2+} flux from bursting [18], normal DA neuron activity might entail relatively high intraneuronal Ca^{2+}, which might induce oxidative stress and possibly excitotoxicity. Mitochondria buffer Ca^{2+} levels, and so mitochondrial damage could directly lead to increased vulnerability. Ca^{2+} homeostasis is further regulated by ATP, including plasma membrane, mitochondrial and endoplasmic reticulum pumps. In this regard, it might be important that SN and VTA neurons expressing calbindin are generally spared in PD [19] and from 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) toxicity [20] because this protein buffers cytosolic Ca^{2+}.
- K^+ influx returns neurons to hyperpolarized states and controls the timing of firing [18,21], blocks excitotoxicity and is neuroprotective. The function of the Na^+/K^+ ATPase is to maintain normal K^+ gradients, and it is said to be the major consumer of neuronal ATP [22]. Ventral midbrain DA neurons possess the largest Na^+/K^+ ATPase transporter current yet found in any neuron [23]. Decreased pump function could thus contribute to selective midbrain DA neuron vulnerability. Homomeric G-protein-coupled inwardly rectifying K^+ (GIRK) channels are responsible for the hyperpolarization by DA D2 and GABA-B receptors in SN neurons [24]. Homomeric GIRK2 is expressed at much higher levels in SN DA than in VTA DA neurons. In the *weaver* line of mutant mice, a point mutation in the pore-forming region of GIRK2 causes the death of SN and cerebellar neurons. The ability of the G-protein-coupled receptors to hyperpolarize the mutant SN neurons is lost, and instead they maintain a depolarized resting potential [24]. Dysfunction of homomeric GIRK2 channels might contribute to selective SN vulnerability in PD. ATP-sensitive K^+ (K-ATP) channels have also been suggested to mediate PD pathogenesis. The probability of K-ATP channel opening is lowered with a high ATP:ADP ratio and thus enhanced by decreased mitochondrial function. In *weaver* mice, K-ATP channels are constantly open owing to loss of ATP, providing a partial compensation for the tonic depolarization [24]. MPTP activates K-ATPase channels selectively in SN but not VTA neurons [25], although the channels seem identical in both regions. SN neurons are rescued from MPTP toxicity through inactivation of the K-ATP channel pore, although the means by which enhanced K^+ flux, which would normally be protective, kills SN neurons is unclear.

In summary, the particular requirements of K^+ and Ca^{2+} channel function during normal physiological function of SN neurons could underlie cellular stress and in some cases, as with the Ca^{2+} flux required for tonic firing, could contribute to specificity of SN neuronal death. Several of these potentially toxic processes could be particularly exacerbated by inadequate mitochondrial ATP production.

Cytosolic DA-related stress

DA readily oxidizes to react with proteins, lipids and nucleic acids. It forms neurotoxic derivatives, including 6-hydroxy-DA, and interacts with intracellular iron or products of monoamine oxidase to form toxic oxygen radicals [26]. DA neurons have evolved multiple mechanisms to protect themselves from cytosolic DA stress, including feedback inhibition of tyrosine hydroxylase, synaptic vesicle sequestration of DA by the vesicular monoamine transporter-2 (VMAT2), metabolism by monoamine oxidase, and reduction of DA quinone by glutathione. The tyrosine hydroxlase cofactor, tetrahydrobiopterin, scavenges superoxide and is protective in DA neurons [27].

These defense mechanisms could be overwhelmed by the redistribution of synaptic vesicle DA to the cytosol, because there is approximately fivefold order of magnitude higher concentration of DA within the vesicle. A vesicular H^+-ATPase in synaptic vesicles generates the H^+ electrochemical force that drives accumulation of DA against its concentration gradient; DA in the vesicle can reach nearly molar levels [28]. Collapse of this gradient redistributes DA from the vesicle [29], with resulting oxidative damage. Thus, loss of mitochondrial function and ATP could redistribute DA to the cytosol and have a part in selective DA neuronal loss.

Cytosolic DA levels reach ~15 μM DA in chromaffin cells [30] but presumably much lower levels in neurons. Whether cytosolic DA *in vivo* ever reaches toxic levels in the SN is not known, and treatment of patients with L-DOPA does not exacerbate development of PD [31], perhaps because toxic levels are not reached, although damage from high cytosolic DA might have already taken place at a stage before diagnosis and L-DOPA exposure.

Multiple hit hypotheses

The low penetrance of PD and the involvement of α-syn and LBs suggest that a combination of insults is required for

SN neuron death, which is similar to the notion in cancer biology that 'multiple hits', including loss of proliferation control, migration and invasion, are required for tumorigenesis [32]. In this way, PD differs from some other forms of parkinsonism, such as Segawa disease and DOPA-responsive dystonia, which are caused by a 'single hit' from a low level of DA synthesis. In this section, we list examples of primary hits that might lead to SN neuronal stress and secondary hits by loss or inhibition of stress-induced protective pathways that together could cause massive SN death in PD.

Neuromelanin as an inducible protective response

Neuromelanin (NM) is composed of oxyradical DA derivatives of lipids and proteins that have been sequestered within autophagic vacuoles (AVs) [33]. The protein components have not been characterized, and it is not known whether DA-modified α-syn is a component. The presence of NM in SN and LC neurons indicates that these neurons are under ongoing oxidative stress from cytosolic DA even in normal humans. (LC neurons release norepinephrine, which is converted within synaptic vesicles from cytosolic DA).

AVs provide a protective mechanism for all eukaryotic cells and are induced by many forms of stress, including starvation and infection. DA neurons, in particular, respond to various cellular stressors, including methamphetamine and L-DOPA, by forming AVs [34]. This process entails the synthesis and transport of AVs to fuse with lysosomes, where the contents are degraded. AVs degrade mitochondria and large protein aggregates, perhaps including LBs or α-syn aggregates that would otherwise develop into LBs. Autophagic degradation in general is reduced during aging [34], providing a clue to the age dependence of PD.

Normal humans accumulate NM over a lifetime [35], and AVs containing NM eventually occupy a substantial fraction of SN and LC cell bodies; it seems that NM is not effectively broken down by neurons. In PD patients, however, NM loss seems to occur after neuronal death from the phagocytosis and degradation of extracellular NM deposits by microglia. Although normal AV sequestration prevents NM from reacting with other cellular constituents, NM sequesters iron and other reactive substances [35], and these could exacerbate damage when NM is released from a dying SN neuron.

Lipofuscin, the primary morphological sign of aging in neurons, is a compound in AVs that is similar to NM but lacks DA derivatives. NM and lipofuscin can be observed within the same SN autophagic vacuoles. There is no NM in nucleus basalis neurons, which are also lost in PD, but PD patients have abnormally high levels of lipofuscin in these cells [36].

In contrast to the SN, the neighboring DAergic VTA neurons, which are relatively spared in PD, contain little NM, indicating less DA-related oxidative stress during the life of the neuron. A clue to the reason for differential NM accumulation by ventral midbrain DA neurons is that VMAT2 overexpression blocks NM synthesis [33], apparently by packaging DA into synaptic vesicles, and there is an inverse relationship between VMAT2 expression and NM levels in the human midbrain [37]. If AV sequestration of NM and lipofuscin were damaged, it might lead to selective SN cell loss in PD.

LBs as an inducible protective response

The ubiquitin–proteasome system is responsible for the bulk of turnover of short-lived cytosolic proteins. It was initially reported that proteasome inhibition alone could produce specific DA SN neuronal damage [38], although this was not replicated by other investigators [39].

Alternatively, formation of ubiquitinated protein aggregates might be an inducible stress response, and cytosolic aggregates in HD seem to protect the cell by removing toxic huntingtin fragments [40]. Although LBs are an important sign of PD, it is plausible that PD proceeds when aggregation leading to LB formation is no longer sufficient to remove toxic proteins.

The familial PD gene, parkin, is an E3 ubiquitin ligase, and patients with homozygous parkin mutations develop a juvenile form of parkinsonism without LBs [41], and a single parkin mutation seems to predispose adults to PD. As ubiquitination and marking proteins for proteasomal or autophagic degradation provide a stress response, mutant parkin might produce a deficient stress response. Although parkin-deficient mice do not lose SN neurons [42], parkin overexpression exerts neuroprotection from various forms of stress, including that owing to *PINK1* mutation [43] (see below), which is consistent with parkin functioning as an inducible stress response. The lack of LBs in the homozygous form of parkin-associated juvenile PD [41] also suggests that parkin might be required for LB formation.

An attractive hypothesis would be that if parkin activity were required for α-syn degradation, patients with a single parkin mutation would have α-syn build-up and form LBs, whereas homozygotes with no wild-type parkin would have α-syn build up with no protective LBs. This would explain both early onset disease and PD in heterozygotes. Parkin-mediated degradation of α-syn has, however, been found only for a particular glycosylated form of α-syn [44], and others found no effect of proteasome inhibition on α-syn degradation [45,46].

Perhaps parkin-mediated ubiquitination of proteins other than α-syn is required for LB formation. For example, α-syn degradation or aggregation could depend on parkin-mediated ubiquitination of synphilin-1, an α-syn-interacting protein in LBs, or chaperone-like proteins, such as 14-3-3 proteins, that might inhibit α-syn aggregation or parkin function. LRRK2 is another possible parkin-interacting protein and a potential candidate for an inducible protective response pathway. Finally, cytosolic DA has been implicated in ubiquitin–proteasome dysfunction and can inhibit parkin activity [47].

In summary, loss of parkin function, by mutation of itself or its interacting proteins, or through interaction with cytosolic DA products, might block degradation or protective sequestration through LB aggregation and lead to PD.

α-Syn interactions with chaperone-mediated autophagic protein degradation

α-Syn protein can be degraded by autophagy [45], that is, degradation within lysosomes. This is to be expected, as

mentioned above, following α-syn aggregation leading to an AV stress response. Before aggregation, however, α-syn can undergo degradation through an alternative stress-induced autophagy pathway: chaperone-mediated autophagy (CMA). CMA entails recognition by the chaperone hsc-70 and targeting to the lysosomal membrane [48]. CMA blockade causes cell death following cellular stress [48].

The A30P and A53T α-syn mutants associated with familial PD block lysosomal translocation of α-syn and other proteins degraded by CMA [48]. Moreover, high levels of wild-type α-syn competitively inhibit uptake of other protein substrates for CMA. A defect in lysosomal degradation produced by α-syn might thus tie together multiple observations, including the presence of α-syn in LBs, a role for pathogenic α-syn mutations, an explanation for disease arising from α-syn gene duplications, and the requirement for α-syn expression in MPTP models. However, it is not clear why SN neurons are particularly vulnerable.

Interactions of cytosolic DA with α-syn

Not surprisingly, products resulting from the interaction between DA and α-syn have been extensively explored for toxicity. Originally presumed to be a covalent bond, recent results suggest that there is an ionic interaction between DA–quinone and residues 125–129 in the protein [49]. DA modification of α-syn seems to maintain small α-syn oligomers in a reactive protofibril conformation by inhibiting progression to a less reactive aggregate. Protofibrils damage the membrane and do so more effectively with the pathogenic α-syn mutations [50].

Micromolar concentrations of α-syn cause proton leakage from isolated chromaffin secretory vesicles [51], and α-syn molecules with pathogenic mutations are particularly effective at causing leakage. It is not known whether the leak is caused by protofibril or native forms, as α-syn is amphiphilic and might have detergent-like properties. If DA synaptic vesicles are damaged by α-syn, a vicious cycle of dysregulated cytosolic DA and further α-syn damage could ensue.

DA-modified α-syn might block CMA in a manner similar to that of the pathogenic mutants. If so, when neurons are stressed, CMA would be induced as a protective pathway, but for SN neurons with reactive levels of DA, the resulting α-syn modification would block CMA and could lead to specific DA neuronal death.

Interactive roles for mitochondrial dysfunction

Mitochondrial dysfunction as a cause of PD is suspected, because rotenone and possibly MPTP in PD models kill DA neurons through complex 1 inhibition [52] independently of DA [53]. It has been suggested that SN neurons might be particularly prone to the accumulation of mitochondrial mutations during aging [52], and PD patients seem to express damaged mitochondria with low complex 1 activity in a variety of tissues [52].

Some products of the genes causing familial PD (DJ-1, POLG, and PINK1) are expressed in mitochondria. PINK1 is required for normal mitochondrial function in *Drosophila* and exerts protection against various forms of mitochondrial stress [43]. PINK1 expression might be involved in the protective effects of parkin, although parkin overexpression can overcome the effects of PINK1 loss. DJ-1 might have a similar protective mechanism in mitochondria, and α-syn might affect mitochondria [54].

On this evidence, it has been suggested that the recessive *PARK* genes protect against mitochondrial toxicity, although the mechanism remains unclear. As described above, there are multiple properties of SN neurons that might require particularly high demands on ATP. Perhaps loss of protective mitochondrial functions in tandem with demands particular to SN neuron structure, function and control of cytosolic DA might provide selective cell death.

An interaction with inflammation

Constantin Tretiakoff noted an inflammatory response in PD nearly 90 years ago [12], and inflammation has been implicated in many neurodegenerative disorders. Although toxicity through T cells or microglia has been demonstrated in various PD models [52], it is not clear why they should be specific for SN DA neurons.

One clue is that SN neurons can express the classic proinflammatory cyclooxygenase cox-2 [52]. Thus, it is possible that neuronal infections, such as viral encephalitis leading to parkinsonism, or possibly misfolded or aggregated proteins, directly trigger microglia or T cells to kill cells. Similarly, SN neurons and brain-stem neurons under some conditions produce MHC1 and β microglobulin [55]. Perhaps misfolded or aggregated proteins or protein fragments are presented directly by MHC, leading to DA neuron killing.

NM, which is released from dying neurons in PD, are phagocytosed by microglia, and such a microglial activation would elicit a vicious cycle of NM release followed by inflammation. Misfolded or aggregated proteins from diseased SN neurons, such as those found in LBs, could similarly activate a local immune response. Perhaps inflammation in SN provides a tertiary hit required for PD to spread to neighboring neurons.

Concluding remarks

With PD, we seem to be dealing with a syndrome stemming from multiple etiologies that arise from a variety of interactions. Some interactions might be relatively specific for DA neurons compared with other neurons, and others might overlap with diseases such as AD and synucleinopathies in which DA neurons are relatively spared. The common features of PD, nevertheless, eventually converge at massive loss of SN DA neurons and LBs. In analogy, the pathogenic pathways of other complex CNS diseases with multiple genetic causes, for example, autophagic vacuolar myopathies and hereditary spastic paraplegia, seem to converge to inhibit lysosomal and cytoskeletal functions, respectively.

Although there are many clues to the particular vulnerability of SN DA neurons, the decisive factors are not known, in part owing to the lack of adequate PD animal models. Incorporating multiple-hit hypotheses into the design of improved animal models in which multiple steps can be independently regulated could assist in understanding PD pathogenesis and exploring therapy.

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Supplementary data

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