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Commentary

Patients with Parkinson's disease (PD) show selective degeneration of dopaminergic neurons in the substantia nigra and cholinergic neurons in the dorsal motor nucleus (DMnX), but the drivers of this specific susceptibility are unknown. In this issue of the *JCI*, Musgrove et al. report on their use of an impressive array of in vivo and ex vivo tools for interrogating DMnX neurons and demonstrate that this population exhibits enhanced sensitivity to oxidative stress. Remarkably, this sensitivity was amplified by the overexpression of α-Synuclein (α-Syn), a pathological protein in PD. They further show that oxidative stress augments cell-cell transfer of α-Syn, which may be an important mechanism underlying the development and progression of PD.

Oxidative stress and α-synuclein conspire in vulnerable neurons to promote Parkinson's disease progression

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Patients with Parkinson's disease (PD) show selective degeneration of dopaminergic neurons in the substantia nigra and cholinergic neurons in the dorsal motor nucleus (DMnX), but the drivers of this specific susceptibility are unknown. In this issue of the *JCI*, Musgrove et al. report on their use of an impressive array of in vivo and ex vivo tools for interrogating DMnX neurons and demonstrate that this population exhibits enhanced sensitivity to oxidative stress. Remarkably, this sensitivity was amplified by the overexpression of α-Synuclein (α-Syn), a pathological protein in PD. They further show that oxidative stress augments cell-cell transfer of α-Syn, which may be an important mechanism underlying the development and progression of PD.

Selective susceptibility to stress

Selective neuronal loss is a characteristic feature of neurodegenerative diseases. In patients with Parkinson's disease (PD), for example, targeted degeneration of dopaminergic neurons of the substantia nigra pars compacta (SNpc) is well documented (1). Yet the relative sparing of dopamine-producing ventral tegmental area neurons in these individuals indicates that not all neurons with the same neurotransmitter are equally vulnerable (2). While the precise reasons for the selective vulnerability of the SNpc remain to be fully defined, intrinsic properties, such as high energy demand, extensive axonal arborization, and elevated cytosolic calcium levels, have emerged as factors that differentiate cells that succumb to or withstand neurodegenerative insults (3). The presence of α-synuclein (α-Syn), a central component of Lewy bodies and neurites in PD brains, also contributes to neuronal stress, as knockout or reduction of this protein ameliorates toxicity in several models of SNpc cell death (4–6).

Analogous to SNpc dopaminergic neurons, cholinergic neurons also show differential vulnerability in PD, with projection neurons of the dorsal motor nucleus (DMnX) representing one of the earliest populations to accumulate in α-Syn pathology in the CNS (1, 7). Like SNpc neurons, DMnX neurons stand out from other cholinergic populations in the hypoglossal nucleus, medial septum, and striatum, in which cell loss is limited.

In this issue, Musgrove et al. (8) report on their investigation of the sensitivity of cholinergic DMnX neurons to intraperitoneal injection of paraquat, a bipirydyl herbicide that catalyzes the formation of ROS and induces the degeneration of SNpc neurons in mice (9). They show that paraquat results in a significant increase in ROS in cholinergic DMnX neurons within days of treatment. Importantly, ROS levels monitored using the superoxide-sensitive indicator dihydroethidium (DHE) remained unchanged in cholinergic neurons in the neighboring hypoglossal nucleus, medial septal nucleus, and the striatum of wild-type mice, pointing to the selective sensitivity in the DMnX among cells expressing this neurotransmitter. However, in contrast to SNpc dopamine neurons, cholinergic neurons did not degenerate following paraquat treatment alone in the regions examined.

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Effect of α-Syn on oxidative stress

Since vagal cholinergic neurons are one of the earliest populations to accumulate α-Syn containing Lewy bodies in PD (1), the authors examined whether overexpression of α-Syn could also modulate oxidative stress in these cells. Unilateral injection of adeno-associated virus (AAV) particles encoding human α-Syn (hα-Syn) into the vagus nerve increased basal ROS levels in DMnX neurons that expressed hα-Syn. While only 40% of DMnX neurons were immunoreactive for hα-Syn, oxidized DHE levels were approximately 30% higher in these cells than in neurons lacking hα-Syn. Treatment with paraquat further increased ROS levels by 1.5-fold and reduced the total number of surviving DMnX neurons by 25% after 7 days, suggesting a toxic synergy between oxidative stress and α-Syn in these cells.

To test whether these combined insults in DMnX neurons also generated higher levels of modified α-Syn typical of human synucleinopathies, Musgrove et al. probed DMnX neurons with antibodies raised against oxidized or nitrated hα-Syn. Consistent with the oxidative environment induced by paraquat and hα-Syn AAV, modified hα-Syn could be detected in both neuronal processes and cell bodies by immunostaining and ELISA. Curiously, the accumulated α-Syn species were also detectable with an antibody recognizing both oligomeric and fibrillar recombinant hα-Syn, but not with an α-Syn fibril-specific antibody (10). This differs somewhat from human PD pathology, where fibrillar α-Syn species predominate (6), although this observation may be a func-

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tion of the short treatment durations used; whether these α-Syn species later mature into intraneuronal pathology resembling classical Lewy bodies and neurites after prolonged exposure to oxidative conditions remains to be seen.

Oxidative stress promotes α-Syn transfer

Although the cholinergic DMnX neurons do not project to more rostral CNS regions, they receive input from neurons in the pons and ventral midbrain (11). Previous work from the Di Monte group had shown that AAV-expressed hα-Syn can be transferred in a retrograde fashion from the vagus to more rostral regions, such as pons and midbrain (12). In the present study, Musgrove et al. show that treatment with paraquat more than doubled the density of hα-Syn immunoreactive processes in these rostral regions. Moreover, proximity ligation assay labeling with a 3-nitrotyrosine–specific antibody confirmed that the hα-Syn contained within these axons was nitrated, though it was unclear whether this modification occurred while in the donor (i.e., DMnX) neurons or after transfer to recipient axons. To further address this question, the authors turned to a cell-based model to measure the effect of oxidative stress on intercellular α-Syn transfer in cocultures of two SH-SY5Y cell lines expressing complementary fragments of the Venus fluorescent protein (13). Both paraquat and hydrogen peroxide dramatically increased the proportion of cells with bimolecular fluorescence without altering total α-Syn levels. Intriguingly, this transfer could be blocked with antibodies specifically recognizing nitrated α-Syn. Further studies are required to resolve whether nitrated α-Syn species are more readily released into the extracellular space or whether nitration facilitates α-Syn uptake by neurons. Nonetheless, this observation suggests that oxidative stress enhances the cell-to-cell transmission of α-Syn and is consistent with the in vivo finding of increased nitrated hα-Syn in CNS regions connected to the DMnX.

What is the source of ROS in DMnX neurons following paraquat exposure? Past studies have shown that NADPH oxidase is a potent catalyst for ROS formation from paraquat. Interestingly, mice lacking gp91phox, a subunit of the membrane-bound NADPH oxidase present in microglia, are protected against SNpc neuron loss after paraquat treatment (14). Here, Musgrove et al. demonstrated that the ROS accumulation induced by paraquat and hα-Syn overexpression was absent in the cholinergic DMnX neurons of gp91phox$^{-/-}$ mice, thus implicating surrounding microglia as the major source of ROS in these neurons. While this further supports the notion that the source of ROS in neurons originates from neighboring glial cells, it also raises the question of whether microglia surrounding DMnX and SNpc neurons differ functionally from other microglia or whether vulnerable neurons provide prooxidative signals to the local environment.

Conclusions and future directions

In summary, these provocative findings provide a fresh perspective on how a triad of processes frequently implicated in PD (i.e., oxidative stress, α-Syn, and microglia) converge at a subpopulation of neurons, leading to their selective degeneration. One possible scenario is that chronic exposure to environmental toxins such as paraquat primes DMnX cholinergic and other vulnerable neurons, causing them to later exhibit increased α-Syn pathology formation and dissemination. Alternatively, misfolded α-Syn is a potent trigger for microglia, and early accumulation of Lewy pathology in DMnX neurons could trigger a neuroinflammatory cascade, leading to oxidative stress that disproportionately affects these cells (15, 16). These processes are also not mutually exclusive. Establishing the sequence in which each of these events (co-)occur will therefore be a crucial next step to translating these findings into a better understanding of human disease.

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