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The mechanisms that drive neuronal death in Parkinson's disease (PD) are incompletely understood, presenting a critical obstacle to the development of effective treatments to prevent disease progression. Two central themes have emerged from research on the causes and mechanisms of PD. First, dysfunction of neuronal mitochondria – resulting from either exogenous chemical toxicants such as MPTP, or Mendelian gene mutations that abrogate expression of key mitochondrial proteins, including Parkin and PINK1 – can cause neurodegeneration. Neuronal groups affected by PD, such as the dopaminergic neurons of the substantia nigra, seem particularly vulnerable to mitochondrial dysfunction. Second, aberrant cellular proteostasis causes accumulation and aggregation of the neuronal protein α-Synuclein, resulting in the formation of Lewy Bodies, the pathological hallmark of PD. This process is common to both sporadic PD, and rare Mendelian PD phenocopies caused by mutation or over-expression of α-Synuclein. Recent research at the University of Pittsburgh has started to resolve the link between mitochondrial function and α-Synuclein in the pathophysiology of PD.

Rotenone is a potent mitochondrial complex I inhibitor, frequently used as a pesticide. Occupational exposure to rotenone more than doubles the risk of PD in epidemiological studies. Previous work found that rats exposed chronically to rotenone developed motor deficits and degeneration of substantia nigra neurons1. Some surviving cells showed formation of inclusion bodies that contained α-Synuclein and resembled Lewy Bodies ultrastructurally. Several interpretations of these data are possible. First, α-Synuclein accumulation might be a non-specific marker of neuronal mitochondrial damage, its appearance simply indicating that cells are unhealthy or degenerating. Alternatively, α-Synuclein could be involved in the mechanisms that drive pathophysiology in the presence of mitochondrial inhibitors. We showed in a recent study that the second interpretation is correct2. We generated a gene transfer vector derived from a harmless defective virus – Adeno-Associated Virus (AAV) – to introduce a small interfering RNA into dopaminergic neurons, in order to specifically decrease expression of α-Synuclein. Rats exposed to rotenone characteristically show symmetric loss of substantia nigra neurons and bilateral motor deficits. However, after decreasing α-Synuclein expression on one side of the brain only, we found that rotenone produced a highly asymmetric lesion, in which the side of the brain lacking α-Synuclein was strongly protected, and the contralateral limbs showed no motor deficit. These findings indicate unequivocally that α-Synuclein is involved in mediating the pathogenic consequences of mitochondrial dysfunction in dopaminergic neurons.

We discovered vital clues concerning the underlying mechanism in our new study3. Disruption of mitochondrial respiration causes multiple effects, including the generation of reactive oxygen species (ROS). The mitochondrial respiratory chain normally shuttles electrons from metabolic fuel substates, to form water through the four-electron reduction of molecular oxygen at complex IV. However, electrons can 'leak' from the respiratory chain proximal to

complex IV to react with oxygen and form ROS such as hydrogen peroxide. These chemically-reactive species can damage cellular macromolecules and trigger multiple signaling events. At low concentrations, mitochondrial respiratory chain inhibitors enhance ROS production without causing other consequences such as ATP depletion or cell death signaling. Previous work showed this is the primary mechanism by which rotenone exposure causes neurodegeneration4. In our new study, we found that protein oxidation, a marker of ROS abundance, was strongly mitigated by α-Synuclein knockdown in the substantia nigra neurons of rotenone-exposed rats3. This suggests that ROS production triggered by mitochondrial inhibitors is amplified by cellular α-Synuclein. In order to test this idea directly, we developed a novel experimental model that allows us to visualize ROS in dopaminergic neurons within the living brain for the first time. Zebrafish are used commonly as an experimental model in neurobiology because they are small, easily manipulated genetically, and have a vertebrate nervous system that is organized similarly to the human CNS. Importantly, larval zebrafish are transparent and can be immobilized on the stage of a microscope, uniquely allowing direct observation of labeled cells in the living brain. We generated novel transgenic zebrafish that express a fluorescent protein in their dopaminergic neurons3. When the protein encounters hydrogen peroxide its fluorescence properties change, allowing us to measure ROS flux in dopaminergic neurons directly and dynamically, at cellular resolution in the intact brain. We further generated zebrafish that express human α-Synuclein in their dopaminergic neurons and crossed these with the fluorescent ROS reporter zebrafish. Using these unique transgenic lines (see figure), we discovered that α-Synuclein strongly augments ROS flux in dopaminergic neurons of the intact CNS following exposure to mitochondrial inhibitors. This key finding places two major molecular mechanisms implicated in PD – mitochondrial dysfunction and α-Synuclein – in the same pathogenic cascade.

How do these findings facilitate efforts to develop new treatments for PD? First, our observations suggest that decreasing α-Synuclein levels in the human brain could disrupt PD pathophysiology. However, α-Synuclein is expressed strongly in dopaminergic neurons and its physiological functions are incompletely understood, raising concerns about the potential toxicity of knockdown approaches. This concern was partly addressed in another recent study, in which we found that α-Synuclein expression can be decreased significantly in the adult rat CNS for an entire year without deleterious consequences5. This is reassuring that α-Synuclein knockdown is likely to be safe, a key consideration for the future deployment of α-Synuclein-targeting reagents (such as antisense oligonucleotides) in the clinic. Second, our current work is focused on defining the mechanisms through which α-Synuclein promotes cellular ROS fluxes, since the underlying biochemistry may provide targets for small molecule inhibitors that prevent disease progression. This approach might mitigate pathophysiology without the use of gene targeting reagents that have to be delivered directly to the brain or CSF, potentially providing a simpler and more accessible option. Future work will determine whether either strategy proves effective.

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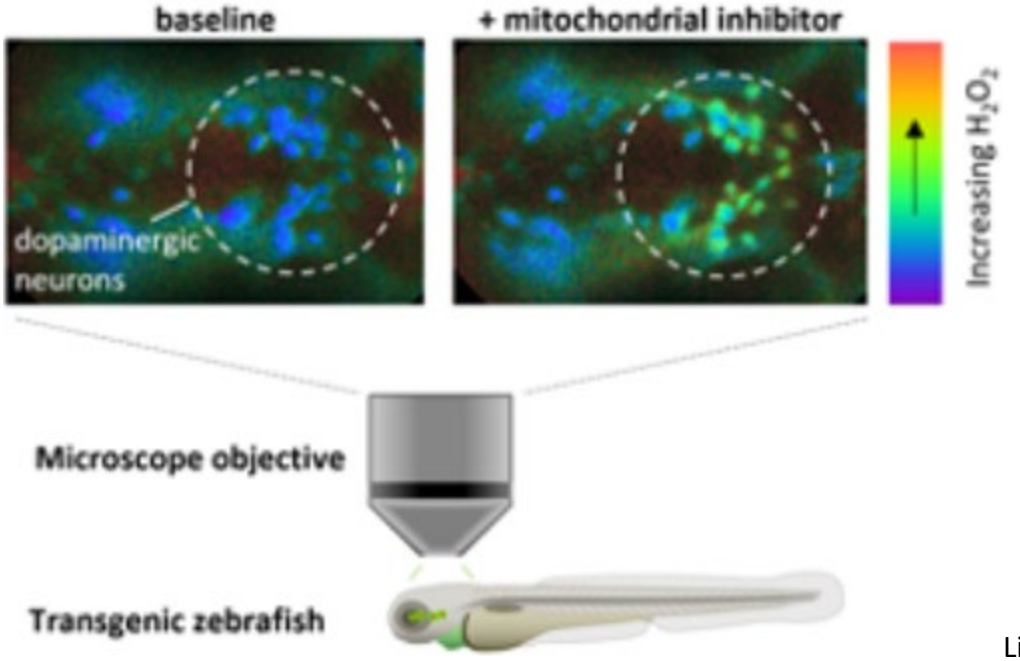

Live imaging of hydrogen peroxide flux in the dopaminergic neurons of transgenic zebrafish.
Novel transgenic zebrafish express a fluorescent protein sensor of hydrogen peroxide, along with human α-Synuclein, in their dopaminergic neurons. The signal from the sensor protein (shown as a color scale) increases within the dopaminergic

neurons when the zebrafish is exposed to a mitochondrial inhibitor. The change in signal was restricted to dopaminergic neurons and was much larger in the presence of α-Synuclein.