JCB Journal of Cell Biology

SPOTLIGHT

Synapses have autophagy under control

Claudia Guimas Almeida

Regulation of autophagy in neurons remains unclear. In this issue, Kulkarni et al. (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202002084) show with elegant live imaging that in dendrites, but not in axons, autophagosome motility and function is regulated by synaptic activity.

Macroautophagy is a type of autophagy that refers to the capacity to form double membrane compartments called autophagosomes that engulf large protein aggregates and defective organelles. Autophagosomes fuse with lysosomes, forming degradative autolysosomes (1). Autophagosome formation depends on the conjugation of LC3-I (cytosolic) to phosphatidylethanolamine, generating LC3-II, which remains bound to autolysosomes (1). In neurons, inactivation of autophagy genes impacts neurodevelopment, axon growth and guidance, synapse formation and pruning, ultimately leading to neurodegeneration. Particularly, in motor neurons and cerebellum Purkinje cells, autophagy gene knockout leads to the accumulation of intracellular protein aggregates and degeneration, impacting movement coordination (1). Interestingly, stimulation of memory up-regulates autophagy, and while reducing autophagy reduces memory, activating it has the opposite effect on memory (2). What triggers macroautophagy in neurons remains unclear. In this issue, Kulkarni et al. test whether synaptic activity regulates autophagy and detail the impact of synaptic activity on autophagosome motility (3).

Kulkarni et al. used multiple strategies to manipulate synaptic activity. They stimulated synaptic activity by depolarizing neurons with high potassium, treating them with a cocktail of antagonists of voltage-gated potassium channels and inhibitory gamma-aminobutyric A receptors, and using uncaging of the excitatory neurotransmitter glutamate. To inhibit synaptic activity, the researchers treated neurons with antagonists of excitatory α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-D-aspartate receptors (4). To image autophagosomes and autolysosomes (here globally termed autophagic vacuoles [AVs]) in live neurons, the authors expressed LC3 tagged with fluorescent proteins. They elegantly imaged the same neuronal compartment before and after depolarization, or under basal, increased, or reduced synaptic activity, and used kymograph analysis (via Kymoanalyser; 5) to quantify the mean speeds of AVs in both dendrites and axons. An increase in intracellular calcium measured with a genetically encoded calcium sensor, GCAMP3, indicated synaptic activity. Kulkarni et al. observed that, in dendrites, AVs stop with synaptic activity and move with synaptic inhibition (Fig. 1). This AV movement change was swift and unaltered by co-culture with astrocytes, and reversible. One key finding is that this change in AV movement occurred in dendrites, but not in axons. Interestingly, AVs stopped at or near synapses, which were identified with PSD-95-GFP.

The authors further characterized the AVs in terms of acidity (lysotracker labelling of acidic organelles) and of degradative capacity (DQ-BSA fluorescence accumulation upon lysosomal degradation). Lysotracker motility changed similarly with synaptic activity. Interestingly, the lysotracker density increased with synaptic stimulation. The higher number of acidic organelles (likely autolysosomes) indicated increased autophagy or acidification with synaptic activity, which could underlie increased degradative activity. Indeed, about half of the LC3-positive AVs were degradative in dendrites, while in axons there was virtually no degradative AV, supporting the requirement for transport to the soma for degradation of autophagic cargo (6). Finally, Kulkarni et al. show that degradative AVs increase with synaptic activity, correlating with the reduced motility of LC3-positive AVs.

An intriguing observation is that the autophagic vacuoles identified by LC3-mCherry were virtually all positive for LAMP1, a marker of late endosomes and lysosomes, indicating that dendrites mainly contain autolysosomes and no or very few autophagosomes (LC3-positive and LAMP1-negative) and late endosomes/lysosomes (LC3-negative and LAMP1-positive). One is left wondering if it results from LC3 overexpression and overflooding to interconnected organelles. An alternative possibility is that LC3 may not always label autophagosomes, in which case complementary electron microscopy is necessary for confirmation. Where are dendritic autolysosomes formed? In axons, a fraction of the LC3 autophagic vacuoles was LAMP1 negative, and the formation of autophagosomes at axon terminals has been well documented (7). Thus, do autophagosomes

iNOVA4Health, CEDOC, NOVA Medical School, Universidade Nova de Lisboa, Lisboa, Portugal.

Correspondence to Claudia Guimas Ameida: claudia.almeida@nms.unl.pt.

© 2021 Guimas Almeida. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms/). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 4.0 International license, as described at https://creativecommons.org/licenses/by-nc-sa/4.0/).

Check for updates

Downloaded from http://rupress.org/jcb/article-pdf/220/6/e202105008/1838726/jcb_202105008.pdf by guest on 09 February 2026

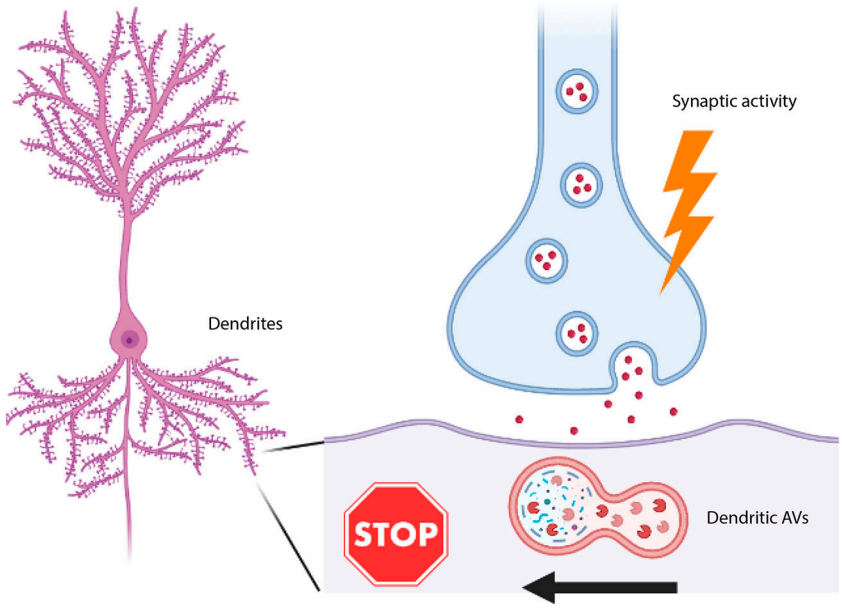

Figure 1. **In dendrites, AVs stop at synapses upon synaptic activity.**

form in axons, fuse with LAMP1-positive late endosomes/lysosomes, and only after are they transported to dendrites? Alternatively, autophagosomes may form in dendrites and fuse with late endosomes/lysosomes, preventing their detection unless fusion is inhibited (8).

Another interesting observation concerns the similar change in the motility of early endosomes, identified by Rab5, an early endosome GTPase, with synaptic activity. Other organelles, post-ER vesicles (4), and proteasomes (9) similarly display a change in motility in dendrites upon synaptic activity. In contrast, mitochondria stop moving in axons with synaptic activity (10). The significance of this arrest of several dendritic organelles with synaptic activity is an attractive area for research.

Neuronal autophagy dysfunction is implicated in many neurodegenerative diseases (1). At least early in the disease, increasing autophagy improves neuronal function and synapse activity (1). Genetic risk factors include lysosomal proteins, whose defective function leads to the accumulation of nondegraded autophagic vacuoles and recapitulate neurodegenerative phenotypes (11). Lysosomal dysfunction is a mechanism of cellular aging. Moreover, synapses become dysfunctional with aging and lost in neurodegenerative diseases (12). Based on this study, synapse dysfunction and thus reduced synaptic activity could increase AV motility and reduce acidification and the degradative capacity of autolysosomes. Similarly, neuronal overexcitability, as in early Alzheimer's disease patients with seizures, could cause excessive AV motility and degradative activity.

What is the mechanism that stops AV movement? Do early endosomes, secretory vesicles, or proteasomes change motility using similar mechanisms? For post-ER vesicles, the CAMKII dependent phosphorylation of the microtubule motor Kif17 was sufficient to arrest movement (4). Alternatively, could it be the actin cytoskeleton that forms patches in the dendritic shaft at the base of postsynaptic glutamatergic synapses to halt microtubule-dependent transport of organelles (13)? More work is needed to tackle these questions and define the cell biological mechanisms by which synaptic activity controls AV function and dynamics in different neuronal compartments. Understanding the mechanisms underlying the regulation of autophagy and autophagosome maturation and degradation provides an exciting opportunity for therapeutic development in neurodegenerative diseases.

Acknowledgments

The author thanks Tatiana Burrinha, Mariana Barata, and Jorge Castanheira from the Neuronal Trafficking in Aging Laboratory for critically reading this Spotlight.

The author is supported by Fundação para a Ciência e a Tecnologia (CEECIND/00410/2017).

The author declares no competing financial interests.

References

1. Fleming, A., and D.C. Rubinsztein. 2020. *Trends Neurosci.* https://doi.org/10.1016/j.tins.2020.07.003
2. Glatigny, M., et al. 2019. *Curr. Biol.* https://doi.org/10.1016/j.cub.2018.12.021
3. Kulkarni, V.V., et al. 2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202002084
4. Hanus, C., et al. 2014. *Cell Rep.* https://doi.org/10.1016/j.celrep.2014.05.028
5. Neumann, S., et al. 2017. *Traffic.* https://doi.org/10.1111/tra.12456
6. Yap, C.C., et al. 2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201711039
7. Ferguson, S.M. 2018. *Curr. Opin. Neurobiol.* https://doi.org/10.1016/j.conb.2018.02.020
8. Shehata, M., et al. 2012. *J. Neurosci.* https://doi.org/10.1523/JNEUROSCI.4533-11.2012
9. Bingol, B., and E.M. Schuman. 2006. *Nature.* https://doi.org/10.1038/nature04769
10. Wang, X., and T.L. Schwarz. 2009. *Cell.* https://doi.org/10.1016/j.cell.2008.11.046
11. Platt, F.M., et al. 2012. *J. Cell Biol.* https://doi.org/10.1083/jcb.201208152
12. Gouras, G.K. 2013. *Int. J. Cell Biol.* https://doi.org/10.1155/2013/141083
13. van Bommel, B., et al. 2019. *EMBO J.* https://doi.org/10.15252/embj.2018101183

Downloaded from http://rupress.org/jcb/article-pdf/220/6/e202105008/1838726/jcb_202105008.pdf by guest on 09 February 2026