Spatiotemporal Dynamics of Single Serotonergic Axons in *Ex Vivo* Systems

M.T. HINGORANI[1], A.M. VIVIANI[1], J.C. CHIU[1], A.M. VARGAS[1], R. STOWERS[2], C.M. BATES[3], S. JANUSONIS[1]; *Departments of [1]Psychological & Brain Sciences, [2]Mechanical Engineering, [3]Materials, University of California Santa Barbara, Santa Barbara, CA*

Previous work in our group has shown that the self-organization of the serotonergic matrix depends in part on the decisions made by individual fibers. Evidence suggests that the trajectories of serotonergic fibers are strongly stochastic and can be well described by step-wise random walks or fractional Brownian motion (a time-continuous process). The success of these modeling efforts crucially depends on experimental data which are necessary to validate the general structure of a model and to constrain the values of its parameters. In particular, further progress crucially depends on the experimental capability to track individual serotonergic axons in *time* and *space*. However, visualizing this dynamic behavior *in vivo* is currently extremely difficult. In this study, we cultured primary mouse brainstem neurons in several *in vitro* and *ex vivo* systems. Using a combination of methods such as confocal microscopy, digital holotomography, false fluorescent neurotransmitters, and novel 3D hydrogel systems, we investigated serotonergic neurons and their axons with unprecedented spatiotemporal precision. The dynamics of axon growth cones, branching events, and other key processes were analyzed with respect to the properties of the tunable extracellular environment and to the refractive index-sensitive intracellular movements. These experimental data include the first holotomographic images of serotonergic axons and provide essential information for the predictive modeling of the serotonergic matrix. In addition to the importance of these findings for fundamental neuroscience, they may also support future efforts in the restoration of brain tissue (serotonergic fibers are almost unique in the mammalian brain in their ability to robustly regenerate). Some of the novel approaches developed in this study may be applicable to other axons in the ascending reticular activating system.

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