Transcriptomic Analysis of the Zebrafish Optic Tectum: A Step Towards Comprehensive Characterization

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Abstract

The optic tectum (OT) is the non-mammalian homologue to the superior colliculus (SC), an important structure in the midbrain that receives and coordinates responses to visual, auditory, and other sensory stimuli. Recently, researchers have started looking at the development of the OT and are divided on if visual stimulus is truly necessary for the OT to develop fully. While some believe that the OT has a well refined retinotopic map from the outset of development, others argue that without visual stimulus during development, zebrafish show a decreased sensitivity to light, reduced prey capture abilities, and reduced number of nuclear assemblies in the OT . Using single cell RNA sequencing, we aim to compare the neural populations of normally reared zebrafish (LR) against fish reared without visual stimuli (DR) and map genetic changes. To supplement this, confocal imaging on both types of zebrafish, combined with image analysis through ImageJ and Huygens Software will enable comprehensive cell counts in both LR and DR fish to compare physical changes in the OT based on light stimuli.

Background

The SC is a highly laminated sensorimotor structure located in the mammalian midbrain. Despite SC studies persisting back as far as the 1970s, the knowledge concerning specific cell types and circuitry involved in those roles has historically been suboptimal due to the innate genetic inaccessibility of most mammals . The homologous OT, found in non-mammalian vertebrate species, provides an excellent opportunity to study the SC in more accessible organisms. The OT, similar to the mammalian SC, is the primary retinorecipient region and has the critical role of receiving sensory input used in determining complex visually guided behaviors . Due to lack of a visual cortex, the OT is especially enhanced in zebrafish which, in recent years, has emerged as an ideal model organism for optogenetic studies due to advances in driving higher levels of transgenic expression in defined neural population and precise control over sample illumination. Large clutch sizes, optical clarity during development-which enables deep imaging of early neural systems, and expanding ease of gene editing and advancement of specific labeling tools also contribute to the zebrafish rise in popularity. A common belief is that zebrafish develop spatially structured neural circuitry through spontaneous activity in the brain without the need for sensory input. Conversely other researchers have found that DR fish exhibited decreased photoreceptor shedding, normally mediated by the light dark cycle, consistent with patterns shown by chemical inhibitors and reduced neuronal survival in the medial periventricular gray area of the OT when visual experience was restricted. These differing results drive our interest in the subject. Single-cell RNA sequencing (scRNA-seq) technology is a novel method for transcriptional analysis that has been gaining favor over the last several years . We previously established a protocol for the sequencing of single cells via manual harvest, dissociation, and FAC sorting of 7dpf zebrafish OT cells. The targeted cells are labeled by the transgenic Y304: kaede line, a green to red photoconvertible fluorescent protein, in tectal, olfactory, and habenular cell regions. Combining scRNA-seq with the zebrafish OT creates a powerful new approach to discovering more about neural gene expression, an approach that has been increasingly popular amongst studies aiming to characterize specific cell types within the zebrafish brain. Our flexible protocol allows for the targeting and exclusion of particular subregions; a valuable tool when working with transgenic lines with broad labeling.

Results

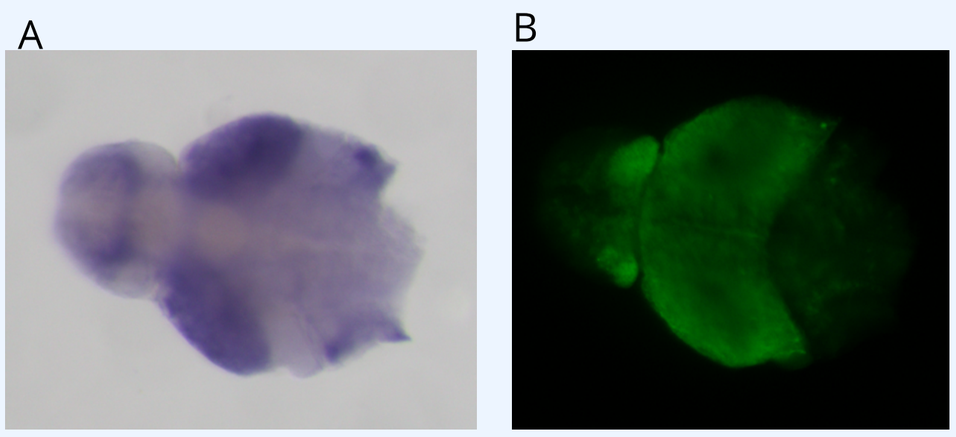

Figure2. In Situ hybridization of Nrxn3a with BM Purple visualization (A) and Kaede staining (B). Nrxn3a was chosen as a target for in situ hybridization as it is a marker gene in one of our cell clusters. Nrxn3a is localized in a Kaede + area confirming the bioinformatical processing, that our neurons are in the areas that we claimed they would be. It also gives us interesting insight into the structure of the tectum in that Nrxn3a seems to be localized in the NP, which is made upf of mostly cell extensions and few cell bodies.

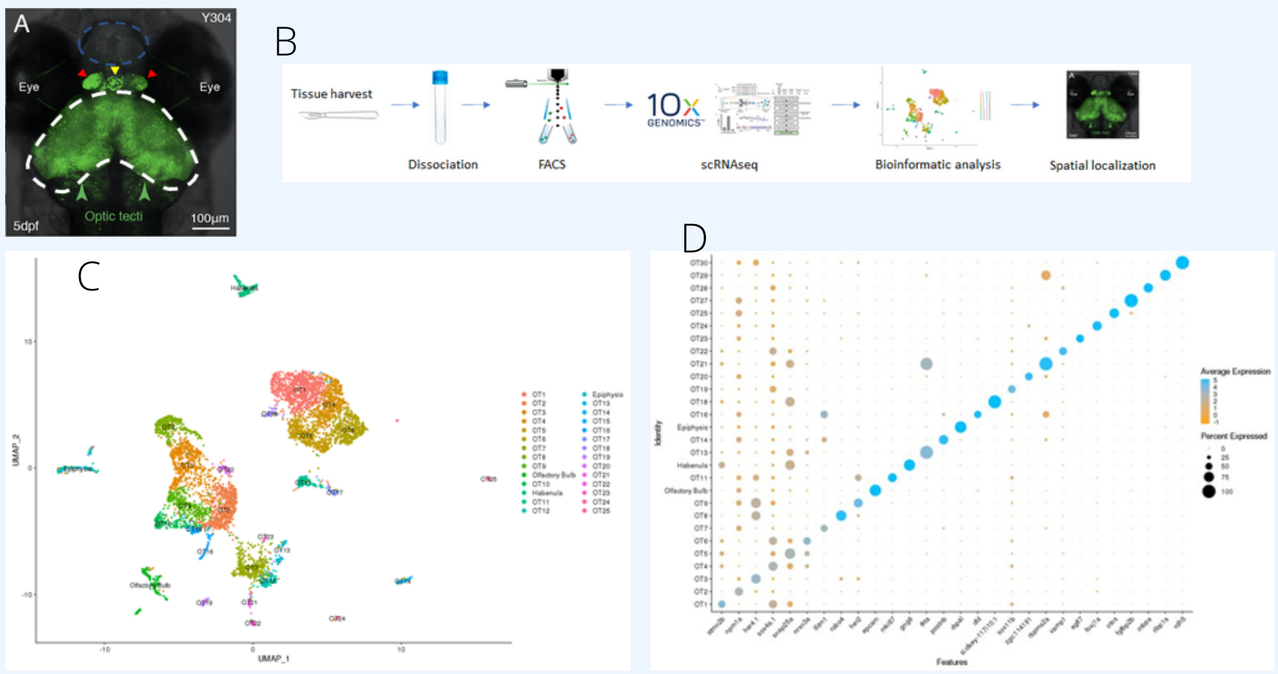

Figure1. Analysis of scRNAseq Data Reveals 25 Distinct Populations Within the Larval Zebrafish Optic Tectum. A: Representative image of the UAS:Gal4;Y304:Kaede transgenic line used to obtain tectal cells. The line simultaneously labels the optic tectum (white dashes), habenula (red arrows), epiphysis (yellow arrows), and olfactory system (blue dashes). B: Experimental workflow for the collection and isolation of tectal cells. Transgenic heads exhibiting kaede expression were collected, enzymatically dissociated, and kaede positive cells were isolated via FACS. 6,629 single-cell libraries were obtained using 10X Genomics droplet-based protocol, raw reads were demultiplexed and aligned to prepare a gene expression matrix. Quality control removed 254 cells, and 6,375 cells were obtained for downstream analysis. PCA and Leiden graph-based clustering were utilized to sort cells into clusters and identify candidate marker genes for each. Neurotransmitter profiles, gene ontology analysis, and KEGG pathway analysis characterized each tectal population. Bioinformatic validation and spatial mapping was completed via flourescent RNA in situ hybridization (FISH) of select markers (see Methods). C: 2D visualization of 25 tectal cell populations identified via scRNAseq analysis of 6,375 kaede + cells using uniform manifold approximation and projection (UMAP). Each point represents a single cell that is colored according to cluster identity as determined by Leiden graph-based clustering. Two-dimensional spatial arrangement of clusters is a product of genetic similarity as similar clusters are represented as closer together. UMAP embedding was used for visualization purposes only and not to define the clusters. D: Gene expression profiles of putative tectal populations. Candidate marker genes (columns) for each cluster (rows) were nominated by differential expression analysis (DEA) wherein candidate genes must: (1) be expressed in at least 50% of cells within that cluster, and (2) show specificity to the cluster compared to all other cells by exhibiting ≥1 log fold-change in expression with at least 25% difference in gene presence.

Disscussion

We diverged from the initial plan of a comparison between LR and DR tecti because we realized that there was simply not enough knowledge about the tecti to make it feasible. For that purpose we decided to concentrate solely on the normally raised LR tectum

Although efforts have been made to characterize cells within the zebrafish optic tectum, access to the transcriptomes of previously identified morphologies (Robles, Nevin, id2b paper) are unavailable. Our research presents a novel advantage in these efforts by providing molecular transcriptomes for tectal cell populations via individual gene expression profiles. Through single-cell RNA sequencing at 2X cellular coverage, we showcase the complex heterogeneity of tectal cell types by identifying 25 molecularly distinct populations

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From the dataset we extracted 5 marker genes of important populations for in situ hybridization. These included exclusion genes for non tectal clusters (habenula and olfactory bulb) as well as groups of interest. Because acquiring this data took so long only one in-situ hybridization is completed on nrxn3a. The other 4 probes are underway and are in the process of being completed. Furthermore, as we believed that some of the clusters would become more defined with more data, we started another run and are currently processing the combined data from the two runs to obtain a more wholistic view of the cells that make up the zebrafish brain.

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