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SPOTLIGHT

Stress balls for the brain: How beading protects axons from mechanical damage

Christophe Leterrier¹ 

The slender shape of axons makes them uniquely susceptible to mechanical stress. In this issue, Pan, Hu et al. (<https://doi.org/10.1083/jcb.202206046>) use a microfluidic axon-on-chip device to reveal how actomyosin protects axons from mild mechanical stress, by transiently adopting a beaded shape that helps limit the spread of damaging calcium waves.

Axons are slender projections that allow neurons to communicate with each other and the other cells of the body. Their diameter is typically under a micron for a length that can reach centimeters or even a meter for myelinated axons of the peripheral nervous system (1). This elongated shape makes them uniquely susceptible to mechanical damage. In the central nervous system, the fine unmyelinated axons must withstand stress due to head movements or shocks (2). Following the discovery of a scaffold of actin rings regularly spaced by spectrin tetramers lining the axonal membrane (3, 4), it has been proposed that this membrane-associated periodic scaffold (MPS) has a crucial role in protecting axons against mechanical stress (5). The MPS contains myosins, and its contractile nature has been shown to regulate the axon diameter (6) as well as allow for the transport of large organelles such as mitochondria (7). How the MPS actomyosin contractility is involved in the response to mechanical stress where axons switch from a mostly cylindrical to a “beads on a string” morphology (8) was, however, still unknown. This is now explored by Pan, Hu et al. in this issue (9): they show that MPS-embedded myosins drive reversible beading along the axon, restricting calcium wave propagation to protect the non-stressed parts of the axon.

Pan, Hu et al. (9) used a recently developed “axon-on-chip” microfluidic device

that isolates axons and subjects them to shearing stress via fluid flow (10), with validation of their key results in an *in vivo* model of shock-induced brain injury. Application of a mild stress in the axonal chamber of the device led to the formation of “beads” along the axon, as previously described in response to local puffs (8). 15 min after the application of flow, this beading reaction is reversed along axons but not dendrites, suggesting that stress-induced damage to the gray matter might be more irreversible. Detailed visualization of morphological change during and after application of the flow by confocal and structured illumination microscopy showed that the beading response consisted of the dilatation of bead regions (from 0.4 to about 1 μm in diameter every 10 μm on average), while the segments between beads constricted from 0.4 to about 0.25 μm . Reversibility was not complete, as axons were on average slightly thinner after recovery from the mechanical stress. *In vivo*, two-photon microscopy through a cranial window allowed visualization of axonal beading after a lateral shock to the head of mice, with a slower reversibility over 2–3 h.

Pan, Hu et al. (9) then carefully examined the interplay between this morphological change and axonal components such as transported organelles and cytoskeletal structures. Beads preferentially formed around mitochondria, stopping their transport,

and at axonal branches. This is interesting given the implication of transient opening of actin rings to allow for the transport of mitochondria (7), and the topological necessity—despite poor characterization—of MPS rearrangement from a cylindrical geometry at branches. This warranted a close examination of how the MPS is rearranged in response to flow-induced stress, which the authors performed using structured illumination microscopy on living neurons. They could visualize the dilatation of actin rings at beads, and their constriction between the beads, with 75% of the diameter changes being reversible (Fig. 1). The next step was to assess the localization of myosins, which were found by stimulated emission-depletion microscopy to be present along the MPS in both parallel and perpendicular orientations relative to the axon, as described previously (6, 11). Given that a bipolar myosin filament is ~ 300 nm in length, which is similar to the full diameter of most axons in these cultures, a better picture of the exact arrangement and orientation of myosin filaments will be necessary to clearly understand how their contraction can drive changes in axonal diameter, either by acting within a single actin ring or by a combined radial and longitudinal movement between two rings.

To demonstrate that actomyosin contractility is necessary for the flow-induced

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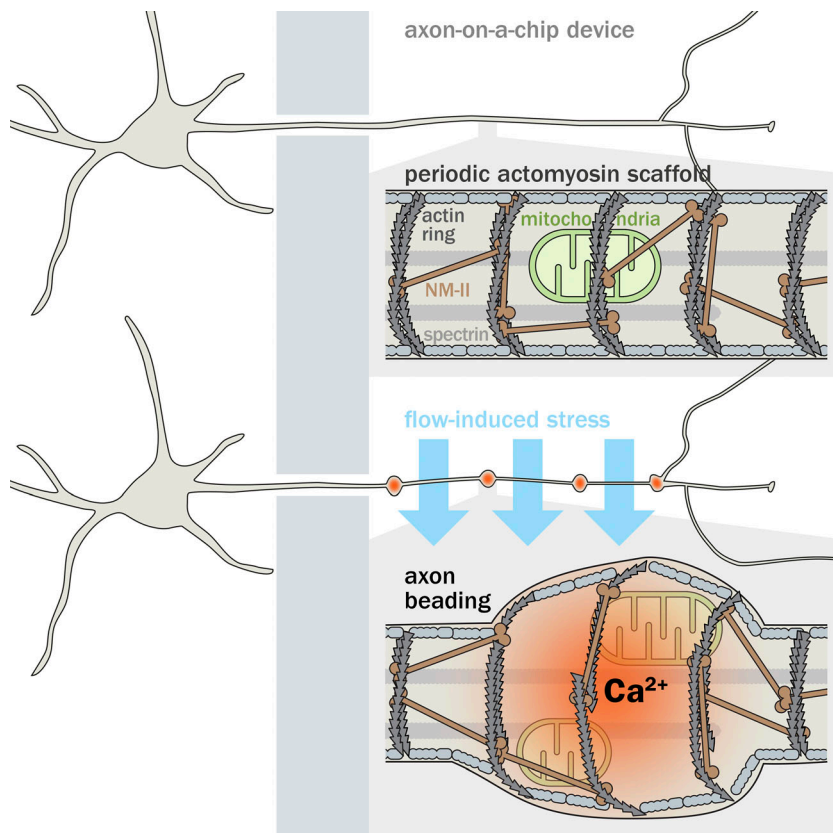


Figure 1. Stress induces actomyosin-driven beading of axons that limits the propagation of calcium waves. An axon-on-a-chip device (gray wall) permits isolation of axons and subjects them to flow-induced stress (blue arrows). This results in rearrangement of the periodic actomyosin scaffold (gray actin rings and brown non-muscular myosin II [NM-II] filaments) that drives the reversible appearance of beads along the axon. This morphological transformation limits the propagation of elevated calcium (red) to non-stressed regions and the downstream axonal degeneration.

beading of axons, the authors used drugs and myosin mutants that result in inhibition or activation of the contractility. This revealed that a precise level of contractility was necessary for the reversible beading, as both inhibition and activation of myosin led to attenuated responses to flow-induced stress. Structured illumination microscopy of axons labeled for the phosphorylated form of the myosin light chain (p-MRLC, indicative of active contractility) showed that p-MRLC was dissolving from beads and concentrating between beads, indicating preferential activation in the constricting regions of the axon.

What is the function of this beading morphological change? It could be a consequence of the stress and the first step

toward damage, as is the case for the irreversible focal axonal swellings observed in other models of axonal degeneration (12). However, a key point in this new work is that reversible beading is rather a protective response that promotes axonal recovery. Imaging of calcium dynamics using fluorescent reporters during and after flow-induced stress shows that beading is indeed induced by the calcium elevation caused by mechanical stress, but in turn helps contain this elevation to the stressed area (Fig. 1). The presence of beads prevents the propagation of elevated calcium concentration to unstressed parts of the axon, as inhibition of beading via manipulation of myosin activity results in further spread of calcium waves across the axonal arborization. Moreover,

such unrestricted calcium propagation caused by contractility inhibition leads to more downstream irreversible damage in the stressed culture, whereas a constitutively active myosin light chain mutant protects against irreversible damage. In a final validation of their model, the authors express the myosin light chain mutants in vivo, and show that a constitutively active MRLC has a protective role against traumatic brain injury.

Overall, this study is a compelling demonstration that reinforces the role of the MPS as a protective scaffold against mechanical stress along axons, highlighting the specific implication of actomyosin contractility in this function. An interesting direction for further studies would be to explore the link between axonal damage, calcium elevation, and activation of myosin contractility. Furthermore, as axon beading is also a phenomenon observed in physiological conditions (13, Preprint) as well as in a range of neurodegenerative contexts (14), stress-induced response could be a useful acute model to explore the role of axonal beading beyond mechanical protection.

References

1. Prokop, A. 2020. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201912081>
2. Funk, J.R., et al. 2011. *Ann. Biomed. Eng.* <https://doi.org/10.1007/s10439-010-0183-3>
3. Xu, K., et al. 2013. *Science.* <https://doi.org/10.1126/science.1232251>
4. Letierrier, C. 2021. *Curr. Opin. Neurobiol.* <https://doi.org/10.1016/j.conb.2020.12.015>
5. Krieg, M., et al. 2014. *Nat. Cell Biol.* <https://doi.org/10.1038/ncb2915>
6. Costa, A.R., et al. 2020. *Elife.* <https://doi.org/10.7554/eLife.55471>
7. Wang, T., et al. 2020. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201902001>
8. Gu, Y., et al. 2017. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201606065>
9. Pan, X., et al. 2024. *J. Cell Biol.* <https://doi.org/10.1083/jcb.202206046>
10. Pan, X., et al. 2022. *Lab Chip.* <https://doi.org/10.1039/D2LC00730D>
11. Zhou, R., et al. 2022. *Nat. Commun.* <https://doi.org/10.1038/s41467-022-30720-x>
12. Tang-Schomer, M.D., et al. 2010. *FASEB J.* <https://doi.org/10.1096/fj.09-142844>
13. Griswold, J.M., et al. 2023. *bioRxiv.* <https://doi.org/10.1101/2023.07.20.549958> (Preprint posted July 20, 2023).
14. Datar, A., et al. 2019. *Biophys. J.* <https://doi.org/10.1016/j.bpj.2019.07.046>