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# The zebrafish as a model system for assessing the reinforcing properties of drugs of abuse

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**Abstract**

Recent reports make use of the zebrafish to study complex behavior such as addiction, anxiety, or learning and memory. We have established reliable tests and appropriate controls to measure these behavioral parameters in the zebrafish adult. Our assays are robust enough to permit the detection of dominant mutations affecting drug-induced reward, and therefore can be used in forward genetic screens. We provide the reader with the technical details of these tests, as well as their appropriate and crucial, although often overlooked, control assays. In particular, our results make it possible to use the zebrafish as a promising model to identify new genetic components of the reward pathway, or other measurable behaviors.


*Keywords:* Zebrafish; Addiction; Conditioned place preference (CPP); Aversion; Learning and memory; D-Amphetamine; Behavior

## 1. Introduction

Behavioral disorders are among the most widespread and costly brain diseases in modern societies [24]. However, our understanding of the molecular networks underlying these disorders is still limited, preventing adequate therapies. A number of animal models have been used to investigate the role of different brain structures in disease development, but the identification of the genes involved has been difficult, both because of the complexity of the disorders, which involve environmental factors, and because of a limited number of candidate genes. The candidate gene approach, used mainly in rodents, has two main disadvantages: (i) it requires that the genes of interest are known and very well characterized; (ii) the production of adequate sample size is costly. On the contrary, forward genetics methods where the genome is mutagenized, the resulting phenotypes characterized and the affected gene subsequently cloned, enables the researcher to detect both new and known factors controlling the behavior of interest. Forward genetics has been successfully employed in several large-scale screens [25,14], aimed to determine the genes controlling the early development of zebrafish. Researchers have used either embryos or juveniles and utilized the main advantages of the zebrafish: (i) Zebrafish possess a good balance between simplicity and complexity of its organs and systems. For example, the fish nervous system is simpler than in rodents, but is still able to control a variety of complex behaviors such as learning, addiction, aggression, locomotion, etc.; (ii) Zebrafish is an excellent model organism for use in forward genetics. Protocols exist for efficient mutagenesis, and mutations can be rapidly cloned thanks to comprehensive genetic maps [31,17]; (iii) They have relatively short generation time and large progeny sizes that facilitate large-scale screens;

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(iv) they are relatively simple and cheap to raise and/or breed.

When compared to the assays designed for embryos and juveniles, the use of adult fish in large-scale screens appears to be cumbersome. It requires extra breeding, extended housing, and increased time for mutation recovery. Additionally, adult fish are very sensitive to stress caused by being handled [26,22] and great care has to be taken when behavioral assays are performed [26]. Nevertheless, it is obvious that adult animals have to be used when the behavior of interest requires the full functionality of a mature nervous system. Recently, considerable efforts have been placed into the development of robust, rapid, and reproducible assays for adult zebrafish behavior, which can be used in large-scale screens. Table 1 summarizes the published behavioral assays.

This review will highlight three assays addressing complex behaviors in the adult zebrafish: (i) addiction (reward); (ii) basal level of aversion (a likely measurement of anxiety); (iii) learning and memory. It summarizes the results of several years of investigations in our laboratory, to eventually achieve tests and controls sufficiently robust to permit their use in large-scale genetic screens. We will detail our experimental protocols, discuss important pitfalls, and demonstrate that our tests are suitable for large-scale screens for dominant modifiers of the reward response.

## 2. Measuring addiction in zebrafish using the conditioned place preference paradigm

Addiction is characterized by the uncontrollable and chronically relapsing compulsion to take drugs in spite of their dramatically negative effect on normal brain function. Addiction was studied in animal models, mainly rodents, using two experimental protocols: the conditioned place preference (CPP) paradigm and different protocols of drug self-administration. In the CPP paradigm, the primary motivational properties of a drug serve as a conditioning stimulus that is repeatedly paired with a set of environmental cues. During the course of conditioning, these cues acquire secondary motivational properties.

There are a number of excellent reviews on CPP (Carr et al. [10]; Hoffman, [19]; Schechter and Calcagnetti [29]; Bardo et al. [3]; Bozarth [6]; van der Kooy [36]; White et al. [37]; Calcagnetti et al. [8]; Tzschentke [34]) and the reader is referred to these publications for detailed discussions and considerations of basic methodological issues of the CPP paradigm.

In this article, we will focus on the methodology we have developed to use CPP in large-scale ENU-screens, aimed at recovering mutations affecting the molecular pathway(s) of drug-induced reward in zebrafish adults. We will point out the crucial aspects of CPP experiment design and the necessary specific controls, such as the assessment of the animal's stress, learning capacity, and memory. To validate

Table 1
Behavioral paradigms addressing complex behavior in zebrafish

| Complex behavior | Paradigm | Reference |
|---|---|---|
| Addiction | Conditioned place preference | Gerlai et al. [16] Ninkovic et al. [26] |
| Anxiety/exploratory behavior | Locomotor activity | Gerlai et al. [16] |
| | Group preference | Gerlai et al. [16] |
| | Place preference | Serra et al. [30] Ninkovic et al. [26] |
| | Light/dark preference test | Gerlai et al. [16] |
| | Time in enriched chamber of T-maze | Swain et al. [32] |
| | Exit latency test | Serra et al. [30] |
| Aggression | Mirror image test | Gerlai et al. [16] |
| | Pigment response | Gerlai et al. [16] |
| | Startle reaction | Dlugos and Rabin [13] |
| Memory | T-maze | Darland and Dowling [12] Swain et al. [32] Ninkovic et al. [26] |
| | Spatial alternation learning and memory | Williams et al. [38] Carvan et al. [11] |
| | Delayed spatial alternation | Levin and Chen [21] |
| | Active avoidance conditioning | Pradel et al. [27] Pradel et al. [28] |
| | Learned alarm reactions | Hall and Suboski [18] |
| Locomotor activity | Number of lines crossed | Gerlai et al. [16] Bretaud et al. [7] Swain et al. [32] |
| | Total distance moved | Anichtchik et al. [1] |
| | Mean velocity | Anichtchik et al. [1] |
| | Turning angle | Anichtchik et al. [1] |
| Social preference | Group preference | Gerlai et al. [16] |
| | Shoaling | Wright et al. [39] Engeszer et al. [15] |
| | Nearest neighbor distance | Dlugos and Rabin [13] |
| | Area occupied | Dlugos and Rabin [13] |
| Mate choice | Video-stimulus techniques | Turnell et al. [33] |
| Boldness/antipredatory behavior | Predator simulation | Gerlai et al. [16] Wright et al. [39] |