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Parkinson's Disease: Cells Succumbing to Lifelong Dopamine-related Oxidative Stress and Other Bioenergetic Challenges

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Review

Parkinson's Disease: Cells Succumbing to Lifelong Dopamine-related Oxidative Stress and Other Bioenergetic Challenges

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Abstract: The core pathological event in Parkinson's disease (PD) is the specific dying of dopamine (DA) neurons of the substantia nigra pars compacta (SNc). Why SNc DA neurons are especially vulnerable and why idiopathic PD has only been found in humans is still puzzling. The two main underlying factors of SNc DA neuron vulnerability appear related to high DA production, namely (i) the toxic effects of cytoplasmic DA metabolism and (ii) continuous cytosolic Ca^{2+} oscillations in the absence of the Ca^{2+}-buffer protein calbindin. Both factors cause oxidative stress by producing highly reactive quinones and increasing intra-mitochondrial Ca^{2+} concentrations, respectively. High DA expression in human SNc DA neuron cell bodies is suggested by the abundant presence of the DA-derived pigment neuromelanin, which is not found in such abundance in other species and has been associated with toxicity at higher levels. The oxidative stress created by their DA production system, despite that the SN does not use unusually high amounts of energy, explains why SNc DA neurons are sensitive to various genetic and environmental factors that create mitochondrial damage and thereby promote PD. Aging increases multiple of the risk factors for PD, and, to a large extent, PD is accelerated aging. To prevent PD neurodegeneration, possible approaches that are discussed here are (1) reducing cytoplasmic DA accumulation, (2) blocking cytoplasmic Ca^{2+} oscillations, and (3) providing bioenergetic support.

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Introduction

Parkinson's disease (PD) is defined as an age-related, clinically evident Parkinsonism, and pathologically neurodegenerative disease with specific neuronal loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNc) and norepinephrine (NE) neurons in the locus coeruleus (LC) that are rich in neuromelanin (NM), a decrease in NM, and formation of toxic misfolded oligomers of α-synuclein [1]. At least temporarily, treatment with L-DOPA (also known as "levodopa"), which provides a replacement for decreasing natural DA, can ameliorate symptoms but not the progression of the disease [2]. The most significant risk factor for PD is aging. It is estimated that the number of PD patients worldwide doubled to more than 6 million between 1990 and 2015, mainly due to aging, and it is projected to exceed 12 million by 2040 [3]. PD, essentially being the preferential dying of DA neurons, is believed to be caused by the sensitivity of those neurons to harmful endogenous and/or exogenous factors. Those factors can vary, be singular or multiple, and are the topic of debate [4,5]. The present review focuses on two main underlying reasons causing SNc DA neurons' specific vulnerability: (i) their bioenergetic demands and (ii) their high DA production. It also provides a historical perspective, showing that whereas initially, the bioenergetic demands were simply portrayed as "high metabolism," gradually, a more nuanced understanding of mitochondria under particular oxidative stress emerged. We discuss how several risk factors appear

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to act on PD vulnerabilities and how understanding these vulnerabilities may lead to strategies against the disease.

Progression of PD pathology

Normal age-related loss of SNc DA neurons.

Even in healthy aging, SNc DA neurons show their specific vulnerability by substantially higher losses (roughly 5–10% per decade) than many other types of neurons [6]. This is probably caused by several risk factors that increase with aging, as discussed below. PD, with few exceptions, is a disease of the elderly and, to a large extent, appears to involve an acceleration/worsening of the normal age-related deterioration of the nigrostriatal DA system. The healthy nigrostriatal DA system seems to provide considerable surplus DA for maintaining "normal" motor functioning. It is commonly estimated that only when nigral DA neuron counts and striatal DA levels are diminished by about 50% and 80%, respectively, Parkinsonian motor symptoms appear [7–9]. However, it should be considered that some motor function abnormalities tend to appear already several years before classic PD symptoms manifest [10]. Furthermore, in a discussion on how much DA is necessary for normal function, it should be realized that the brain appears to have compensatory mechanisms for dealing with some levels of DA deficiencies [11].

The Braak model of PD staging.

The SNc DA neuron vulnerability and enhanced degeneration are common aspects among PD cases, but the triggers and anatomical spreading routes of PD vary [5]. In most—though not all—cases, the progression of PD includes the generation of Lewy bodies (LB), which are aggregates that have α-synuclein proteins as their primary components [12], and toxic α-synuclein oligomers are believed to spread as prions between neurons [13]. LBs are also increased in healthy aging and do not always lead to PD or other distinguishable neurological diseases [14]. However, LBs, on average, are significantly enhanced in PD, and a frequently observed sequential order of their appearance in different neuronal regions in postmortem PD brains led Braak and co-workers to propose what has become known as the "Braak hypothesis" or "dual-hit hypothesis" for PD staging [15,16]. The dual-hit hypothesis postulated that the pathology—potentially induced by a neurotropic pathogen—enters the brain simultaneously via a nasal and a gastric route, based on the observations of initial LB lesions in "stage 1" PD in the olfactory bulb, anterior olfactory nucleus, the dorsal nucleus of the vagus nerve (which connects with the gastrointestinal tract), and the intestine and implies retrograde transport from the environment to the central brain [17]. However, later studies have proposed the single-hit hypothesis (brain-first PD or body-first PD) [18,19] since few PD cases simultaneously present with peripheral nerve and olfactory tract lesions. Most cases can be divided into two types: one with lesions localized to the peripheral autonomic nervous system and the other with lesions localized to the olfactory tract [18]. Based on the location of the initial LB lesions, the outside world seems to be a logical suspect for being the instigator of PD in both the dual-hit and single-hit hypotheses.

A contributing role of Lewy bodies.

Roughly, only about half of PD cases present the pattern of occurrence of LB consistent with the Braak hypothesis [20], and it has been shown that many cellular and molecular neuropathological changes occur before the appearance of LB [21]. It should also be noted that some forms of familial PD, such as those resulting from mutations in LRRK2 [22] or PARK2 [23], lack a common association with LB. Furthermore, in a study of idiopathic PD brains, the severity of dorsal vagal nucleus lesions did not correlate with the severity of cortical lesions in semiquantitative assessments [24]. Overall, no theory has yet been proposed that can explain all of the pathophysiology of LB.

Because LB can be found without PD, and vice versa, it is tempting to speculate that LB is only a bystander phenomenon and is not the direct cause of PD. However, multiple different mutations in the gene for α-synuclein, *SNCA*, are associated with familial PD [25], providing evidence that α-

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synuclein-related phenomena can directly contribute to PD. The most straightforward way to interpret all observations is that LB/α-synuclein spreading is one of the factors that can contribute to PD but does not always lead to PD and is not necessary for inducing PD. A crucial aspect of the Braak model is the realization that PD can start in different brain regions and may pathologically spread toward the SNc from there.

Cellular and regional differences within the SNc.

The SNc is not homogeneous, and not all SNc neurons die equally in PD. They are mostly the dopaminergic neurons—identified by being positive for tyrosine hydroxylase (TH)—that die [26,27]. Among the TH-positive neurons, mostly the neurons with (DA-derived) neuromelanin (NM) pigment die [26,27], and, among those, predominantly the neurons with most NM die [28]. NM amounts increase with the concentration of cytosolic DA [29] and, roughly, with a person's age [30].

The SNc can be divided into subregions, of which one method is based on calbindin D28K immunohistochemistry. This method distinguishes between calbindin-rich "matrix" areas and calbindin-poor "nigrosomes." In the nigrosomes, the neurons have more NM and are more densely packed, and here the PD-induced neurodegeneration starts earlier and is more severe [31].

The axonal arbor degenerates first.

SNc DA neurons have a massive axonal arbor in the striatum (see below). At both PD motor symptom onset and death, the loss of striatal DA markers exceeds that of SNc DA neurons [32]. This suggests a "dying-back process" (retrograde degeneration) in PD of SNc DA neurons [7,33].

Degeneration of other catecholamine (CA) neurons in the midbrain.

Because PD is believed to be predominantly caused by the degeneration of SNc DA neurons (A9 area), this article will mainly focus on those cells. However, it is essential to realize that, in PD, DA neurons in the SN pars reticulata (SNr), the retrorubral field (A8 area), and the ventral tegmental area (VTA, A10 area) also preferentially die, although to a lesser extent than in the SNc [26,27,34,35]. The LC shows similarities with the SNc in that its catecholaminergic (NE neurons in this case) NM-producing neurons preferentially die [26,36,37], and an effect of this neurodegeneration on the SNc has been proposed [37,38]. In the Braak model of PD staging, LBs are formed in the LC before they are formed in the SNc [15].

The nucleus accumbens (NAc).

The axons of DA neurons whose cell bodies reside in the SNc predominantly project to the dorsal striatum, while DA supply to the NAc mainly derives from neurons with their cell bodies in the VTA, a brain region directly adjacent to the SNc [39,40]. However, this specialization is not absolute; VTA DA neurons also innervate the dorsal striatum, and SNc DA neurons also innervate the NAc [39]. Although to a lesser degree and at a later stage than in the SNc, DA neurons in the VTA are affected by PD as well [34,41]. In PD patients with cognitive disorders, the NAc shows atrophy and a statistically significant decrease in volume [42,43], which is thought to be primarily caused by the dying of DA neurons.

Summary.

PD does, at least in most cases, not start in SNc DA neurons. However, it results in their preferential degeneration, especially of those that reside in the "nigrosome" regions of the SNc and starting with their axonal arbors in the striatum. CA neurons in brain regions other than the SNc also preferentially die. Among SNc DA neurons, those with higher NM concentrations die, suggesting a critical toxic role of CA production in PD susceptibility. However, there is no evidence that biological differences in CA production are sufficient to induce PD, and CA production rather appears to create a common vulnerability that, in the case of aging and PD, is acted on by other factors. In the majority of PD patients, Lewy bodies/ α-synuclein appear to contribute to the initiation and anatomical spread

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of the disease. However, they do not always lead to PD and are not always found in PD. Thus, critical questions in PD are: (1) why are SNc DA neurons especially vulnerable, and (2) which factors can act on this vulnerability and thereby induce neurodegeneration?

Comparison of SNc DA neurons between humans and other species

An evolutionary ancient system.

DA is a neurotransmitter that promotes motor activity/control and learning in a wide variety of animals [44–46], including the primitive nematode *C. elegans* [47]. Already from the evolutionary level of jawless vertebrates, in the basal ganglia, SNc/VTA DA neurons projecting to the striatum are found, and from the level of amniotes (reptiles/birds/mammals) separate SNc and VTA regions can be distinguished that predominantly project to the dorsal and ventral striatum, respectively [48]. Conserved properties from *C. elegans* to humans also seem to be that DA concentrations decrease with the age of the animals (for *C. elegans*, see [49] and that DA is an immunomodulator that is importantly released by means of extra-synaptic "volume transmission" [50].

In non-human mammals, SNc DA neurons preferentially die upon aging like in humans, but natural PD may hardly exist.

Upon aging, also in non-human primates, the SNc DA neurons preferentially die, as summarized by Stark and Pakkenberg [6], and can reach a 50% decrease [51], which is similar to what can be seen in healthy elderly humans. Aged rhesus monkeys display significant impairments in performing delicate motor tasks, and the clinical rating scale correlates with their loss of TH-positive neurons [51]. Also, in aged mice, the combination of locomotor impairments, loss of DA neurons (although mainly in the VTA) or their degeneration (characterized by fragmented mitochondria), and a reduction in striatal DA levels has been observed [52]. However, even though DA neuron vulnerability upon aging is common among mammals, PD is common only in humans. Only recently, the first case of natural PD was found outside humans, namely in a cynomolgus monkey that showed the classical signs of PD, which could be ameliorated by treatment with L-DOPA [53]. However, this case may be more similar to familial PD than idiopathic PD in humans, as this monkey had mutations in the *LRRK2* gene [53,54]. Li and coworkers in 2021, calculated that the incidence rate of PD cases in monkeys and humans may be similar but that in monkeys, because of their low numbers in captivity, the chance of detection is minimal [54]. Whether that is correct reasoning can be debated, but for animals that are abundantly kept as pets, like cats and dogs, it appears reasonable to assume that true PD is absent. More animal individuals with PD will likely be found, but the overall data indicate that the incidence rate in humans is much higher than in most, if not all, other species.

Only in humans neuromelanin (NM) has abundantly been found.

Another at least relatively unique feature of humans appears to be the abundant NM in SNc DA neurons. However, NM is also found in the SNc of non-human mammals [55,56], and non-human primates may not become old enough or may not have been sufficiently investigated at old age for a valid quantitative comparison. Nevertheless, given that NM is produced upon excess cytosolic DA [57] and that the concentration of NM is positively correlated with the degree of neurodegeneration [28], it is attractive to assume that there is a connection between the seemingly uniquely high frequencies of PD and high NM concentrations in humans. This is corroborated by a study showing that recombinant expression of tyrosinase in the rat and mouse SN, leading to the gradual production of NM-like pigment in a multiple-month period, also leads to PD phenomena [58].

Some studies suggested that humans have higher levels of DA in their striatum than other animals, including apes, but that conclusion seems to be questionable because those studies were only based on analysis of TH transcripts or proteins (the latter by immunohistochemistry) in the striatum [59–62]; those observed species-specific differences may have been caused by differences in the abundance of non-DA dopaminergic TH^+ interneurons [63] and species-specific differences in anti-TH antibody reactivities upon immunohistochemistry.

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Artificial animal PD model systems.

By means of neurotoxins, usually 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), PD model animals can be created in which the animals show many classical PD signs [64]. However, compared to human PD, these systems tend to lack the slowness of progression and to not replicate the "axonal arbor first" sequence of SNc DA neuron degeneration. In contrast, the group of Surmeier established a transgenic mouse system in which gradual loss of mitochondria in SNc DA neurons not only induced PD phenomena but also showed slowness of progression and "axonal arbor first" phenotypes reminiscent of human PD [65]. Interestingly, combined studies imply that sufficient DA production in either the striatum (by release from axons of SNc DA neurons) or SNc (by somatodendritic release from SNc DA neurons) is sufficient for prohibiting Parkinsonism [66], suggesting a critical role of the substantia nigra pars reticulata (SNr) neurons on which both DA pathways are believed to have an overall inhibitory effect [67].

Summary.

In summary, the vulnerability of DA neurons appears to be evolutionary ancient, including their preferential degeneration upon animal aging. Why natural idiopathic PD has only been observed in humans is unclear, and it may be related to the same unknown reasons that also cause NM to be the most abundant in humans. Artificial animal model systems, for example by using neurotoxins, show that animals can exhibit PD symptoms if SNc DA neurons are sufficiently damaged, suggesting that quantitative rather than qualitative factors cause the differences between humans and other species in acquiring natural PD.

The bioenergetic demands of SNc DA neurons, and a role for calcium in their vulnerability

Shared features among neurons susceptible to PD.

By studying the different neuron populations with LB in PD, Braak, and co-workers concluded all of them to be projection neurons with extensive axon length and poor axonal myelination among their common factors [16] (Table 1). For example, in PD, the parasympathetic unmyelinated preganglionic fibers are the predominant site of lesions in the vagus nerve. In the olfactory nerve, α-synuclein accumulation is found in the olfactory bulb, olfactory tract, and anterior olfactory nucleus, with the olfactory tract being constructed mainly from unmyelinated axons [68]. Furthermore, in PD, in cardiac sympathetic nerves, unmyelinated axons account for the overwhelming majority (98.2%) of axons with α-synuclein aggregates, and they are lost at a higher frequency [69,70]. In the skin of PD patients, α-synuclein deposits were found in unmyelinated nerve fibers of the autonomic nervous system in association with length-dependent nerve fiber loss [71]. Braak and colleagues [15,16] postulated that poor axonal myelination is a cause of stress because it is associated with a higher requirement of energy for the transmission of impulses [72].

The vulnerability concept by Braak et al. [16] was later modified by Sulzer and Surmeier, 2013 [29], who concluded common factors in different populations of PD-susceptible neurons to be "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" (Table 1). Similar to the proposal by Braak et al. 2003 [16], Sulzer and Surmeier [29] postulated that energy demands play a major role in the cell vulnerabilities underlying PD, but instead of the focus on poor myelination, they focused on the energy burdens on the cell body of maintaining a massive axon system and continuous firing (autonomous pace-making). Surmeier especially strongly expressed PD vulnerability through high bioenergetic demands in a presentation in 2016, in which the type of sustained (tonic) firing by SNc DA neurons (see below) was considered "bioenergetically expensive" and to cause these cells to be "close to a bioenergetic cliff"[73]. Also, in later studies, when discussing PD vulnerability, the Surmeier group kept emphasizing that "SN DA neurons have a high basal bioenergetic demand" [74], which may be true but at least deserves some nuance as the demand may not be higher than in PD-resistant neurons (see below).

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Although gradually modifying the original concept of PD vulnerability by Braak and co-workers, in 2013, Sulzer and Surmeier [29] still assumed that SNc DA neurons were "unmyelinated or thinly myelinated" and that this contributed to their vulnerability. However, we are not aware of primary literature showing evidence for the postulated poor myelination of SNc DA neurons, and the Surmeier group, while in their later studies continuously improving the knowledge of other stressors of SNc DA neurons, appears to not mention poor myelination of these neurons anymore [75].

Compared to many other neurons, SNc DA neurons are unusual by having unmyelinated varicose "bead" structures within their extensive axonal arbor from which they can release DA [76,77], but this may not have been referred to in the early studies by Braak when mentioning "poor myelination."

SNc DA neurons have a massive axonal arbor in the striatum involved in "volume transmission" and tonic releasing of DA.

Compared to some other types of neurons, the number of SN dopaminergic (DA) neurons is relatively small, with only about 200,000 to 420,000 in adult humans [78]. However, these neurons have a large volume as they are extensively branched in the striatum—the volume of the combined axons of a single neuron can be larger than 1 mm^3—and individually exert influence over a large number of striatal neurons (75,000 on average in rat) while innervating both the striatum striosomes and matrix compartments [79] (Figure 1). Extrapolating these numbers to humans, it was estimated that one single human SNc DA neuron may have 1 to 2.5 million striatal synaptic sites [80]. The fact that SNc DA neurons in humans are over 4 m in total length and have over 1 million synapses presumably leads to high energy costs and makes them vulnerable to energy deprivation [81]. Besides being involved in specific signaling at synapses, DA is also a neuromodulator released by "volume transmission," in which release is followed by diffusion for widespread activation of many target cells, and—especially given that DA is released by volume transmission from abundant varicose structures within the SNc DA axonal arbor [76,77]—the number of striatal sites affected by a single SNc DA neuron is even higher than the synaptic contacts alone. Overall, the SNc DA system does not seem to be developed for selectively targeting precise circuitries, and this "broadcasting" (spatially nonselective action) effect [82] in the striatum is further enhanced by SNc DA neurons being in communication with each other and tending to be synchronized (fire together) [83–85].

Figure 1

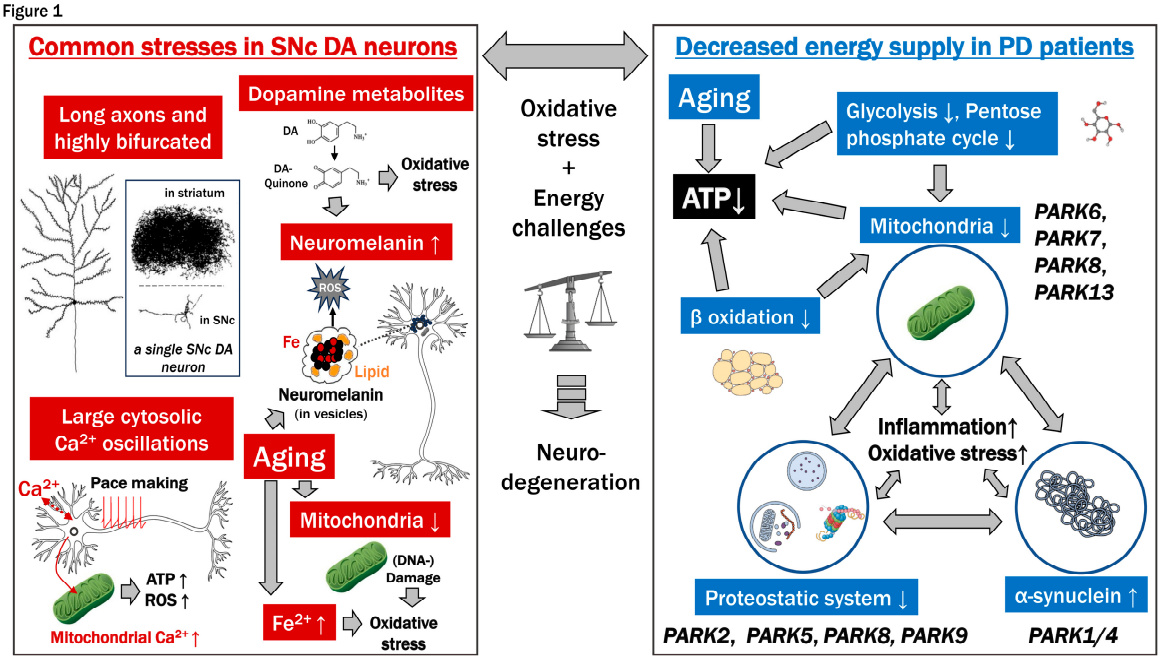

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Figure 1. Specific Vulnerability of SNc DA Neurons: High DA Production Causes Oxidative Stress and Energy Challenges that Contribute to Parkinson's Disease. Dopamine neurons have long, highly branched axons. The boxed figure showing the part of a single SNc DA neuron in the SNc and its axonal arbor in the striatum is modified from Matsuda et al. 2009. DA-quinone derived from DA is an important source of oxidative stress. DA metabolites are building blocks of the melanin component of neuromelanin (NM), which forms an association within membraned organelles with proteins, lipids, and metals. Aging increases the amount of NM, as are the accumulation of DNA damage in mitochondria and the concentration of iron, both contributing to oxidative stress. In order to generate a robust pace making system with abundant ATP, SNc DA neurons have large Ca^{2+} oscillations that increase the intra-mitochondrial Ca^{2+} concentrations and thereby increase both ATP and ROS production. In PD, the energy metabolism is generally impaired. Dysfunction of the glycolytic system, pentose phosphate pathway, mitochondria, and β-oxidation can commonly cause a decrease in ATP. Glycolysis, the pentose phosphate pathway, and β-oxidation interact with or constitute mitochondrial functions. Proteostatic functions (protein metabolism involving building and degradation) require ATP and directly interact with mitochondrial functions, and in PD and aging deficiencies in all three aspects are observed. The misfolding of α-synuclein also affects mitochondria and proteostatic competence. Abnormalities in mitochondria, the proteostatic system, and α-synuclein can all lead to neuroinflammation and elevated oxidative stress. Genes responsible for familial PD generally affect one of these factors. Aging is associated with a decrease in ATP. Ultimately, excessive oxidative stress and energy challenges outweigh compensatory mechanisms and can lead to neurodegeneration. Most drawings in this figure are obtained from "Mind the Graph" (https://mindthegraph.com/my-creations/).

By continuous firing through pace-making activity (see below), SNc DA neurons continuously release a tonic level of DA in the striatum, which is the background against which increases or decreases in striatal DA concentrations are interpreted as validation for learning and starting activities [85,86]. It is believed that besides through slow and diffuse ("broadcasting") signaling by volume transmission, DA from SNc DA neurons can also participate in the stimulation of neural circuits with more spatiotemporal precision [77,87]. Regardless, for understanding the energy demands of SNc DA cells, their massive axonal arborization, in combination with tonic firing and volume transmission release of DA, appears to be the most important.

Pacemaking activity.

Neurons in the SNc are continuously active in vivo, with very broad spikes that dissipate ionic gradients, especially calcium gradients [29]. Other autonomic neurons, especially those of the enteric nervous system [88], are also spontaneously active and have a wide range of spikes. Compared to DA neurons in the VTA, DA neurons in the SNc maintain striatal DA concentrations by the generation of regular (oscillatory) action potentials via a process that also involves L-type Cav1.3 Ca^{2+} channels, even in the absence of synaptic input, and need to pump intracellular Ca^{2+} back into the extracellular space against a huge concentration gradient which requires considerable energy [89]. This Ca^{2+} system does not affect the pacemaker frequency of signal spiking, which was shown to be determined by TRPC3 and NALCN channels [90], but improves pacemaking robustness by providing a "feed-forward stimulation" system that ensures a sufficient level of ATP for spiking [74,91]. For a mechanical discussion of the effect of Ca^{2+} influx on ATP generation, see the paragraph on mitochondrial dysfunction below.

The Surmeier group postulated that this oscillatory Ca^{2+} pumping, at the interface of energy demands and oxidative stress, is an important contributor to PD vulnerability, and stated: "One neuronal trait implicated in PD selective neuronal vulnerability is the engagement of feed-forward stimulation of mitochondrial oxidative phosphorylation (OXPHOS) to meet high bioenergetic demand, leading to sustained oxidant stress and ultimately degeneration" [92].

The presence versus absence of the calcium-binding protein calbindin-D28K appears to confer relative protection to the DA neurons in the SNc matrix compared to the ones in the SNc nigrosome [93,94]. Calbindin-D28K is believed to work as a Ca^{2+} buffer that protects against Ca^{2+} toxicity, but the

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trade-off appears to be a reduction in activity; namely, when Calbindin-D28K expression was genetically blocked, VTA DA neurons that otherwise express this protein released significantly more DA [95]. Therefore, the fact that Calbindin-D28K is absent in the SNc nigrosomal DA neurons is probably indicative of a higher demand on Ca^{2+} mediated signaling for increasing DA release.

Despite that the SNc DA neurons do not seem to have higher bioenergy requirements than many PD-resistant neurons (see below), there are compelling indications that their PD vulnerability does relate to bioenergy factors such as mitochondria and Ca^{2+} pumping.

Mitochondrial dysfunction

Mitochondria in the SNc DA neuron cell bodies; energy demands in the SNc DA neurons are not especially high.

Mitochondria are the energy suppliers of the cell, providing the cell's bulk of ATP through a process called oxidative phosphorylation (OXPHOS). During this process, mitochondria also produce reactive oxygen species (ROS) that have a variety of functions but in excess, and in case of mitochondrial damage/leakage, can harm the cell [96]. Apart from ATP generation, mitochondria also function in other processes relevant to this review, such as calcium signaling [97] and iron homeostasis [98,99]. One cell has many mitochondria, each with a limited lifespan, and their turnover includes processes like fusion/fission and mitophagy [100–104]. Liang et al. [105] found that compared to other midbrain neurons, including VTA DA neurons and non-DA neurons in the SN, SNc DA neurons in mice have relatively low mitochondria mass in their somata and dendritic areas [105]. Therefore, and because the SN exhibits low glucose utilization compared to many other brain regions [106], and SNc DA neuron action potentials are at a slow rate [107,108], Liang et al. argued [105]: "Such low metabolic demands may predispose these neurons to contain a low mitochondrial mass, which in turn may predispose these neurons to degeneration when mitochondria function is impaired." Thus, although the concepts by Liang et al., 2007, and of the Surmeier group differ in claiming low versus high bioenergy demands by the cell bodies of SNc DA neurons, they share the viewpoint that PD vulnerability involves mitochondria being stressed.

Mitochondria in the SNc DA neuron axonal arbor in the striatum.

The SNc DA neuron axonal arbor in the striatum has its own mitochondria and is also locally supplied with energy, although it depends on the cell body for nuclear and other functions [109]. In the striatum of PD patients, when the volume of SNc DA neuron axonal arborizations is decreased, their concentration of mitochondria is increased, suggesting an attempt to compensate [110]. Many factors that affect the quality of mitochondria in the cell body also do so in the axonal arbor [109]. The common idea that in PD, the pathology of neurodegeneration in SNc DA neurons starts in their axonal arbor in the striatum and only later reaches their cell bodies in a "dying-back process" [7,33] does not necessarily correspond to the mechanistic route of events. Namely, malfunctioning in the cell body may have a more immediate impact on the maintenance of the axonal arbor than on the intactness of the cell body itself.

Calcium and mitochondria.

In the SNc, the cytoplasm of SNc DA neurons undergoes continuous oscillations in Ca^{2+} concentration related to the pacemaking that includes Cav1 channel signaling (see above). The increased cytoplasmic Ca^{2+} concentrations also lead to increased Ca^{2+} concentrations within mitochondria, stimulating OXPHOS and ATP production and increasing ROS [91] (Figure 1). Studies showed that the oxidative stress in mitochondria of SNc DA neurons is considerably higher than in VTA DA neurons, which is caused by the Cav1 channel signaling [111,112]. A therapeutically promising line of evidence is that the drug isradipine, which diminishes cytosolic Ca^{2+} oscillations in SNc DA neurons without altering autonomous spiking or expression of Ca^{2+} channels, reduced mitochondrial stress and increased mitochondrial numbers in the SNc DA neurons of mice [112]. However, in clinical trials, isradipine has arguably not been a significant breakthrough yet in treating

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PD in humans, although some promising observations were made [113–115]. It should also be noted that isradipine, conditionally-dependent, was found to reduce DA release in the striatum [116].

Familial PD types mediated by mitochondrial dysfunction.

Many, if not all, of the significant types of familial PD are characterized by gene mutations that directly or indirectly, often by creating proteostatic dysfunction, affect mitochondrial functions [117] (Figure 1, Table 2). For example, PINK1 (PARK6) and PARKIN (PRKN or PARK2), associated with hereditary latent PD, play essential roles in mitophagy process [100,118]. PINK1 and PARKIN mutations disrupt mitochondrial function and lead to the generation of ROS as well as inflammatory responses [119]. Phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1) is a mitochondrial serine/threonine kinase that targets damaged mitochondria for degradation. Parkin is an E3 ubiquitin ligase that, as one of its functions, recognizes proteins on the outer membrane of damaged mitochondria and can target these mitochondria for destruction. Parkin deficiencies lead to oxidative stress [120] and more than half of the investigated PARKIN PD cases show an absence of LB pathology, which is one of the arguments that LBs are not necessary for inducing PD.

Animal PD models based on mitochondrial dysfunction.

The majority of animal models for PD are based on rather direct targeting of mitochondrial functions, i.e., by inhibition of complex I [121], and include the two examples mentioned earlier in this manuscript. MPTP, which causes PD symptoms in both humans and animal models, is metabolized in the brain by glial cells to a mitochondrial toxin, MPP^+, that is taken up selectively by monoaminergic neurons [122,123]. In the earlier mentioned study by the Surmeier group, a genetic mouse PD model was created in which the nuclear gene for the core subunit NDUFS2 of mitochondrial complex I could be deleted by adenovirus vector injection in the SNc region, creating a slow decrease in the number of mitochondria because of the stability and longevity of NDUSF2 protein [124]; this mouse model provides yet another line of evidence that mitochondrial dysfunction alone can be sufficient for causing SNc DA neuron degeneration and PD symptoms.

Intracellular toxicity directly related to DA or its derivates

Vulnerability of catecholaminergic neurons in PD.

The vulnerability of catecholaminergic neurons (DA and NE neurons), such as SNc DA neurons and locus coeruleus (LC) NE neurons, combined with the general resistance of GABAergic neurons, suggests that the type of neurotransmitter is an important determinant of neuron susceptibility in PD [125]. However, the type of neurotransmitter is not a necessary determinant, as exemplified by the PD vulnerability of cholinergic, glutamatergic, substance P, GABAergic, and glycinergic neurons in the pedunculopontine nucleus [126,127].

Dopamine (DA).

DA and its metabolites containing 2 hydroxyl residues also have cytotoxic properties, primarily due to the generation of highly reactive DA- and DOPA-quinones that enhance oxidative stress [128,129]. A standard theory for the incorporation of DA metabolites into NM is that this is a safe way to store excess free DA molecules in the cytoplasm and thereby reduce oxidative stress. Sulzer et al. [57] showed in vitro that increasing the intracellular DA concentration (by adding L-DOPA) led to enhancements in NM production and neurodegenerative symptoms, which could be reversed by decreasing the cytosolic DA concentration through enhanced uptake of DA into synaptic vesicles by increasing expression of vesicular monoamine transporter molecule VMAT2. This experiment by Sulzer et al. [57] provided straightforward evidence of the cytotoxicity of either cytoplasmic DA itself or its derivate products (including NM).

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The pigment neuromelanin (NM).

NM in human SNc DA neurons is a dark black/brown melanin pigment that starts to accumulate from early childhood and is enclosed in membranes where it forms aggregates with other molecules [30,130,131] (Figure 1). NM is formed from the oxidation of DA via DAquinone, followed by interaction with other cellular components such as small thiols (cysteine and glutathione), proteins (also α-synuclein), lipids, and metals, etc. As mentioned above, common theory for the incorporation of DA metabolites into NM is that this is a safe way to discard excess free DA molecules from the cytoplasm and thereby reduce oxidative stress.

NM is so abundant (in humans) that it gave the word "nigra" (Latin for black) to the name "substantia nigra" [132], and in micrographs of SNc DA neurons in elderly people, the average NM area was found to be >40% of the total cell body area [133]. This high NM content should already interfere with normal cellular processes by presence and steric hindrance alone.

NM accumulates so much iron that it can even be detected by magnetic resonance imaging [134]. Complexation by NM is believed to shield the metal from the cytoplasm and to also lower the one-electron reduction potential of the iron ions, making such ions more difficult to reduce by mild reductants; while this helps to protect against iron-induced oxidative stress, under extreme conditions the iron may be released from NM and become cytotoxic [135,136].

As mentioned above, NM amounts increase with the amount of cytosolic DA [57], with DA being a source for the melanic component of NM [28]. However, the biological function of NM—if it has a biological "function" at all—is not very clear [28], and, depending on conditions, NM can both enhance and reduce neurodegeneration [137]. Critical observations are that (i) those SNc DA neurons which have most NM preferentially die in PD [28], and that (ii) a rodent PD model system based on recombinant expression of tyrosinase in SNc DA neurons shows a coincident development of PD symptoms and NM-like pigment [58]. Thus, either (a) NM as a material, (b) the steric hindrance by NM presence, and/or (c) a molecule of which the abundance correlates with NM abundance increases neurodegeneration if at high concentration. After SNc DA neurons have died, the released NM can enhance local toxicity through the stimulation of microglia and inducing neuroinflammation, as reported by Zecca et al., 2008 [138].

Humans have more NM in their SNc DA neurons than other species (see above), which may represent a higher degree of toxicity and thereby explain why only in humans PD is common, either by the toxicity of NM (-abundance) itself or by the higher concentrations of intracellular DA that give rise to NM. Importantly, it should be realized that NM generation is in the SNc DA neuron cell bodies and not in their axonal arbor in the striatum where DA is produced as well, concluding that the PD vulnerability of humans may have to do with a high demand for local DA production in the SNc.

Summary.

The high cytoplasmic DA expression in human SNc DA neuron cell bodies, evidenced by NM formation, leads to enhanced oxidative stress from DA metabolites and probably to enhanced metabolic stress by steric and possibly other effects from abundant NM. This should make it harder for mitochondria to fulfill their tasks and NM probably is an important contributors to PD development.

Risk factors of PD

PD risk factors, general.

Among the known risk factors other than aging are, amongst others, male sex, routine pesticide exposure, occupational solvent exposure, caffeine nonintake, nonsmoking, type 2 diabetes, lack of exercise, and low plasma uric acid levels in men [139]. Several genetic factors increase the risk of PD, with the ones best-known and conferring the highest risk being named as PARK genes (Table 2). Although these genes contribute to a variety of cellular processes, they generally have rather direct effects on mitochondrial or proteostatic functions [117,140] (Figure 1); in addition to the PARK genes,

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many other potential genetic risk loci have been identified as well [141]. However, it is essential to realize that most PD cases are considered idiopathic, thus primarily determined by factors other than patient-specific genetic susceptibilities.

Exposure to toxins.

Environmental toxins can also cause or contribute to PD development [142]. The most famous case is the above-mentioned agent MPTP (1-methyl-4-phenyl-1,2, 3, 6-tetrahydropyridine). MTPT was discovered around 1980 when drug users, first a case in Maryland and then more cases in California, started using 1-methyl-4-phenyl-propionoxypiperidine, a meperidine analog and a new synthetic illicit drug supposedly having "heroin-like" qualities but containing MPTP as a by-product from synthesis; this MPTP was found to induce PD-like symptoms that could be alleviated by L-DOPA treatment [143,144]. Nowadays, various toxins, including metals and a variety of agricultural pesticides such as rotenone, have been associated with increasing susceptibility to PD [142,145]. As mentioned earlier in this article, many of these toxins are believed to promote PD through their detrimental effect on mitochondria, and the PD type and progression may somewhat differ from other causes. To give one more example, very recent epidemiological studies have reported that exposure to trichloroethylene (used as industrial degreasing solvent), which disrupts mitochondrial complex I, increases the prevalence of PD but produces a clinical form with more frequent resting tremor and less frequent rapid eye movement (REM) sleep behavior disturbances, olfactory loss, constipation, and urinary disturbance [146].

Reduction in the level of reduced glutathione causes oxidative stress.

Glutathione is an antioxidant, and a decreased concentration of reduced glutathione in the brain regions indicates oxidative stress. One of the pronounced biochemical changes seen in PD is a reduction in reduced and total glutathione levels in several brain regions including in the SN [147–149]. Rather than only indicating oxidative stress, decreases in reduced glutathione levels are also thought to directly enhance PD vulnerability [149].

Increased iron levels cause oxidative stress.

Iron is also a significant component of oxidative stress and is considered an essential player in the pathogenesis of PD [150]. In PD patients, there is a significant increase of iron in the SN, while not in several other brain regions, suggesting a causative relationship between iron and PD [151–154]. Ferroptosis is a type of programmed cell death characterized by iron dependency and the accumulation of lipid peroxides, and it is genetically and biochemically distinct from other regulated cell deaths, such as apoptosis [155]. Ferroptosis is initiated by the failure of glutathione-dependent antioxidant defenses, leading to unchecked lipid peroxidation, ultimately resulting in cell death. It has been reported that ferroptosis is also associated with the death of DA neurons in PD [156].

Aging increases various risk factors.

The average age of PD onset in populations that are primarily Caucasian is 58.9 years old [157]. Aging is characterized by a general deterioration of functions, including those predisposing to PD, such as deteriorations of proteostasis and mitochondrial functions [158]. Mitochondrial dysfunction in aging is characterized by an accumulation of genetic damage, increased oxidative stress, and decreased in the number of mitochondria [159,160]. In particular, in the SN of the aging brain—although to lesser extents than in age-matched PD patients—oxidative stress is increased by substantial increases in iron concentration [161] and decreases in the levels of reduced glutathione [162]. Furthermore, the aging brain has an enhanced immune status (low-grade inflammation, also known as "inflammaging"), which additionally interferes with brain homeostasis and puts cells under stress [163]. The fact that neurons, most of which cannot 't replicate or be replaced, can live for many decades is remarkable in itself, and it appears only logical that under the increased stresses

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from aging, they will eventually deteriorate. Probably all people, if they would live long enough, would eventually develop PD(-like symptoms).

Is non-secretion of DA a risk factor, and a reason why regular smoking may be protective against PD?

Interestingly, various mutations are believed to decrease DA release before the cells degenerate [164]. If this occurs without a decrease in DA synthesis, this might lead to increased cytotoxicity by accumulated intracellular DA [57]. Therefore, we have speculated that regular DA release by regular physical exercise may be beneficial against PD [28]. Similarly, we here speculate that it may explain why smokers, who regularly release DA through stimulation by nicotine, are, on average, better protected against PD than nonsmokers [165]. Nonsmoking has even been described as one of the most substantial risk factors [166].

Energy status and PD

Metabolic alterations in PD.

With mitochondrial functions having such a prominent role in PD vulnerabilities, it is only logical that various bioenergetic factors may impact PD, including nutritional status. Several questions must be addressed to understand energy deficits in neurodegeneration, such as its role in disease onset and whether restoring energy may halt cell death. Minor energy shortfalls can hamper synapses, while significant deficits can trigger cell death [167].

Early PD is associated with various metabolic abnormalities, such as a decline in mitochondrial β-oxidation and reduced long-chain acylcarnitines [168]. In epidemiological studies, weight loss has been observed in PD patients before disease onset [169], and fat reduction is the primary driver of this pre-onset weight loss [170]. A systematic review of multiple studies found that malnutrition prevalence in PD varies from 0-24%, with the malnutrition risk ranging from 3-60% [171]. Moreover, progressive weight loss is observed during PD [172] and has been associated with increases in cognitive decline [173] and mortality [174]. In a human brain imaging study, in PD, a widespread reduction in cerebral glucose uptake was observed by [18F] FDG PET along with cognitive decline [175]. More specifically, analysis by [18F] FDG PET also indicated, in PD, a reduced glucose uptake in the substantia nigra [175,176].

On the other hand, suggesting that different types of energy unbalances can promote PD, there is a heightened incidence of type 2 diabetes in PD patients [177].

Disruption of the ATP-producing system in PD.

Several lines of evidence support that adenosine triphosphate (ATP), or sufficient levels of ATP (energy), is protective against PD. For example, phosphorus and proton magnetic resonance spectroscopy studies have reported decreased ATP levels in the PD midbrain, putamen, and muscle [178]. Moreover, various mutations causing PD, including in PINK1 and SNCA, have been associated with an impaired ATP production [118,179,180]. Furthermore, research underscores ATP's protective potential against protein aggregation [181] and α-synuclein toxicity [182].

Also, evidence suggests that energy replenishment may prevent cell death, as maintaining a balance between ATP production and ROS appears to be vital for neuronal survival. For example, interventions enhancing ATP levels have demonstrated therapeutic potential in PD models [183]. An underlying reason may be that PD-associated mitochondrial dysfunction may prompt inefficient ATP production, exacerbating ROS generation and activating aerobic glycolysis (neuronal Warburg effect) [184]. Interestingly, it has been reported that α1-adrenoceptor blockers (terazosin, doxazosin, alfuzosin), which potentiate glycolysis, appear to help prevent PD-associated cognitive decline [185].

Purine nucleotides are synthesized by two pathways: a "de novo" synthetic pathway that biosynthesizes purine nucleotides using ribose-5-phosphate provided by the pentose phosphate pathway as material and a nucleotide re-synthesis (“salvage”) pathway using bases and nucleosides as materials (Figure 2). Autopsy brain studies have reported that the pentose monophosphate circuit may be extensively impaired in early PD [22]. The salvage synthesis pathway, which uses degraded

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nucleotides (IMPs) from ATP to produce ATP, is highly developed in humans and is essential for efficiently maintaining ATP [186]. In the salvage circuit, the process by which hypoxanthine, produced in the pathway where uric acid is synthesized from IMP, is recovered as IMP by hypoxanthine phosphoribosyltransferase (HPRT) is important. We have found that augmentation of this salvage synthesis pathway by administering febuxostat and inosine to patients can increase their plasma hypoxanthine levels and may improve the clinical manifestations of PD [187]. Figure 2 summarizes the energy impairments reported in PD.

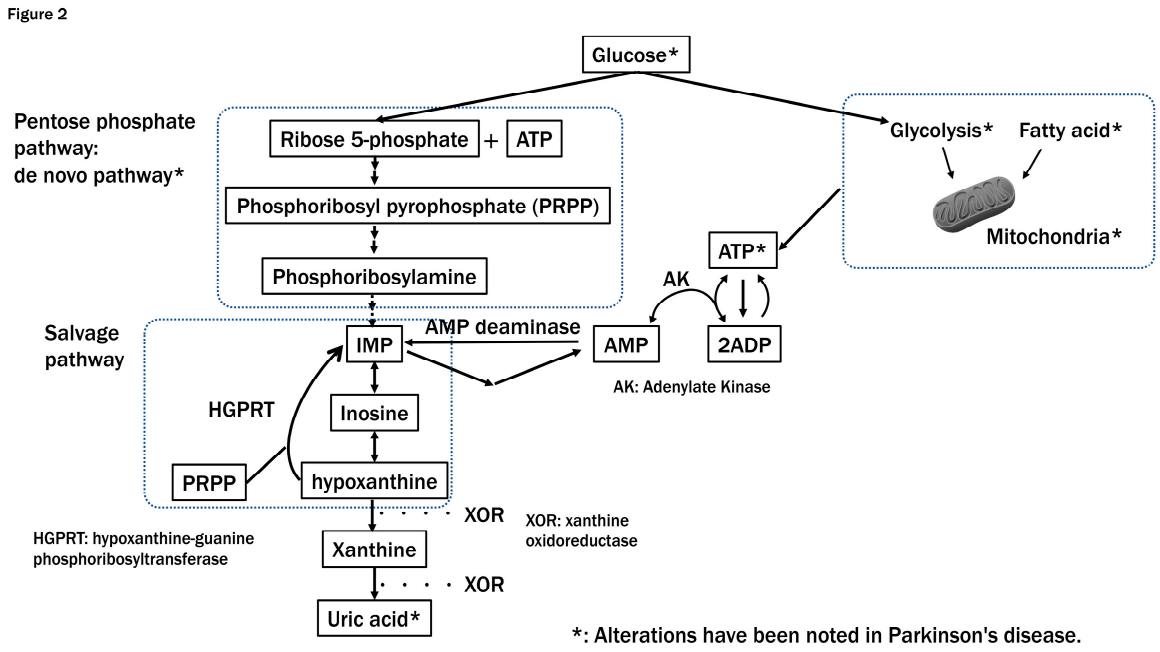

Figure 2. Major ATP synthesis pathways and their impairment in Parkinson's disease. In Parkinson's disease, extensive impairment of pathways that synthesize ATP, such as the pentose phosphate pathway, which plays a vital role in producing NADPH and purine synthesis, the mitochondrial and glycolytic systems, and fatty acids, has been reported. Decreased levels of uric acid, a purine metabolite, have also been reported. Figure modified from Johnson et al. [186].

Conclusion

This review discusses two main underlying causes of SNc DA neurons' specific vulnerability: (i) their high bioenergetic demands, and (ii) their high DA production. We summarized our discussions in Figure 1.

To a large degree, as shown by animals naturally showing similar phenomena, the preferential dying of SNc DA neurons upon aging seems unavoidable. In humans, however, the stresses on these neurons seem to be exceptionally high, as only humans have abundant NM and idiopathic PD. Although the first degeneration symptoms of SNc DA neurons in PD are believed to start in the axonal arbor in the striatum, there is the possibility that the cause of PD starts in the cell bodies in the SNc. Namely, this is the location where two main stressors, NM formation and cytosolic Ca^{2+} oscillations, occur. Although we are not aware of studies that addressed this, we speculate that in human SNc the local somatodendritic DA release may be exceptionally high compared to other species, possibly to enhance the within-SN pathway for DA from SNc to SNr.

The DA production system of SNc DA neurons seems to be tailored towards high DA production, such as by involving cytosolic Ca^{2+} oscillations without the protective presence of Calbindin-D28K, thereby creating a lot of oxidative stress for the mitochondria. That the mitochondria of these neurons are already stressed under non-PD conditions appears to be proven by the fact that various factors that damage mitochondrial functions seem to tip them over the edge

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and enhance PD. Mitochondria are the energy providers of the cell, so it is only logical that energy factors like nutrition also affect PD.

Logical approaches against PD progression include (i) reducing intracellular DA accumulation, (ii) blocking cytosolic Ca^{2+} oscillations, and (iii) providing bioenergetic support to PD patients.

As for reducing intracellular DA accumulation, especially in the SNc DA neuron cell bodies, we are not aware of any current treatments aimed at this. However, we assume that the regular DA release may explain the lesser PD risk of smokers. We also have speculated that regular exercise may increase protection against PD by releasing DA [28]. It is puzzling why isradipine, which blocks the cytosolic Ca^{2+} oscillations, has not been more successful against PD yet. We wonder if the benefit of protection against Ca^{2+} influx may not be masked by a decrease in DA release, possibly leading to increased DA-derived cytotoxicity. Although it is not still determined whether nutrition and other bioenergetic factors can be the primary drivers of PD or not, they include risk factors, and it has been shown that supporting the bioenergetics of PD patients is beneficial to them.

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Abbreviations

PD	Parkinson's disease
SNc	Substantia nigra pars compacta
DA	Dopamine
NE	Norepinephrine
LC	Locus coeruleus
NM	Neuromelanin
LB	Lewy bodies
TH	Tyrosine hydroxylase
CA	Catecholamine
VTA	Ventral tegmental area
Nac	Nucleus accumbens
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
OXPHOS	Oxidative phosphorylation
ROS	Reactive oxygen species
FDG	Fluorodeoxyglucose
ATP	Adenosine triphosphate

References

1. Berg, D.; Postuma, R.B.; Bloem, B.; Chan, P.; Dubois, B.; Gasser, T.; Goetz, C.G.; Halliday, G.M.; Hardy, J.; Lang, A.E.; et al. Time to Redefine PD? Introductory Statement of the MDS Task Force on the Definition of Parkinson's Disease. Mov. Disord. 2014, 29, 454–462.
2. Hornykiewicz, O. A Brief History of Levodopa. J. Neurol. 2010, 257, S249–S252.
3. Dorsey, E.R.; Sherer, T.; Okun, M.S.; Bloem, B.R. The Emerging Evidence of the Parkinson Pandemic. J. Parkinsons. Dis. 2018, 8, S3–S8.
4. Brichta, L.; Greengard, P. Molecular Determinants of Selective Dopaminergic Vulnerability in Parkinson's Disease: An Update. Front. Neuroanat. 2014, 8, 152.
5. Riederer, P.; Nagatsu, T.; Youdim, M.B.H.; Wulf, M.; Dijkstra, J.M.; Sian-Huelsmann, J. Lewy Bodies, Iron, Inflammation and Neuromelanin: Pathological Aspects Underlying Parkinson's Disease. J. Neural Transm. 2023, 130, 627–646.
6. Stark, A.K.; Pakkenberg, B. Histological Changes of the Dopaminergic Nigrostriatal System in Aging. Cell Tissue Res. 2004, 318, 81–92.

7. Bernheimer, H.; Birkmayer, W.; Hornykiewicz, O.; Jellinger, K.; Seitelberger, F. Brain Dopamine and the Syndromes of Parkinson and Huntington. Clinical, Morphological and Neurochemical Correlations. J. Neurol. Sci. 1973, 20, 415–455.
8. Riederer, P.; Wuketich, S. Time Course of Nigrostriatal Degeneration in Parkinson's Disease. A Detailed Study of Influential Factors in Human Brain Amine Analysis. J. Neural Transm. 1976, 38, 277–301.
9. Burke, R.E.; O'Malley, K. Axon Degeneration in Parkinson's Disease. Exp. Neurol. 2013, 246, 72–83.
10. Schalkamp, A.-K.; Peall, K.J.; Harrison, N.A.; Sandor, C. Wearable Movement-Tracking Data Identify Parkinson's Disease Years before Clinical Diagnosis. Nat. Med. 2023, 29, 2048–2056.
11. Blesa, J.; Foffani, G.; Dehay, B.; Bezard, E.; Obeso, J.A. Motor and Non-Motor Circuit Disturbances in Early Parkinson Disease: Which Happens First? Nat. Rev. Neurosci. 2021, doi:10.1038/s41583-021-00542-9.
12. Spillantini, M.G.; Schmidt, M.L.; Lee, V.M.; Trojanowski, J.Q.; Jakes, R.; Goedert, M. Alpha-Synuclein in Lewy Bodies. Nature 1997, 388, 839–840.
13. Watts, J.C. Calling α-Synuclein a Prion Is Scientifically Justifiable. Acta Neuropathol. 2019, 138, 505–508.
14. Markesbery, W.R.; Jicha, G.A.; Liu, H.; Schmitt, F.A. Lewy Body Pathology in Normal Elderly Subjects. J. Neuropathol. Exp. Neurol. 2009, 68, 816–822.
15. Braak, H.; Del Tredici, K.; Rüb, U.; de Vos, R.A.I.; Jansen Steur, E.N.H.; Braak, E. Staging of Brain Pathology Related to Sporadic Parkinson's Disease. Neurobiol. Aging 2003, 24, 197–211.
16. Braak, H.; Rüb, U.; Gai, W.P.; Del Tredici, K. Idiopathic Parkinson's Disease: Possible Routes by Which Vulnerable Neuronal Types May Be Subject to Neuroinvasion by an Unknown Pathogen. J. Neural Transm. 2003, 110, 517–536.
17. Hawkes, C.H.; Del Tredici, K.; Braak, H. Parkinson's Disease: A Dual-Hit Hypothesis. Neuropathol. Appl. Neurobiol. 2007, 33, 599–614.
18. Borghammer, P.; Just, M.K.; Horsager, J.; Skjærbæk, C.; Raunio, A.; Kok, E.H.; Savola, S.; Murayama, S.; Saito, Y.; Myllykangas, L.; et al. A Postmortem Study Suggests a Revision of the Dual-Hit Hypothesis of Parkinson's Disease. NPJ Parkinsons Dis 2022, 8, 166.
19. Horsager, J.; Andersen, K.B.; Knudsen, K.; Skjærbæk, C.; Fedorova, T.D.; Okkels, N.; Schaeffer, E.; Bonkat, S.K.; Geday, J.; Otto, M.; et al. Brain-First versus Body-First Parkinson's Disease: A Multimodal Imaging Case-Control Study. Brain 2020, 143, 3077–3088.
20. Parkkinen, L.; Pirttilä, T.; Alafuzoff, I. Applicability of Current Staging/categorization of Alpha-Synuclein Pathology and Their Clinical Relevance. Acta Neuropathol. 2008, 115, 399–407.
21. Koeglsperger, T.; Rumpf, S.-L.; Schließer, P.; Struebing, F.L.; Brendel, M.; Levin, J.; Trenkwalder, C.; Höglinger, G.U.; Herms, J. Neuropathology of Incidental Lewy Body & Prodromal Parkinson's Disease. Mol. Neurodegener. 2023, 18, 32.
22. Zimprich, A.; Biskup, S.; Leitner, P.; Lichtner, P.; Farrer, M.; Lincoln, S.; Kachergus, J.; Hulihan, M.; Uitti, R.J.; Calne, D.B.; et al. Mutations in LRRK2 Cause Autosomal-Dominant Parkinsonism with Pleomorphic Pathology. Neuron 2004, 44, 601–607.
23. Madsen, D.A.; Schmidt, S.I.; Blaabjerg, M.; Meyer, M. Interaction between Parkin and α-Synuclein in PARK2-Mediated Parkinson's Disease. Cells 2021, 10, doi:10.3390/cells10020283.
24. Kingsbury, A.E.; Bandopadhyay, R.; Silveira-Moriyama, L.; Ayling, H.; Kallis, C.; Sterlacci, W.; Maeir, H.; Poewe, W.; Lees, A.J. Brain Stem Pathology in Parkinson's Disease: An Evaluation of the Braak Staging Model. Mov. Disord. 2010, 25, 2508–2515.
25. Deng, H.; Yuan, L. Genetic Variants and Animal Models in SNCA and Parkinson Disease. Ageing Res. Rev. 2014, 15, 161–176.
26. Hirsch, E.; Graybiel, A.M.; Agid, Y.A. Melanized Dopaminergic Neurons Are Differentially Susceptible to Degeneration in Parkinson's Disease. Nature 1988, 334, 345–348.
27. Hirsch, E.C.; Graybiel, A.M.; Agid, Y. Selective Vulnerability of Pigmented Dopaminergic Neurons in Parkinson's Disease. Acta Neurol. Scand. Suppl. 1989, 126, 19–22.
28. Nagatsu, T.; Nakashima, A.; Watanabe, H.; Ito, S.; Wakamatsu, K.; Zucca, F.A.; Zecca, L.; Youdim, M.; Wulf, M.; Riederer, P.; et al. The Role of Tyrosine Hydroxylase as a Key Player in Neuromelanin Synthesis and the Association of Neuromelanin with Parkinson's Disease. J. Neural Transm. 2023, 130, 611–625.
29. Sulzer, D.; Surmeier, D.J. Neuronal Vulnerability, Pathogenesis, and Parkinson's Disease. Mov. Disord. 2013, 28, 715–724.
30. Zecca, L.; Fariello, R.; Riederer, P.; Sulzer, D.; Gatti, A.; Tampellini, D. The Absolute Concentration of Nigral Neuromelanin, Assayed by a New Sensitive Method, Increases throughout the Life and Is Dramatically Decreased in Parkinson's Disease. FEBS Lett. 2002, 510, 216–220.
31. Damier, P.; Hirsch, E.C.; Agid, Y.; Graybiel, A.M. The Substantia Nigra of the Human Brain. II. Patterns of Loss of Dopamine-Containing Neurons in Parkinson's Disease. Brain 1999, 122 (Pt 8), 1437–1448.
32. Cheng, H.-C.; Ulane, C.M.; Burke, R.E. Clinical Progression in Parkinson Disease and the Neurobiology of Axons. Ann. Neurol. 2010, 67, 715–725.
33. Hornykiewicz, O. Biochemical Aspects of Parkinson's Disease. Neurology 1998, 51, S2–S9.

34. Kish, S.J.; Shannak, K.; Hornykiewicz, O. Uneven Pattern of Dopamine Loss in the Striatum of Patients with Idiopathic Parkinson's Disease. Pathophysiologic and Clinical Implications. N. Engl. J. Med. 1988, 318, 876–880.
35. Alberico, S.L.; Cassell, M.D.; Narayanan, N.S. The Vulnerable Ventral Tegmental Area in Parkinson's Disease. Basal Ganglia 2015, 5, 51–55.
36. Mann, D.M.; Yates, P.O.; Hawkes, J. The Pathology of the Human Locus Ceruleus. Clin. Neuropathol. 1983, 2, 1–7.
37. Paredes-Rodriguez, E.; Vegas-Suarez, S.; Morera-Herreras, T.; De Deurwaerdere, P.; Miguelez, C. The Noradrenergic System in Parkinson's Disease. Front. Pharmacol. 2020, 11, 435.
38. Gesi, M.; Soldani, P.; Giorgi, F.S.; Santinami, A.; Bonaccorsi, I.; Fornai, F. The Role of the Locus Coeruleus in the Development of Parkinson's Disease. Neurosci. Biobehav. Rev. 2000, 24, 655–668.
39. Watabe-Uchida, M.; Zhu, L.; Ogawa, S.K.; Vamanrao, A.; Uchida, N. Whole-Brain Mapping of Direct Inputs to Midbrain Dopamine Neurons. Neuron 2012, 74, 858–873.
40. Morales, M.; Margolis, E.B. Ventral Tegmental Area: Cellular Heterogeneity, Connectivity and Behaviour. Nat. Rev. Neurosci. 2017, 18, 73–85.
41. Ueda, S.; Sakakibara, S.; Watanabe, E.; Yoshimoto, K.; Koibuchi, N. Vulnerability of Monoaminergic Neurons in the Brainstem of the Zitter Rat in Oxidative Stress. Prog. Brain Res. 2002, 136, 293–302.
42. Mavridis, I.; Boviatsis, E.; Anagnostopoulou, S. The Human Nucleus Accumbens Suffers Parkinsonism-Related Shrinkage: A Novel Finding. Surg. Radiol. Anat. 2011, 33, 595–599.
43. Hanganu, A.; Bedetti, C.; Degroot, C.; Mejia-Constain, B.; Lafontaine, A.-L.; Soland, V.; Chouinard, S.; Bruneau, M.-A.; Mellah, S.; Belleville, S.; et al. Mild Cognitive Impairment Is Linked with Faster Rate of Cortical Thinning in Patients with Parkinson's Disease Longitudinally. Brain 2014, 137, 1120–1129.
44. Wise, R.A. Dopamine, Learning and Motivation. Nat. Rev. Neurosci. 2004, 5, 483–494.
45. Puig, M.V.; Rose, J.; Schmidt, R.; Freund, N. Dopamine Modulation of Learning and Memory in the Prefrontal Cortex: Insights from Studies in Primates, Rodents, and Birds. Front. Neural Circuits 2014, 8, 93.
46. Tolstenkov, O.; Mikhaleva, Y.; Glover, J.C. A Miniaturized Nigrostriatal-like Circuit Regulating Locomotor Performance in a Protochordate. Curr. Biol. 2023, 33, 3872–3883.e6.
47. Ardiel, E.L.; Rankin, C.H. An Elegant Mind: Learning and Memory in Caenorhabditis Elegans. Learn. Mem. 2010, 17, 191–201.
48. Pérez-Fernández, J.; Barandela, M.; Jiménez-López, C. The Dopaminergic Control of Movement-Evolutionary Considerations. Int. J. Mol. Sci. 2021, 22, doi:10.3390/ijms222011284.
49. Yin, J.-A.; Liu, X.-J.; Yuan, J.; Jiang, J.; Cai, S.-Q. Longevity Manipulations Differentially Affect Serotonin/dopamine Level and Behavioral Deterioration in Aging Caenorhabditis Elegans. J. Neurosci. 2014, 34, 3947–3958.
50. Atkinson, L.E.; Liu, Y.; McKay, F.; Vandewyer, E.; Viau, C.; Irvine, A.; Rosa, B.A.; Li, Z.; Liang, Q.; Marks, N.J.; et al. Ascaris Suum Informs Extrasynaptic Volume Transmission in Nematodes. ACS Chem. Neurosci. 2021, 12, 3176–3188.
51. Emborg, M.E.; Ma, S.Y.; Mufson, E.J.; Levey, A.I.; Taylor, M.D.; Brown, W.D.; Holden, J.E.; Kordower, J.H. Age-Related Declines in Nigral Neuronal Function Correlate with Motor Impairments in Rhesus Monkeys. J. Comp. Neurol. 1998, 401, 253–265.
52. Noda, S.; Sato, S.; Fukuda, T.; Tada, N.; Hattori, N. Aging-Related Motor Function and Dopaminergic Neuronal Loss in C57BL/6 Mice. Mol. Brain 2020, 13, 46.
53. Li, H.; Su, L.-Y.; Yang, L.; Li, M.; Liu, Q.; Li, Z.; Hu, Y.; Li, H.; Wu, S.; Wang, W.; et al. A Cynomolgus Monkey with Naturally Occurring Parkinson's Disease. Natl Sci Rev 2021, 8, nwaa292.
54. Li, H.; Yao, Y.-G.; Hu, X.-T. Biological Implications and Limitations of a Cynomolgus Monkey with Naturally Occurring Parkinson's Disease. Zool Res 2021, 42, 138–140.
55. Adler, A. Melanin Pigment in the Brain of the Gorilla. J. Comp. Neurol. 1942, 76, 501–507.
56. Marsden, C.D. Pigmentation in the Nucleus Substantiae Nigrae of Mammals. J. Anat. 1961, 95, 256–261.
57. Sulzer, D.; Bogulavsky, J.; Larsen, K.E.; Behr, G.; Karatekin, E.; Kleinman, M.H.; Turro, N.; Krantz, D.; Edwards, R.H.; Greene, L.A.; et al. Neuromelanin Biosynthesis Is Driven by Excess Cytosolic Catecholamines Not Accumulated by Synaptic Vesicles. Proc. Natl. Acad. Sci. U. S. A. 2000, 97, 11869–11874.
58. Carballo-Carbajal, I.; Laguna, A.; Romero-Giménez, J.; Cuadros, T.; Bové, J.; Martinez-Vicente, M.; Parent, A.; Gonzalez-Sepulveda, M.; Peñuelas, N.; Torra, A.; et al. Brain Tyrosinase Overexpression Implicates Age-Dependent Neuromelanin Production in Parkinson's Disease Pathogenesis. Nat. Commun. 2019, 10, 973.
59. Raghanti, M.A.; Edler, M.K.; Stephenson, A.R.; Wilson, L.J.; Hopkins, W.D.; Ely, J.J.; Erwin, J.M.; Jacobs, B.; Hof, P.R.; Sherwood, C.C. Human-Specific Increase of Dopaminergic Innervation in a Striatal Region Associated with Speech and Language: A Comparative Analysis of the Primate Basal Ganglia. J. Comp. Neurol. 2016, 524, 2117–2129.

60. Raghanti, M.A.; Edler, M.K.; Stephenson, A.R.; Munger, E.L.; Jacobs, B.; Hof, P.R.; Sherwood, C.C.; Holloway, R.L.; Lovejoy, C.O. A Neurochemical Hypothesis for the Origin of Hominids. Proc. Natl. Acad. Sci. U. S. A. 2018, 115, E1108–E1116.
61. Sousa, A.M.M.; Zhu, Y.; Raghanti, M.A.; Kitchen, R.R.; Onorati, M.; Tebbenkamp, A.T.N.; Stutz, B.; Meyer, K.A.; Li, M.; Kawasawa, Y.I.; et al. Molecular and Cellular Reorganization of Neural Circuits in the Human Lineage. Science 2017, 358, 1027–1032.
62. Gibbons, A. Dopamine May Have given Humans Our Social Edge over Other Apes. Science 2018.
63. Xenias, H.S.; Ibáñez-Sandoval, O.; Koós, T.; Tepper, J.M. Are Striatal Tyrosine Hydroxylase Interneurons Dopaminergic? J. Neurosci. 2015, 35, 6584–6599.
64. Blandini, F.; Armentero, M.-T. Animal Models of Parkinson's Disease. FEBS J. 2012, 279, 1156–1166.
65. Gonzalez-Rodriguez, P.; Zampese, E.; Surmeier, D.J. Selective Neuronal Vulnerability in Parkinson's Disease. Prog. Brain Res. 2020, 252, 61–89.
66. Surmeier, D.J. From Causes of Selective Neuronal Vulnerability in Parkinson's Disease to a Phase III Clinical Trial. C. David Marsden Award Lecture. 20th International Congress of Parkinson's Disease and Movement Disorders Available online: https://www.youtube.com/watch?v=h1WGbC2vMtQ.
67. Rommelfanger, K.S.; Wichmann, T. Extrastriatal Dopaminergic Circuits of the Basal Ganglia. Front. Neuroanat. 2010, 4, 139.
68. Ubeda-Bañon, I.; Saiz-Sanchez, D.; Flores-Cuadrado, A.; Rioja-Corroto, E.; Gonzalez-Rodriguez, M.; Villar-Conde, S.; Astillero-Lopez, V.; Cabello-de la Rosa, J.P.; Gallardo-Alcañiz, M.J.; Vaamonde-Gamo, J.; et al. The Human Olfactory System in Two Proteinopathies: Alzheimer's and Parkinson's Diseases. Transl. Neurodegener. 2020, 9, 22.
69. Orimo, S.; Uchihara, T.; Kanazawa, T.; Itoh, Y.; Wakabayashi, K.; Kakita, A.; Takahashi, H. Unmyelinated Axons Are More Vulnerable to Degeneration than Myelinated Axons of the Cardiac Nerve in Parkinson's Disease. Neuropathol. Appl. Neurobiol. 2011, 37, 791–802.
70. Goldstein, D.S.; Holmes, C.; Sullivan, P.; Lopez, G.; Gelsomino, J.; Moore, S.; Isonaka, R.; Wu, T.; Sharabi, Y. Cardiac Noradrenergic Deficiency Revealed by 18F-Dopamine Positron Emission Tomography Identifies Preclinical Central Lewy Body Diseases. J. Clin. Invest. 2023, doi:10.1172/JCI172460.
71. Han, Y.; Wu, D.; Wang, Y.; Xie, J.; Zhang, Z. Skin Alpha-Synuclein Deposit Patterns: A Predictor of Parkinson's Disease Subtypes. EBioMedicine 2022, 80, 104076.
72. Nieuwenhuys, R. Structure and Organization of Fibre Systems. In The central nervous system of vertebrates; Ten Donkelaar H. J. Nicholson C., N.R., Ed.; 1999; Vol. 1, pp. 113–157.
73. Zampese, E.; Surmeier, D.J. Calcium, Bioenergetics, and Parkinson's Disease. Cells 2020, 9, doi:10.3390/cells9092045.
74. Surmeier, D.J.; Obeso, J.A.; Halliday, G.M. Selective neuronal vulnerability in Parkinson disease. Nat. Rev. Neurosci. 2017, 18, 101–113.
75. Liu, C.; Kershberg, L.; Wang, J.; Schneeberger, S.; Kaeser, P.S. Dopamine Secretion Is Mediated by Sparse Active Zone-like Release Sites. Cell 2018, 172, 706–718.e15.
76. Liu, C.; Goel, P.; Kaeser, P.S. Spatial and Temporal Scales of Dopamine Transmission. Nat. Rev. Neurosci. 2021, 22, 345–358.
77. Chinta, S.J.; Andersen, J.K. Dopaminergic Neurons. Int. J. Biochem. Cell Biol. 2005, 37, 942–946.
78. Matsuda, W.; Furuta, T.; Nakamura, K.C.; Hioki, H.; Fujiyama, F.; Arai, R.; Kaneko, T. Single Nigrostriatal Dopaminergic Neurons Form Widely Spread and Highly Dense Axonal Arborizations in the Neostriatum. J. Neurosci. 2009, 29, 444–453.
79. Bolam, J.P.; Pissadaki, E.K. Living on the Edge with Too Many Mouths to Feed: Why Dopamine Neurons Die. Mov. Disord. 2012, 27, 1478–1483.
80. Pissadaki, E.K.; Bolam, J.P. The Energy Cost of Action Potential Propagation in Dopamine Neurons: Clues to Susceptibility in Parkinson's Disease. Front. Comput. Neurosci. 2013, 7, 13.
81. Arbuthnott, G.W.; Wickens, J. Space, Time and Dopamine. Trends Neurosci. 2007, 30, 62–69.
82. Vandecasteele, M.; Glowinski, J.; Venance, L. Electrical Synapses between Dopaminergic Neurons of the Substantia Nigra Pars Compacta. J. Neurosci. 2005, 25, 291–298.
83. Joshua, M.; Adler, A.; Prut, Y.; Vaadia, E.; Wickens, J.R.; Bergman, H. Synchronization of Midbrain Dopaminergic Neurons Is Enhanced by Rewarding Events. Neuron 2009, 62, 695–704.
84. Eshel, N.; Tian, J.; Bukwich, M.; Uchida, N. Dopamine Neurons Share Common Response Function for Reward Prediction Error. Nat. Neurosci. 2016, 19, 479–486.
85. Wang, Y.; Toyoshima, O.; Kunimatsu, J.; Yamada, H.; Matsumoto, M. Tonic Firing Mode of Midbrain Dopamine Neurons Continuously Tracks Reward Values Changing Moment-by-Moment. Elife 2021, 10, doi:10.7554/eLife.63166.
86. Hamid, A.A.; Frank, M.J.; Moore, C.I. Wave-like Dopamine Dynamics as a Mechanism for Spatiotemporal Credit Assignment. Cell 2021, 184, 2733–2749.e16.
87. Travagli, R.A.; Gillis, R.A.; Rossiter, C.D.; Vicini, S. Glutamate and GABA-Mediated Synaptic Currents in Neurons of the Rat Dorsal Motor Nucleus of the Vagus. Am. J. Physiol. 1991, 260, G531–G536.

88. Trist, B.G.; Hare, D.J.; Double, K.L. Oxidative Stress in the Aging Substantia Nigra and the Etiology of Parkinson's Disease. Aging Cell 2019, 18, e13031.
89. Um, K.B.; Hahn, S.; Kim, S.W.; Lee, Y.J.; Birnbaumer, L.; Kim, H.J.; Park, M.K. TRPC3 and NALCN Channels Drive Pacemaking in Substantia Nigra Dopaminergic Neurons. Elife 2021, 10, doi:10.7554/eLife.70920.
90. Zampese, E.; Wokosin, D.L.; Gonzalez-Rodriguez, P.; Guzman, J.N.; Tkatch, T.; Kondapalli, J.; Surmeier, W.C.; D'Alessandro, K.B.; De Stefani, D.; Rizzuto, R.; et al. Ca2+ Channels Couple Spiking to Mitochondrial Metabolism in Substantia Nigra Dopaminergic Neurons. Sci Adv 2022, 8, eabp8701.
91. Tubert, C.; Zampese, E.; Pancani, T.; Tkatch, T.; Surmeier, D.J. Feed-Forward Metabotropic Signaling by Cav1 Ca2+ Channels Supports Pacemaking in Pedunculopontine Cholinergic Neurons. Neurobiol. Dis. 2023, 188, 106328.
92. Yamada, T.; McGeer, P.L.; Baimbridge, K.G.; McGeer, E.G. Relative Sparing in Parkinson's Disease of Substantia Nigra Dopamine Neurons Containing Calbindin-D28K. Brain Res. 1990, 526, 303–307.
93. Inoue, K.-I.; Miyachi, S.; Nishi, K.; Okado, H.; Nagai, Y.; Minamimoto, T.; Nambu, A.; Takada, M. Recruitment of Calbindin into Nigral Dopamine Neurons Protects against MPTP-Induced Parkinsonism. Mov. Disord. 2019, 34, 200–209.
94. Brimblecombe, K.R.; Vietti-Michelina, S.; Platt, N.J.; Kastli, R.; Hnieno, A.; Gracie, C.J.; Cragg, S.J. Calbindin-D28K Limits Dopamine Release in Ventral but Not Dorsal Striatum by Regulating Ca2+ Availability and Dopamine Transporter Function. ACS Chem. Neurosci. 2019, 10, 3419–3426.
95. Zhao, R.-Z.; Jiang, S.; Zhang, L.; Yu, Z.-B. Mitochondrial Electron Transport Chain, ROS Generation and Uncoupling (Review). Int. J. Mol. Med. 2019, 44, 3–15.
96. Raffaello, A.; Mammucari, C.; Gherardi, G.; Rizzuto, R. Calcium at the Center of Cell Signaling: Interplay between Endoplasmic Reticulum, Mitochondria, and Lysosomes. Trends Biochem. Sci. 2016, 41, 1035–1049.
97. Spinelli, J.B.; Haigis, M.C. The Multifaceted Contributions of Mitochondria to Cellular Metabolism. Nat. Cell Biol. 2018, 20, 745–754.
98. Dietz, J.V.; Fox, J.L.; Khalimonchuk, O. Down the Iron Path: Mitochondrial Iron Homeostasis and Beyond. Cells 2021, 10, doi:10.3390/cells10092198.
99. Eldeeb, M.A.; Thomas, R.A.; Ragheb, M.A.; Fallahi, A.; Fon, E.A. Mitochondrial Quality Control in Health and in Parkinson's Disease. Physiol. Rev. 2022, 102, 1721–1755.
100. Youle, R.J.; van der Bliek, A.M. Mitochondrial Fission, Fusion, and Stress. Science 2012, 337, 1062–1065.
101. Gottlieb, R.A.; Stotland, A. MitoTimer: A Novel Protein for Monitoring Mitochondrial Turnover in the Heart. J. Mol. Med. 2015, 93, 271–278.
102. Eisner, V.; Picard, M.; Hajnóczky, G. Mitochondrial Dynamics in Adaptive and Maladaptive Cellular Stress Responses. Nat. Cell Biol. 2018, 20, 755–765.
103. Onishi, M.; Nagumo, S.; Iwashita, S.; Okamoto, K. The ER Membrane Insertase Get1/2 Is Required for Efficient Mitophagy in Yeast. Biochem. Biophys. Res. Commun. 2018, 503, 14–20.
104. Liang, C.-L.; Wang, T.T.; Luby-Phelps, K.; German, D.C. Mitochondria Mass Is Low in Mouse Substantia Nigra Dopamine Neurons: Implications for Parkinson's Disease. Exp. Neurol. 2007, 203, 370–380.
105. Sokoloff, L.; Reivich, M.; Kennedy, C.; Des Rosiers, M.H.; Patlak, C.S.; Pettigrew, K.D.; Sakurada, O.; Shinohara, M. The [14C]deoxyglucose Method for the Measurement of Local Cerebral Glucose Utilization: Theory, Procedure, and Normal Values in the Conscious and Anesthetized Albino Rat. J. Neurochem. 1977, 28, 897–916.
106.
107. Bunney, B.S.; Aghajanian, G.K. D-Amphetamine-Induced Inhibition of Central Dopaminergic Neurons: Direct Effect or Mediated by a Striatonigral Feedback Pathway? Adv. Biochem. Psychopharmacol. 1977, 16, 577–582.
108. Shepard, P.D.; German, D.C. Electrophysiological and Pharmacological Evidence for the Existence of Distinct Subpopulations of Nigrostriatal Dopaminergic Neuron in the Rat. Neuroscience 1988, 27, 537–546.
109. Cheng, X.-T.; Huang, N.; Sheng, Z.-H. Programming Axonal Mitochondrial Maintenance and Bioenergetics in Neurodegeneration and Regeneration. Neuron 2022, 110, 1899–1923.
110. Reeve, A.K.; Grady, J.P.; Cosgrave, E.M.; Bennison, E.; Chen, C.; Hepplewhite, P.D.; Morris, C.M. Mitochondrial Dysfunction within the Synapses of Substantia Nigra Neurons in Parkinson's Disease. NPJ Parkinsons Dis 2018, 4, 9.
111. Guzman, J.N.; Sanchez-Padilla, J.; Wokosin, D.; Kondapalli, J.; Ilijic, E.; Schumacker, P.T.; Surmeier, D.J. Oxidant Stress Evoked by Pacemaking in Dopaminergic Neurons Is Attenuated by DJ-1. Nature 2010, 468, 696–700.
112. Guzman, J.N.; Ilijic, E.; Yang, B.; Sanchez-Padilla, J.; Wokosin, D.; Galtieri, D.; Kondapalli, J.; Schumacker, P.T.; Surmeier, D.J. Systemic Isradipine Treatment Diminishes Calcium-Dependent Mitochondrial Oxidant Stress. J. Clin. Invest. 2018, 128, 2266–2280.

113. Surmeier, D.J.; Nguyen, J.T.; Lancki, N.; Venuto, C.S.; Oakes, D.; Simuni, T.; Wyse, R.K. Re-Analysis of the STEADY-PD II Trial-Evidence for Slowing the Progression of Parkinson's Disease. Mov. Disord. 2022, 37, 334–342.
114. Venuto, C.S.; Yang, L.; Javidnia, M.; Oakes, D.; James Surmeier, D.; Simuni, T. Isradipine Plasma Pharmacokinetics and Exposure-Response in Early Parkinson's Disease. Ann Clin Transl Neurol 2021, 8, 603–612.
115. Parkinson Study Group STEADY-PD III Investigators Isradipine Versus Placebo in Early Parkinson Disease: A Randomized Trial. Ann. Intern. Med. 2020, 172, 591–598.
116. Brimblecombe, K.R.; Connor-Robson, N.; Bataille, C.J.R.; Roberts, B.M.; Gracie, C.; O'Connor, B.; Te Water Naude, R.; Karthik, G.; Russell, A.J.; Wade-Martins, R.; et al. Inhibition of Striatal Dopamine Release by the L-Type Calcium Channel Inhibitor Isradipine Co-Varies with Risk Factors for Parkinson's. Eur. J. Neurosci. 2023, doi:10.1111/ejn.16180.
117. Moon, H.E.; Paek, S.H. Mitochondrial Dysfunction in Parkinson's Disease. Exp. Neurobiol. 2015, 24, 103–116.
118. Malpartida, A.B.; Williamson, M.; Narendra, D.P.; Wade-Martins, R.; Ryan, B.J. Mitochondrial Dysfunction and Mitophagy in Parkinson's Disease: From Mechanism to Therapy. Trends Biochem. Sci. 2021, 46, 329–343.
119. Scarffe, L.A.; Stevens, D.A.; Dawson, V.L.; Dawson, T.M. Parkin and PINK1: Much More than Mitophagy. Trends Neurosci. 2014, 37, 315–324.
120. Palacino, J.J.; Sagi, D.; Goldberg, M.S.; Krauss, S.; Motz, C.; Wacker, M.; Klose, J.; Shen, J. Mitochondrial Dysfunction and Oxidative Damage in Parkin-Deficient Mice. J. Biol. Chem. 2004, 279, 18614–18622.
121. Luo, Y.; Hoffer, A.; Hoffer, B.; Qi, X. Mitochondria: A Therapeutic Target for Parkinson's Disease? Int. J. Mol. Sci. 2015, 16, 20704–20730.
122. Nicklas, W.J.; Vyas, I.; Heikkila, R.E. Inhibition of NADH-Linked Oxidation in Brain Mitochondria by 1-Methyl-4-Phenyl-Pyridine, a Metabolite of the Neurotoxin, 1-Methyl-4-Phenyl-1,2,5,6-Tetrahydropyridine. Life Sci. 1985, 36, 2503–2508.
123. Javitch, J.A.; D'Amato, R.J.; Strittmatter, S.M.; Snyder, S.H. Parkinsonism-Inducing Neurotoxin, N-Methyl-4-Phenyl-1,2,3,6 -Tetrahydropyridine: Uptake of the Metabolite N-Methyl-4-Phenylpyridine by Dopamine Neurons Explains Selective Toxicity. Proc. Natl. Acad. Sci. U. S. A. 1985, 82, 2173–2177.
124. González-Rodríguez, P.; Zampese, E.; Stout, K.A.; Guzman, J.N.; Ilijic, E.; Yang, B.; Tkatch, T.; Stavarache, M.A.; Wokosin, D.L.; Gao, L.; et al. Disruption of Mitochondrial Complex I Induces Progressive Parkinsonism. Nature 2021, 599, 650–656.
125. Hijaz, B.A.; Volpicelli-Daley, L.A. Initiation and Propagation of α-Synuclein Aggregation in the Nervous System. Mol. Neurodegener. 2020, 15, 19.
126. Hirsch, E.C.; Graybiel, A.M.; Duyckaerts, C.; Javoy-Agid, F. Neuronal Loss in the Pedunculopontine Tegmental Nucleus in Parkinson Disease and in Progressive Supranuclear Palsy. Proc. Natl. Acad. Sci. U. S. A. 1987, 84, 5976–5980.
127. Chambers, N.E.; Lanza, K.; Bishop, C. Pedunculopontine Nucleus Degeneration Contributes to Both Motor and Non-Motor Symptoms of Parkinson's Disease. Front. Pharmacol. 2019, 10, 1494.
128. Greenamyre, J.T.; Hastings, T.G. Biomedicine. Parkinson's--Divergent Causes, Convergent Mechanisms. Science 2004, 304, 1120–1122.
129. Miyazaki, I.; Asanuma, M. Dopaminergic Neuron-Specific Oxidative Stress Caused by Dopamine Itself. Acta Med. Okayama 2008, 62, 141–150.
130. Zecca, L.; Bellei, C.; Costi, P.; Albertini, A.; Monzani, E.; Casella, L.; Gallorini, M.; Bergamaschi, L.; Moscatelli, A.; Turro, N.J.; et al. New Melanic Pigments in the Human Brain That Accumulate in Aging and Block Environmental Toxic Metals. Proc. Natl. Acad. Sci. U. S. A. 2008, 105, 17567–17572.
131. Zucca, F.A.; Segura-Aguilar, J.; Ferrari, E.; Muñoz, P.; Paris, I.; Sulzer, D.; Sarna, T.; Casella, L.; Zecca, L. Interactions of Iron, Dopamine and Neuromelanin Pathways in Brain Aging and Parkinson's Disease. Prog. Neurobiol. 2017, 155, 96–119.
132. Parent, M.; Parent, A. Substantia Nigra and Parkinson's Disease: A Brief History of Their Long and Intimate Relationship. Can. J. Neurol. Sci. 2010, 37, 313–319.
133. Halliday, G.M.; Ophof, A.; Broe, M.; Jensen, P.H.; Kettle, E.; Fedorow, H.; Cartwright, M.I.; Griffiths, F.M.; Shepherd, C.E.; Double, K.L. Alpha-Synuclein Redistributes to Neuromelanin Lipid in the Substantia Nigra Early in Parkinson's Disease. Brain 2005, 128, 2654–2664.
134. Horowitz, M.P.; Greenamyre, J.T. Mitochondrial Iron Metabolism and Its Role in Neurodegeneration. J. Alzheimers. Dis. 2010, 20 Suppl 2, S551–S568.
135. Zareba, M.; Bober, A.; Korytowski, W.; Zecca, L.; Sarna, T. The Effect of a Synthetic Neuromelanin on Yield of Free Hydroxyl Radicals Generated in Model Systems. Biochim. Biophys. Acta 1995, 1271, 343–348.
136. Zecca, L.; Zucca, F.A.; Albertini, A.; Rizzio, E.; Fariello, R.G. A Proposed Dual Role of Neuromelanin in the Pathogenesis of Parkinson's Disease. Neurology 2006, 67, S8–S11.

137. Zecca, L.; Wilms, H.; Geick, S.; Claasen, J.-H.; Brandenburg, L.-O.; Holzknecht, C.; Panizza, M.L.; Zucca, F.A.; Deuschl, G.; Sievers, J.; et al. Human Neuromelanin Induces Neuroinflammation and Neurodegeneration in the Rat Substantia Nigra: Implications for Parkinson's Disease. Acta Neuropathol. 2008, 116, 47–55.
138. Sulzer, D.; Cassidy, C.; Horga, G.; Kang, U.J.; Fahn, S.; Casella, L.; Pezzoli, G.; Langley, J.; Hu, X.P.; Zucca, F.A.; et al. Neuromelanin detection by magnetic resonance imaging (MRI) and its promise as a biomarker for Parkinson's disease. npj Parkinson's Disease 2018, 4, 11.
139. Heinzel, S.; Berg, D.; Gasser, T.; Chen, H.; Yao, C.; Postuma, R.B.; MDS Task Force on the Definition of Parkinson's Disease Update of the MDS Research Criteria for Prodromal Parkinson's Disease. Mov. Disord. 2019, 34, 1464–1470.
140. Belin, A.C.; Westerlund, M. Parkinson's Disease: A Genetic Perspective. FEBS J. 2008, 275, 1377–1383.
141. Blauwendraat, C.; Nalls, M.A.; Singleton, A.B. The Genetic Architecture of Parkinson's Disease. Lancet Neurol. 2020, 19, 170–178.
142. Goldman, S.M. Environmental Toxins and Parkinson's Disease. Annu. Rev. Pharmacol. Toxicol. 2014, 54, 141–164.
143. Langston, J.W.; Ballard, P.; Tetrud, J.W.; Irwin, I. Chronic Parkinsonism in Humans due to a Product of Meperidine-Analog Synthesis. Science 1983, 219, 979–980.
144. Davis, G.C.; Williams, A.C.; Markey, S.P.; Ebert, M.H.; Caine, E.D.; Reichert, C.M.; Kopin, I.J. Chronic Parkinsonism Secondary to Intravenous Injection of Meperidine Analogues. Psychiatry Res. 1979, 1, 249–254.
145. Ullah, I.; Zhao, L.; Hai, Y.; Fahim, M.; Alwayli, D.; Wang, X.; Li, H. "Metal Elements and Pesticides as Risk Factors for Parkinson's Disease - A Review." Toxicol Rep 2021, 8, 607–616.
146. Goldman, S.M.; Weaver, F.M.; Stroupe, K.T.; Cao, L.; Gonzalez, B.; Colletta, K.; Brown, E.G.; Tanner, C.M. Risk of Parkinson Disease Among Service Members at Marine Corps Base Camp Lejeune. JAMA Neurol. 2023, 80, 673–681.
147. Riederer, P.; Sofic, E.; Rausch, W.D.; Schmidt, B.; Reynolds, G.P.; Jellinger, K.; Youdim, M.B. Transition Metals, Ferritin, Glutathione, and Ascorbic Acid in Parkinsonian Brains. J. Neurochem. 1989, 52, 515–520.
148. Sofic, E.; Lange, K.W.; Jellinger, K.; Riederer, P. Reduced and Oxidized Glutathione in the Substantia Nigra of Patients with Parkinson's Disease. Neurosci. Lett. 1992, 142, 128–130.
149. Martin, H.L.; Teismann, P. Glutathione--a Review on Its Role and Significance in Parkinson's Disease. FASEB J. 2009, 23, 3263–3272.
150. Riederer, P.; Monoranu, C.; Strobel, S.; Iordache, T.; Sian-Hülsmann, J. Iron as the Concert Master in the Pathogenic Orchestra Playing in Sporadic Parkinson's Disease. J. Neural Transm. 2021, 128, 1577–1598.
151. Youdim, M.B.; Ben-Shachar, D.; Riederer, P. The Possible Role of Iron in the Etiopathology of Parkinson's Disease. Mov. Disord. 1993, 8, 1–12.
152. Dexter, D.T.; Wells, F.R.; Agid, F.; Agid, Y.; Lees, A.J.; Jenner, P.; Marsden, C.D. Increased Nigral Iron Content in Postmortem Parkinsonian Brain. Lancet 1987, 2, 1219–1220.
153. Sofic, E.; Riederer, P.; Heinsen, H.; Beckmann, H.; Reynolds, G.P.; Hebenstreit, G.; Youdim, M.B. Increased Iron (III) and Total Iron Content in Post Mortem Substantia Nigra of Parkinsonian Brain. J. Neural Transm. 1988, 74, 199–205.
154. Wang, J.-Y.; Zhuang, Q.-Q.; Zhu, L.-B.; Zhu, H.; Li, T.; Li, R.; Chen, S.-F.; Huang, C.-P.; Zhang, X.; Zhu, J.-H. Meta-Analysis of Brain Iron Levels of Parkinson's Disease Patients Determined by Postmortem and MRI Measurements. Sci. Rep. 2016, 6, 36669.
155. Dixon, S.J.; Lemberg, K.M.; Lamprecht, M.R.; Skouta, R.; Zaitsev, E.M.; Gleason, C.E.; Patel, D.N.; Bauer, A.J.; Cantley, A.M.; Yang, W.S.; et al. Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death. Cell 2012, 149, 1060–1072.
156. Do Van, B.; Gouel, F.; Jonneaux, A.; Timmerman, K.; Gelé, P.; Pétrault, M.; Bastide, M.; Laloux, C.; Moreau, C.; Bordet, R.; et al. Ferroptosis, a Newly Characterized Form of Cell Death in Parkinson's Disease That Is Regulated by PKC. Neurobiol. Dis. 2016, 94, 169–178.
157. Grover, S.; Kumar Sreelatha, A.A.; Pihlstrom, L.; Domenighetti, C.; Schulte, C.; Sugier, P.-E.; Radivojkov-Blagojevic, M.; Lichtner, P.; Mohamed, O.; Portugal, B.; et al. Genome-Wide Association and Meta-Analysis of Age at Onset in Parkinson Disease: Evidence From the COURAGE-PD Consortium. Neurology 2022, 99, e698–e710.
158. López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. The Hallmarks of Aging. Cell 2013, 153, 1194–1217.
159. Lima, T.; Li, T.Y.; Mottis, A.; Auwerx, J. Pleiotropic Effects of Mitochondria in Aging. Nat Aging 2022, 2, 199–213.
160. Zhang, R.; Wang, Y.; Ye, K.; Picard, M.; Gu, Z. Independent Impacts of Aging on Mitochondrial DNA Quantity and Quality in Humans. BMC Genomics 2017, 18, 890.

161. Zecca, L.; Stroppolo, A.; Gatti, A.; Tampellini, D.; Toscani, M.; Gallorini, M.; Giaveri, G.; Arosio, P.; Santambrogio, P.; Fariello, R.G.; et al. The Role of Iron and Copper Molecules in the Neuronal Vulnerability of Locus Coeruleus and Substantia Nigra during Aging. Proc. Natl. Acad. Sci. U. S. A. 2004, 101, 9843–9848.
162. Venkateshappa, C.; Harish, G.; Mythri, R.B.; Mahadevan, A.; Bharath, M.M.S.; Shankar, S.K. Increased Oxidative Damage and Decreased Antioxidant Function in Aging Human Substantia Nigra Compared to Striatum: Implications for Parkinson's Disease. Neurochem. Res. 2012, 37, 358–369.
163. Finger, C.E.; Moreno-Gonzalez, I.; Gutierrez, A.; Moruno-Manchon, J.F.; McCullough, L.D. Age-Related Immune Alterations and Cerebrovascular Inflammation. Mol. Psychiatry 2022, 27, 803–818.
164. Cramb, K.M.L.; Beccano-Kelly, D.; Cragg, S.J.; Wade-Martins, R. Impaired Dopamine Release in Parkinson's Disease. Brain 2023, 146, 3117–3132.
165. Chen, H.; Huang, X.; Guo, X.; Mailman, R.B.; Park, Y.; Kamel, F.; Umbach, D.M.; Xu, Q.; Hollenbeck, A.; Schatzkin, A.; et al. Smoking Duration, Intensity, and Risk of Parkinson Disease. Neurology 2010, 74, 878–884.
166. Noyce, A.J.; Bestwick, J.P.; Silveira-Moriyama, L.; Hawkes, C.H.; Giovannoni, G.; Lees, A.J.; Schrag, A. Meta-Analysis of Early Nonmotor Features and Risk Factors for Parkinson Disease. Ann. Neurol. 2012, 72, 893–901.
167. Pathak, D.; Berthet, A.; Nakamura, K. Energy Failure: Does It Contribute to Neurodegeneration? Ann. Neurol. 2013, 74, 506–516.
168. Saiki, S.; Hatano, T.; Fujimaki, M.; Ishikawa, K.-I.; Mori, A.; Oji, Y.; Okuzumi, A.; Fukuhara, T.; Koinuma, T.; Imamichi, Y.; et al. Decreased Long-Chain Acylcarnitines from Insufficient β-Oxidation as Potential Early Diagnostic Markers for Parkinson's Disease. Sci. Rep. 2017, 7, 7328.
169. Chen, H.; Zhang, S.M.; Hernán, M.A.; Willett, W.C.; Ascherio, A. Weight Loss in Parkinson's Disease. Ann. Neurol. 2003, 53, 676–679.
170. Song, S.; Luo, Z.; Li, C.; Huang, X.; Shiroma, E.J.; Simonsick, E.M.; Chen, H. Changes in Body Composition Before and After Parkinson's Disease Diagnosis. Mov. Disord. 2021, 36, 1617–1623.
171. Sheard, J.M.; Ash, S.; Silburn, P.A.; Kerr, G.K. Prevalence of Malnutrition in Parkinson's Disease: A Systematic Review. Nutr. Rev. 2011, 69, 520–532.
172. Yong, V.W.; Tan, Y.J.; Ng, Y.-D.; Choo, X.Y.; Sugumaran, K.; Chinna, K.; Md Shah, M.N.; Raja Aman, R.R.A.; Moy, F.M.; Mohd Ramli, N.; et al. Progressive and Accelerated Weight and Body Fat Loss in Parkinson's Disease: A Three-Year Prospective Longitudinal Study. Parkinsonism Relat. Disord. 2020, 77, 28–35.
173. Kim, H.J.; Oh, E.S.; Lee, J.H.; Moon, J.S.; Oh, J.E.; Shin, J.W.; Lee, K.J.; Baek, I.C.; Jeong, S.-H.; Song, H.-J.; et al. Relationship between Changes of Body Mass Index (BMI) and Cognitive Decline in Parkinson's Disease (PD). Arch. Gerontol. Geriatr. 2012, 55, 70–72.
174. Park, K.; Oeda, T.; Kohsaka, M.; Tomita, S.; Umemura, A.; Sawada, H. Low Body Mass Index and Life Prognosis in Parkinson's Disease. Parkinsonism Relat. Disord. 2018, 55, 81–85.
175. Andersen, K.B.; Hansen, A.K.; Schacht, A.C.; Horsager, J.; Gottrup, H.; Klit, H.; Danielsen, E.H.; Poston, K.L.; Pavese, N.; Brooks, D.J.; et al. Synaptic Density and Glucose Consumption in Patients with Lewy Body Diseases: An [11 C]UCB-J and [18 F]FDG PET Study. Mov. Disord. 2023, 38, 796–805.
176. Schröter, N.; Blazhenets, G.; Frings, L.; Jost, W.H.; Weiller, C.; Rijntjes, M.; Meyer, P.T.; Brumberg, J. Nigral Glucose Metabolism as a Diagnostic Marker of Neurodegenerative Parkinsonian Syndromes. NPJ Parkinsons Dis 2022, 8, 123.
177. Cullinane, P.W.; de Pablo Fernandez, E.; König, A.; Outeiro, T.F.; Jaunmuktane, Z.; Warner, T.T. Type 2 Diabetes and Parkinson's Disease: A Focused Review of Current Concepts. Mov. Disord. 2023, 38, 162–177.
178. Hattingen, E.; Magerkurth, J.; Pilatus, U.; Mozer, A.; Seifried, C.; Steinmetz, H.; Zanella, F.; Hilker, R. Phosphorus and Proton Magnetic Resonance Spectroscopy Demonstrates Mitochondrial Dysfunction in Early and Advanced Parkinson's Disease. Brain 2009, 132, 3285–3297.
179. Nakano, M.; Imamura, H.; Sasaoka, N.; Yamamoto, M.; Uemura, N.; Shudo, T.; Fuchigami, T.; Takahashi, R.; Kakizuka, A. ATP Maintenance via Two Types of ATP Regulators Mitigates Pathological Phenotypes in Mouse Models of Parkinson's Disease. EBioMedicine 2017, 22, 225–241.
180. Tang, B.L. Glucose, Glycolysis, and Neurodegenerative Diseases. J. Cell. Physiol. 2020, 235, 7653–7662.
181. Patel, A.; Malinovska, L.; Saha, S.; Wang, J.; Alberti, S.; Krishnan, Y.; Hyman, A.A. ATP as a Biological Hydrotrope. Science 2017, 356, 753–756.
182. Takaine, M.; Imamura, H.; Yoshida, S. High and Stable ATP Levels Prevent Aberrant Intracellular Protein Aggregation in Yeast. Elife 2022, 11, doi:10.7554/eLife.67659.
183. McKenzie, M.; Liolitsa, D.; Akinshina, N.; Campanella, M.; Sisodiya, S.; Hargreaves, I.; Nirmalananthan, N.; Sweeney, M.G.; Abou-Sleiman, P.M.; Wood, N.W.; et al. Mitochondrial ND5 Gene Variation Associated with Encephalomyopathy and Mitochondrial ATP Consumption. J. Biol. Chem. 2007, 282, 36845–36852.
184. Dunn, L.; Allen, G.F.; Mamais, A.; Ling, H.; Li, A.; Duberley, K.E.; Hargreaves, I.P.; Pope, S.; Holton, J.L.; Lees, A.; et al. Dysregulation of Glucose Metabolism Is an Early Event in Sporadic Parkinson's Disease. Neurobiol. Aging 2014, 35, 1111–1115.

185. Weber, M.A.; Sivakumar, K.; Tabakovic, E.E.; Oya, M.; Aldridge, G.M.; Zhang, Q.; Simmering, J.E.; Narayanan, N.S. Glycolysis-Enhancing α1-Adrenergic Antagonists Modify Cognitive Symptoms Related to Parkinson's Disease. NPJ Parkinsons Dis 2023, 9, 32.
186. Johnson, T.A.; Jinnah, H.A.; Kamatani, N. Shortage of Cellular ATP as a Cause of Diseases and Strategies to Enhance ATP. Front. Pharmacol. 2019, 10, 98.
187. Watanabe, H.; Hattori, T.; Kume, A.; Misu, K.; Ito, T.; Koike, Y.; Johnson, T.A.; Kamitsuji, S.; Kamatani, N.; Sobue, G. Improved Parkinsons disease motor score in a single-arm open-label trial of febuxostat and inosine. Medicine 2020, 99, e21576.