Research Week 2023

Distinct transcriptional identity of pioneer neurons

Benjamin Woodruff

Keywords

Zebrafish, Nerve Growth Factor Receptors, Axons, Neurons, Neurogenesis

Abstract

Pioneer neurons are the first to extend their axons into a target tissue during nervous system development. Pioneer axon tracts act as a scaffold for subsequent extension of follower neuron axons. Pioneer neurons have been well documented in the central and peripheral nervous systems of both vertebrates and invertebrates, but their molecular make-up remains poorly understood. We conducted single-cell-RNA-sequencing (scRNA-seq) on neurons of the zebrafish posterior lateral line (pLL). Using a priori knowledge that a neurotrophic factor receptor, ret, is specifically enriched in pioneer neurons of the zebrafish pLL, we determined that ret+ pLL pioneer neurons have a transcriptional profile distinct from that of ret- follower neurons. Differential expression (DE) analysis revealed 30 DE genes which we validated via fluorescent in situ hybridization (FISH). Using scRNA-seq and FISH, we also found that all pLL neuron precursors express follower markers during early neurogenesis. In contrast, pioneer genes are activated later, just before the onset of axon outgrowth. Many follower markers are factors involved in retinoic acid (RA) signaling and their target genes. RA signaling appears active in pLL precursors and follower neurons, but not in pioneers. To test the role of RA signaling, we treated embryos with RA and found an increase in RA-associated genes, but a decrease in ret expression. We next expressed dominant negative or constitutively active RA receptor in individual pLL neurons during neurogenesis. Inhibition of RA biased pLL axons to distal targets (normally innervated by pioneers), while activation of RA biased pLL axons to innervate proximal targets (normally innervated by followers). Overall, our data show that pLL pioneer neurons have a distinct transcriptional profile and that pioneer versus follower axon targeting is regulated by RA signaling.