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Calcium, cellular aging, and selective neuronal vulnerability in Parkinson's disease

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Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disease in developed countries. The core motor symptoms are attributable to the degeneration of dopamine (DA) neurons in the substantia nigra pars compacta (SNc). Why these neurons, and other restricted sets of non-dopamine neuron, succumb in PD is not clear. One potential clue has come from the observation that the engagement of L-type Ca^{2+} channels during autonomous pacemaking elevates the sensitivity of SNc DA neurons to mitochondrial toxins used to create animal models of PD, suggesting that Ca^{2+} entry is a factor in their selective vulnerability. Epidemiological data also supports a linkage between L-type Ca^{2+} channels and the risk of developing PD. This review examines the hypothesis that the primary factor driving neurodegenerative changes in PD is the metabolic stress created by sustained Ca^{2+} entry, particularly in the face of genetic or environmental factors that compromise oxidative defenses or proteostatic competence.

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 formation) [4], the motor symptoms of PD, including bradykinesia, rigidity, and resting tremor, are clearly linked to the degeneration and death of SNc DA neurons [5, 6]. Although there are pathological changes in other select regions of the brains [4], the efficacy of the clinical gold-standard treatment of L-DOPA – a DA precursor – is testament to the centrality of DA neurons in the motor symptoms of PD.

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 [7, 8]. However, there are reasons to doubt this type of cellular stress *alone* is responsible for 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 [9-13].

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Moreover, L-DOPA administration (which relieves symptoms by elevating DA levels in PD patients) does not appear to accelerate disease progression [14], suggesting that DA is not a significant source of reactive oxidative stress, at least in the short term. Sulzer and colleagues have recently reported that Ca^{2+} entry through L-type channels stimulates DA metabolism in SNc DA neurons, pushing cytosolic DA concentrations into a toxic range with L-DOPA loading [15]. For this mechanism to be relevant to selective vulnerability, one would have to posit that modest elevations in cytosolic DA over decades leads to an accumulation of cellular defects that ultimately produces cell death. Although plausible, the hypothesis is not readily testable. It does suggest that treating patients in the early stages of the disease with direct acting agonists, rather than L-DOPA, should lead to a slower progression of the disease. That said, the frank death or phenotypic decline of a variety of non-dopaminergic neurons in PD argues that DA itself is not likely to be the principal culprit in the disease.

If not DA, then what? In recent years, attention has turned to the role of mitochondrial dysfunction in PD [16-18]. In addition to the ability of several toxins that target mitochondria to create a parkinsonian phenotype [19, 20], 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) [21]; this deficit is specific to PD patients [22] and appears to reflect oxidative damage to complex I [23]. Oxidative damage to other cellular components such as lipids, proteins and DNA also has been found in the SNc of PD brains [24]. The source of this oxidative stress is likely to be mitochondrial. Reactive oxygen species (ROS) and other radicals are generated by inefficiencies in the ETC, which is responsible for creating the electrochemical gradient across the inner mitochondrial membrane that drives ATP synthase and the conversion of adenosine diphosphate to ATP [25]. ROS also are thought to be responsible for the high level of somatic DNA and mitochondrial DNA mutations in SNc DA neurons [26-28]. Lastly, although deficits in the complex I activity of platelets, skeletal muscle, fibroblasts, and lymphocytes have been reported in some PD patients [29], this is not a consistent feature of the disease, arguing that mitochondrial dysfunction is regionally selective [30]. Further support for a mitochondrial link in PD comes from the rapidly expanding literature on genetic mutations associated with familial forms of PD [16].

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 [31]. One of the hallmarks of PD is the formation of Lewy bodies (LBs), an abnormal protein aggregate found in SNc DA neurons and elsewhere in the brain [32]. These depositions reflect a deficiency in proteostasis that is accompanied by signs of ER stress and an attempt to sequester cytotoxic proteins [33]. In part, LBs in PD must reflect the decline in proteostatic competence that accompanies normal aging [31]. What appears to distinguish PD is the presence of an additional proteostatic burden that causes an aged DA neuronal ensemble to fail *en masse* (see below) [18, 34, 35].

Taken together, the evidence for the *involvement* of mitochondria and ER in the PD pathogenesis is unequivocal. The critical question is whether the disease begins with the dysfunction of these organelles. The striking regional distribution of deficits argues against this proposition. 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 [36]. 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 regularly (2-4 Hz) in the absence of synaptic input [37]. This pacemaking activity is believed to be important in maintaining ambient DA levels in regions that are innervated by these neurons, particularly the striatum [38]. While most neurons rely exclusively on monovalent cation channels to drive pacemaking, SNc DA neurons also engage ion channels that allow Ca^{2+} to enter the cytoplasm [39-41], leading to elevated intracellular Ca^{2+} concentrations [42, 43]. The L-type Ca^{2+} channels used by SNc DA neurons in pacemaking have a distinctive Cav1.3 pore-forming subunit encoded by *Cacna1d* [43, 44]. Cav1.3 Ca^{2+} channels are relatively rare, constituting only about 10% of the all the L-type Ca^{2+} channels found in the brain [45]. Channels with this subunit differ from other L-type Ca^{2+} channels in that they open at relatively hyperpolarized potentials, allowing them to contribute to the mechanisms driving the membrane potential to spike threshold underlying autonomous pacemaking [41, 43, 46].

Until recently, it was thought that L-type Ca^{2+} channels were essential for pacemaking in SNc DA neurons making them less than ideal drug targets if pacemaking was necessary to maintain physiologically important levels of DA in target structures [47, 48]. This inference was based upon the ability of L-type channel antagonists (dihydropyridines) to halt pacemaking. However, we know now that at the concentrations of dihydropyridine (DHP) necessary to stop pacemaking, other ion channels are being antagonized, complicating the interpretation of previous studies. This discovery was made possible by the integration of traditional patch clamp electrophysiology and two photon laser scanning microscopy in brain slices. The combination of approaches allowed dendritic Ca^{2+} oscillations to be monitored with high temporal resolution at the same time as somatic voltage, revealing that at lower, channel-specific DHP concentrations, pacemaking continues in SNc DA neurons, unaltered in rate and regularity, even when dendritic L-type Ca^{2+} channels were effectively antagonized (Fig. 1) [46]. The ability of SNc DA neurons to continue pacemaking under these circumstances reflects the robustness of the multi-channel pacemaking mechanism and that Cav1.3 Ca^{2+} channels play a supportive, but not necessary role. In addition to this role, Cav1.3 Ca^{2+} channels participate in the postsynaptic response to activation of glutamatergic synapses and burst spiking in response to reward prediction errors [M. Bevan, unpublished observations]. However, as with pacemaking, this role is supportive, rather than necessary.

The sustained engagement of Cav1.3 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 100 nM – 10,000 times lower than the concentration of Ca^{2+} in the extracellular space [49-51]. 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. 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 Cav1.3 Ca^{2+} channels are open much of the time, the magnitude and the spatial extent of Ca^{2+} influx are much larger [42].

Another distinctive feature of SNc DA neurons, and many of the other neurons that succumb in PD (e.g., locus ceruleus neurons), is their enormous axonal field. Recent anatomical work has estimated that a typical SNc DA neuron has mean axonal length of 470,000 μm [52]. Furthermore, each axon supports $\sim 370,000$ synapses, orders of magnitude higher than the number supported by cortical pyramidal neurons for example [53]. Although the oxidative stress experienced by each of these terminals might very well be normal (this has yet to be determined), it could be that the proteostatic burden created by maintaining this extraordinary axonal tree creates a substantial metabolic load in and of itself. Moreover, the need to supply this axonal field with mitochondria to maintain ionic gradients and exocytosis could create an axonal sink for these organelles, potentially depriving the somatic region of an adequate mitochondrial complement. In fact, mitochondrial density in the somatodendritic region of SNc DA neurons appears to be abnormally low [54]. Asking a depleted mitochondrial complement in the somatodendritic region to meet the challenge posed by sustained Ca^{2+} entry and this large axonal tree should lead to a sustained elevation in oxidative phosphorylation and the production of superoxide and ROS.

Is PD a manifestation of Ca^{2+} -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 [55, 56]. 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. Is then PD simply a reflection of accelerated aging in neurons that rely too heavily upon Ca^{2+} channels to do their business? Age is undoubtedly the single strongest risk factor for PD [57, 58]. 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 environmental toxins or pathogens*, as they are lost at a significantly higher rate than many other types of neurons (some of which show no appreciable loss over a 6-7 decade span) [59]. In mammals with significantly shorter life-spans, loss of SNc DA neurons with age has not been seen reliably, but there is a clear decline in phenotypic markers with age that matches that seen in PD, as well as an increased susceptibility to toxins [60-65]. Taken together, these studies make a case that SNc DA neurons age more rapidly than the vast majority of the neurons in the brain.

This ‘wear and tear’ theory suggests that PD is first and foremost a consequence of aging. Why then do some people become symptomatic in their 50's, others in their 60's or 70's or not at all in an 80 or 90 year life? Genetic factors certainly could account for a large part of this variation [8, 36, 57]. These factors could increase the rate at which vulnerable neurons age by compromising mitochondrial function or Ca^{2+} homeostasis. Several of the genetic mutations associated with early onset forms of PD are directly linked to mitochondrial dysfunction [16]. Unfortunately, animal models of these forms of PD have failed to recapitulate the disease. For example, mutations of *DJ-1 (PARK7)* are associated with an early-onset form of PD. It has been putatively categorized as an atypical mitochondrial “peroxiredoxin-like peroxidase,” decreasing the accumulation of hydrogen peroxide and damage due to ETC superoxide generation [66]. But, how this happens is not clear. DJ-1 is redox sensitive, giving it the capacity to signal oxidative challenges and potentially coordinate a variety of mitochondrial oxidative defense mechanisms [67]. Although deleting

DJ-1 increases the sensitivity of cells generally to heroic oxidative challenge, it remains to be determined how it compromises SNc DA neurons under more physiological conditions. Studies of *DJ-1* knockouts have not revealed any loss of SNc DA neurons or a fundamental shift in their physiology, only a modest deficit in DA release during burst stimulation that is difficult to tie to an increased vulnerability [68]. But there have been no studies in these mice of mitochondrial physiology in mature SNc DA neurons where Ca^{2+} influx during pacemaking has fully developed. This could prove to be critical to an understanding of the mechanisms that could accelerate the loss of these cells over decades.

Two other genes linked to familial PD have unequivocal linkages to mitochondria. One is Parkin (*PARK2*). Fruit flies with functional deletions of Parkin have fragmented and apoptotic mitochondria [69]; knockout mice have a less dramatic but a clear mitochondrial phenotype (including decreased mitochondrial (respiratory) function, decreased metabolic drive, and increased lipid and protein phosphorylation) [70]. Another is PTEN-induced putative kinase 1 or *PINK1* (*PARK6*). Its deletion leads to an identical phenotype in *Drosophila* as does Parkin deletion – fragmented cristae and apoptotic mitochondria; this phenotype can be rescued by Parkin over-expression, suggesting involvement in some common biochemical pathway [71, 72]. Although found both in cytosolic and mitochondrial preparations, PINK1 has an N-terminus mitochondrial targeting sequence [73]. PINK1 deletion also compromises DA release during burst stimulation, like *DJ-1* [74]. It is not difficult to infer that loss of function mutations in either of these genes should have their biggest impact in a cell type that had a high basal level of metabolic activity, creating a mitochondrial oxidative stress. But to date, this has not been convincingly demonstrated.

Can the Ca^{2+} -mediated cellular aging hypothesis account for the vulnerability of other cell types in PD? Other regions of the brain that have cell loss paralleling that of the SNc are the locus ceruleus (LC) and hypothalamic tuberomamillary nucleus [32, 75]. The neurons of the LC and the tuberomamillary neurons are similar to SNc DA neurons in several respects. Like SNc DA neurons, both LC and tuberomamillary neurons are autonomous pacemakers that depend upon L-type Ca^{2+} channels [76-78]. In contrast, DA neurons in the VTA do not rely upon L-type Ca^{2+} channels for pacemaking and are relatively intact in PD patients and in animal models of PD [11, 43, 79-81]. DA neurons in the olfactory bulb also are autonomous pacemakers and rely upon Ca^{2+} channels (although not L-type channels) [81]. While olfactory deficits have been associated with PD [82], there is no obvious loss of olfactory bulb DA neurons [83]. 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 [84].

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 as well as slow its progression. This might be possible with orally deliverable, DHP L-type channel antagonists shown to be safe in humans [43]. Adult SNc DA neurons readily compensate for the antagonism of L-type Cav1.3 Ca^{2+} channels and continue pacemaking at a normal rate [46]. 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 [43]. SNc DA neurons that express the Ca^{2+} binding protein calbindin also have a diminished sensitivity to PD toxins [79, 85]. Furthermore, at neuroprotective doses of an L-type channel antagonist, mice have no obvious motor, learning or cognitive deficits, suggesting that the patterned activity of SNc DA neurons is functionally unchanged [39, 46, 86].

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 to treat hypertension, creating a potential database to be mined. A 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 [87]. More recently, a large Danish data set has been examined [88]. The authors agreed with the main conclusions of the Becker et al. study but extended their findings by showing that only DHPs that cross the blood-brain barrier (BBB) are associated with reduced PD risk (~30%). Given the short period of treatment in many cases (~2 years), variable dosing, and low relative affinity of DHPs for Cav1.3 Ca²⁺ channels (compared to Cav1.2 channels) [89-91], this is a surprisingly strong association and lends further credence to the proposition that a BBB permeable and potent Cav1.3 antagonist could be a very effective neuroprotective agent.

That said, these studies are not a substitute for a controlled clinical trial. In the absence of a selective Cav1.3 Ca²⁺ channel antagonist, the DHP isradipine is the most attractive drug for such a trial. Isradipine has a relatively higher affinity for Cav1.3 Ca²⁺ channels than the other known DHP and has good brain bioavailability [92, 93]. At the doses used to treat hypertension, isradipine has relatively minor side effects [94]. 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, Dynacirc CR), suggesting that neuroprotection is achievable.

It is also worth considering how DHPs might be used in combination 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 [95], 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 appears compromised in PD patients [96] and is neuroprotective in animal models of PD [97]. Creatine is a substrate for ATP production that can both improve mitochondrial efficiency and reduce oxidative stress by buffering fluctuations in cellular energy production [98]. Both approaches are aimed at improving mitochondrial function rather than attacking the source of stress on mitochondria. Rasagline or deprenyl also could prove to have neuroprotective effects by virtue not of its ability to inhibit monoamine oxidase B, but by their ability to induce the expression of antioxidant defenses [99, 100]. Because their sites of action differ within the chain of events leading to oxidative stress and mitochondrial dysfunction, a combination therapy could prove more effective than any one therapy alone.

Gaps in our understanding of the role of Ca²⁺ in the etiology of PD

There are a number of major gaps in our understanding of the role Ca²⁺ might play in the neuronal pathology seen in PD. Filling these gaps not only could provide novel therapeutic strategies for preventing or slowing the progression of the disease but could also help to create strategies for 'successful aging' generally. Figure 2 graphically summarizes some of the key points of the review and points of uncertainty.

One major gap is the mechanistic linkage between activity dependent Ca²⁺ entry into neurons and mitochondrial oxidative stress. In spite of its plausibility, there is no direct evidence that plasma membrane Ca²⁺ influx elevates mitochondrial oxidative phosphorylation and the production of superoxide. Limiting plasma membrane Ca²⁺ influx

certainly diminishes the sensitivity of SNc DA neurons to mitochondrial toxins, but this effect could be indirect. The development of redox sensitive optical probes [101-103] and two photon laser scanning microscopy to allow imaging of mitochondria *in situ* puts this question within reach. A related, albeit more difficult question, is whether the role of mitochondria in intracellular Ca²⁺ buffering contributes to neuronal apoptosis in slowly progressing neurodegenerative diseases, like PD. Although mitochondria do not normally flux Ca²⁺ from the cytoplasm at physiological concentrations, Ca²⁺ released from the ER through inositol trisphosphate (IP3) or ryanodine receptors can enter mitochondria at points of apposition between the organelles, which form functional Ca²⁺ microdomains in which Ca²⁺ concentrations can rise into the micromolar range [104-106]. Through these junctions, 'dumping' of ER Ca²⁺ stores into mitochondria could trigger apoptosis in marginally competent mitochondria [107]. However, the vast majority of the studies demonstrating the existence of close interactions between mitochondria and the ER have been performed in cell lines, none have been performed in SNc dopaminergic neurons where the functional relationship between these organelles could be quite different. That said, mechanisms like this seem to be in play in Alzheimer's disease [108].

A closely related question is whether non-autonomous oxidative stress could synergize with that created by the Ca²⁺ phenotype. There is ample evidence that inflammation and the production of ROS and reactive nitrogen species (RNS) could accelerate the loss of SNc DA neurons and the progression of PD. Recent work has identified another possible player in translating extrinsic oxidative stress into mitochondria dysfunction. ROS mediated activation of protein kinase C beta (PKC β) phosphorylates 66-kilodalton isoform of the growth factor adapter Shc (p66^{shc}), promoting transport into mitochondria where it alters Ca²⁺ responses and promotes apoptosis [109]. Because it controls lifespan [110], p66^{shc} could be particularly important in regulating the aging of neurons, like SNc DA neurons, that are stressed by Ca²⁺ flux across the plasma membrane.

A second major gap in our understanding is how genetic mutations associated with PD interact with Ca²⁺ triggered events in SNc DA neurons. Although DA could be a factor [8], Ca²⁺ must be considered the prime suspect in making these mutations lethal. As outlined here, this is most likely mediated by metabolic, oxidative stress. It is easy to imagine that a negative dominant mutation of a gene like *DJ-1* could exacerbate the basal oxidative stress in an SNc DA neuron and accelerate mitochondrial and proteostatic collapse, leaving neurons lacking the same basal stress largely unaffected. But, these types of interaction between mutations and cellular phenotype have not been pursued.

A third gap in our understanding is whether non-dopaminergic neurons that are vulnerable in PD share the Ca²⁺ phenotype. As mentioned above, there is some evidence that at least a subset of the neurons that die or functionally decline are pacemakers that engage L-type Ca²⁺ channels. However, the phenotypic characterization must be explored more systematically in those cell types at greatest risk (dorsal motor nucleus of the vagus, locus ceruleus, basal forebrain cholinergic neurons, dorsal raphe, etc.). Moreover, this should be done *in situ* (either with *in vivo* recording or in brain slices from adult mice) where the behavior of the neurons is as close as possible to that found in humans.

Conclusions

Ca²⁺ mediated cellular stress 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 suggests that the mitochondrial stress created by sustained Ca²⁺ entry could be responsible for their selective vulnerability, rather

than simply a late stage consequence. This hypothesis is consistent with the centrality of mitochondria in prevailing models of pathogenesis in PD. Genetic mutations and environmental challenges could easily synergize with this basal stress, hastening cellular aging and eventual death. Although plausible and consistent with regional deficits seen in normal aging and PD, the proposition that Ca^{2+} entry during pacemaking compromises mitochondrial function remains to be fully untested. The tools necessary to conduct this test are now becoming available. Nevertheless, given the plausibility of the link between Ca^{2+} and the loss of SNc DA neurons, the absence of any proven neuroprotective therapy in PD and the availability of Ca^{2+} 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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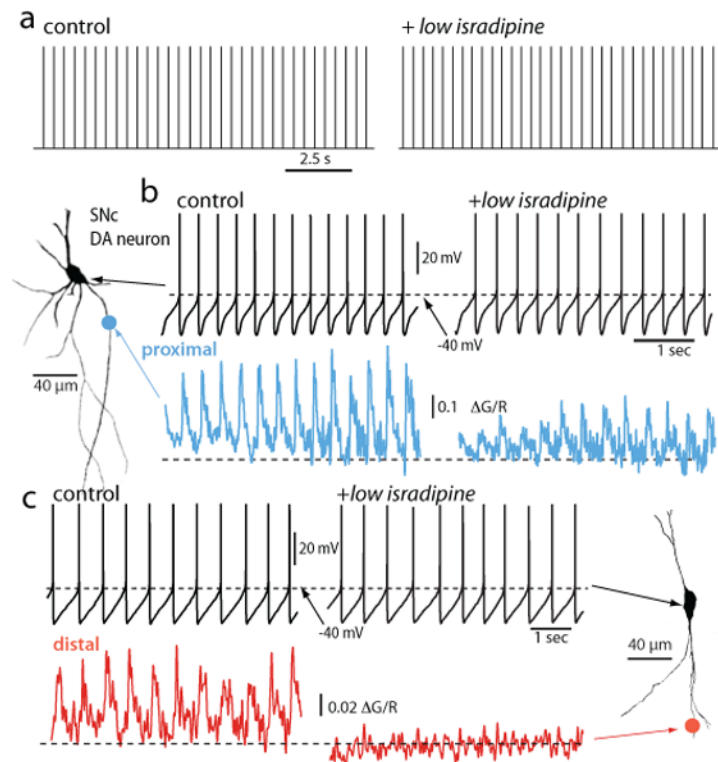


Figure 1. Low concentrations of DHPs suppress dendritic Ca^{2+} oscillations but do not slow pacemaking

a. Digitized cell-attached patch recordings from an SNc DA neuron before and after application of isradipine (5 μM). The median discharge rate before isradipine application was 2.2 Hz and 2.4 Hz after ($p > 0.05$, $n = 4$). **b.** Whole cell recording from the cell shown to the left (projection image) before and after isradipine (5 μM) application; there was no significant change in discharge rate in this cell or in 10 others. At the bottom, 2PLSM measurements of Fluo-4 fluorescence (G) at a proximal dendritic location ($\sim 40 \mu\text{m}$ from the soma) normalized by the fluorescence of the red Alexa dye used to image the cell. **c.** Somatic recording during imaging at a more distal dendritic location ($\sim 120\text{-}200 \mu\text{m}$ from the soma). Note the complete elimination of the spike associated dendritic Ca^{2+} transient at the distal imaging site. Similar results were obtained in 6 other neurons. From [46]

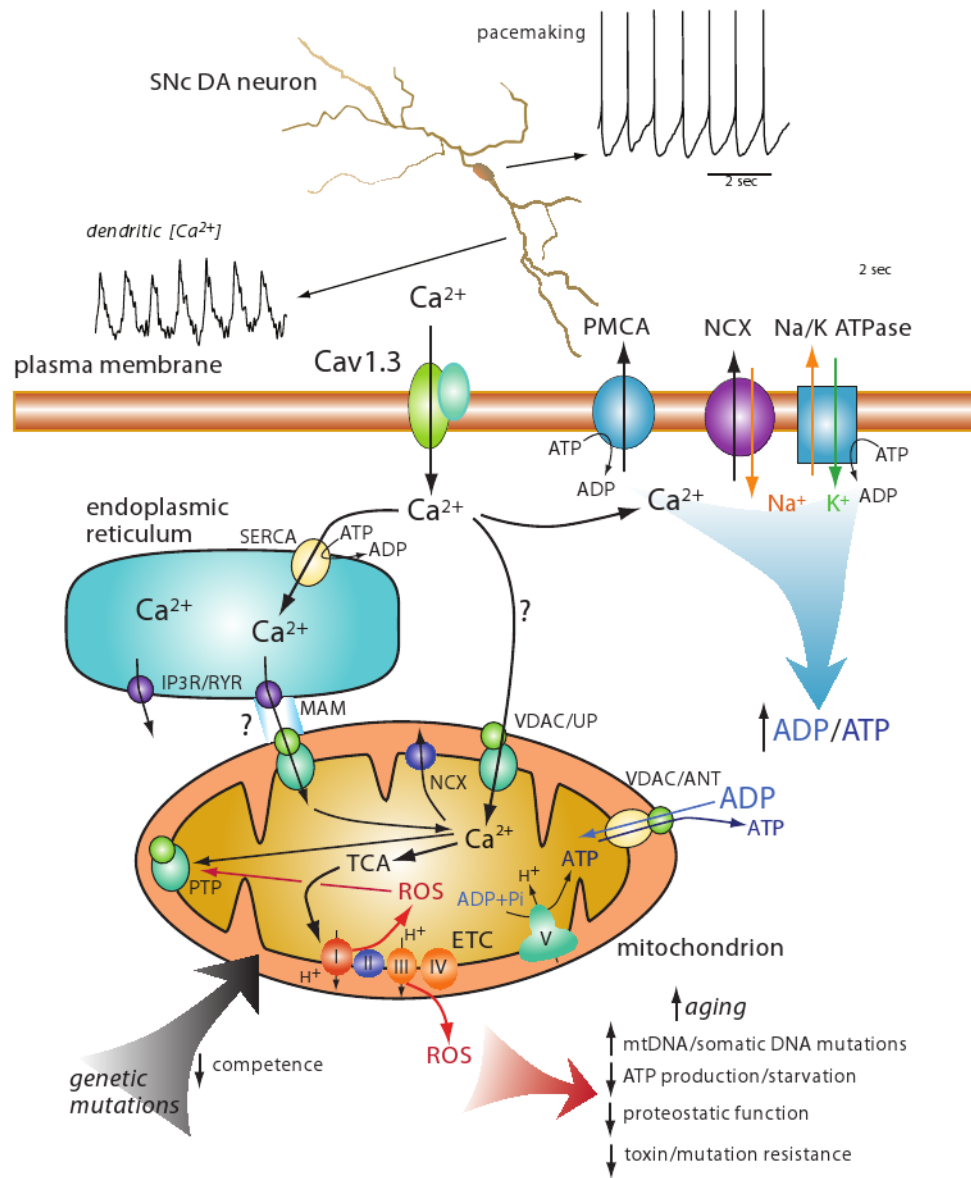


Figure 2. A schematic summarizing a model of how Ca^{2+} entry during pacemaking in SNc DA neurons might lead to mitochondrial oxidative stress, accelerated aging and eventual cell death At the top is an image of a reconstructed SNc DA neuron showing the pacemaker driven somatic spiking and dendritic Ca^{2+} oscillations associated with pacemaking. This dendritic Ca^{2+} influx is attributable almost entirely to flux through Cav1.3 Ca^{2+} channels. At the bottom is a cartoon depicting the hypothetical mechanisms involved in elevating mitochondrial oxidative stress. Ca^{2+} influx through Cav1.3 channels is either sequestered in the endoplasmic reticulum by uptake through smooth endoplasmic reticulum Ca^{2+} (SERCA) pumps or taken up by mitochondria through channels created by voltage dependent anion channels (VDAC) and Ca^{2+} uniporter (UP); the extent to which this pathway is important is in question (hence the question mark on the arrow). Ca^{2+} could also enter mitochondria through this VDAC/UP channel at points of apposition between the ER and mitochondria where inositol trisphosphate receptors (IP3) and/or ryanodine receptors (IP3R/RYR) are positioned at specializations – mitochondrial associated membrane (MAM). This entry route has not been established in SNc DA neurons, hence the question mark. Ca^{2+} entering

through Cav1.3 channels is moved back across the plasma membrane through either the Ca^{2+} -ATPase (PMCA) or through a $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) that relies upon the Na^+ gradient maintained by the Na/K ATPase at energetic cost; leading to conversion of ATP to ADP. ADP and ATP are exchanged by mitochondria with the VDAC and adenine nucleotide transporter (ANT). ADP stimulates oxidative phosphorylation. Ca^{2+} entering the mitochondrial matrix can stimulate enzymes of the tricarboxylic acid (TCA) cycle that produces reducing equivalents for the electron transport chain (ETC); complex I through V are shown at the inner mitochondrial membrane; complex V (ATP synthase) uses the electrochemical gradient to convert adenosine diphosphate (ADP) and inorganic phosphate to adenosine triphosphate (ATP). Electron movement along the ETC generates superoxide, leading to the production of reactive oxygen species (ROS) that can produce a variety of deleterious effects that can be viewed as accelerated aging (red arrow at the bottom). One other action of ROS is to promote opening of the mitochondrial permeability transition pore (mPTP); irreversible opening of the mPTP leads to release of pro-apoptotic factors into the cytosol. A basic question is whether *reversible* opening of the mPTP is possible under conditions of mild oxidative stress; mPTP opening and depolarization of the inner mitochondrial membrane could serve to diminish ROS production. Ca^{2+} is removed from mitochondrial through mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchangers (NCXs). Lastly, genetic mutations associated with Parkinson's disease, like those to DJ-1 or PINK1, might directly compromise the competence of mitochondria, leading to accelerated aging or increased oxidative stress.