Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Evolutionarily Conserved Mechanisms in Calcium Handling May Underlie Intrinsic Sensitivity to Dopaminergic Neuron Death

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Review of Nagarajan et al.

Mammalian dopaminergic neurons of the substantia nigra pars compacta (SNc) within the CNS are complex neurons with expansive arborization. Projecting from the SNc to the striatum, the average total axonal length of these neurons in the rat has been estimated to be ~467 mm, with each axon supporting upwards of 370,000 synapses (Matsuda et al., 2009). This massive network contributes to our ability to execute movement and degeneration of these neurons has catastrophic effects, as seen in Parkinson's disease (PD).

Many hypotheses have been put forth to explain the death of dopaminergic neurons in PD, including pathological aggregation of α-synuclein (a component of Lewy bodies), calcium toxicity, and lysosomal and mitochondrial dysfunction (Sulzer, 2007). Furthermore, dopamine/α-synuclein interactions have been suggested as a potential pathogenic mechanism in which dopamine can modify α-synuclein to increase its reactivity and aggregative properties. In turn, the reactive aggregation may disrupt the membrane of dopamine-containing vesicles, leading to higher cytoplasmic dopamine levels and further α-synuclein modification, or potentially reach cytotoxic levels of dopamine, although this hypothesis has just begun to be rigorously tested (Sulzer, 2007; Mosharov et al., 2009). Recent observations in mouse models of PD suggest that interplay between these pathologies might be responsible for dopamingeric cell death of the SNc (Sulzer, 2007). Interestingly, although dopaminergic neurons in both the SNc and the ventral tegmental area (VTA) exhibit Lewy bodies, the neurons of the SNc tend to undergo degradation while those of the VTA are relatively spared (Dauer and Przedborski, 2003).

Why are VTA neurons spared the degenerative fate of SNc neurons? Calcium toxicity has long been proposed as a mechanism in neuronal cell death, including necrosis and apoptosis. Maintenance of low intracellular calcium levels is essential for cellular function and survival. Interestingly, the calcium binding protein, calbindin, is differentially expressed between VTA and SNc neurons, and single nucleotide polymorphisms in the human calbindin gene, *CALB1*, have been associated with sporadic PD in a Japanese cohort (Mizuta et al., 2008). Calbindin contains five EF-hand protein motifs that are thought to buffer cytoplasmic calcium levels, thus serving a neuroprotective function. High expression of calbindin is observed in the VTA while the ventral SNc is calbindin-negative (German et al., 1992). This suggests that neurons of the VTA are better equipped to handle fluctuations in intracellular calcium. Differences in other calcium-binding proteins, such as calretinin, also appears to convey a neuroprotective function in some dopaminergic neurons (Mouatt-Prigent et al., 1994).

Caenorhabditis elegans has emerged as a powerful tool to address the molecular mechanisms underlying PD and has provided essential information regarding dopaminergic neuronal degeneration (Harrington et al., 2010). In a recent edition of *The Journal of Neuroscience*, Nagarajan et al. (2014) performed a mutagenesis screen in *C. elegans* to identify genes that are involved in the progressive loss of dopaminergic neurons. Nagarajan et al. (2014) identified a mutation in an essential pore forming subunit of a transient receptor potential (TRP) channel that allowed excess calcium to enter dopaminergic neurons, leading to necrotic-like cell death. Nagarajan et al.'s (2014) findings may therefore shed light on the vulnerability of dopaminergic neurons.

Nagarajan et al. (2014) found that *C. elegans* mutants that had gain-of-function mutations in the *trp-4* gene (which they termed *trp-4(d)*) displayed normal development of dopaminergic neurons, but dopaminergic cell death during adulthood. Although there are no direct mammalian homologs of TRP4, many TRP channels are present in mammalian dopaminergic neurons of the SNc, suggesting common functionality may exist (Riccio et al., 2002). The role of TRP channels in

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PD is still unclear, but several observations suggest TRPC1 may be involved in dopaminergic endoplasmic reticulum (ER) stress and death (Selvaraj et al., 2010).

As most all TRPs are permeable to calcium ions, Nagarajan et al. (2014) hypothesized that dysregulation of calcium signaling and calcium handling caused the neuronal degeneration. They noted that the phenotype of *trp-4(d)* mutants was strikingly similar to a previously described gain-of-function mutation in a degenerin/epithelial sodium channel subunit, *mec-4* (Royal et al., 2005). In nematodes with an A713V mutation in mec-4 (termed *mec-4(d)*), specific mechanosensory neurons develop normally, but undergo degeneration via calcium-mediated necrosis by adulthood (Royal et al., 2005). Growth of *mec-4(d)* mutants in the presence of a calcium chelator and genetic alterations of calcium handling pathways restored neuronal survival. Therefore, Nagarajan et al. (2014) grew *trp-4(d)* mutants in the presence of a calcium chelator and observed a mild rescue of neuronal degeneration. Furthermore, double mutants of *trp-4(d)* and calreticulin (*crt-1*), a calcium-binding endoplasmic reticulum chaperone, significantly increased neuronal survival. *trp-4(d);itr-1* and *trp-4(d); unc-68* double mutants, which abolish inositol triphosphate (IP3) and ryanodine receptors of the ER, respectively, also displayed a mild increase in survival. Although the authors did not directly measure intracellular calcium levels, these findings indicate that intracellular calcium and, in particular, the mobilization of intracellular stores are necessary for the observed degeneration.

The work of Nagarajan et al. (2014) clearly illustrates that *trp-4(d)* dopaminergic neurons undergo a form of necrotic degeneration similar to that of *mec-4(d)* mutants. As a next step, it would be interesting to confirm the role of the *trp-4(d)* in calcium permeability by expressing in *Xenopus* embryos and performing electrophysiological measurements, as done previously for the *mec-4(d)* mutant channel (Royal et al., 2005). Alternatively, calcium imaging within *C. elegans* using a genetically encoded calcium indicator could be used to directly measure calcium mishandling as the causal factor. As mitochondria are also an important store of intracellular calcium, it would be interesting to address their role in this calcium-mediated toxicity as well.

As mentioned previously, one feature of PD is the specific degeneration of dopaminergic neurons in the SNc while neurons of the VTA, and other populations, are relatively spared. Likewise, Nagarajan et al. (2014) show that not all dopaminergic neurons are equally sensitive to degeneration. While the head neurons [cephalic (CEP) and anterior deirid (ADE) neurons] of *trp-4(d)* mutants often underwent necrosis, the tail neurons of males and posterior deirid (PDE) neurons tended not to degenerate. This difference is somewhat mitigated by overexpression of the *trp-4(d)* mutant gene, which can induce cell death in nearly all dopaminergic cells. Nonetheless, PDE neurons remain more resilient even in transgenic *trp-4(d)*-overexpressing nematodes, with less frequent degeneration occurring than in the CEP neurons. Similarly, CEP neurons were much more susceptible than PDE neurons to 6-OHDA, a synthetic reagent that selectively kills dopaminergic neurons and is often used to induce PD-like symptoms in animal models (Nass et al., 2002). What cellular differences cause this difference in sensitivity? Nagarajan et al. (2014) suggest that calcium regulation may lie at the heart of this effect. Further elucidation of calcium-mediated degeneration pathways should begin to address differential vulnerability of specific neurons to calcium. In addition, examining the expression patterns of different calcium-binding proteins in different neurons in *C. elegans* will be an essential follow up experiment.

The finding that dopaminergic neurons are sensitive to fluctuations in intracellular calcium is not new. In fact, calcium transients have been recognized as a potential cause of mitochondrial stress in dopaminergic neurons of the mammalian SNc (Chan et al., 2007). The dopaminergic neurons of the SNc display autonomous pacemaking behavior in the absence of presynaptic influence with a firing frequency between 1 and 4 Hz (Grace and Bunney, 1984). In conjunction with neuronal firing, intracellular levels of calcium spike by activation of L-type voltage-gated calcium channels (Guzman et al., 2010). The function of the pacemaking activity is likely to sustain dopamine concentrations in the striatum (Romo and Schultz, 1990). The oscillations in intracellular calcium challenge dopaminergic neurons, because calcium levels must be tightly regulated. This challenge can be observed in mice with a mitochondrial-specific green fluorescent protein that fluoresces during oxidative stress. Dopaminergic neurons of the SNc display much higher basal levels of oxidative stress than neurons of the VTA. Furthermore, treatment of SNc neurons with isradipine, a calcium-channel blocker, reduces the levels of oxidative stress indicating that increases in intracellular calcium are responsible (Chan et al., 2007). On top of this constant pacemaking activity, dopaminergic neurons are stimulated by glutamatergic inputs, which further drives calcium levels within the cell through the NMDA receptor (Deister et al., 2009). It is easy to envision that even a slight dysregulation in calcium handling can be potentially toxic to these cells and differential sensitivity between dopaminergic neurons may influence the decision to undergo cell death.

By demonstrating that dopaminergic neurons in *C. elegans* display differential sensitivity to *trp-4(d)*-mediated cell death, Nagarajan et al. (2014) provide a potential window into calcium-mediated degeneration of dopaminergic neurons. It is important to note that despite the relative simplicity of *C. elegans* dopaminergic neurons, including lack of action potentials, simple morphologies, and synaptic connections, their function remains somewhat conserved, as both mammalian and *C. elegans* dopaminergic neurons play an integral role in coordinating locomotion (Chase and Koelle, 2007). The findings by Nagarajan et al. (2014) suggest that evolutionarily conserved mechanisms may underlie the intrinsic sensitivity of dopaminergic neurons and this sensitivity is greatly modulated by neuronal-specific calcium handling abilities. Future work on differential calcium sensitivity of neuronal populations using this *trp-4(d)* mutant will have the advantage of the high-throughput, genetically accessible *C. elegans* model system to further our understanding of the process of dopaminergic neuronal degeneration. This will undoubtedly provide exciting insights into the vulnerability of certain neurons to cell death.

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