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Calcium homeostasis, selective vulnerability and Parkinson's disease

C. Savio Chan*, Tracy S. Gertler*, and D. James Surmeier

Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA

Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder of which the core motor symptoms are attributable to the degeneration of dopamine (DA) neurons in the substantia nigra pars compacta (SNc). Recent work has revealed that the engagement of L-type Ca^{2+} channels during autonomous pacemaking renders SNc DA neurons susceptible to mitochondrial toxins used to create animal models of PD, indicating that homeostatic Ca^{2+} stress could be a determinant of their selective vulnerability. This view is buttressed by the central role of mitochondria and the endoplasmic reticulum (linchpins of current theories about the origins of PD) in Ca^{2+} homeostasis. Here, we summarize this evidence and suggest the dual roles had by these organelles could compromise their function, leading to accelerated aging of SNc DA neurons, particularly in the face of genetic or environmental stress. We conclude with a discussion of potential therapeutic strategies for slowing the progression of PD.

Introduction

Parkinson's disease (PD) is a disabling neurodegenerative disorder that is strongly associated with aging, increasing exponentially in incidence above the age of 65 [1,2]. The incidence of PD is expected to rise dramatically worldwide in the next 25 years with the extension of life expectancy by improved health care [3]. Although there are signs of distributed neuropathology, as judged by Lewy body (LB) formation, the motor symptoms of PD, including bradykinesia, rigidity and resting tremor, are clearly linked to the degeneration and death of substantia nigra pars compacta (SNc) dopamine (DA) neurons [4,5]. The efficacy of the clinical gold-standard treatment of L-DOPA (3,4-dihydroxy-L-phenylalanine; a DA precursor) is testament to the centrality of these neurons in PD. Here, we examine the evidence that the selective vulnerability of these neurons is attributable to their expression of a physiological phenotype that creates a sustained challenge to Ca^{2+} homeostasis.

Corresponding author: Surmeier D.J. (j-surmeier@northwestern.edu).

*These authors contributed equally.

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What causes SNc DA neurons to die in PD?

The mechanisms responsible for the preferential loss of DA neurons in PD have been debated for decades. A widely held theory implicates DA itself, suggesting that oxidation of cytosolic DA (and its metabolites) leads to the production of cytotoxic free radicals [6]. However, there are reasons to doubt this type of cellular stress is responsible for either normal aging or the loss of DA neurons in PD. For example, there is considerable regional variability in the vulnerability of DA neurons in PD, with some being devoid of pathological markers [7–11]. Moreover, L-DOPA administration (which relieves symptoms by elevating DA levels in PD patients) does not accelerate disease progression [12], indicating that DA is not a substantial source of reactive oxidative stress.

In recent years, attention has turned to the role of mitochondrial dysfunction in PD [13–15]. In addition to the ability of several toxins that target mitochondria to create a parkinsonian phenotype [16,17], compelling evidence for mitochondrial involvement in PD comes from the study of human PD patients. In postmortem tissue samples of the SNc from sporadic PD patients, there is a substantial decrease in the activity of mitochondrial NADH ubiquinone reductase, referred to as complex I of the electron transport chain (ETC) [18]; this deficit is specific to PD patients [19] and seems to reflect oxidative damage to complex I [20]. Oxidative damage to other cellular components such as lipids, proteins and DNA also has been found in the SNc of PD brains [21]. The source of this oxidative stress is largely mitochondrial: reactive oxygen species (ROS) and other radicals are generated by inefficiencies in the ETC; the ETC is responsible for creating the electrochemical gradient across the inner mitochondrial membrane that drives ATP synthase and the conversion of adenosine diphosphate to ATP [22]. ROS also are thought to be responsible for the high level of somatic DNA mutations in SNc DA neurons [23]. The physical proximity of mitochondrial DNA (mtDNA) to the site of ROS generation probably makes them an even more vulnerable target. The mitochondrial genome encodes 13 proteins involved in the mitochondria respiratory chain, 7 of which are involved in the formation of complex I [13]. The number of mtDNA mutations present in clonally expanded clusters of SNc mitochondria is positively correlated with age and negatively correlated with cytochrome oxidase activity (a marker for functional respiratory activity) [24]. Their clonal nature argues that these mutations are due to the expansion of a somatic mutation, not a genetic mutation present at birth. Because many of these mtDNA mutations impair ETC function [25], they are likely to contribute to the loss of SNc DA neurons. Lastly, although deficits in the complex I activity of platelets, skeletal muscle, fibroblasts and lymphocytes have been reported in some PD patients [26], this is not a consistent feature of the disease, arguing that mitochondrial dysfunction is regionally selective [27].

Further support for a mitochondrial link in PD comes from the rapidly expanding literature on genetic mutations associated with familial forms of PD [28]. Although much remains to be done in defining the roles of the proteins coded for by these genes, an extraordinary number of them are localized in or interact with mitochondria (Box 1).

Box 1**Genetic PD mutations and mitochondria**

Several of the genetic mutations associated with familial PD are directly linked to mitochondrial dysfunction. DJ-1 (*PARK7*), a genetic mutation associated with early-onset PD, has been putatively categorized as an atypical mitochondrial ‘peroxiredoxin-like peroxidase’, decreasing the accumulation of hydrogen peroxide and, thus, damage from downstream ROS production [106]. Parkin (*PARK2*) is a ubiquitin ligase tied to mitochondrial function in knockout studies in both mice and *Drosophila*. Fruit flies with functional deletions of Parkin possess fragmented and apoptotic mitochondria with compromised structural integrity [107]; knockout mice have a less dramatic but noteworthy syndromic phenotype (including decreased mitochondrial [respiratory] function, decreased metabolic drive and increased lipid and protein phosphorylation), indicative of functional mitochondrial impairment [108]. Phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1; *PARK6*), which also is associated with familial PD, leads to an identical phenotype in *Drosophila* loss-of-function mutants as does Parkin: fragmented cristae and apoptotic mitochondria; this phenotype can be rescued by Parkin overexpression, indicating involvement in some common biochemical pathway [109,110]. Although found both in cytosolic and mitochondrial preparations, PINK1 has an N-terminus mitochondrial targeting sequence [111]. Mutations of leucine-rich repeat kinase (LRRK2; *PARK8*) are associated with a much larger fraction of PD cases in some ethnic groups [112]; LRRK2 is largely cytosolic but a fraction of the protein is associated with mitochondria [113].

Another organelle that has been widely linked to pathogenesis in PD is the endoplasmic reticulum (ER). The ER is an integral component of the cellular machinery responsible for the production, delivery and degradation of proteins, a process referred to as proteostasis [29]. One of the hallmarks of PD is the formation of LBs, an abnormal protein aggregate found in SNc DA neurons and elsewhere in the brain [30]. These depositions reflect a deficiency in proteostasis that is accompanied by signs of ER stress and an attempt to sequester cytotoxic proteins [31]. In part, LBs in PD reflect the decline in proteostatic competence that accompanies normal aging [29]. What seems to distinguish PD is the presence of an additional proteostatic burden that causes an aged DA neuronal ensemble to fail *en masse* (see later) [15,32,33].

Taken together, the evidence for the involvement of mitochondria and ER in the PD pathogenesis is unequivocal. The crucial question is whether or not the disease begins with the dysfunction of these organelles. The striking regional distribution of deficits argues against the primacy of mitochondrial or ER dysfunction in PD. There is no evidence that mitochondria in cortical pyramidal neurons (which show little to no sign of pathology in PD) differ in any important respect from mitochondria in SNc DA neurons. There is no evidence for substantive variation in the ER (or other proteostatic elements) between vulnerable and resistant neurons either. Furthermore, there is no evidence of selective regional expression of genes associated with familial forms of PD that would be predictive of disease progression [28]. The most straightforward conclusion to be drawn from the evidence at hand is that the

cellular environment in which these organelles find themselves accelerates their decline with age, making them more vulnerable to genetic or environmental stress. What then is distinctive about the organelle environment created by SNc DA neurons?

SNc DA neurons have a distinctive physiological phenotype

SNc DA neurons have an unusual physiological phenotype. Unlike the vast majority of neurons in the brain, adult SNc DA neurons are autonomously active, generating action potentials in a clock-like (2–4 Hz) manner in the absence of synaptic input [34]. This pacemaking activity is believed to be important in maintaining ambient DA levels in regions that are innervated by these neurons, particularly the striatum [35]. Whereas most neurons rely on monovalent cation channels to drive pacemaking, SNc DA neurons also engage ion channels that enable Ca^{2+} to enter the cytoplasm [36–38], leading to elevated intracellular Ca^{2+} concentrations [39,40]. The L-type Ca^{2+} channels used by SNc DA neurons in pacemaking have a distinctive $\text{Ca}_v1.3$ pore-forming subunit encoded by *Cacna1d* [40,41]; channels with this subunit differ from other L-type Ca^{2+} channels in that they open at relatively hyperpolarized potentials, enabling them to help push the cell to spike threshold [38,40]. $\text{Ca}_v1.3$ Ca^{2+} channels are relatively rare, constituting only ~10% of the all the L-type Ca^{2+} channels found in the brain [42]. Not only are they rare but in many of the other neurons where they are found they have a different role than the one in SNc DA neurons; for example, in striatal medium spiny neurons, $\text{Ca}_v1.3$ Ca^{2+} channels are positioned near to synapses where they are only activated episodically in response to synaptic depolarization or dendritic action potentials [43].

The sustained engagement of $\text{Ca}_v1.3$ Ca^{2+} channels during pacemaking comes at an obvious metabolic cost to SNc DA neurons. Because of its involvement in cellular processes ranging from the regulation of enzyme activity to programmed cell death, Ca^{2+} is under very tight homeostatic control, with a cytosolic set point near to 100 nM; that is 10 000 times lower than the concentration of Ca^{2+} in the extracellular space [44–46]. Ca^{2+} entering neurons is rapidly sequestered or pumped back across the steep plasma membrane concentration gradient; this process requires energy stored in ATP or in ion gradients that are maintained with ATP-dependent pumps (Figure 1). In most neurons, Ca^{2+} channel opening is a rare event, occurring primarily during very brief action potentials. This makes the task and the metabolic cost to the cell readily manageable. But in SNc DA neurons, where $\text{Ca}_v1.3$ Ca^{2+} channels are open much of the time, the magnitude and the spatial extent of Ca^{2+} influx is much larger [39]. The two organelles most responsible for handling Ca^{2+} crossing the plasma membrane are the two organelles most closely linked to PD: the ER and the mitochondrion.

The ER and mitochondria partner in Ca^{2+} homeostasis

The ER sits squarely at the center of the machinery responsible for Ca^{2+} homeostasis [47]. The ER forms a continuous, intracellular network, enabling it to regulate both local and global Ca^{2+} signals. As in other neurons, the ER network in SNc DA neurons extends throughout the somatodendritic tree [48,49]. High-affinity ATP-dependent transporters move Ca^{2+} from the cytoplasm into the ER lumen. The absence of high-affinity, anchored

intraluminal Ca^{2+} buffers and the physical continuity of the lumen within the cell [50,51] enables the ER to rapidly ($\sim 30 \mu\text{m/s}$) redistribute Ca^{2+} between intracellular compartments, thus avoiding pro-apoptotic accumulations in the cytosol [49]. Ca^{2+} sequestered in the ER is released at sites where it can be pumped back across the plasma membrane or where it can be used to modulate cellular function [52–56]. However, the storage capacity of the ER is limited. BCL-2 family proteins, which are implicated in apoptosis, control the ER Ca^{2+} concentration and are capable of adjusting it in response to stress [57]. Movement away from this set point can compromise proteostasis. In part, this is a consequence of the fact that Ca^{2+} is an allosteric regulator of protein processing and folding [58,59]. Depleting ER Ca^{2+} stores induces ER stress and the unfolded protein response [58,59]. Conversely, proteostatic deficits in Alzheimer's disease have been associated with high ER Ca^{2+} concentrations and large changes in cytosolic Ca^{2+} concentration upon ER release [60], mirroring what has been found in wild-type SNc DA neurons [61].

Ca^{2+} released from the ER through inositol trisphosphate (IP_3) or ryanodine receptors can enter mitochondria at points of apposition between the organelles which form functional Ca^{2+} microdomains [62,63]. The steep potential gradient ($\sim -200 \text{ mV}$) across the inner mitochondrial membrane drives Ca^{2+} into the matrix through a pore called the Ca^{2+} uniporter [64]. This ER–mitochondrial system not only serves a role in Ca^{2+} buffering but also increases ATP production by stimulating enzymes of the tricarboxylic acid (TCA) cycle [65], helping to meet the metabolic demands associated with electrical activity and influx of Ca^{2+} . However, it is possible that, despite this normally stimulatory role, the large Ca^{2+} -buffering burden created by pacemaking in SNc DA neurons ultimately compromises mitochondrial ATP synthesis. In isolated brain mitochondria, elevating the Ca^{2+} concentration in the media induces a transient collapse of the matrix potential, reflecting the reversible opening of an inner membrane pore in response to the rise in matrix Ca^{2+} concentration [66]; elevating the concentration of Ca^{2+} further induces an irreversible mitochondrial depolarization attributable to the opening of the mitochondrial permeability transition pore (mPTP) [22]. During these transient periods of depolarization there is no electrochemical gradient to power ATP synthase, and ATP production halts [22]. Unpublished studies by our group have revealed that mitochondrial potential in SNc DA neurons oscillates during normal pacemaking, much like that of isolated mitochondria challenged by Ca^{2+} , indicating that mitochondrial ATP production is compromised by elevations in cytosolic Ca^{2+} concentration during pacemaking.

Judging by aging-related changes in phenotypic markers [67], the stress created by playing two roles seems to be inconsequential for either the ER or mitochondria in most neurons. However, it is not clear that this is true for SNc DA neurons. As outlined earlier, elevated intraluminal ER Ca^{2+} concentration could compromise the ability of the ER to properly fold and process proteins (Figure 2). Passing this Ca^{2+} on to mitochondria could compromise their ability to generate ATP, despite the concomitant increased demand for ATP posed by the need to move Ca^{2+} out of the cell. Attempts to meet this demand by accelerating ETC activity between periods of depolarization brought on by Ca^{2+} buffering might overwhelm oxidative defenses, leading to the escape of damaging ROS.

Is PD a manifestation of Ca²⁺-accelerated aging?

One of the oldest and most popular theories of aging is that it is a direct consequence of accumulated mtDNA and organelle damage produced by ROS and related reactive molecules generated by the ETC in the course of oxidative phosphorylation [68,69]. A corollary of this hypothesis is that the rate of aging is directly related to metabolic rate. There is no obvious reason not to extend this organismal postulate to individual cells. The reliance of SNc DA neurons on a metabolically expensive strategy to generate autonomous activity that taxes mitochondria should mean that they age more rapidly than other types of neuron. The added homeostatic weight on mitochondria and the ER by Ca²⁺ should exacerbate this metabolic stress.

Is PD simply a reflection of accelerated aging in neurons that rely too heavily upon Ca²⁺ channels to do their business? Age is undoubtedly the single strongest risk factor for PD [70,71]. Stereological estimates of normal aging-related cell death in humans argue that SNc DA neurons are at a higher risk than other neurons in the absence of disease, because they are lost at a higher rate (5–10% per decade), than many other types of neurons (some of which show no appreciable loss over a 6–7 decade span) [67]. In mammals with substantially shorter lifespans, loss of SNc DA neurons with age has not been seen reliably, but there is a clear decline in phenotypic markers with age and an increased susceptibility to toxins, arguing that they are on the same road traversed by human neurons [72–74]. Furthermore, the spatial pattern of the decline in phenotypic markers with age matches that seen in PD [75–77], arguing that they are closely related phenomena.

This ‘wear and tear’ theory of PD does not require a pathogenic agent. Moreover, it argues that there is no disease ‘onset’ other than the one created by the emergence of symptoms when the surviving SNc DA neurons are incapable of fully compensating for the loss of neighboring neurons [78]. It also predicts that we will all develop symptoms if we live long enough. Why then do some people become symptomatic in their 50s, others in their 60s or 70s or not at all? Genetic and environmental factors certainly could account for this variation [28,70,79]. These factors could increase (or decrease) the rate at which vulnerable neurons age by compromising (or enhancing) ER or mitochondrial function. Furthermore, because the rate of cell loss should increase with accumulated age-dependent organelle damage, it is entirely possible that at some point an inflammatory threshold is crossed, triggering microglia activation and further neuron loss [80].

Can the Ca²⁺-mediated cellular aging hypothesis account for the vulnerability of other cell types in PD? Certainly it is consistent with the diminished vulnerability of SNc DA neurons that express the Ca²⁺-binding protein calbindin [81,82]. Other regions of the brain that have cell loss paralleling that of the SNc are the locus ceruleus (LC) and hypothalamic tuberomammillary nucleus [30,83]. The neurons of the LC and the tuberomammillary neurons are similar to SNc DA neurons in several respects. Like SNc DA neurons, both LC and tuberomammillary neurons are autonomous pacemakers that depend upon L-type Ca²⁺ channels [84–86]. In contrast, DA neurons in the ventral tegmental area do not rely upon L-type Ca²⁺ channels for pacemaking and are relatively intact in PD patients and in animal models of PD [9,40,81,87,88]. DA neurons in the olfactory bulb also are autonomous

pacemakers and rely upon Ca^{2+} channels (although not L-type channels) [88]. Olfactory deficits have been associated with PD [89]; however, there is no obvious loss of olfactory bulb DA neurons [90]. Although this would seem to run counter to the Ca^{2+} hypothesis, this could simply be a consequence of the capacity of this region for adult neurogenesis [91].

Can PD be prevented?

If PD is a consequence of Ca^{2+} -accelerated aging in SNc DA neurons (and in those neurons with a similar phenotype) then reducing Ca^{2+} flux should delay the onset of PD symptoms and slow its progression. This might be possible with orally deliverable, dihydropyridine (DHP) L-type channel antagonists shown to be safe in humans [40]. Adult SNc DA neurons readily compensate for the antagonism of L-type $\text{Ca}_v1.3$ Ca^{2+} channels and continue pacemaking at a normal rate. More importantly, although the impact on mitochondrial and ER stress can only be inferred at this point, reducing Ca^{2+} influx during pacemaking dramatically diminishes the sensitivity of SNc DA neurons to toxins used to generate animal models of PD [40]. Furthermore, at neuroprotective doses of an L-type channel antagonist, mice have no obvious motor, learning or cognitive deficits, indicating that the patterned activity of SNc DA neurons is functionally unchanged [36,92].

Is there evidence that this strategy might work in humans to prevent or slow PD? Calcium channel antagonists (CCAs), including the DHPs used in animal studies, are commonly used in clinical practice, creating a potential database to be mined. A recent case-control study of hypertensive patients found a significant reduction in the observed risk of PD with CCA use, but not with medications that reduce blood pressure in other ways [93]. This finding is consistent with a retrospective examination of patients treated for hypertension with DHPs [94]. Given that many of the CCAs currently in use for hypertension are weak antagonists of $\text{Ca}_v1.3$ Ca^{2+} channels or have low lipophilicity and are unlikely to cross the blood–brain barrier effectively [95–97], the apparent neuroprotective effect of CCAs as a group indicates that those drugs overcoming these obstacles might be very potent neuroprotective agents.

That said, these studies are not a substitute for a controlled clinical trial. In the absence of a selective $\text{Ca}_v1.3$ Ca^{2+} channel antagonist, DHPs are the most attractive drug for such a trial. DHPs are more selective modulators of L-type channels than other CCAs approved for human use and many have good brain bioavailability [95]. However, most members of this drug class, including nimodipine (Nimotop) and nifedipine (Procardia, Adalat), are more potent negative modulators of $\text{Ca}_v1.2$ than $\text{Ca}_v1.3$ channels [98]. This is also true of isradipine (DynaCirc), but less so [99,100]. At the doses used to treat hypertension, isradipine has relatively minor side effects [101]. The question is whether it will prove neuroprotective at doses tolerated by the general population. Pharmacokinetic studies by our group have found that serum concentrations of isradipine achieved in mice that are protected (~2 ng/ml) against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 6-hydroxydopamine toxicity are very close to those achieved in humans with a very well tolerated daily dose (10 mg/day, isradipine), indicating that neuroprotection is achievable.

It is also worth considering how CCAs compare with other drugs that are being tested in clinical neuroprotection trials for PD. Although early trials with creatine, coenzyme Q10 and

other antioxidant supplements have been disappointing [102], they share the hypothesis that oxidative stress exacerbates the symptoms and progression of PD. Coenzyme Q10 is an electron acceptor for complexes I and II that seems to be compromised in PD patients [103] and is neuroprotective in animal models of PD [104]. Creatine is a substrate for ATP production that can both improve mitochondrial efficiency and reduce oxidative stress by buffering fluctuations in cellular energy production [105]. Both approaches are aimed at improving mitochondrial function rather than attacking the source of stress on mitochondria. This contrasts with the rationale for CCAs, in which the goal is to reduce the source of stress responsible for mitochondrial and ER dysfunction. Nevertheless, the two approaches could prove complementary, pointing to a combination therapy.

Conclusions

Ca²⁺ dyshomeostasis has long been thought to be important in neurodegeneration, but it usually is envisioned as a late stage consequence of organelle damage inflicted by some other challenge. The unusual reliance of SNc DA neurons on voltage-dependent L-type Ca²⁺ channels in autonomous pacemaking indicates that Ca²⁺ entry and dyshomeostasis could be a cause of their selective vulnerability rather than simply a late stage consequence. This hypothesis is consistent with the centrality of the ER and mitochondria (which are key organelles in Ca²⁺ homeostasis) in prevailing models of pathogenesis in PD. The additional stress placed upon these organelles by the demands associated with sustained Ca²⁺ entry could accelerate their aging and enhance the vulnerability of SNc DA neurons to genetic and environmental challenges. Although plausible and consistent with regional deficits seen in normal aging and PD, the proposition that Ca²⁺ entry during pacemaking compromises mitochondrial and ER function remains to be fully tested. The tools necessary to conduct this test are now becoming available. Nevertheless, given the plausibility of the link between Ca²⁺ and the loss of SNc DA neurons, the absence of any proven neuroprotective therapy in PD and the availability of Ca²⁺ channel antagonists that are well tolerated and approved for human use, it would seem that the human experiment should proceed now in the form of clinical neuroprotection trials.

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References

1. de Lau LM, et al. Incidence of parkinsonism and Parkinson disease in a general population: the Rotterdam Study. *Neurology*. 2004; 63:1240–1244. [PubMed: 15477545]
2. de Rijk MC, et al. Prevalence of parkinsonism and Parkinson's disease in Europe: the EUROPARKINSON Collaborative Study. European Community Concerted Action on the Epidemiology of Parkinson's disease. *J Neurol Neurosurg Psychiatry*. 1997; 62:10–15. [PubMed: 9010393]
3. Dorsey ER, et al. Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology*. 2007; 68:384–386. [PubMed: 17082464]
4. Hornykiewicz O. Dopamine (3-hydroxytyramine) and brain function. *Pharmacol Rev*. 1966; 18:925–964. [PubMed: 5328389]

5. 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. [PubMed: 956814]
6. Greenamyre JT, Hastings TG. Biomedicine. Parkinson's – divergent causes, convergent mechanisms. *Science.* 2004; 304:1120–1122. [PubMed: 15155938]
7. Damier P, et al. The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain.* 1999; 122:1437–1448. [PubMed: 10430830]
8. Ito H, et al. Calbindin-D28k in the basal ganglia of patients with parkinsonism. *Ann Neurol.* 1992; 32:543–550. [PubMed: 1456738]
9. Kish SJ, et al. 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. [PubMed: 3352672]
10. Matzuk MM, Saper CB. Preservation of hypothalamic dopaminergic neurons in Parkinson's disease. *Ann Neurol.* 1985; 18:552–555. [PubMed: 4073850]
11. Saper CB, et al. Medullary catecholaminergic neurons in the normal human brain and in Parkinson's disease. *Ann Neurol.* 1991; 29:577–584. [PubMed: 1892359]
12. Fahn S. Does levodopa slow or hasten the rate of progression of Parkinson's disease? *J Neurol.* 2005; 252(Suppl 4):IV37–IV42. [PubMed: 16222436]
13. Schapira AH. Mitochondria in the aetiology and pathogenesis of Parkinson's disease. *Lancet Neurol.* 2008; 7:97–109. [PubMed: 18093566]
14. Vila M, et al. Mitochondrial alterations in Parkinson's disease: new clues. *J Neurochem.* 2008; 107:317–328. [PubMed: 18680555]
15. Henchcliffe C, Beal MF. Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. *Nat Clin Pract Neurol.* 2008; 4:600–609. [PubMed: 18978800]
16. Przedborski S, et al. MPTP as a mitochondrial neurotoxic model of Parkinson's disease. *J Bioenerg Biomembr.* 2004; 36:375–379. [PubMed: 15377875]
17. Betarbet R, et al. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci.* 2000; 3:1301–1306. [PubMed: 11100151]
18. Mann VM, et al. Complex I, iron, and ferritin in Parkinson's disease substantia nigra. *Ann Neurol.* 1994; 36:876–881. [PubMed: 7998774]
19. Gu M, et al. Mitochondrial respiratory chain function in multiple system atrophy. *Mov Disord.* 1997; 12:418–422. [PubMed: 9159739]
20. Keeney PM, et al. Parkinson's disease brain mitochondrial complex I has oxidatively damaged subunits and is functionally impaired and misassembled. *J Neurosci.* 2006; 26:5256–5264. [PubMed: 16687518]
21. Zhang J, et al. Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. *Am J Pathol.* 1999; 154:1423–1429. [PubMed: 10329595]
22. Nicholls, DG.; Ferguson, SJ. *Bioenergetics.* 1. Vol. 3. Academic Press; 2002.
23. Soong NW, et al. Mosaicism for a specific somatic mitochondrial DNA mutation in adult human brain. *Nat Genet.* 1992; 2:318–323. [PubMed: 1303287]
24. Kraytsberg Y, et al. Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. *Nat Genet.* 2006; 38:518–520. [PubMed: 16604072]
25. Bender A, et al. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nat Genet.* 2006; 38:515–517. [PubMed: 16604074]
26. Tretter L, et al. Initiation of neuronal damage by complex I deficiency and oxidative stress in Parkinson's disease. *Neurochem Res.* 2004; 29:569–577. [PubMed: 15038604]
27. Jenner P. Parkinson's disease, pesticides and mitochondrial dysfunction. *Trends Neurosci.* 2001; 24:245–247. [PubMed: 11311359]
28. Moore DJ, et al. Molecular pathophysiology of Parkinson's disease. *Annu Rev Neurosci.* 2005; 28:57–87. [PubMed: 16022590]
29. Balch WE, et al. Adapting proteostasis for disease intervention. *Science.* 2008; 319:916–919. [PubMed: 18276881]

30. Del Tredici, K.; Braak, H. Idiopathic Parkinson's disease: staging an α -synucleinopathy with a predictable pathoanatomy. In: Kahle, P.J.; Haas, C., editors. *Molecular Mechanisms of Parkinson's Disease*. Landes Bioscience; 2004. p. 1-32.
31. Ryu EJ, et al. Endoplasmic reticulum stress and the unfolded protein response in cellular models of Parkinson's disease. *J Neurosci*. 2002; 22:10690–10698. [PubMed: 12486162]
32. Kaushik S, Cuervo AM. Chaperone-mediated autophagy. *Methods Mol Biol*. 2008; 445:227–244. [PubMed: 18425454]
33. Wang HQ, Takahashi R. Expanding insights on the involvement of endoplasmic reticulum stress in Parkinson's disease. *Antioxid Redox Signal*. 2007; 9:553–561. [PubMed: 17465880]
34. Grace AA, Bunney BS. Intracellular and extracellular electrophysiology of nigral dopaminergic neurons – 2. Action potential generating mechanisms and morphological correlates. *Neuroscience*. 1983; 10:317–331. [PubMed: 6633864]
35. Romo R, Schultz W. Dopamine neurons of the monkey midbrain: contingencies of responses to active touch during self-initiated arm movements. *J Neurophysiol*. 1990; 63:592–606. [PubMed: 2329363]
36. Bonci A, et al. L-Type calcium channels mediate a slow excitatory synaptic transmission in rat midbrain dopaminergic neurons. *J Neurosci*. 1998; 18:6693–6703. [PubMed: 9712641]
37. Ping HX, Shepard PD. Apamin-sensitive Ca^{2+} -activated K^{+} channels regulate pacemaker activity in nigral dopamine neurons. *Neuroreport*. 1996; 7:809–814. [PubMed: 8733751]
38. Puopolo M, et al. Roles of subthreshold calcium current and sodium current in spontaneous firing of mouse midbrain dopamine neurons. *J Neurosci*. 2007; 27:645–656. [PubMed: 17234596]
39. Wilson CJ, Callaway JC. Coupled oscillator model of the dopaminergic neuron of the substantia nigra. *J Neurophysiol*. 2000; 83:3084–3100. [PubMed: 10805703]
40. Chan CS, et al. 'Rejuvenation' protects neurons in mouse models of Parkinson's disease. *Nature*. 2007; 447:1081–1086. [PubMed: 17558391]
41. Striessnig J, et al. Role of voltage-gated L-type Ca^{2+} channel isoforms for brain function. *Biochem Soc Trans*. 2006; 34:903–909. [PubMed: 17052224]
42. Sinnegger-Brauns MJ, et al. Expression and 1,4-dihydropyridine-binding properties of brain L-type calcium channel isoforms. *Mol Pharmacol*. 2008; 75:407–414. [PubMed: 19029287]
43. Olson PA, et al. G-protein-coupled receptor modulation of striatal $\text{Ca}_v1.3$ L-type Ca^{2+} channels is dependent on a Shank-binding domain. *J Neurosci*. 2005; 25:1050–1062. [PubMed: 15689540]
44. Rizzuto R. Intracellular Ca^{2+} pools in neuronal signalling. *Curr Opin Neurobiol*. 2001; 11:306–311. [PubMed: 11399428]
45. Orrenius S, et al. Regulation of cell death: the calcium-apoptosis link. *Nat Rev Mol Cell Biol*. 2003; 4:552–565. [PubMed: 12838338]
46. Berridge MJ, et al. The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol*. 2000; 1:11–21. [PubMed: 11413485]
47. Berridge MJ. The endoplasmic reticulum: a multifunctional signaling organelle. *Cell Calcium*. 2002; 32:235–249. [PubMed: 12543086]
48. Schwyn RC, Fox CA. The primate substantia nigra: a Golgi and electron microscopic study. *J Hirnforsch*. 1974; 15:95–126. [PubMed: 4135903]
49. Choi YM, et al. Regional interaction of endoplasmic reticulum Ca^{2+} signals between soma and dendrites through rapid luminal Ca^{2+} diffusion. *J Neurosci*. 2006; 26:12127–12136. [PubMed: 17122037]
50. Park MK, et al. The endoplasmic reticulum as one continuous Ca^{2+} pool: visualization of rapid Ca^{2+} movements and equilibration. *EMBO J*. 2000; 19:5729–5739. [PubMed: 11060024]
51. Mogami H, et al. Ca^{2+} flow via tunnels in polarized cells: recharging of apical Ca^{2+} stores by focal Ca^{2+} entry through basal membrane patch. *Cell*. 1997; 88:49–55. [PubMed: 9019404]
52. Cui G, et al. Spontaneous opening of T-type Ca^{2+} channels contributes to the irregular firing of dopamine neurons in neonatal rats. *J Neurosci*. 2004; 24:11079–11087. [PubMed: 15590924]
53. Bardo S, et al. The role of the endoplasmic reticulum Ca^{2+} store in the plasticity of central neurons. *Trends Pharmacol Sci*. 2006; 27:78–84. [PubMed: 16412523]

54. Rose CR, Konnerth A. Stores not just for storage. intracellular calcium release and synaptic plasticity. *Neuron*. 2001; 31:519–522. [PubMed: 11545711]
55. Verkhratsky A. Physiology and pathophysiology of the calcium store in the endoplasmic reticulum of neurons. *Physiol Rev*. 2005; 85:201–279. [PubMed: 15618481]
56. Park MK, et al. The endoplasmic reticulum as an integrator of multiple dendritic events. *Neuroscientist*. 2008; 14:68–77. [PubMed: 17911213]
57. Hetz CA. ER stress signaling and the BCL-2 family of proteins: from adaptation to irreversible cellular damage. *Antioxid Redox Signal*. 2007; 9:2345–2355. [PubMed: 17854276]
58. Toescu EC. Normal brain ageing: models and mechanisms. *Philos Trans R Soc Lond B Biol Sci*. 2005; 360:2347–2354. [PubMed: 16321805]
59. Paschen W, Mengesdorf T. Endoplasmic reticulum stress response and neurodegeneration. *Cell Calcium*. 2005; 38:409–415. [PubMed: 16087231]
60. LaFerla FM. Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. *Natl Rev Neurosci*. 2002; 3:862–872.
61. Morikawa H, et al. Inositol 1,4,5-triphosphate-evoked responses in midbrain dopamine neurons. *J Neurosci*. 2000; 20:RC103. [PubMed: 11027254]
62. Rizzuto R, Pozzan T. Microdomains of intracellular Ca^{2+} : molecular determinants and functional consequences. *Physiol Rev*. 2006; 86:369–408. [PubMed: 16371601]
63. Csordas G, et al. Structural and functional features and significance of the physical linkage between ER and mitochondria. *J Cell Biol*. 2006; 174:915–921. [PubMed: 16982799]
64. Kirichok Y, et al. The mitochondrial calcium uniporter is a highly selective ion channel. *Nature*. 2004; 427:360–364. [PubMed: 14737170]
65. McCormack JG, et al. Role of calcium ions in regulation of mammalian intramitochondrial metabolism. *Physiol Rev*. 1990; 70:391–425. [PubMed: 2157230]
66. Vergun O, Reynolds IJ. Distinct characteristics of Ca^{2+} -induced depolarization of isolated brain and liver mitochondria. *Biochim Biophys Acta*. 2005; 1709:127–137. [PubMed: 16112074]
67. Stark AK, Pakkenberg B. Histological changes of the dopaminergic nigrostriatal system in aging. *Cell Tissue Res*. 2004; 318:81–92. [PubMed: 15365813]
68. Harman D. The biologic clock: the mitochondria? *J Am Geriatr Soc*. 1972; 20:145–147. [PubMed: 5016631]
69. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet*. 2005; 39:359–407. [PubMed: 16285865]
70. Calne DB, Langston JW. Aetiology of Parkinson's disease. *Lancet*. 1983; 2:1457–1459. [PubMed: 6140548]
71. Gibb WR, Lees AJ. Anatomy, pigmentation, ventral and dorsal subpopulations of the substantia nigra, and differential cell death in Parkinson's disease. *J Neurol Neurosurg Psychiatry*. 1991; 54:388–396. [PubMed: 1865199]
72. McCormack AL, et al. Aging of the nigrostriatal system in the squirrel monkey. *J Comp Neurol*. 2004; 471:387–395. [PubMed: 15022260]
73. Ishikawa T, et al. Comparative nigrostriatal dopaminergic imaging with iodine-123- β CIT-FP/ SPECT and fluorine-18-FDOPA/PET. *J Nucl Med*. 1996; 37:1760–1765. [PubMed: 8917170]
74. Backman L, et al. Age-related cognitive deficits mediated by changes in the striatal dopamine system. *Am J Psychiatry*. 2000; 157:635–637. [PubMed: 10739428]
75. Collier TJ, et al. Aging-related changes in the nigrostriatal dopamine system and the response to MPTP in nonhuman primates: diminished compensatory mechanisms as a prelude to parkinsonism. *Neurobiol Dis*. 2007; 26:56–65. [PubMed: 17254792]
76. Kanaan NM, et al. Age-related accumulation of Marinesco bodies and lipofuscin in rhesus monkey midbrain dopamine neurons: relevance to selective neuronal vulnerability. *J Comp Neurol*. 2007; 502:683–700. [PubMed: 17436290]
77. Kanaan NM, et al. Age-related changes in dopamine transporters and accumulation of 3-nitrotyrosine in rhesus monkey midbrain dopamine neurons: relevance in selective neuronal vulnerability to degeneration. *Eur J Neurosci*. 2008; 27:3205–3215. [PubMed: 18598263]

78. Zigmond MJ, et al. Compensations after lesions of central dopaminergic neurons: some clinical and basic implications. *Trends Neurosci.* 1990; 13:290–296. [PubMed: 1695406]
79. Sulzer D. Multiple hit hypotheses for dopamine neuron loss in Parkinson's disease. *Trends Neurosci.* 2007; 30:244–250. [PubMed: 17418429]
80. McGeer PL, McGeer EG. Glial reactions in Parkinson's disease. *Mov Disord.* 2008; 23:474–483. [PubMed: 18044695]
81. German DC, et al. Midbrain dopaminergic cell loss in Parkinson's disease and MPTP-induced parkinsonism: sparing of calbindin-D28k-containing cells. *Ann N Y Acad Sci.* 1992; 648:42–62. [PubMed: 1353337]
82. Yamada T, et al. Relative sparing in Parkinson's disease of substantia nigra dopamine neurons containing calbindin-D28K. *Brain Res.* 1990; 526:303–307. [PubMed: 2257487]
83. German DC, et al. Disease-specific patterns of locus coeruleus cell loss. *Ann Neurol.* 1992; 32:667–676. [PubMed: 1449247]
84. Williams JT, et al. Membrane properties of rat locus coeruleus neurones. *Neuroscience.* 1984; 13:137–156. [PubMed: 6493483]
85. Stevens DR, Haas HL. Calcium-dependent prepotentials contribute to spontaneous activity in rat tuberomammillary neurons. *J Physiol.* 1996; 493:747–754. [PubMed: 8799896]
86. Taddese A, Bean BP. Subthreshold sodium current from rapidly inactivating sodium channels drives spontaneous firing of tuberomammillary neurons. *Neuron.* 2002; 33:587–600. [PubMed: 11856532]
87. Belzunegui S, et al. The number of dopaminergic cells is increased in the olfactory bulb of monkeys chronically exposed to MPTP. *Synapse.* 2007; 61:1006–1012. [PubMed: 17853434]
88. Pignatelli A, et al. Functional properties of dopaminergic neurones in the mouse olfactory bulb. *J Physiol.* 2005; 564:501–514. [PubMed: 15731185]
89. Postuma RB, et al. Potential early markers of Parkinson disease in idiopathic REM sleep behavior disorder. *Neurology.* 2006; 66:845–851. [PubMed: 16567700]
90. Huisman E, et al. Gender-related changes in increase of dopaminergic neurons in the olfactory bulb of Parkinson's disease patients. *Mov Disord.* 2008; 23:1407–1413. [PubMed: 18581481]
91. Pignatelli A, et al. A potential reservoir of immature dopaminergic replacement neurons in the adult mammalian olfactory bulb. *Pflugers Arch.* 2009:899–915. [PubMed: 19011893]
92. Grace AA, Bunney BS. The control of firing pattern in nigral dopamine neurons: single spike firing. *J Neurosci.* 1984; 4:2866–2876. [PubMed: 6150070]
93. Becker C, et al. Use of statins and the risk of Parkinson's disease: a retrospective case-control study in the UK. *Drug Saf.* 2008; 31:399–407. [PubMed: 18422380]
94. Rodnitzky RL. Can calcium antagonists provide a neuroprotective effect in Parkinson's disease? *Drugs.* 1999; 57:845–849. [PubMed: 10400399]
95. Kupsch A, et al. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity in non-human primates is antagonized by pretreatment with nimodipine at the nigral, but not at the striatal level. *Brain Res.* 1996; 741:185–196. [PubMed: 9001722]
96. Mannhold R, et al. Comparative evaluation of the predictive power of calculation procedures for molecular lipophilicity. *J Pharm Sci.* 1995; 84:1410–1419. [PubMed: 8748322]
97. Eisenberg MJ, et al. Calcium channel blockers: an update. *Am J Med.* 2004; 116:35–43. [PubMed: 14706664]
98. Xu W, Lipscombe D. Neuronal $\text{Ca}_v1.3\alpha_1$ L-type channels activate at relatively hyperpolarized membrane potentials and are incompletely inhibited by dihydropyridines. *J Neurosci.* 2001; 21:5944–5951. [PubMed: 11487617]
99. Koschak A, et al. $\alpha_1\text{D}$ ($\text{Cav}1.3$) subunits can form L-type Ca^{2+} channels activating at negative voltages. *J Biol Chem.* 2001; 276:22100–22106. [PubMed: 11285265]
100. Scholze A, et al. Functional expression and characterization of a voltage-gated $\text{Ca}_v1.3$ ($\alpha_1\text{D}$) calcium channel subunit from an insulin-secreting cell line. *Molecular endocrinol.* 2001; 15:1211–1221.

101. Fitton A, Benfield P. Isradipine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in cardiovascular disease. *Drugs*. 1990; 40:31–74. [PubMed: 2143980]
102. Hung AY, Schwarzschild MA. Clinical trials for neuroprotection in Parkinson's disease: overcoming angst and futility? *Curr Opin Neurol*. 2007; 20:477–483. [PubMed: 17620885]
103. Shults CW, et al. Coenzyme Q10 levels correlate with the activities of complexes I and II/III in mitochondria from parkinsonian and nonparkinsonian subjects. *Ann Neurol*. 1997; 42:261–264. [PubMed: 9266740]
104. Beal MF, et al. Coenzyme Q10 attenuates the 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP) induced loss of striatal dopamine and dopaminergic axons in aged mice. *Brain Res*. 1998; 783:109–114. [PubMed: 9479058]
105. Matthews RT, et al. Creatine and cyclocreatine attenuate MPTP neurotoxicity. *Exp Neurol*. 1999; 157:142–149. [PubMed: 10222117]
106. Andres-Mateos E, et al. DJ-1 gene deletion reveals that DJ-1 is an atypical peroxiredoxin-like peroxidase. *Proc Natl Acad Sci U S A*. 2007; 104:14807–14812. [PubMed: 17766438]
107. Greene JC, et al. Mitochondrial pathology and apoptotic muscle degeneration in *Drosophila parkin* mutants. *Proc Natl Acad Sci U S A*. 2003; 100:4078–4083. [PubMed: 12642658]
108. Palacino JJ, et al. Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. *J Biol Chem*. 2004; 279:18614–18622. [PubMed: 14985362]
109. Clark IE, et al. *Drosophila pink1* is required for mitochondrial function and interacts genetically with *parkin*. *Nature*. 2006; 441:1162–1166. [PubMed: 16672981]
110. Park J, et al. Mitochondrial dysfunction in *Drosophila PINK1* mutants is complemented by *parkin*. *Nature*. 2006; 441:1157–1161. [PubMed: 16672980]
111. Exner N, et al. Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin. *J Neurosci*. 2007; 27:12413–12418. [PubMed: 17989306]
112. Ishihara L, et al. Screening for Lrrk2 G2019S and clinical comparison of Tunisian and North American Caucasian Parkinson's disease families. *Mov Disord*. 2007; 22:55–61. [PubMed: 17115391]
113. West AB, et al. Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc Natl Acad Sci U S A*. 2005; 102:16842–16847. [PubMed: 16269541]

Figure 1.

Ca²⁺ transport in SNc DA neurons. The steep concentration gradient for Ca²⁺ enables it to cross the plasma membrane readily into cells through open pores such as L-type Ca²⁺ channels. Once inside neurons, it is either transported back across the plasma membrane or sequestered in intracellular organelles. Ca²⁺ is transported across the plasma membrane through either the Ca²⁺-ATPase (PMCA) or through a Na⁺/Ca²⁺ exchanger (NCX) that relies upon the Na⁺ gradient. Ca²⁺ is rapidly sequestered either by ionic interactions with buffering proteins or by transport into cytosolic organelles (i.e. the mitochondria and the ER). The ER uses high-affinity smooth ER Ca²⁺ (SERCA) pumps that depend upon ATP to take Ca²⁺ from the cytoplasm into the ER lumen. Ca²⁺ flows back into the cytoplasm after the opening of inositol trisphosphate receptors (IP₃R) and ryanodine receptors (RyR) studding the ER membrane. Mitochondria are often found in close apposition to the ER and plasma membrane, creating a region of high (but localized) Ca²⁺ concentration that drives Ca²⁺ into the matrix of mitochondria through a Ca²⁺ uniporter. Ca²⁺ can leave the

mitochondrion through a number of mechanisms. The dominant mitochondrial Ca^{2+} -efflux path in neurons is through mitochondrial NCXs. Ca^{2+} release through higher conductance ion channels, such as the mitochondrial permeability transition pore (mPTP), has also been proposed. The mPTP is posited to have two conductance states: a low-conductance state that is reversible and participates in physiological Ca^{2+} handling, and a high-conductance state that is irreversible and leads to mitochondrial swelling and loss of molecules such as cytochrome *c* that trigger apoptosis (Figure 2). Also shown schematically are elements of the tricarboxylic acid (TCA) cycle that produces reducing equivalents for the electron transport chain; complexes I–IV are shown in red. The electrochemical gradient created drives ATP synthase and the conversion of ATP from adenosine diphosphate (ADP) delivered to the matrix by the adenine nucleotide translocator (ANT).

Figure 2.

The role played by the ER and mitochondria in Ca²⁺ homeostasis could contribute to Lewy body (LB) formation and premature death of SNc DA neurons. Ca²⁺ entry through plasma membrane Ca_v1.3 Ca²⁺ channels during activity is either pumped back across the plasma membrane or rapidly sequestered in the ER or mitochondria (Figure 1). Both processes require energy stored in the form of ATP (red 'ATP' labels denote ATP requirement). The metabolic demand created by these ATP-dependent steps in Ca²⁺ homeostasis should increase oxidative phosphorylation in mitochondria and the production of damaging ROS. ROS damage mitochondrial proteins such as complex I and mtDNA, reducing the efficiency of oxidative phosphorylation (negative consequences are symbolized by red circles; positive or augmenting consequences are symbolized by red arrows). In extreme cases, the stress on

mitochondria induces mPTP opening, swelling and the release of cytochrome *c* and other pro-apoptotic proteins such as apoptosis-inducing factor (AIF). In parallel, ROS are capable of damaging ER proteins, elevating the concentration of misfolded proteins that need to be degraded by proteasomes and autophagosomes. The unfolded protein response (UPR) triggered by this elevation in misfolded proteins should further reduce ER production of proteins and potentially lead to the release of pro-apoptotic factors such as C/EBP homologous protein (CHOP). The role of mitochondria in Ca^{2+} homeostasis could further compromise their ability to generate ATP, leading to a functionally important drop in cytosolic ATP levels. Such a drop would compromise both ER and proteasome and autophagosome function, also promoting the formation of protein aggregates such as LBs. Genetic mutations (Box 1) or environmental toxins such as rotenone could further compromise mitochondrial or ER function, rendering them more vulnerable to Ca^{2+} -induced stress. By hastening the decline in ER and mitochondrial function and the accelerated loss of SNc DA neurons, these genetic and environmental factors could be seen as ‘causing’ PD.