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Institut: Zoologisches Institut der Christian-Albrechts-Universität
Ort: Kiel
Arbeitsgruppe: Bosch
Betreuer: Christopher Noack (M.Sci.)
Thema: N4 Neuron Population Count in Hatchling Stages of *Hydra vulgaris*

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Contents

List of Abbreviations

BSA Bovine Serum Albumin

DABCO 1,4-Diazabicyclo[2.2.2]octane

GFP Green Fluorescent Protein

PBS Phosphate-Buffered Saline

PBT Phosphate-Buffered Saline with Tween-20

RT Room Temperature

1 Introduction

1.1 Personal Background and Internship Overview

My name is Lino Riepenhausen, I am eighteen years old and I attend a school with a focus on Information and communications technology. Thanks to a prize from the International Biology Olympiad, I had the opportunity to do a scientific internship this summer. I have been interested in biology, especially biochemistry and developmental biology, for some time now because of the diverse and (sometimes) practically applicable research avenues. It was nice to apply in practice what I had previously only learned in theory. I completed my internship at the Zoological Institute of Christian-Albrechts-University in Kiel, specifically in the research group led by Prof. Thomas Bosch and supervised by Christopher Noack (M.Sc.). The primary focus of this research group is on the study of the freshwater polyp *Hydra*, particularly in the context of textit*Hydra* as a metaorganism. A metaorganism encompasses the host organism, in this case *Hydra*, as well as its microbiome, virome, and all other organisms that contribute to the overall functioning of the system. In their research, special emphasis is currently placed on the neurobiology of *Hydra*. During my stay, I was able to learn and participate in multiple research aspects of the group, but I will explicitly focus on just one project here.

Figure 1: location of the research group: "Bioturm" [1].

1.2 Abstract

The primary aim of this experiment was to count the N4 neuron population in transgenic N4-GFP *Hydra vulgaris* hatchlings through the use of antibody staining techniques. The N4-GFP neurons were stained using a primary chicken antibody against GFP, followed by a secondary antibody against chicken IgY. The resulting data revealed an increase in N4 neuron population numbers up to the hypostome stage, and potentially even up to the tentacle stage; however, this latter observation may be an artifact as neurons at the hypostome stage are easier to count. The N4 neuron population in *Hydra vulgaris* hatchlings appears to slightly increase over the course of development stages, particularly up to the hypostome stage, warranting further investigation to verify the observations.

2 Project Information and Theoretical Background

2.1 General Subject Area

The research is embedded in the disciplines of biochemistry, developmental biology and neurobiology with a particular focus on *Hydra*, a cnidarian. It is formulated in the context of an expanding knowledge base concerning the nervous system in non-bilaterian animals (radiata), such as *Hydra*.

2.2 Transgenic *Hydra vulgaris*

Jörg Wittlieb from the Bosch Lab was the first person to develop a protocol for the generation of transgenic *Hydra vulgaris*. The protocol consists of three stages. The first stage involves the formulation and assembly of a genetic construct specifically designed for transgenic applications. This initial preparation typically takes around two weeks to complete. The second stage centers on the microinjection of the prepared construct into the embryos of *Hydra vulgaris*. Specialized microinjection equipment is utilized to accurately insert the construct into the embryo, thereby minimizing damage and maximizing the chances of successful genome integration. The final stage involves the selection and propagation of animals that have successfully integrated the transgenic construct. These selected polyps are then cultured under controlled conditions to develop into uniformly transgenic clonal lines. The completion of this last stage takes approximately three months [2].

2.3 Ca-Imaging with Inverted Light Microscopes

Although calcium imaging wasn't used in this project, the microscope utilized for capturing the images was an inverted light microscope with a specialized setup for calcium imaging. Inverted light microscopes are commonly used in calcium imaging to monitor cellular activity in biological specimens. Unlike traditional upright microscopes, inverted microscopes allow for easier access to the sample, making them ideal for live-cell imaging experiments. The setup for calcium (Ca) imaging includes a high-sensitivity camera to capture the fluorescence signals, as well as a laser source for excitation of calcium-sensitive fluorescent dyes or like in this project, genetically encoded calcium indicators like GCaMP. The laser source is chosen to match the excitation wavelength of the calcium indicator used. The emitted fluorescence is then captured by the camera, which is often a high-speed, high-resolution CCD or CMOS camera.

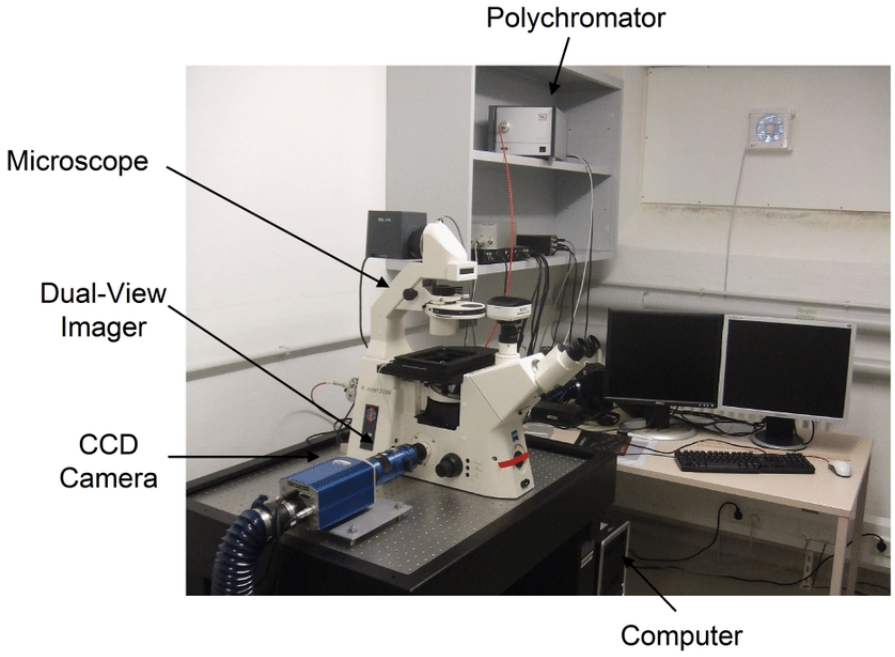

Figure 2: inverted light microscope setup [3]

3 Materials and Methods

Transgenic N4-GFP *Hydra vulgaris* embryos and hatchlings (further only described as hatchlings) from the stages bilayer, hatching, cigar, hypostome and tentacle were taken for the experiment. Also, transgenic N4-GFP *Hydra* vulgaris adult animals were used to check for a working N4-GFP stain. First medium was removed, then they were fixed in 2% urethane in *Hydra* S-Medium at RT for 2min. Urethane solution was removed and $400\mu l$ Zamboni added, then incubated for 2-3h at RT. Then Zamboni was removed and the fixed hatchlings were either directly further used or washed 1-2x with PBT (0.1% Tween) + NaN_3 and stored in the solution at 4°C until used. The hatchlings which were used directly after fixation were washed 4x for 15min with PBT (0,1% Tween) at RT. The hatchlings in NaN_3 were only 2x for 15min washed with PBT (0.1% Tween) at RT, then

treated the same as the others. PBS + 0.5% Triton X-100 was added and incubated at 30min at RT. Then anti-GFP (chicken) antibody diluted 1:1000 in PBT (0.1% Tween) + 1% BSA was added and incubated overnight at 4°C. The next day, hatchlings were washed with PBT (0.1% Tween) + 1% BSA, 1x for 3min and then 2x for 30min. After that, an anti-Chicken antibody in PBT (0.1% Tween) + 1% BSA was added and incubated at RT dark for 2h. Then washed with PBT (0.5% Tween) + 1% BSA, 1x for 3min and then 2x for 30min. After this the hatchlings were stored at 4°C until embedding. For embedding, the hatchlings were put on a microscope slide with spacers. Then about 100μl Moviol / DABCO were added and covered with cover glass. The hatchlings were imaged with a confocal fluorescence microscope. The counting of the antibody-stained N4 neurons was done with the program ImageJ [4], using the plugin cell counter. The counted number was doubled to account for the fact that only one side of the hatchling was counted.

4 Experimental Results

Qualitatively, one-third of the hatchlings and adults were positive for the GFP-antibody staining. Quantitatively, the count of the N4 neurons increased up to the hypostome stage but showed a decrease at the tentacle stage.

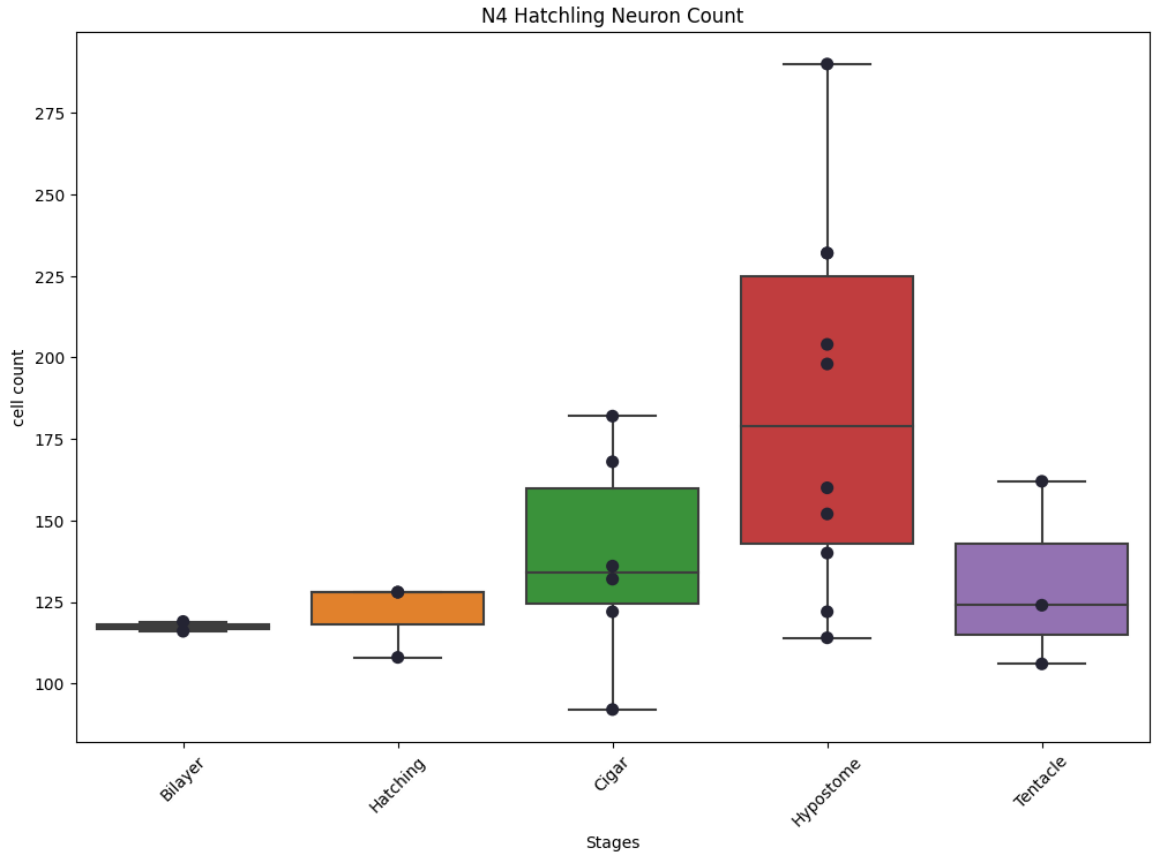

Figure 3: neuron counting results

5 Discussion

The observed pattern shows an increase in the number of N4 neurons up to the hypostome stage, followed by a decrease at the tentacle stage. This decrease at the tentacle stage is likely not a biologically meaningful phenomenon and may be attributed to an artifact or error. The data could be skewed to show an artificially low number of N4 neurons at this stage. The reason for this could be not fully transgenic animals, a too small number of counted animals or a mistake in the protocol.

6 Conclusion

In conclusion, the data showed specific trends in N4 neuron counts through GFP-antibody staining across different hatchling developmental stages. The deviation in the number of counted neurons at the tentacle stage is likely attributable to some form of error, rather than representing a genuine trend. To get more robust and representative data that could hold biological significance, repeating these experiments is essential.

7 Acknowledgments

Thanks to Christopher, Jörg, Ornina, Lisa, Christoph, Ehsan, Alexander, Doris, Jinru, Tim, Thomas, and all other members of the Bosch Lab, working in the lab and with *Hydra* has been incredibly interesting. I've had a great time and learned so much. I'm really glad to have had the opportunity to complete this internship.

Figure 4: photo taken by Jörg Wittlieb

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