Description of a Multivariate Behavioral Test Arena for Zebrafish – the Zebrafish Multivariate Concentric Square Field Test

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Introduction

There is a strong need to develop new behavioral tests in order to gain progress in generating valid animal models for the understanding of pathophysiology in complex human disorders and in development of new treatment strategies [8].

The zebrafish, *Danio rerio*, possess many advantages as a model organism including low maintenance costs, short generation time and high homology to humans [4]. Many conventional behavioral tests for rodents [2] and zebrafish [5] have limited reproducibility and are intended for interpretation of specific predetermined mental states (e.g. "anxiety-like" behavior), which results in limited opportunities for more extended analyses of the various processes that presumably interact to establish complex behavioral patterns.

The multivariate concentric square field™ (MCSF) test for rats and mice, developed at Uppsala University, is unique in its design on the basis that the various areas should provide different elements of risk versus shelter and explorative incentives [6]. The guiding principle for the test is that it is unprejudiced, i.e. not designed for interpretation of specific predetermined mental states but instead enables a behavioral profiling of the animal. Thus, the MCSF performance is based on evolutionary conserved innate behaviors in a novel environment where areas of potential risk and shelter are incorporated [6, 7].

The aim of this study is to establish a MCSF test arena suitable for zebrafish. The novel tank diving (NTD) test was used as a reference test.

Material and methods

Male and female zebrafish from three different strains were used (n = 20 per sex and strain); AB, which is common lab strain, as well as wild-caught and pond-reared fish both originating from the Calcutta area, India. The experimental protocol and use of animals in this study was approved by the Uppsala Animal Ethical Committee, and was consistent with the Swedish Legislation on Animal Experimentation (Animal Welfare Act SFS1998:56) and the European Union Directive on the Protection of Animals Used for Scientific Purposes (2010/63/EU).

The fish were separated by sex 4 days before the experiments started. In this experiment, the fish were first tested in the NTD test followed by the MCSF test. They spent the night between the two tests in solitary tanks.

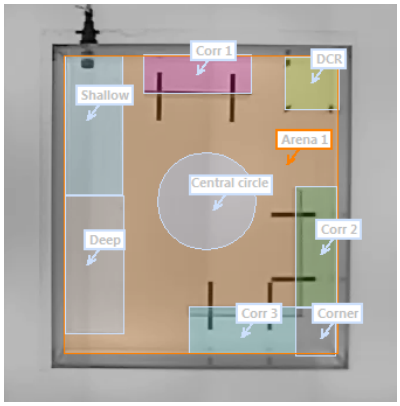

Figure 1. The MCSF arena with the different zones marked out.

The MCSF consists of a tank made of Plexiglas (50 × 50 × 30 cm; 13 cm water depth). The walls of the arena are sandpapered to prevent different cues in the room to affect the behavior. The different parts in the MCSF arena were a Plexiglas ramp with weights, a roof made of IR-transparent plastic, and three walls with weights. These parts were placed in the arena to form the various zones: dark corner room (DCR), central circle, corridor 1, corridor 2, corridor 3, corner and the ramp which was divided into deep and shallow (Figure 1). An IR-light table was placed underneath the arena and an IR-sensitive camera recorded the fish from above. The fish were placed in the arena with a net and allowed to freely explore for 30 minutes.

The NTD test consists of a rectangular Plexiglas tank (5 × 15 × 20 cm) divided into three zones; bottom, middle and top. The bottom and sides of the tank were covered with white plastic. An IR-sensitive camera was placed in front of the tank to capture the location and activity of the fish and an IR-light table was placed behind the tank. The fish was poured in to the tank from a transport beaker and a lid was placed on top. The testing time was 6 minutes.

The fish were tracked using Ethovision® XT 11.0 (Noldus Information Technology, Wageningen, The Netherlands). The number of visits, latency (s) to first visit, total time (s) sent, duration per visit (D/F, s) and distance travelled (cm) in each zone was registered, as well as mean velocity (cm/s) and distance travelled (cm) in the total arena during the trial.

Non-parametric statistics and multivariate data analysis (SIMCA-P+ 12.0, Umetrics AB, Umeå, Sweden) was used to examine MCSF and NTD performance. A principal component analysis was used to obtain an overview of the data, e.g. groups of observations, trends and outliers, and also to uncover the relationships between observations and variables.

Results

The chosen testing time (30 min) was enough for all fish to explore the MCSF arena, and individual differences in explorative strategies were evident. Moreover, using the zebrafish MCSF test we were able to detect clear differences in behavioral profiles between all three strains tested. As expected AB fish were more active, showed higher risk taking and were more explorative than fish of the two wild-type strains. We also detected significant differences in behavioral profile between males and females; males being more risk taking, interpreted as bolder, than females. This sex difference was most obvious in the AB strain.

The fish tested in the MCSF test were also monitored in the NTD, a more established behavioral assay for screening zebrafish for boldness. In general, the results on risk taking show good correspondence between the tests. Fish being more risk taking in the MCSF test (e.g. high activity in the central circle and on the shallow part of the ramp) were faster to explore the upper zone in the NTD test. However, it was also obvious that the divergence in behavioral profiles of the zebrafish strains tested was more clearly detected by the MCSF than by the NTD test.

Discussion

The results of the present study show that the zebrafish MCSF test is useful for screening for behavioral profiles. The MCSF test has the advantage that individual fish could be screened for multiple behavioral traits (e.g. general activity, risk taking, shelter seeking and exploratory activity) using a single test. In the current study we could show clear behavioral differences between the AB and the two wild-type strains. The AB strain has been kept in the lab for a large number of generations and an increase in boldness, i.e. more explorative and risk taking, has been reported in response to domestication in other teleosts [3]. We also detected clear behavioral differences between male and female zebrafish of the AB strain; males being bolder than females. This is in accordance with the results by Dahlbom et al. [1]. The zebrafish MCSF needs further validation but it clearly has a great potential in screening behavioral profiles in zebrafish.

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