Developing a High-Throughput System for monitoring Zebrafish Behaviour

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Introduction: Neurological disorders are alarming conditions and a major topic of concern worldwide. Challenges of their treatment and diagnosis are present within clinical and research studies, making it difficult to understand the outcomes of brain abnormalities. Human brain itself is a complex organ, so studying disorders associated with the brain can be problematic. Animal models contribute in evaluating the symptoms of neurological disorders, but not entirely and they are not expected to fulfil all the requirements of research studies. However, they still provide insight into human behaviour. The aim of this thesis project was to develop a high-throughput (HT) system for recording the behaviour of zebrafish larvae in response to light stimuli. The zebrafish larvae animal model was selected since it presents several advantages over other animal vertebrate models, such as their availability, fertility, shorter life span, reduced ethical concerns and require less resources to maintain.

Objectives: The first objective of this thesis was the optimisation and scalability of the HT-system for the microcontrollers, cameras, and light source to work together. The second objective was to develop a startle response protocol of zebrafish larvae towards light stimuli, and observe the behavioural responses of the larvae by recording videos in the HT-system. Similarly, the third aim was to develop a prepulse inhibition (PPI) protocol and observe the behavioural responses. PPI is a neurological phenomenon, in which there is a startle response of an organism towards a startling stimulus (pulse). After a certain time delay, a weaker stimulus is introduced (prepulse), and is followed by the same startling stimulus (pulse), to which the organism does not have a startling response, therefore inhibiting the response. PPI is not shown in patients with neurological disorders, for example in schizophrenia, which serves as an important phenomenon when studying neurological disorders.

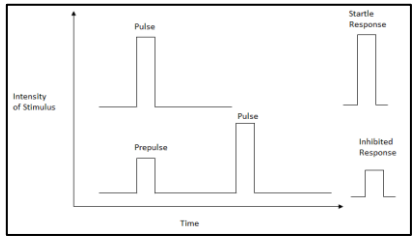

Figure 1 Prepulse Inhibition (PPI): This figure shows the graphical representation of PPI

Methods: The HT-system consists of three cameras, each of them able to image an entire 96-well plate. Each well contained one larva, but for system optimisation and well plate condensation issues the edge wells of the plates were not used. A white light planar source was used to provide light stimuli to the zebrafish larvae. The system is designed in a way, that the well-plate is placed on the light source and the camera images from the top view of the well-plate. The cameras and the light source are controlled using small integrated circuits called microcontrollers. Microcontrollers were used since they allow for easier scripting for automating the system.

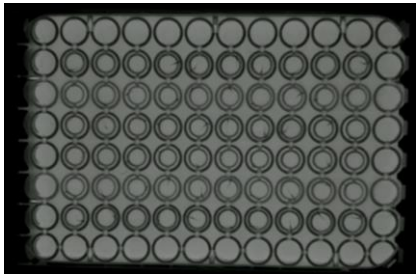

Figure 2 Camera view of the Well-plate: This figure shows how the well-plate and zebrafish larvae appear during a light pulse. Fresnel Lens has been placed on top of the plate as part of system optimisation.

Results and Data Analysis: The recorded videos were converted into images with the conversion software FFmpeg. The set of images are then analysed in ImageJ to observe the movement of zebrafish larvae in different frame by frame in each used well. The result is the difference of pixel values in each well, showing whether the larvae moved or did not move, or simply drifted in the well. The future implications of this project involve making the image analysis process entirely automated.

Keywords: High-Throughput (HT) System, Zebrafish Larvae, Light Stimulus, Startle Response, Prepulse Inhibition (PPI)