PNAS COMMENTARY

Tensed axons are on fire

Kristian Franze[a,b,c,1]

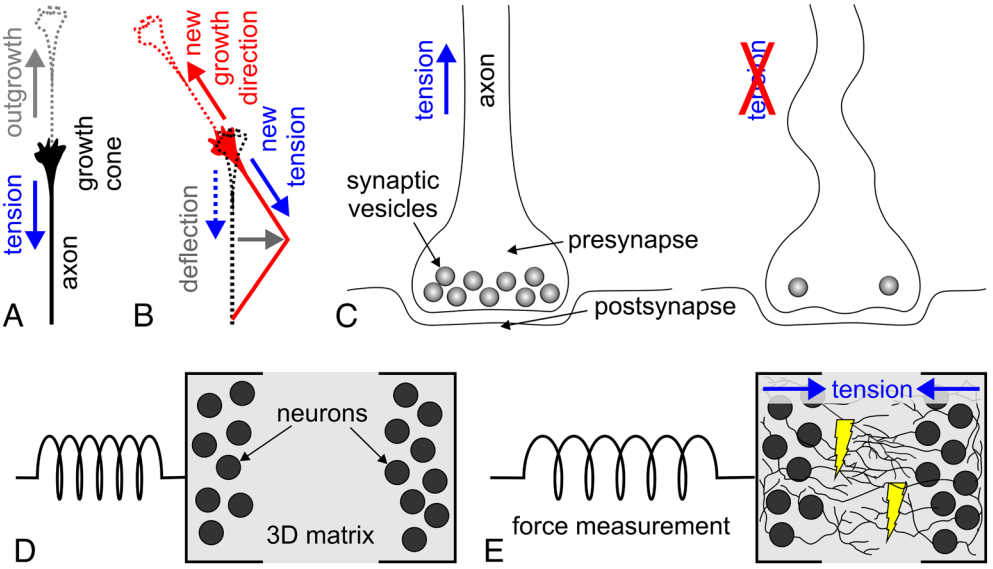

Fig. 1. Tension during neuronal development. (*A*) During axon growth, the growth cone pulls on the axon, thus putting it under tension (blue arrow) (5, 9, 10). (*B*) In 2D cultures, axonal tension can be measured by pulling on the axon with a force sensor like pulling on a guitar string (gray arrow). The resulting change in tension (blue arrow) leads to a change in growth direction (red arrow) (5). (*C*) In vivo, peripheral *Drosophila* axons are under tension (blue arrow). A decrease in axonal tension leads to a decrease in synaptic vesicle clustering at the neuromuscular junction (11). (*D*) Schematic of the device developed by Joy et al. (12). Neurons are cultured in a 3D matrix at opposite ends of a chamber whose walls are connected to a force sensor. (*E*) Over the course of weeks, the neurons extend processes that connect to each other and form synapses, thus building a neuronal network. This process is accompanied by the build-up of tension across the matrix (blue arrows). In 2D cultures, neuronal networks are characterized by spontaneous electrical activity, which depends on actomyosin-based contractility (12).

Already in the late 19th/early 20th century, neuroscientists such as Wilhelm His, Ross Granville Harrison, and Paul Weiss, discussed the role of mechanical signals in neuronal development (1–4). A few decades later, in two seminal papers, Dennis Bray showed that extending neuronal processes ("neurites") are under tension and that the external application of mechanical tension to a neuron is sufficient to generate an axon (5, 6). Other pioneers in the field include Steve Heidemann and Larry Taber, who devoted much of their work to the study of cellular forces and tissue mechanics in the developing nervous system (7–9). Their work has clearly demonstrated that mechanical tension, i.e., a contractile force acting along neurites, is a critical component during brain development (Fig. 1*A*). However, many open questions remain, which are often difficult to address due to a lack of appropriate technology.

For example, axonal tension is difficult to assess in complex environments. In two-dimensional (2D) cultures, a force can be applied to isolated neurites using calibrated microneedles (5) or red blood cells acting as springs (13), similar to pulling on a guitar string (Fig. 1*B*). The amount of deflection of the neurite in response to the applied force can then be used to calculate the tension along the neurite. Alternatively, the tension along the distal end of a neurite can be estimated using 2D traction force microscopy (14). However, when neurons are embedded in a three-dimensional (3D) environment, external forces cannot be easily applied to the neurites, and 3D traction force microscopy (15) is challenging. In PNAS, Joy et al. have developed a new experimental approach that enables straightforward measurements of tissue-scale forces building up within developing neuronal networks over the course of weeks in 3D culture (12).

When neurons are born, their morphology is not very different from that of many other types of tissue cells. Only after they have migrated to their final location, they begin to grow two types of processes to connect with other cells: shorter, branched dendrites, which collect information from surrounding cells, and a usually longer axon, along which information is sent to other cells (e.g., to other neurons or to muscles). The direction and speed of axon and dendrite growth are regulated by the growth cone, an actin-rich

Author affiliations: [a]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge CB2 3DY, United Kingdom; [b]Institute of Medical Physics and Microtissue Engineering, Friedrich-Alexander-Universität Erlangen-Nürnberg, 91052 Erlangen, Germany; and [c]Max-Planck-Zentrum für Physik und Medizin, 91058 Erlangen, Germany

Author contributions: K.F. wrote the paper.

The author declares no competing interest.

See companion article, "Synapses without tension fail to fire in an in vitro network of hippocampal neurons," 10.1073/pnas.2311995120.

[1]Email: kristian.franze@fau.de.

Published January 17, 2024.

structure at the distal end of extending neuronal processes. The growth cone pulls on its neurite (9), thus putting it under tension (5) (Fig. 1*A*). The direction and magnitude of this tension are critical regulators of axon growth (9, 10) (Fig. 1*B*). Axons growing along the same path often form bundles (they fasciculate). Their bundling and unbundling are also regulated by axonal tension (13).

Once extending neurites reach other neurons, they begin to connect with them and form networks. The morphology of these networks is regulated by neurite tension, through the enforcement of axon branches with strong adhesion and the retraction or elimination of axon collaterals whose adhesive forces are smaller than the axonal tension (10, 16).

In PNAS, Joy et al. have developed a new experimental approach that enables straightforward measurements of tissue-scale forces building up within developing neuronal networks over the course of weeks in 3D culture.

Connections between different neurons are called synapses, between neurons and muscles neuromuscular junctions. In many types of neurons, information is sent along axons in the form of action potentials, which are rapidly propagating changes in their membrane potential (the potential gradient across the cell membrane). When the action potential arrives at a synapse, the depolarization of the cell membrane activates voltage-gated calcium channels, leading to an influx of calcium ions along a concentration gradient and thus converting the electrical signal into a chemical signal. The calcium influx causes the release of neurotransmitters—small signaling molecules stored in membrane-bound vesicles in the presynaptic terminal of the axon—into the synaptic cleft, an approximately 20 to 30 nm wide space between the pre- and the postsynaptic terminals (Fig. 1*C*). These neurotransmitters then diffuse across the cleft and bind to receptors on the postsynaptic terminal, leading to a response that depends on the type of neurotransmitter and receptors.

In a groundbreaking study, Taher Saif's laboratory had previously shown that the availability of neurotransmitters at neuromuscular presynaptic terminals in vivo is regulated by the mechanical tension along axons (Fig. 1*C*) (11). Embryonic *Drosophila* axons, which have formed neuromuscular junctions, maintain a rest tension of about 1 nN. Deviations from this axonal tension are accompanied by changes in synaptic vesicle clustering at the neuromuscular junction: Increased tension leads to an increase in vesicle clustering, whereas decreased tension leads to a corresponding decrease in vesicle clustering. Since synaptic vesicles are a prerequisite for synaptic information transmission (17), these data suggested that neuronal network activity may also be regulated by axonal tension.

In PNAS, Joy et al. now show that, in vitro, tissue-scale tension, which builds up during the formation of neuronal networks, may indeed be important for spontaneous neuronal network activity. They developed a new elegant device in which they cultured rodent hippocampal neurons in opposite corners of a 3D matrix that was connected to a custom-built force sensor. The neurons extended axons toward each other, which formed synapses, thus eventually leading to the establishment of a neuronal network. This process was accompanied by a steady increase in tension across the matrix in which the axons grew, until a plateau force of tens of nN was reached after about a week. Blocking the interaction of actin and myosin reduced the measured forces, indicating that they were mainly generated by neuronal contractility. Future work will reveal whether synapses between axons are required to generate the tissue-scale forces measured here, or whether a large number of unconnected axons, which are inherently contractile (5), could generate similar tensile forces.

The authors then investigated how the spontaneous electrical activity of neurons cultured on 2D substrates for 2.5 wk depended on actomyosin-based contractility. Firing rates dropped from ~2 spikes per second in the control condition to ~0.2 spikes per second after global treatment with drugs that interfered with the cells' contractility. While we don't yet know how the contractile forces exerted by neurons in a soft 3D matrix compare to those of neurons cultured on stiff 2D surfaces, and how the firing rates observed in 2D relate to spontaneous neuronal activity in 3D cultures, these experiments provide the first intriguing evidence that actomyosin-based cellular forces are involved in regulating spontaneous neuronal network activity. Given that actomyosin interaction is important not just for the generation of axonal tension but also, for example, for dendritic spine function (18), as discussed by the authors, it will be important in the future to use local perturbations to understand how axonal tension contributes to synaptic information transmission.

Molecular mechanisms by which axonal tension regulates synaptic vesicle clustering and the spontaneous electrical activity of neurons remain poorly understood. One possible pathway could be via the regulation of neurotransmitter exocytosis by membrane tension: alterations in membrane tension could impede or facilitate the fusion of synaptic vesicles with the cell membrane at the presynaptic terminal and thus regulate neurotransmitter release. However, recent experiments have shown that actomyosin-based axonal tension may not scale with neuronal membrane tension (19). Furthermore, when mechanical tension is applied externally to an axon, the axon maintains its normal electrical activity (20), suggesting that axonal tension may not affect the electrical activity of a neuron directly but rather indirectly, in a synapse-specific way. Consistent with this idea, the application of a mechanical force to the presynaptic terminal leads to an increase in neurotransmitter release (21). Therefore, axonal tension could lead to the deformation of presynaptic terminals and thus to their increased activity. Molecularly, this increase could be achieved, for example, by altering the activity of ion channels or other mechanosensitive proteins in the deformed synaptic cell membrane (22). Future work will show how axonal tension contributes to the regulation of neuronal network activity and how this mechanical signal is integrated with chemical signals that also control synapse function.

ACKNOWLEDGMENTS. I would like to thank past and present members of the Franze lab for amazing discussions and a great atmosphere and acknowledge the financial support by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation; 460333672–CRC1540 Exploring Brain Mechanics and 270949263/GRK2162 Neurodevelopment and Vulnerability of the Central Nervous System), the European Research Council (Consolidator Award 772426), and the Alexander von Humboldt Foundation (Alexander von Humboldt Professorship).

1. W. His, Die Entwicklung der ersten Nervenbahnen beim menschlichen Embryo: Uebersichtliche Darstellung. *Arch. Anat. Physiol.* **82**, 368 (1887).
2. R. G. Harrison, The outgrowth of the nerve fibers as a mode of protoplasmic movement. *J. Exp. Zool.* **9**, 787–846 (1910).
3. P. Weiss, In vitro experiments on the factors determining the course of the outgrowing nerve fiber. *J. Exp. Zool.* **68**, 393–448 (1934).
4. P. Weiss, Nerve patterns: The mechanics of nerve growth. *Growth (Suppl. Third Growth Symp.)* **5**, 163–203 (1941).
5. D. Bray, Mechanical tension produced by nerve cells in tissue culture. *J. Cell Sci.* **37**, 391–410 (1979).
6. D. Bray, Axonal growth in response to experimentally applied mechanical tension. *Dev. Biol.* **102**, 379–389 (1984).
7. G. Xu *et al.*, Axons pull on the brain, but tension does not drive cortical folding. *J. Biomech. Eng.* **132**, 071013 (2010).
8. S. R. Heidemann, P. Lamoureux, R. E. Buxbaum, Cytomechanics of axonal development. *Cell Biochem. Biophys.* **27**, 135–155 (1995).
9. P. Lamoureux, R. E. Buxbaum, S. R. Heidemann, Direct evidence that growth cones pull. *Nature* **340**, 159–162 (1989).
10. K. Franze, Integrating chemistry and mechanics: The forces driving axon growth. *Annu. Rev Cell Dev. Biol.* **36**, 61–83 (2020).
11. S. Siechen, S. Yang, A. Chiba, T. Saif, Mechanical tension contributes to clustering of neurotransmitter vesicles at presynaptic terminals. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 12611–12616 (2009).
12. M. S. H. Joy *et al.*, Synapses without tension fail to fire in an in vitro network of hippocampal neurons. *Proc. Natl. Acad. Sci U.S.A.* **120**, e2311995120 (2023).
13. D. Smit, C. Fouquet, F. Pincet, M. Zapotocky, A. Trembleau, Axon tension regulates fasciculation/defasciculation through the control of axon shaft zippering. *Elife* **6**, e19907 (2017).
14. D. Koch, W. J. Rosoff, J. Jiang, H. M. Geller, J. S. Urbach, Strength in the periphery: Growth cone biomechanics and substrate rigidity response in peripheral and central nervous system neurons. *Biophys. J.* **102**, 452–460 (2012).
15. J. Steinwachs *et al.*, Three-dimensional force microscopy of cells in biopolymer networks. *Nat. Methods* **13**, 171–176 (2016).
16. S. Anava, A. Greenbaum, E. Ben Jacob, Y. Hanein, A. Ayali, The regulative role of neurite mechanical tension in network development. *Biophys. J.* **96**, 1661–1670 (2009).
17. S. O. Rizzoli, W. J. Betz, Synaptic vesicle pools. *Nat. Rev. Neurosci.* **6**, 57–69 (2005).
18. J. Ryu *et al.*, A critical role for myosin IIb in dendritic spine morphology and synaptic function. *Neuron* **49**, 175–182 (2006).
19. E. Kreysing *et al.*, Effective cell membrane tension is independent of polyacrylamide substrate stiffness. *PNAS Nexus* **2**, pgac299 (2023).
20. J. R. Loverde, B. J. Pfister, Developmental axon stretch stimulates neuron growth while maintaining normal electrical activity, intracellular calcium flux, and somatic morphology. *Front. Cell Neurosci.* **9**, 308 (2015).
21. H. Ucar *et al.*, Mechanical actions of dendritic-spine enlargement on presynaptic exocytosis. *Nature* **600**, 686–689 (2021).
22. E. K. Pillai, K. Franze, Mechanics in the nervous system: From development to disease. *Neuron*, 10.1016/j.neuron.2023.10.005 (2023).