High-resolution spatiotemporal dynamics of serotonergic axons in primary brainstem cultures

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Recent experimental and theoretical work by our group has shown that the self-organization of the brain serotonergic matrix is strongly driven by the spatiotemporal dynamics of single serotonergic axons (fibers). The trajectories of these axons are often stochastic in character and can be described by step-wise random walks or time-continuous processes (e.g., fractional Brownian motion). The success of these modeling efforts depends on experimental data that can validate the proposed mathematical frameworks and constrain their parameters. In particular, further progress requires reliable experimental tracking of individual serotonergic axons in time and space. Visualizing this dynamic behavior *in vivo* is currently extremely difficult because of the high axon densities and other resolution limitations. In this study, we used *in vitro* systems of mouse primary brainstem neurons to examine serotonergic axons with unprecedented spatiotemporal precision. The high-resolution methods included confocal microscopy, STED super-resolution microscopy, and live imaging with holotomography. We demonstrate that the extension of developing serotonergic axons strongly relies on discrete attachments points on other, non-serotonergic neurons. These membrane anchors are remarkably stable but can be stretched into nano-scale tethers that accommodate the axon's transitions from neuron to neuron, as it advances through neural tissue. We also show that serotonergic axons can be flat (ribbon-like) and produce screw-like rotations along their trajectory, perhaps to accommodate mechanical constraints. We conclude that the stochastic dynamics of serotonergic axons may be conditioned by the stochastic geometry of neural tissue and, consequently, may reflect it. Our current research includes hydrogels to better understand these processes in controlled artificial environments. Since serotonergic axons are nearly unique in their ability to regenerate in the adult mammalian brain and they support neural plasticity, this research not only advances fundamental neuroscience but can also inform efforts to restore injured neural tissue.

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