An experimental toolbox for the analysis of single serotonergic axons in the mouse brain

K.C. MAYS, J.H. HAIMAN, S. JANUSONIS
Department of Psychological & Brain Sci., Univ. of California, Santa Barbara, CA

The self-organization of the serotonergic matrix in the brain is a key unsolved problem in neuroscience. This matrix is composed of extremely long axons (fibers) that originate in the brainstem, invade nearly all brain regions, and accumulate in remarkably high densities in many of them. Serotonergic fibers possess a number of intriguing properties, including the ability to robustly regenerate in the adult brain, the strongly stochastic trajectories, and the poorly understood but consistent association with neural plasticity. We developed several experimental methods that can be used to capture the individual trajectories of serotonergic fibers in the mouse brain, including regions with high fiber densities. These data are essential for stochastic modeling efforts that currently utilize two different frameworks (a step-wise random walk based on the von Mises-Fisher directional distribution and the superdiffusive fractional Brownian motion). In one approach, we show that serotonergic fibers can be experimentally isolated by using transgenic mice with the inducible Cre (under the Tph2-promoter), crossed with a Cre reporter line. While the overall labeling intensity falls below that of the best constitutive model in the field (Migliarini et al., 2013), the inducible Cre allows for control over how many fibers are labeled in high-density regions, thus facilitating their semi-automated tracing. A particularly powerful approach is based on the Brainbow toolbox (Cai et al., 2013) which can be used to randomly "color-code" individual axons. We have developed the first implementation of Brainbow-tagging in the serotonergic system (based on intracranial AAV-injections) and demonstrate its potential in downstream stochastic analyses. In particular, we show that some apparent branching points are different fibers crossing at distances below the limit of optical resolution (even in high-power confocal imaging). Finally, we demonstrate the feasibility of imaging single serotonergic fibers with CUBIC-based tissue clearing and high-resolution light-sheet microscopy (with a 20X objective). This experimental toolbox, integrated with stochastic modeling, can advance the current understanding of the dynamics, robustness, and plasticity of the brain serotonergic system.

This research was funded by NSF CRCNS (#1822517 and #2112862), NIMH (#MH117488), and the California NanoSystems Institute.