Synuclein and dopamine: the Bonnie and Clyde of Parkinson's disease

Subhojit Roy

Mouse models have generally failed to recapitulate the dopaminergic neurodegeneration seen in Parkinson's disease. Expressing mutant α-synuclein in a background of elevated dopamine generates mice with nigrostriatal degeneration.

Parkinson's disease is characterized by loss of dopaminergic neurons in the substantia nigra and aggregation of the 140-kDa protein α-synuclein in cell bodies and cell processes as Lewy bodies and Lewy neurites. The precise pathologic role of α-synuclein in neurodegeneration is of great interest, and many animal models expressing wild-type α-synuclein or familial Parkinson's disease mutants have been generated. Although these animal models mimic some aspects of the disease, surprisingly, they have largely failed to recapitulate dopaminergic neuron loss. In this issue of *Nature Neuroscience*, Mor *et al.*[1] report a mouse model in which expression of mutant α-synuclein in the background of elevated dopamine causes degeneration of substantia nigra neurons, including a dramatic loss of projecting striatal synapses (**Fig. 1**). Furthermore, their data suggest that the underlying pathology in these animals is likely related to toxic α-synuclein oligomers in the substantia nigra and that the interaction of α-synuclein with dopamine is critical for pathogenesis.

To develop this model, the authors first generated mice with elevated dopamine in substantia nigra neurons. The rationale here is that dopamine has long been implicated as the neurotransmitter responsible for the vulnerability of substantia nigra neurons. Specifically, dopamine oxidation generates toxic products, including reactive oxygen species, making nigral neurons especially susceptible to injury[2]. Thus, the authors reasoned that raising α-synuclein levels in a background of elevated dopamine might recapitulate the nigral neuron loss seen in human disease. To generate mice with elevated dopamine, Mor *et al.*[1] injected the animals with a lentiviral vector containing a mutant *TH* (tyrosine hydroxylase) gene. TH is the rate-limiting enzyme in dopamine biosynthesis and is in turn inhibited by dopamine as a part of a feedback loop, helping maintain homeostatic levels of the neurotransmitter in neurons. The mutant *TH* gene injected by the researchers encodes a TH protein in which the sequence $R_{37}R_{38}$ is replaced by $E_{37}E_{38}$ (TH-RREE), making the enzyme insensitive to feedback inhibition by dopamine. Thus, mice injected with TH-RREE cannot regulate dopamine levels in the substantia nigra, leading to higher levels of this neurotransmitter. Using this strategy, the researchers were able to raise striatal dopamine in mouse brains by over 50%.

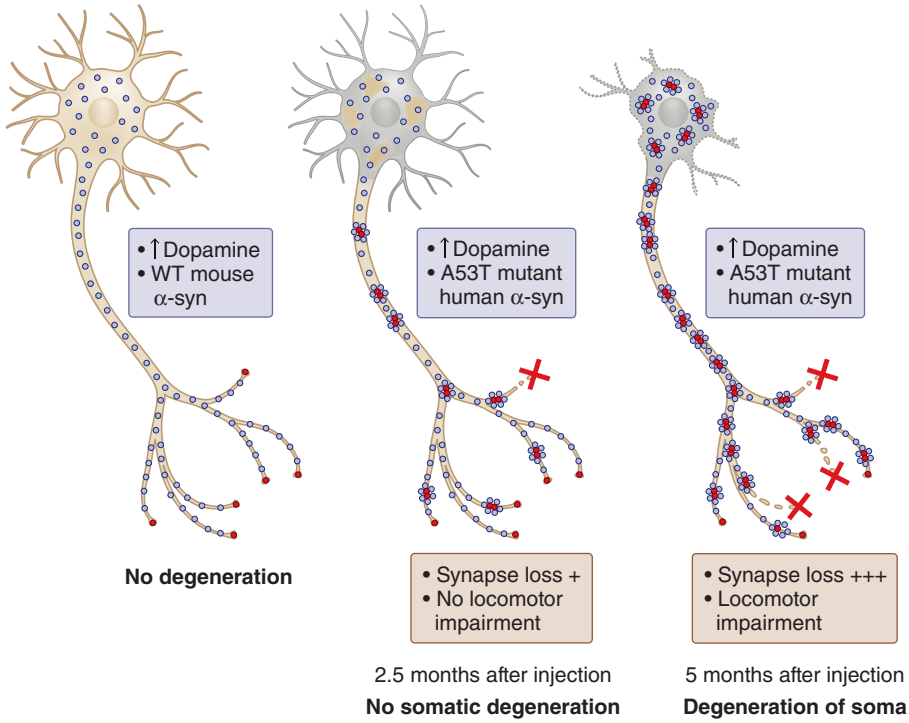

Figure 1 Schematic of a dopaminergic neuron with striatal synaptic terminals. While increasing dopamine (blue dots) in the wild-type (WT) setting is benign (left), similar increases in the setting of mutant (A53T) α-synuclein (α-syn) lead to progressive neurodegeneration (middle and right). Synaptic loss (red X marks) in presynaptic striatal terminals precedes somatic degeneration, and toxicity is thought to be mediated by α-synuclein (red) oligomers, which are stabilized by binding to dopamine^.

Subhojit Roy is at the University of Wisconsin-Madison, Wisconsin Institute for Medical Research, Madison, Wisconsin, USA.
e-mail: roy27@wisc.edu

AU3

Although single high-dose injections of dopamine are known to be acutely toxic[3], surprisingly, the gradual increase of dopamine in the TH-RREE model had no effect on substantia nigra neurons in WT mice. However, when the authors injected the TH-RREE construct into mice expressing mutant A53T α-synuclein, an autosomal dominant mutation seen in familial Parkinson's disease, there was a substantial loss of neuronal cell bodies in the substantia nigra, along with degeneration of projecting presynaptic terminals in the striatum. This pathology was most prominent 5 months after injection, with degeneration of more than half of the synapses at this timepoint. Moreover, these mice had locomotor impairment, likely related to the nigrostriatal damage. By contrast, 2.5 months after injection there was mild synaptic damage but no degeneration of cell bodies and no locomotor impairment (**Fig. 1**), suggesting that the synaptic defects might precede the other pathologic changes in these mice. Collectively, the data suggest that the vulnerability of substantia nigra neurons is related to the collective pathologic interplay of dopamine and α-synuclein.

Previous studies have suggested that α-synuclein oligomers promote the degeneration of substantia nigra neurons. To explore mechanisms by which α-synuclein and dopamine might conspire to kill these neurons, the authors looked at α-synuclein oligomers in the A53T TH-RREE mice. Studies suggest that oxidized dopamine helps stabilize toxic α-synuclein oligomers[4], though the pathophysiological roles of these oligomers remain unclear. The authors found that tissue from the substantia nigra of A53T TH-RREE mice contained several oligomeric α-synuclein species, suggesting that the initiation and progression of pathology in these mice might be related to oligomeric α-synuclein. Finally, the authors recapitulated the cooperative effect of α-synuclein and dopamine in a *Caenorhabditis elegans* model of dopaminergic neuronal degeneration, showing that the physical interaction of dopamine and α-synuclein is critical to pathogenesis. Specifically, expression of CAT-2, a *C. elegans* TH homolog expected to increase dopamine levels, also enhanced the degeneration of dopaminergic neurons expressing A53T. Furthermore, mutating the A53T synuclein at residues known to mediate the interaction of α-synuclein with dopamine-products ($_{125}$YEMPS$_{129}$) abrogated the A53T-induced degeneration, highlighting the importance of this association in disease pathogenesis.

The importance of α-synuclein to the pathogenesis of Parkinson's disease is unquestionable, yet until now there has been no α-synuclein mouse model that can robustly recapitulate the loss of substantia nigra neurons—a cardinal feature of the human disease. The authors have now generated such a model, which should be a useful tool for the community. A noteworthy feature of this model is that the α-synuclein-induced synaptic degeneration precedes the loss of neuronal cell bodies and locomotor impairment. In the physiologic state, α-synuclein is highly enriched at presynapses, maintaining synaptic homeostasis[5–9]. Thus, several groups, including ours, have suggested that the pathology of α-synuclein is initiated at synapses, perhaps starting with the aggregation of α-synuclein here[10–13]. Since oligomerization of α-synuclein seems to be a key feature of the A53T TH-RREE model as well, the early synaptic degeneration in these mice may be induced by α-synuclein oligomers, with eventual degeneration of nigral cell bodies (a 'dying back' consistent with the human neuropathology). However, the spatial and temporal distribution of α-synuclein oligomers in the A53T TH-RREE model is as yet unknown.

The mechanism by which α-synuclein oligomers lead to synaptic toxicity and neuronal loss in this mouse model are unclear. Previous studies suggest that α-synuclein oligomers can directly impair neurotransmitter release[14] and chaperone-mediated autophagy[15], but the underlying cell biology is largely unexplored. Most Parkinson's disease is sporadic rather than familial, and it's unclear whether the mechanisms proposed by Mor *et al.*[1] using the aggregation-prone A53T mutant are generally applicable. Mouse models expressing wild-type α-synuclein in the setting of dysregulated dopamine may shed further light. It is also unclear whether genetic elevation of dopamine levels is truly representative of the pathology seen in human disease. Animal and cellular models that better recapitulate the sporadic pathology are badly needed, not just for Parkinson's but for all neurodegenerative diseases.

COMPETING FINANCIAL INTERESTS^ AU2
The author declares no competing financial interests.

1. Mor, D.E. *et al. Nat. Neurosci.* ^**20**, XXX–XXX PR1 (2017).
2. Graham, D.G. *Mol. Pharmacol.* **14**, 633–643 (1978).
3. Hastings, T.G., Lewis, D.A. & Zigmond, M.J. *Proc. Natl. Acad. Sci. USA* **93**, 1956–1961 (1996).
4. Conway, K.A., Rochet, J.C., Bieganski, R.M. & Lansbury, P.T. Jr. *Science* **294**, 1346–1349 (2001).
5. Wang, L. *et al. Curr. Biol.* **24**, 2319–2326 (2014).
6. Burré, J. *et al. Science* **329**, 1663–1667 (2010).
7. Nemani, V.M. *et al. Neuron* **65**, 66–79 (2010).
8. Vargas, K.J. *et al. Cell Rep.* **18**, 161–173 (2017).
9. Busch, D.J. *et al. Mol. Biol. Cell* **25**, 3926–3941 (2014).
10. Scott, D.A. *et al. J. Neurosci.* **30**, 8083–8095 (2010).
11. Scott, D. & Roy, S. *J. Neurosci.* **32**, 10129–10135 (2012).
12. Kirik, D. *et al. Proc. Natl. Acad. Sci. USA* **100**, 2884–2889 (2003).
13. Kramer, M.L. & Schulz-Schaeffer, W.J. *J. Neurosci.* **27**, 1405–1410 (2007).
14. Choi, B.K. *et al. Proc. Natl. Acad. Sci. USA* **110**, 4087–4092 (2013).
15. Martinez-Vicente, M. *et al. J. Clin. Invest.* **118**, 777–788 (2008).

PRODUCTION Query_NN4660

Synuclein and dopamine: the Bonnie and Clyde of Parkinson's disease

Subhojit Roy

AUTHOR Query

AU2 [AU: Please check that the Competing Financial Interests declaration is correct as stated. If you declare competing interests, please check the full text of the declaration (at the end of the main references section) for accuracy and completeness.]

AU3 [AU: Legend ok as edited?]

PRODUCTION Query

PR1 [Typesetter: add pp]