Inhibitory Avoidance Learning in Zebrafish (*Danio rerio*)

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Introduction

The zebrafish (*Danio rerio*) is increasingly being used as model in behavioural [1,2], neurobiological and genetic research [3]. Underlying reasons are high genetic homology to humans [4] and the many advantages over the use of rodents, such as low cost, easy handling, short reproduction cycle and high fecundity. Furthermore, its genome [5], transcriptome [6] and proteome [7] are well described, making the species a model of choice for behavioural research linked to genetics.

An emerging field addresses learning and memory related to anxiety and fear behaviour [8,9], which has been studied through inhibitory avoidance paradigms [10-12]. Assessment of inhibitory avoidance learning in zebrafish is based on the conflict that arises when a fish wants to enter a dark area to avoid a brightly lit area (innate response; innate anxiety) and avoid this dark area, as it has been associated with an electric shock as negative stimulus. Higher latencies of entering the dark area are indicative of increased inhibitory avoidance learning. Similar paradigms have been studied extensively in rodents. The number of studies on this paradigm in zebrafish is limited.

We therefore studied inhibitory avoidance learning in a series of experiments and assessed whole-body cortisol content and telencephalic expression levels of task-relevant genes. Figure 1 summarises the task and subject variables as well as read-out parameters in relation to the inhibitory avoidance paradigm.
Material and Methods (general)

Zebrafish and housing

For our experiments we used an in-house-reared Tupfel Longfin (also known as Tübingen longfin; Aquarium facilities of the Radboud University Nijmegen) zebrafish strain. Animals were housed according to standard procedures. Four weeks before inhibitory avoidance learning, fish were housed as experimental groups (approx. 20 fish) in one of two equally sized compartments (50 · 50 · 60 cm) created by separating an aquarium (100 · 50 · 60 cm) in two halves by a blackened glass plate. In each aquarium the water level was brought to 30 cm, to realize a total water volume of 75 L per compartment. The two compartments would share a single biological filter (300 L volume) over which the inflowing water had passed. Each compartment received an aeration stone and was provided with independent water inflow and outflow (26°C, pH 8.0); The photoperiod was kept at 12L:12D (lights on from 07:00 to 19:00 hour) with feeding moments at 09:00 hour (artemia) and 15:00 hour (TetraMin; Tetra, Melle, Germany).

Inhibitory avoidance paradigm

Inhibitory avoidance learning was assessed between 11.00 and 13.00 hours under normal light conditions (400 lux; measured at the water surface). The inhibitory avoidance tank was as follows: an aquarium (60 · 30 · 30 cm; 10 cm water level) was split into two equal compartments, separated by a manually operated sliding door. Surfaces of one compartment were white, while surfaces of the other compartment were black. Compartments were not covered by a lid. The black compartment contained two metal plates (covered with black sound box mesh to prevent light reflection) that covered two opposite walls completely. Both plates were wired to a power source that allowed for an electric current to be put between the plates in the water.

On each day of the inhibitory avoidance paradigm, fish were individually placed in the white compartment with the sliding door closed. After 60 s (acclimation period) the sliding door was lifted, giving the fish free access to the black compartment. Once the fish had completely entered the black compartment, the sliding door was...
closed and an electric shock was given for 5 s after which the fish was returned to its home tank or no shock was given and fish were sampled, depending on the day of the experiment. Fish were given a total of 180 seconds to enter or avoid the black compartment.

Operation of the sliding door was done by hand; visual observation was done in real-time and recorded on-site. Recording of latency times was done by stopwatch. All procedures were carried out in a manner that caused the least possible disturbance of fish during the experiment.

Behavioural observations were recorded by camera for the white compartment only. Recordings were analysed post-hoc using EthoVision XT v10.0 tracking software (Noldus, Wageningen, the Netherlands). We assessed fish behaviour both before and after opening of the sliding door.

**Ethical approval**

Experimental procedures were approved by the ethical committee of the Radboud University Nijmegen and were conducted in line with Dutch law (Wet op de Dierproeven 1996) and European regulations (Directive 86/609/EEC).

**Results**

Our data show that manipulation of task-related features, such as increasing shock intensities [13] or the number of shocks [14], increased inhibitory avoidance learning. Furthermore, the effects of environmental manipulations on inhibitory avoidance learning, such as environmental enrichment [15] and unpredictable chronic stress in combination with changes in photoperiod [14], both showed reduced inhibitory avoidance learning. In addition, inhibitory avoidance learning was strongly reduced in aged (24-month old) zebrafish [15]. Recently, we observed strong strain-related (AB strain versus Tupfel longfin strain) differences in inhibitory avoidance learning [16]. In all of these studies we observed two distinct behavioural types. Fish that did not enter the black compartment (latencies > 180 s), marked as avoider fish, and fish that entered the black compartment (latencies < 60 s), marked as nonavoider fish. A selection of these studies will be presented in greater detail, showing how behavioural data may be linked to differences in telencephalic gene expression and whole body cortisol content.

**Conclusion**

The data suggest that the zebrafish is a suitable model to study processes underlying inhibitory avoidance learning. In addition, these data serve as starting point for unravelling individual differences in brain-behaviour relationships underlying learning and memory related to anxiety and fear in zebrafish.

**References**


16. Gorissen, M., Manuel, R., Wolf, de, M., Mes, W., Flik, G., & Bos, van den, R. (in prep.) Differences in inhibitory avoidance learning between two strains of zebrafish (Danio rerio): AB versus Tupfel longfin