Reconstructing the developmental origins and migratory trajectories of the pioneer neurons

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Background

To establish proper connectivity within the brain, neurons migrate from their birthplaces to a set of final locations where they integrate into the surrounding neural circuitry. Motor neurons in particular must migrate correctly to reach the proper muscle targets. We are interested in a class of motor neurons called the facial branchiomotor neurons (FBMNs), which control facial expression and jaw movement in mammals and are conserved across vertebrates. FBMNs migrate from their birthplace in the fourth rhombomere (segment) of the hindbrain to the sixth and seventh rhombomeres, where they coalesce into two distinct nuclei. Our studies in zebrafish identified a novel mechanism contributing to FBMN migration. The first FBMN to exit rhombomere four acts as a pioneer – it actively explores the signaling environment of the hindbrain and leaves a trailing axon behind it to serve as a scaffold for followers. When the pioneer is absent, FBMN migration ceases altogether, demonstrating that the pioneer has unique qualities with respect to its followers.

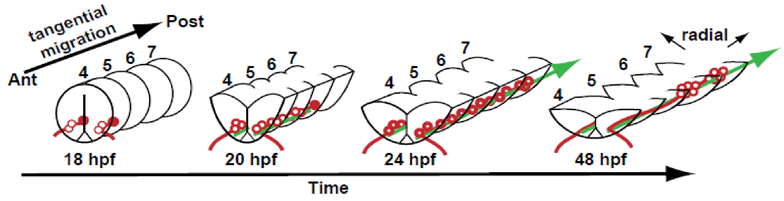

Fig. 1: FBMNs are born in r4 and migrate to r6/7 through the neuroepithelium of the hindbrain.

We are investigating the origins of the FBMNs to better understand how differences between pioneers and followers arise. Using the single-plane illumination microscopy (SPIM), we can image the rapid cell divisions of FBMN progenitors and the migration of the FBMNs themselves. With this data, we aim to reconstruct the cell lineages and movements of the pioneers. However, SPIM generates vast amounts of data, with 1-1.5 TB generated in a single experiment. Manually analyzing this data can take upwards of 20 hours and suffer from human error. We are working on an automated pipeline for drift correction and cropping (Kindlmann, unpublished) plus cell lineage reconstruction and editing (adapted from Amat et. al. 2015) which will allow us to process our data in a more robust and reproducible manner. Additionally, we are pursuing novel data visualization methods which will allow us to check cell lineage accuracy and visualize the migratory trajectories of FBMNs.

The pioneer neuron and its trailing axon are required for successful FBMN migration

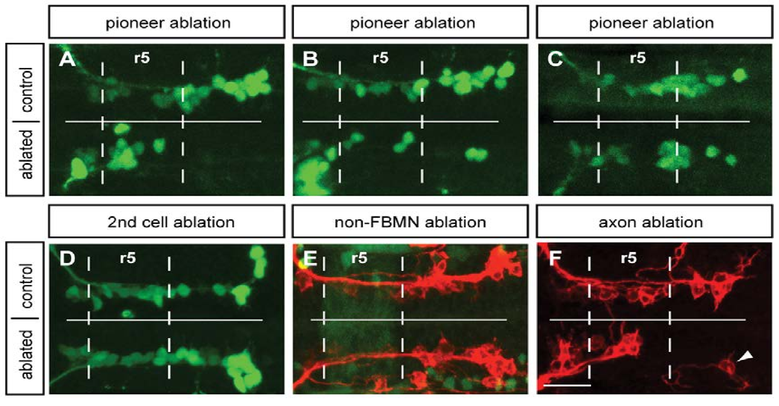

Fig. 2: Ablating the pioneer (A-C) or its trailing axon (F) results in clustering of FBMNs in r4, demonstrating that the pioneer is necessary for successful migration into r6/7. However, ablating the FBMN behind the pioneer does not impact migration (D), nor does ablating the neuroepithelium. Wanner and Prince, 2013.

Central Question

Do the two pioneer neurons (one on either side of the brain) have a common developmental lineage?

SPIM enables data acquisition that is rapid and gentle enough for resolution of FBMN progenitor divisions

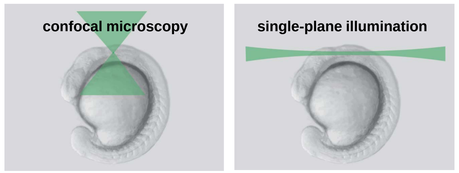

Fig. 3: Schematic of the light paths in confocal and SPIM. SPIM allows the sample to be illuminated along the focal plane only, reducing background and phototoxicity. The detection objective is orthogonal to the plane of illumination and collects fluorescent light emitted by the specimen.

Double transgenic embryos allow for identification of the pioneer and tracking of progenitor nuclei

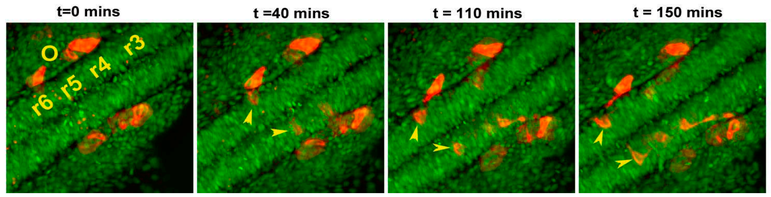

Fig. 4: Maximum intensity projection (MIP) of His:GFP, zCREST:MRFP line which expresses transgenes that label (A) all cell nuclei in green and (B) FBMN cell membranes in red.

Preliminary manual tracking results indicate the bilateral pioneers may not share a common progenitor

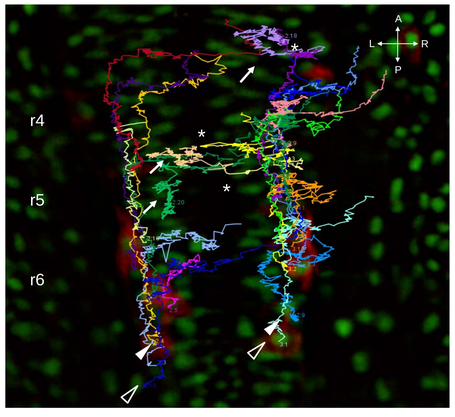

Fig. 5: Single z-slice from the final frame of a time-lapse stopped at 21 hpf. Tracks of 16 neurons are overlaid in their entirety, including those of the pioneers (closed arrowheads). Neurons born in r4 are FBMNs, while neurons born in r5 are presumed to be PLL efferents (open arrowheads). Three divisions (asterisks) are evident though none give rise to a pioneer neuron. Sister cells resulting from these divisions are tracked, each giving rise to a neural progenitor (arrows). Results suggest most cell divisions may be asymmetric and a stochastic mechanism may specify the pioneers rather than a lineage dependent mechanism.

Automated tracking pipeline harnesses GPU acceleration to generate cell tracks in a reproducible manner

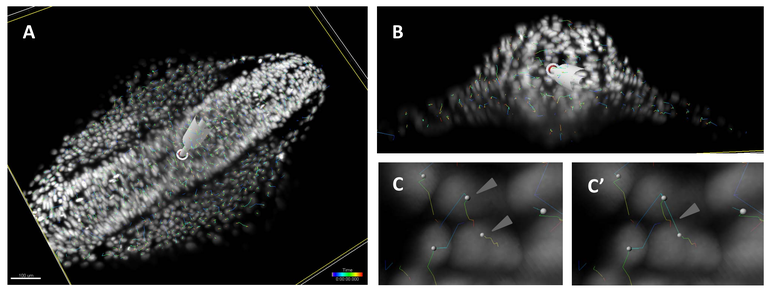

Fig. 6: Automated tracking pipeline adapted from Amat et al, 2015 uses a CUDA-accelerated application to segment cell nuclei, track their movements, and reconstruct their cell lineages over time. 20-slice (A) XY MIP and (B) YZ MIP through the zebrafish hindbrain, showing cell tracks from the first ten time points. (C, C') Tracks can be edited for accuracy after generating.

Rendering cell nuclei based on local peaks in GFP intensity cleans up signal to enable more accurate cell tracking

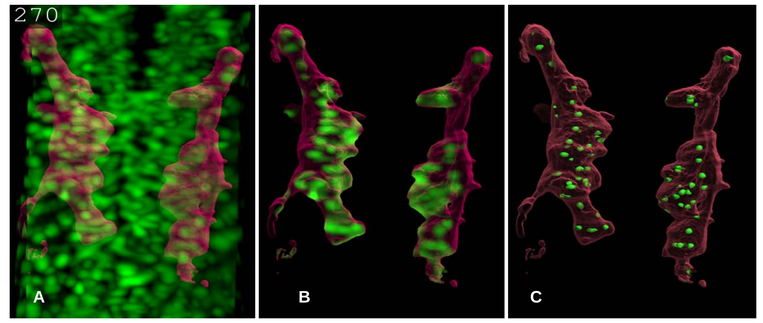

Fig. 7: Rendering nuclei based on local peaks in GFP intensity cleans up signal and could expedite cell tracking. (A) MIP of total GFP signal, with 3D rendering of FBMNs in red. (B) MIP of GFP signal within RFP positive domain. Only nuclei with peaks inside RFP domain are shown, giving a more accurate readout of total FBMN number and increasing ability to manually resolve between adjacent cells. (C) Nuclei rendered as 3D spheres around peaks in GFP signal.

Interactive 3D tracked path rendering with local directional MIP context allows visual confirmation of track accuracy

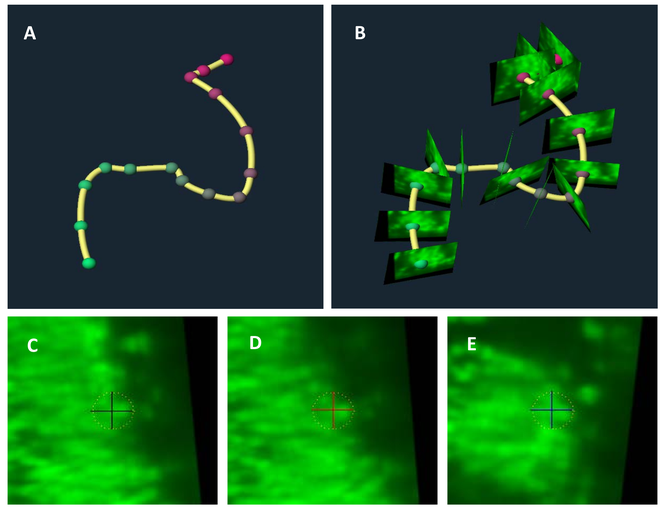

Fig. 8: Rendering of 3D path with local context. (A) 3D (smoothed) tracked path of the neuron, each small sphere is the location of the neuron at each time step, color-coded with green at earlier times, red at later times. (B) Directional MIP within a small slab around the location of the neuron at each time step is overlaid into the path. The slab is orthogonal to the path at each time step, while direction of projection is tangent to the path at that time step. (C,D,E) Sample directional MIPs. The yellow sphere represents the center of the image, which is also the location of the neuron in the smoothed path, while the cross represents the location of the original (not smoothed) path. They are used to check the displacement after smoothing, as well as the accuracy of the tracked path. The cross is color-coded to represent the relative position of the nuclei in the original path with respect to the smoothed path (more red means farther behind, more blue means farther in front, black means falling outside of the slab).

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