ORAL COMMUNICATIONS

# AN EX VIVO GRAFTING PLATFORM TO DISSECT TISSUE-DEPENDENT GUIDANCE OF SEROTONERGIC AXONS

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Serotonergic neurons in the brainstem raphe nuclei give rise to one of the most widespread and structurally complex projection systems in vertebrate brains. In mice, serotonergic neurons begin extending their axons rostrally as early as embryonic day 11.5, reaching their targets by the end of gestation and then arborising extensively to produce massive, region-specific fiber meshworks.

Despite recent advances in the identification of transcriptional programs regulating serotonergic neuron development, the mechanisms that govern axon pathfinding and spatial organisation remain poorly understood. In addition to classical guidance cues, interaction with the local environment is likely to contribute to how serotonergic axons explore and navigate the developing brain.

To investigate these processes, we used 3D holotomography to analyse serotonergic axons in embryonic explants from Tph2-GFP mice, revealing highly motile growth cones and continuous exploratory activity, highlighting the dynamic properties of serotonergic axons in real time. To further explore the role of the tissue environment, we developed an ex vivo grafting assay in which serotonergic explants are transplanted onto foetal brain flat-mounts or onto adult brain slices. Preliminary results indicate that axon growth mode and directionality are strongly influenced by the graft's position in the host tissue, with axons showing a striking tendency to align with and follow pre-existing local fiber architectures.

Together, these findings demonstrate that the local tissue environment is an autonomous determinant of serotonergic axon behaviour, shaping both the mode and direction of growth in a context-dependent manner. This experimental platform advances fundamental neuroscience in dissecting how the geometric and mechanical properties of the local substrate interact with guidance signals to specify axon trajectories, with potential implications for neural repair strategies.