Genetic control of circuit development and function in the zebrafish optic tectum

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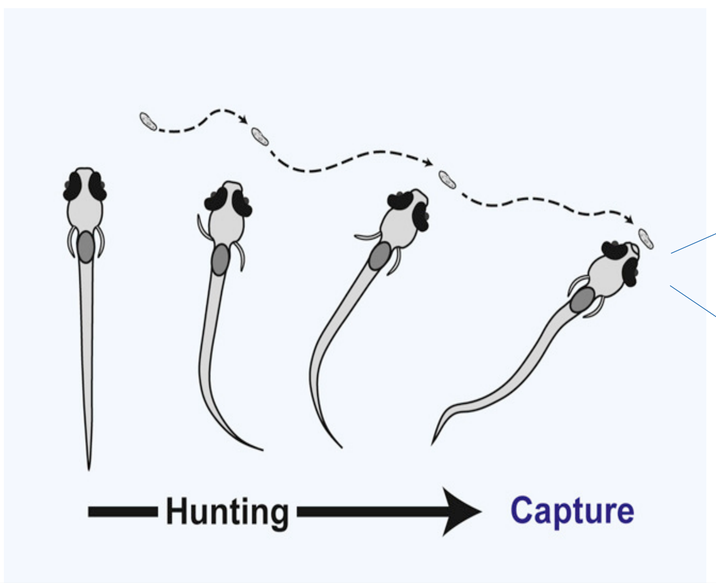
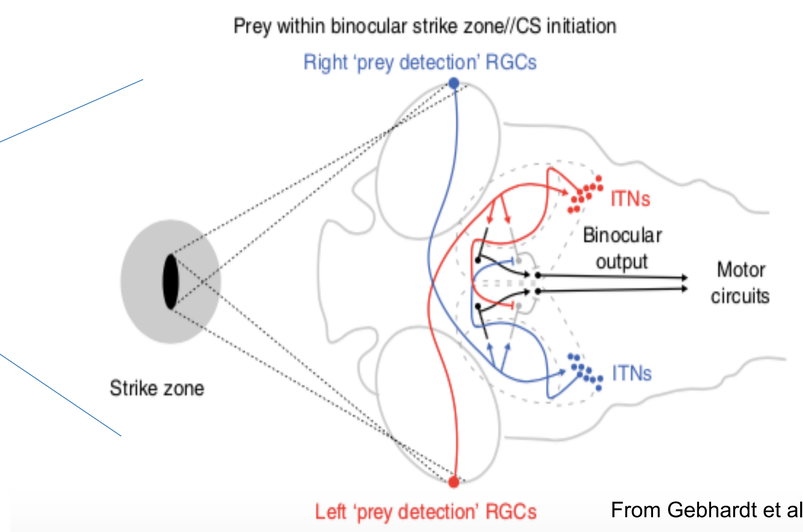
Binocular cues can be processed through the direct superposition of ipsi and contralateral retinal inputs[1]. Although lacking ipsilateral retinal projections, the zebrafish larva shows responses to prey-like stimuli consistent with binocular vision. Previous work of the lab has shown the existence of fundamental neurons essential for this behavior, the intertectal commissural neurons (ITNs)[2]. ITNs connect tectal hemispheres as a ladder-like array of axonal tracts. These commissural interneurons are key to 3D perception in binocular vision, as they form an intertectal circuit that governs the prey-capture behavior, however their precise function and formation remains elusive. The main goal of this project is to investigate the genetic identity, connectivity and mechanism of action of ITNs. We will take advantage of single cell RNA sequencing in order to dissect the genetic identity of ITNs and further define their role in binocular vision during larval development. Classical molecular biology and behavioral experiments will be used to alter the activity and neuromodulation of these neurons. Moreover, calcium and voltage imaging will be employed to dissect the neuronal circuitry controlled by the ITNs[3,4].

ITN mediated prey – hunting behavior

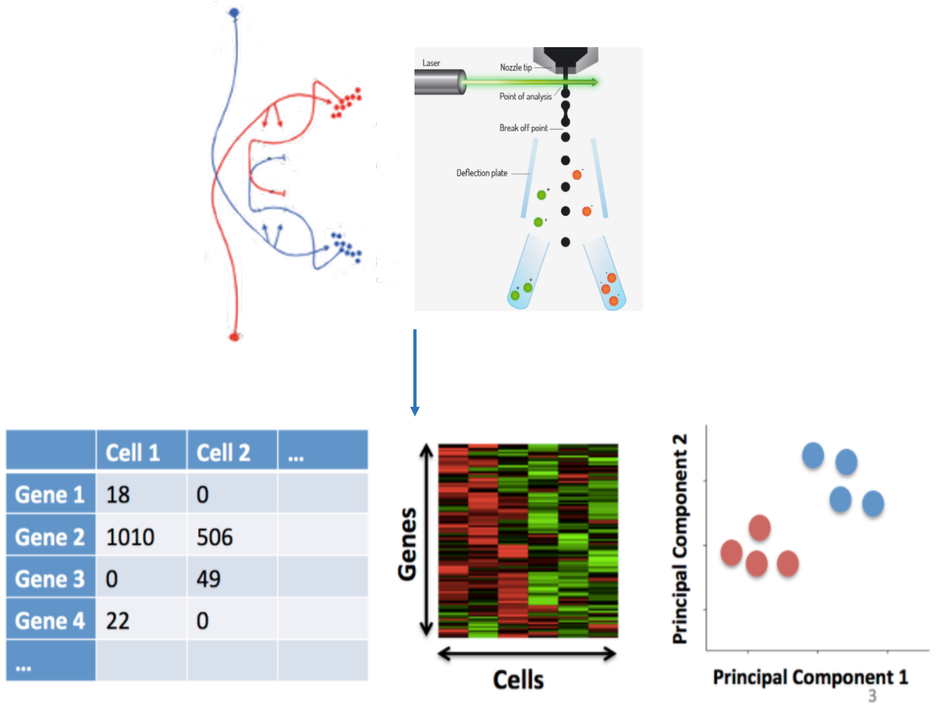




From Gebhardt et al., 2019[2]

The hunting sequence in zebrafish larvae is a complex but well characterized behavior. It consists of an initial foraging step where the larva swims in the water, followed by the detection of the prey and its tracking. The larva then fixates on its target before initiating the capture maneuver. When the prey is detected in the strike zone the animal converges its eye in order to increase the binocular field of view. In the zebrafish larva the processing of binocular cues is achieved through the activation of the ITN mediated circuitry by the contralateral RGCs[2].

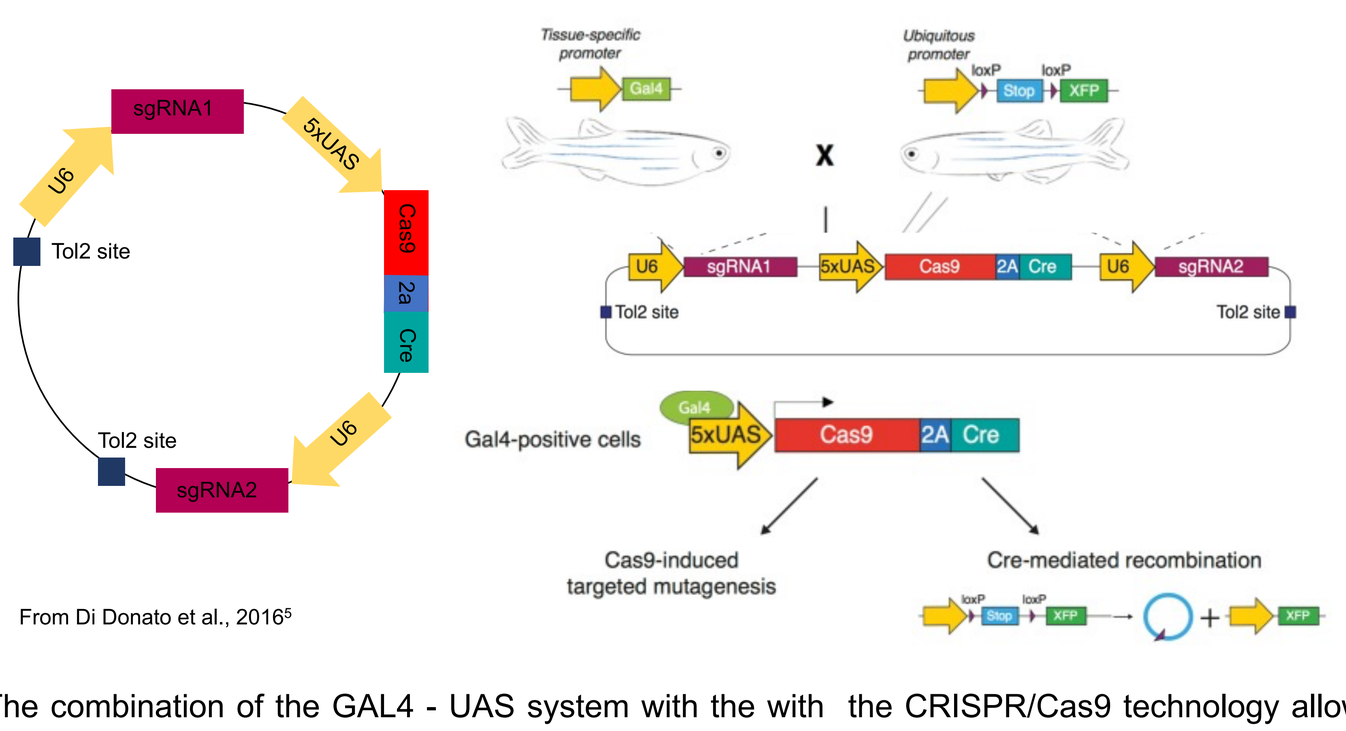
Dissecting ITNs genetic identity



	Cell 1	Cell 2	...
Gene 1	18	0	
Gene 2	1010	506	
Gene 3	0	49	
Gene 4	22	0	
...			

The transcriptome profile of ITNs will be established thorugh FACS sorting and single cell RNA sequencing methodologies.

Altering ITNs activity/neuromodulation



From Di Donato et al., 2016[5]

The combination of the GAL4 - UAS system with the with the CRISPR/Cas9 technology allows targeted mutagenesis in a tissue specific manner, as previously shown in the lab.[5] This tool will be employed to alter ITNs activity and neuromodulation and further assess their involvment in the hunting behavior of larval zebrafish.

2-photon dissection of ITNs neuronal circuitry:

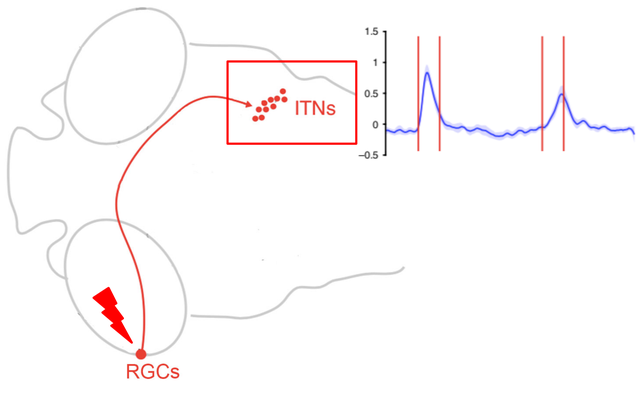
To characterize the neuronal circuitry controlled by the ITNs we are validating new transgenic lines expressing:

1. an opsin at the presynaptic level
2. GECIs (genetically encoded calcium indicators) or GEVIs (genetically encoded voltage indicators) in the post synaptic cells.

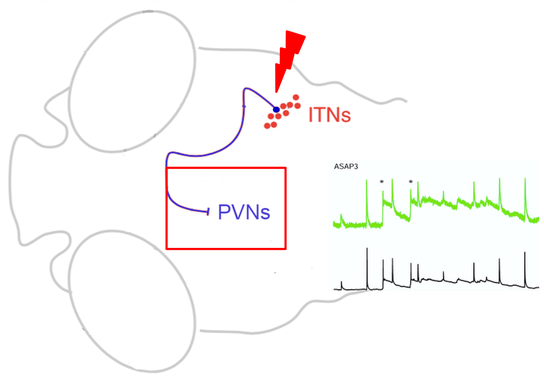
With this approach we aim at defining the connectivity between RGCs and ITNs (1) and between ITNs and PVNs (2).

a. Tg(vglut:gal4;UAS:opsin;elavl3:gCaMP6s)
b. Tg(elavl3:voltageindicator)

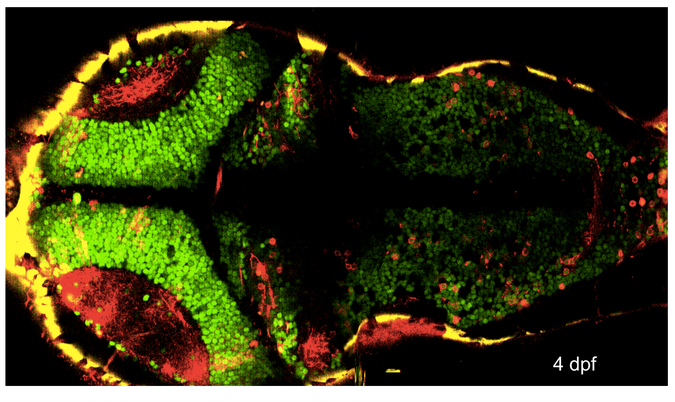
1. from RGCs to ITNs



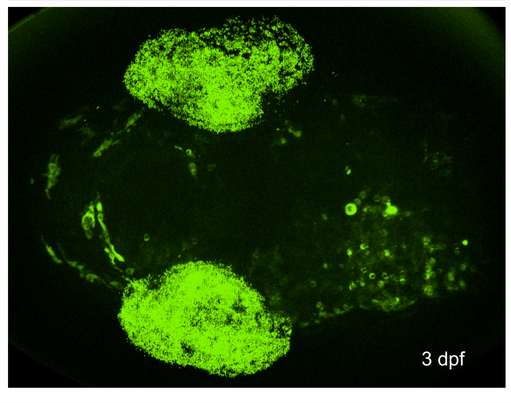
2. from ITNs to PVNs



a) *Tg(vglut:gal4;UAS:opsin;elavl3:gCaMP6s)*



b) *Tg(elavl3:voltageindicator)*



(1) Petros et al., Annu Rev Neurosci, 2008 **(2)** Gebhardt et al., Nat Comm, 2019 **(3)** Del Bene et al., Science, 2010 **(4)** Abdelfattah et al., Science, 2019 **(5)** Di Donato et al., Genome Research, 2016

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