Université Claude Bernard Lyon 1

# HABILITATION A DIRIGER DES RECHERCHES

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Titre des travaux : *« Les rôles des circuits cérébraux, des neurones et des molécules dans le comportement des larves de poisson zèbre »*

## Résumé


**PhD:**

During my PhD I studied the cellular and molecular mechanisms of neuronal morphogenesis in the retina. The retina develops rapidly from a proliferative neuroepithelium into a highly ordered laminated structure, with five distinct neuronal cell types. Like all neurons, these cells need to polarise and extend their axons and dendrites in the appropriate orientations in order to integrate into functional networks. How this transformation takes place, and how these types of orientation decisions are made, was the focus of my PhD project.

My first contribution was a literature review on the mechanisms of morphogenesis in the retina [1]. The vertebrate retina transforms rapidly from a proliferative neuroepithelium into a highly organized laminated structure with five distinct neuronal cell types, which must polarize in specific orientations to assemble into neural circuits. The retina serves as a model for understanding neuronal polarization *in vivo*, indicating that polarization orientation may be specified by a pre-established intrinsic cellular polarity within neuroepithelial cells and external cues acting upon these differentiating neurons [1]. Within this framework, I completed two projects during my PhD:

1) How the site of axon emergence from the cell body is established was not clear, and was thought to be primarily driven by the intrinsic neuroepithelial (apico-basal) polarity of the newborn neuron. Using time-lapse imaging, genetic and surgical manipulations, I showed that Laminin, through direct interaction with retinal ganglion cells, is necessary and sufficient to orient axon emergence *in vivo*. [2]. This was the first demonstration that an extracellular cue is necessary and sufficient to direct the orientation of axon emergence *in vivo*.
2) In collaboration with Ryan McDonald, who was a postdoc in Bill's lab, we studied the genesis of the inner plexiform layer, which is the neuropil layer containing the intermingled processes of the bipolar cells, the neurites of the amacrine cells and Müller glia, and the retinal ganglion cells [3]. We made the surprising discovery that neither presynaptic nor postsynaptic processes are individually essential for IPL formation. We concluded that no single

retinal cell type is critical for the formation of an IPL-like neuropil, and that neuropil formation may result from the coordinated action of multiple autonomously stratifying cell types.

## Postdoc:

For my postdoc work, I transitioned from the study of neuronal development into the functional neuroscience of behaviour, continuing to work with the larval zebrafish system. My original plan was to use Ca2+ imaging to identify neural circuits involved in arousal behaviour, funded by an HFSP long-term fellowship. However, when starting it was apparent that the larval zebrafish neuroscience field lacked standardization in terms of neuronal anatomy, and that Ca2+ imaging had significant limitations related to the requirement of animal restraint and altered behaviours within the microscope environment. To address both of these challenges, I developed a method to map neural activity from freely-swimming larval zebrafish onto a standardized 3D anatomical atlas of its brain [4]. By co-registering the brains of hundreds of larval zebrafish, I built the Z-Brain atlas (zbra.in), complete with molecular labels and definitions of anatomical regions. With the Z-Brain and the immunohistochemical detection of phosphorylated extracellular signal–regulated kinase (ERK) as a neural activity indicator, I developed a system for creating and contextualizing whole-brain maps of neural activity dependent on stimulus and behavior.

In the second phase of my postdoc, I did the foundational work establishing the behavioural paradigm that I am now studying in my independent group [5]. I focused on habituation, which is a learning process where animals lessen their reactions to frequently encountered harmless stimuli, providing a pragmatic model to study the molecular, cellular and circuit properties of plasticity during learning. There are at least two forms of habituation: short-term and long-term. I focus on long-term habituation, which necessitates enduring changes in neural circuits.

I used a visual stimulus consisting of a sudden reduction in illumination (a dark flash). Dark flashes elicit an “O-bend” turning response, and through training with repeated dark flashes this response habituates. Through behavioural, pharmacological and genetic analyses I showed that habituation occurs via a distributed plasticity process, and propose that multiple points within the circuit exhibit plasticity independently, resulting in the adaptation of different components of behaviour, such as the probability of executing the O-bend response, the latency of the response, and the magnitude/vigor of the response. This introduced a more nuanced modular model of habituation, suggesting that each component of a behavior can independently adapt, potentially leading to more precise or adaptable responses based on different stimuli or internal conditions [5].

## Independent:

I started my group at the Institut NeuroMyoGene in Lyon in 2019, funded by an ATIP-Avenir starting grant. Our main focus has been to identify the molecular and neural circuit mechanisms of plasticity underling dark flash habituation. Our strategy stems from a pharmacological screening approach, which identified potential pathways important for habituation. We are using whole-brain functional imaging approaches (pERK/MAP-Mapping, Ca2+ imaging), as well as genetic behavioural analyses to identify the critical receptors/pathways important for habituation, and the functional neuronal types and neural circuit dynamics underlying habituation [6].

This work is done with a focus on open-science practices and open-source hardware and analysis software, as reflected in my recent preprint describing the *pi_tailtrack* system for real-time behavioural monitoring during functional microscopy [7]. We have also begun a collaborative ANR project (TREATCOQ [8]), for which we are using behavioral and

whole-brain functional analysis to characterize a novel model of autosomal recessive ataxia, appling our high-throughput methodologies more directly towards a more translational aim.