

Parkinson's disease: Mitochondria parked at the ER hit the snooze button

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Keywords: Parkinson's disease, Pink1, Parkin, mitochondria, endoplasmic reticulum, *Drosophila*, sleep, circadian rhythms

Abstract

Parkinson's disease patients report sleep disturbances well ahead of motor symptoms. Valadas *et al.* in this issue of *Neuron* report that the disease genes *pink1* and *parkin* exert novel, cell-type-specific effects to modulate ER-mitochondria contacts, neuropeptidergic transmission, and sleep patterns.

Main Text

Parkinson's disease (PD) is the second most common neurodegenerative disorder. Well ahead of the hallmark symptoms of tremor and slowness of movement, the majority of PD patients experience non-motor symptoms that include sleep disruption and impaired olfactory and gastrointestinal function (Khoo *et al.*, 2013). While the underlying aetiology of many symptoms can be attributed to the loss of dopaminergic neurons, the cause of some non-motor symptoms in PD remains elusive. A productive strategy to understand the causes of PD symptoms has been to focus on the minority of PD cases attributable to monogenic inheritance of a mutant disease gene. Now, Verstreken and colleagues (Valadas *et al.*, 2018) have employed this strategy to uncover a mechanism behind sleep-related symptoms in PD.

pink1 and *parkin* are two of the most extensively studied autosomal recessive PD risk genes. Their most well-described function is in mitochondrial quality control. Damaged mitochondria accumulate Pink1 on their outer membrane; Pink1 then recruits the E3 ubiquitin ligase Parkin to ubiquitinate mitochondrial substrates, and thus label the organelles for clearance by autophagic removal (mitophagy) (**Figure 1A**, reviewed in (Pickrell and Youle, 2015)). In addition, functions for Pink1 and Parkin beyond mitophagy have been described, including mitochondrial fission/fusion, transport, and biogenesis (Scarffe *et al.*, 2014). Notably, *in vivo* work with the fruit fly *Drosophila* has been essential to the initial understanding of the connections between both Parkin and mitochondrial dysfunction (Greene *et al.*, 2003) and Pink1 and Parkin (Clark *et al.*, 2006; Park *et al.*, 2006). The current study (Valadas *et al.*, 2018) has used the strength of *Drosophila* genetics to show that Pink1 and Parkin exert mitophagy-independent effects in specific neuronal populations to disrupt sleep and circadian rhythms.

The authors first took advantage of the extensive behavioural toolkit in *Drosophila* to establish the presence of sleep pattern deficits in *pink1* and *parkin* mutants. Among several sleep disruption phenotypes, mutant flies failed to anticipate dawn, and so lacked the wild-type burst of

activity before the dark-to-light transition, and also showed sleep fragmentation. Interestingly, the authors were unable to see similar deficits in *pink1* mutant mice, consistent with previous reports that *pink1* and *parkin* mutant mice do not fully recapitulate deficits seen in PD patients (Whitworth and Pallanck, 2017). The authors thus focused on the *Drosophila* sleep phenotypes to perform a small-scale screen, by driving RNAi for *pink1* or *parkin* in different neuronal subpopulations to pinpoint those responsible for the sleep phenotypes. The morning anticipation phenotype could be attributed to functions of *pink1* and *parkin* in the ventral lateral neurons (LNv) that produce the neuropeptide Pigment Dispersing Factor (PDF) central to the control of circadian rhythms. In these neurons, the authors observed reduced PDF levels at neuron terminals and increased PDF levels in the cell bodies, suggesting a deficit in neuropeptide transport and/or loading into dense-core vesicles (DCVs). Scanning electron microscopy confirmed that DCV levels were indeed reduced in the LNv neurons of *parkin* mutant flies. Importantly, mislocalisation of neuropeptides appears to be a phenotype shared by both fly and human neurons. The authors used human induced pluripotent stem cells (iPSCs) from *pink1* or *parkin* mutant PD patients to generate hypothalamic neurons, where they observed that the circadian coordinator Vasoactive Intestinal Peptide (VIP) was similarly mislocalised away from neurites while accumulating in cell bodies, resulting in reduced VIP secretion into the medium when the neurons were stimulated. This key parallel phenotype suggests that the functions of Pink1 and Parkin in neuropeptidergic neurons are well conserved from invertebrates to humans.

The canonical function of Pink1 and Parkin in mitophagy (Pickrell and Youle, 2015) might suggest that the accumulation of damaged or abnormal mitochondria could underlie these phenotypes in neuropeptidergic neurons. However, surprisingly, the authors observed no obvious changes in mitochondrial volume, morphology, or cristae structure in the LNv neurons of *pink1* or *parkin* mutant flies. Instead, there was increased contact between mitochondria and the endoplasmic reticulum (ER) in LNv neurons of mutant flies, a phenotype that had been previously reported in *pink1* and *parkin* mutant *Drosophila* brains and patient fibroblasts (Celardo et al., 2016). Here again, the authors made the important parallel to human neurons, and found increased ER-mitochondria contacts in iPSC-derived hypothalamic neurons from human *pink1* and *parkin* patients. As ER-mitochondria contacts mediate functions including lipid transport, the authors turned to a lipidomic approach to explore potential mechanisms by which ER-mitochondria contacts could impair neuropeptidergic signalling. They found that *parkin* mutant fly head extracts contained significantly increased phosphatidylserine (PtdSer) levels in the mitochondrial fraction and reduced levels in the ER fraction, suggesting that increased ER-mitochondrial contact may

prolong the transfer of PtdSer from the ER to mitochondria and thus deplete PtdSer from cellular lipid membranes.

To assess whether increased ER-mitochondrial contacts and altered PtdSer distribution directly contribute to deficits in neuropeptidergic transmission and sleep regulation, the authors used two strategies to rescue the phenotypes seen in their fly models. First, they examined a mechanism in which Parkin ubiquitinates (and thus targets for degradation) proteins such as Mitofusin (*Drosophila* Marf) that stabilise ER-mitochondria contacts; *parkin* mutants thus accumulate excess Marf which may contribute to increased ER-mitochondria contacts. The authors found that RNAi knock-down of Marf specifically in LNv neurons was able to fully rescue PDF mislocalisation and partially rescue morning anticipation deficits in *pink1* and *parkin* mutant flies, confirming that Marf accumulation is required for these mutations to exert their full phenotype. Second, the authors examined the link between PtdSer and these *Drosophila* phenotypes. By supplementing the flies' food with PtdSer, the authors again were able to fully rescue PDF mislocalisation in *parkin* mutants and partially rescue morning anticipation deficits in both *pink1* and *parkin* mutants. Taken together, these results suggest a mechanism wherein Pink1 or Parkin loss leads to increased ER-mitochondria contacts, PtdSer imbalance in the cell, reduced DCV generation, and impaired neuropeptidergic transmission to regulate sleep (**Figure 1B**).

Future work building on this study would bolster the translation of these findings to PD therapies. In the short term, it will be important to establish that PtdSer acts locally in neuropeptidergic neurons to rescue disease phenotypes, for example by over-expressing the PtdSer synthase enzyme in specific neuronal subpopulations. It will also be informative to examine additional subtypes of neuropeptidergic neurons in PD patient tissue to determine the extent of DCV deficits and their potential impact on disease symptoms. In the longer term, it will be informative for both basic science and translational research to understand exactly how PtdSer is linked to DCV formation, and whether PtdSer or another part of that pathway could be a gateway for therapy in diseases that disrupt neuropeptidergic neuron function. Additional studies could expand on these findings to determine whether increased ER-mitochondrial contacts unbalance processes other than lipid transfer, either in neuropeptidergic neurons or in other cell types. ER-mitochondrial contacts modulate an extensive number of cellular processes, including calcium homeostasis, ER stress responses, mitochondrial fission, mitochondrial transport, and autophagosome formation (Krols et al., 2016). Moreover, proteins involved in ER-mitochondrial contacts have been implicated in diseases including Alzheimer's disease, frontotemporal dementia, and amyotrophic lateral sclerosis (Krols et al., 2016). This suggests that future work in other cell

types will uncover additional disease-relevant functions of ER-mitochondrial contacts beyond PtdSer transfer.

Finally, the discrepancy among fly, mouse, and human cell models of PD is one that should be addressed by future work in the field of PD research. *Drosophila* research has been at the forefront for understanding the *in vivo* functions of Pink1 and Parkin, and it will be important to understand why mouse models have not fully recapitulated the phenotypes seen in flies or in patients. The possibility remains that compensatory mechanisms in mice could protect against Pink1 or Parkin loss (Whitworth and Pallanck, 2017), so investigating these mechanisms may be a productive line of inquiry for PD research. In addition, the work from this study also exemplifies the strength of validating pathological mechanisms in human cells and/or patient tissue whenever possible.

The current work of Valadas *et al.* (Valadas et al., 2018) is a compelling example of how cell-type-specific pathological mechanisms can explain multiple distinct symptoms arising from a single genetic deficit. Understanding these mechanisms in different cell types will therefore be essential to the development of fully effective therapies. To address non-motor symptoms like sleep dysfunction, the present findings about PtdSer depletion may be one entry point for a future therapeutic approach. Future studies employing cell-type-specific tools are likely to discover additional pathological mechanisms that could be promising therapeutic targets, both for PD and for other neurodegenerative diseases.

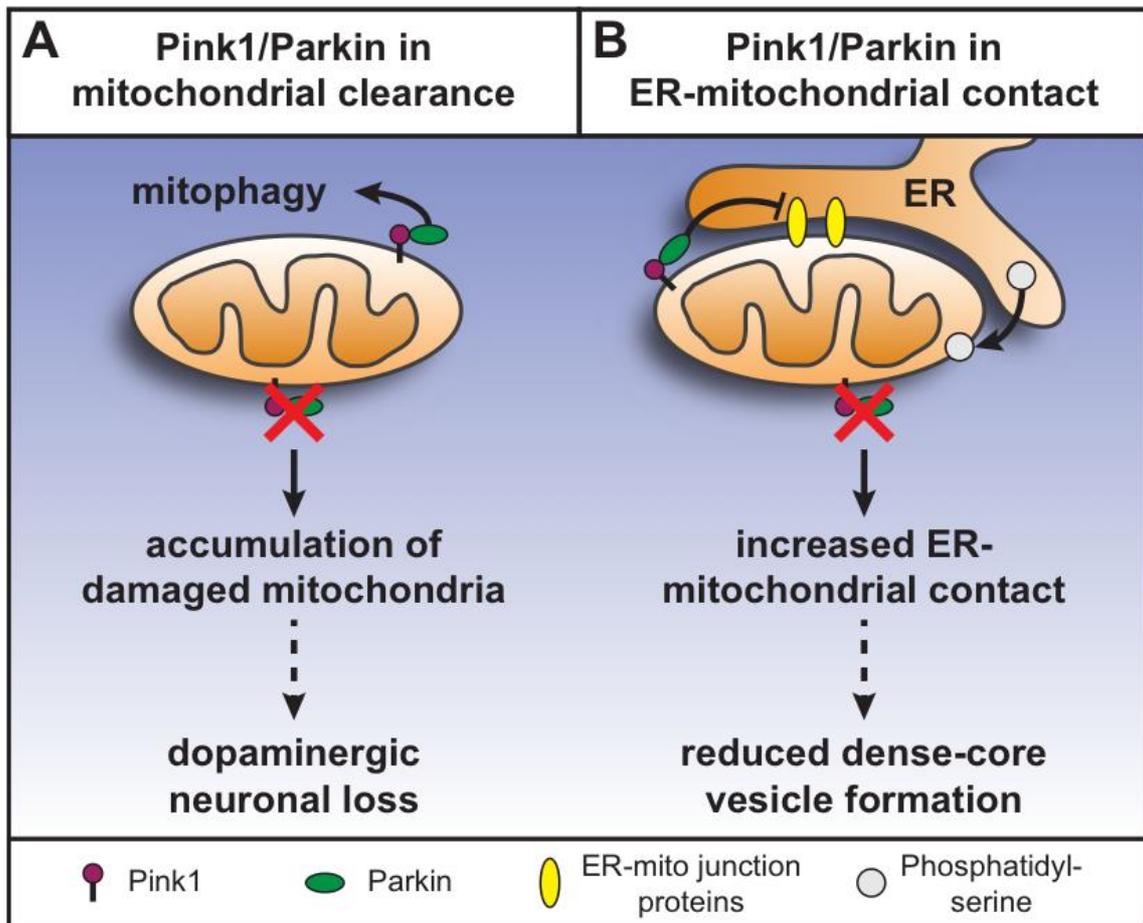


Figure 1. Cell-type-specific consequences of Pink1 or Parkin loss in neurons. (A) Pink1 and Parkin have been best studied in their relevance to mitochondrial quality control. Pink1 accumulates on the outer membrane of damaged mitochondria, where it recruits Parkin to label damaged mitochondria for clearance. Loss of Pink1 or Parkin leads to an accumulation of damaged mitochondria and may eventually cause loss of dopaminergic neurons. (B) A parallel function of Pink1 and Parkin may be necessary in neuropeptidergic neurons to regulate sleep behaviour. In this proposed mechanism, Pink1 and Parkin regulate the clearance of proteins that stabilise ER-mitochondrial contacts. Loss of Pink1 or Parkin leads to increased ER-mitochondrial contacts and increased transfer of phosphatidylserine (PtdSer) to mitochondria. This lipid imbalance may lead to impaired formation of dense-core vesicles (DCVs) and impaired neurotransmission in the neurons that regulate sleep and circadian rhythms.

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