Growth Cone Dynamics and Vesicle Trafficking in Developing Neurons In Vitro

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that is capable of processing inputs and generating an output. Depending on the level of activity, these synapses can then be regulated to increase or decrease their efficiency.

AIM AND OUTLINE OF THIS THESIS

In order to investigate the role of vesicle release in developing neurons, we cultured cells from release-deficient mutants and compared them with wild type controls. First, we investigated the role of vesicle release on outgrowth and motility, and looked at the role of secreted neurotransmitter on outgrowth and motility (stage 3/4 from Dotti et al. (Dotti et al., 1988)). We found a 40% reduction, but not abolishment in neurite outgrowth during the first week. However, this did not result in an impaired morphology at the synaptogenesis stage at 2 or 3 weeks in culture (**Chapter 2**). Using organotypic slice cultures of release-deficient and control animals, we examined the effect of chronic activation or inhibition on outgrowth during development. We found that chronic AMPA application resulted in increased outgrowth for WT and M18 neurons, but a decrease in M13 deficient neurons. Growth cone motility was reduced when AMPA was applied, but only after 3 days in culture (**Chapter 3**).

Secondly, I investigated the transport and release of large dense core vesicles during the final stage of development (stage 5 from Dotti et al. (Dotti et al., 1988)). Using methods in image segmentation and Bayesian statistics (**Appendix A**), we developed a method to automatically quantify the trafficking of labeled vesicles (**Chapter 4**). We then applied this method to study the trafficking and release of a yet uncharacterized LDCV-cargo: CRFBP. We found that CRFBP is localized in a specific subset of LDCVs, and behaves differently than previously characterized LDCV-cargoes. Most importantly, fusion occurred only with stationary vesicles, where the entire cargo was released into the extracellular environment (**Chapter 5**). Then, I describe some optical tools that allow us to manipulate key factors in vesicle release such as calcium using local photolysis and to image these events using a label-free method called interference reflection microscopy (**Chapter 6**).

Finally, I will discuss the main findings of the role of vesicle release during development and the role of vesicle dynamics during synaptogenesis. I will also provide some insights for future research into the field of development and vesicle dynamics (**Chapter 7**).