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Utility of Zebrafish Behavioral Assays in Ecotoxicological and Biomedical Studies: Materials and Methods

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ABSTRACT

The purpose of this review is to provide a qualitative literature review of the latest research on the subject in zebrafish (Danio rerio). Although rodents are the research model used all over the world, the use of the zebrafish model has increased in the scientific community in recent years. This is because this small tropical freshwater bony fish has significant genetic, anatomical and physiological homology with animals. Behavioral testing in zebrafish measures parameters such as motor, cognitive, and social behavior that can help detect drugs, make dosage decisions, and evaluate side effects. By identifying behaviors and manipulating specific genes, researchers can create zebrafish models of human diseases and study their behavioral phenotypes. Because zebrafish are capable of regeneration, zebrafish behavior can also help evaluate treatments and provide insight into regenerative medicine. Overall, Zebrafish serve as an excellent experimental model for behavioral, genetic and toxicological research and reveal mechanisms of many human diseases. As a result, the latest attitude in aquatic toxicology turns from investigative skepticism to exuberant enthusiasm.

Key words: ***Zebrafish, Behavior Repertoire, Biomedical Research, Test Methods, Challenges***

INTRODUCTION

Behavioral monitoring is a kind of biological observing that examines the environmental conditions by reviewing the behavioral responses of biomarker species and studies their associations to the surrounding environments [1] Behavior provides a sole viewpoint connecting the physiology and ecology of an organism and its environment [2] Behavior is both a sequence of quantifiable actions, functioning through the central and peripheral nervous systems [3], and the cumulative exhibition of genetic, biochemical, and physiologic processes vital to life, such as feeding, reproduction and predator avoidance. It permits an organism to adjust to external and internal stimuli in order to finest meet the challenge of surviving in a shifting environment. Contrariwise, behavior is also the consequence of adaptations to environmental in constants. Consequently, behavior is a selective response that is persistently adjusting through direct interaction with physical, chemical, social, and physiological features of the environment. Selective evolutionary progressions have preserved steady behavioral outlines in concert with morphologic and

physiologic adaptations. This stability provides the best opportunity for survival and reproductive attainment by enabling organisms to proficiently exploit resources and define appropriate habitats [2]

Criteria For Water Toxicity Model Organisms: According to (Rand 1985) Indicated that behavioral responses are most beneficial in toxicology should be [4]:

- *High ecological relevance.*
- *Well-defined endpoints that are practical to measure.*
- *Have relatively high reproductive rates and, should have.*
- *Be easy to culture and maintain under laboratory conditions.*
- *Sensitive to a variety of pollutants and adjustable to different species.*
- *Ecologically relevant. To this list, we add endpoints preferably should clarify different modes of action or chemical classes.*
- *Be capable of "stand alone" and be easily assimilated into a suite of assessment.*
- *Vulnerability to the stressor.*
- *Help define ecosystem status i.e., health.*
- *Be simple to systematize in order to maximize their usefulness for a far-reaching choice of applications.*

Even though each of these considerations has excellence, often the application of a particular endpoint, or suite of endpoints, is grounded on the capability of functionally discern exposure-related modifications, using existing techniques, with the most suitable sentinel species [4] The mechanism of action, route of uptake, and behavior of the compound of interest in the aquatic environment must all be understood properly In adult fish, gill and gut epithelia are major means of uptake, nevertheless physiological differences of diverse life stages need to be taken in consideration. Larval fish utilize skin as a respiratory interface and may uptake more complexes than adults of the same species that use gills for respiration. In contrast, for compounds that openly target the gill lamellae, adult fish will have improved sensitivity compared with larvae due to the increased surface area of the gill surface [5]

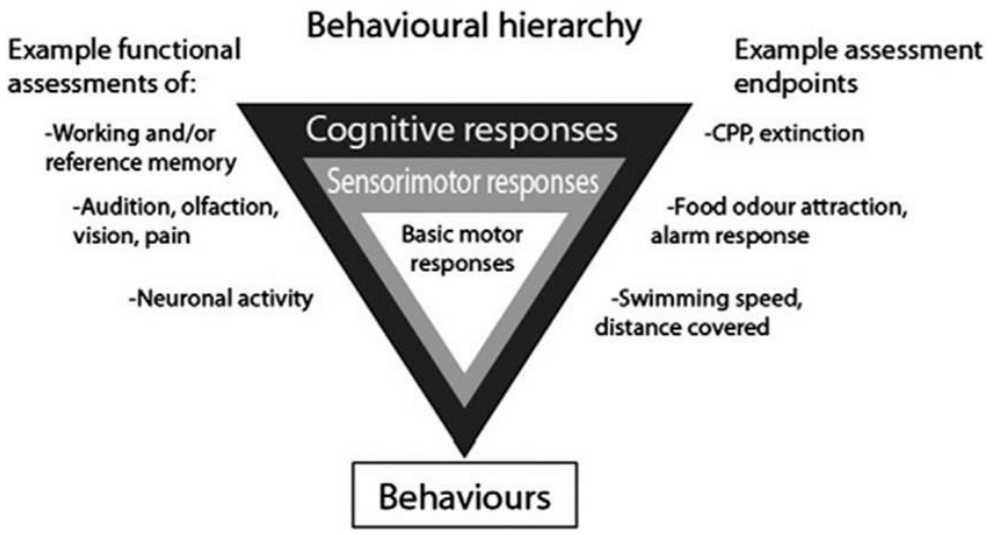

Diagram 1. Behavioral responses can be evoked by internal and external signals and altered by perturbations of the neural state. Evaluation methods in zebrafish to test their neural function in the absence of signals, the ability of their brain to retain and integrate sensory signals from different origins, which allows zebrafish to become a powerful model for testing compounds that make neurons live in the short term. - Long periods of time. CPP = conditioned place preference.

THE IMPORTANCE OF THE FISH MODEL IN BEHAVIOR ANALYSIS

To date, for aquatic behavioral toxicology testing there are no proper species or groups of species used. To stress and toxicant exposure different species often have different behavioral and physiological responses. Consequently, to determine the feasibility of a particular species preliminary observations and assays are required in order, and if abnormal behavioral patterns can be associated with specific exposure scenarios. It is not unreasoning for preliminary study with a novel species to take months, and in some cases, years, in order to develop biologically-relevant endpoints of exposure.

Ideal Sentinels for Behavioral Assays; According to [6, 7] various stressors and toxic chemical exposure fish are ideal sentinels due to their:

- *Constant, direct interaction with the aquatic environment where chemical exposure befalls over the entire body surface.*
- *Ecological relevance in many natural systems.*
- *Have wide geographical distributions.*
- *Include a collection of endpoints that focus on innate behavior of sentinel organisms that can be transformed in association with stress exposure.*
- *Relatively early maturation and easy fertilization in order to produce sufficient numbers of organisms of the proper age and size for testing.*
- *Ease of culture.*
- *Have representation across species (e.g., reproduction, food acquisition) in order to facilitate investigations into the phylogeny and ontogeny of behavior.*
- *Ability to come into reproductive readiness.*
- *Long history of use in behavioral toxicology.*
- *Have environmental relevance to the potential exposure (have been exposed to the test contaminant in the wild.)*
- *Have the ability to yield reproducible data under controlled laboratory conditions Well understood comparative to environmental factors that cause variation in the response.*

The requirement to cautiously document baseline "normal" behavior should be viewed as a strength of behavioral testing [8] Traditional (LC50) tests do not require stringent documentation of baseline behavior, other than visual observations of whether the test subjects were "healthy," and verification that a minimal amount of mortality (i.e., <10%) occurs in the control treatment group(s). Behavioral toxicology studies, however, allows the control group to subsequently be exposed, if careful documentation on baseline behavior is made. By using repeated measures analyses, using each animal as its own control by this method statistical control of behavioral tests can be significantly upgraded. This type of examination greatly lessens the inherent variability between individuals. [5]

ZEBRAFISH AS A BEHAVIORAL MODEL ORGANISM

The over-all fitness of zebrafish as a model organism as well as its utility in the genetic and neuroanatomical analysis of embryo and larval behavior has been expansively described [9]. Adult zebrafish exhibits a full repertoire of mature behavior. Zebrafish (*Danio rerio*) are a typical cyprinid (carp family) schooling fish. zebrafish are naturally social animals that display preference for the existence of conspecifics [10]. Zebrafish are therefore an exceptional model to investigate the genetics of social behavior. Utility of zebrafish as a model organism has various practical advantages such as small size, high fecundity, external fertilization, optical transparency and low housing expense [11]. The zebrafish genome has been completely sequenced and the majority of zebrafish genes are common to humans with 84% of genes known to be linked with human ailments having zebrafish counterparts [12] Biology making zebrafish an attractive model organism in studying human disorders since have a high homology to mammalian morphology and [12, 11] proficient the considerable step to assess the homology between human and zebrafish genomes in 2013. human genome has 25 thousand genes in 23 pairs of chromosomes while the zebrafish genome comprises around 26 thousand protein-coding genes in 26 pairs of chromosomes [12, 13]The researchers detected 70% of human genes have an orthologue in zebrafish.

Zebrafish have proteins with similar functions to human proteins; however, they encoded for different genes in humans and zebrafish. More than 80% of the 3176 genes labelled have an orthologue in zebrafish, allowing the use of zebrafish to examine plentiful human sicknesses [12] The zebrafish model is not only close to morphology and genetics of human beings, but also behaviorally. In particular, zebrafish display an extensive variety of complex behaviors plus learning , social, anxiety, memory, and defensiveness that may be beneficial to modeling neurological and psychiatric diseases [14, 15, 16] The zebrafish and mammalian behavioral patterns are in close parallel signifying the evolutionarily conserved nature of several behaviors across species [17]. Despite the differences between mammals and teleosts, there is evidence that many important regions of the zebrafish brain have homologous functions [18] High-throughput accessions to which zebrafish embryos and larvae have been added recently support behavioral research in the zebrafish field makes research more objective, repeatable and efficient. Studies on zebrafish, which show many behaviors at different developmental stages, are increasing. A zebrafish catalog already exists, describing more than a hundred traits in zebrafish embryonic larvae and adult zebrafish [19] Juvenile zebrafish have the advantages of adult zebrafish such as small size, optical transparency, short production time, ease of manipulation, permeability to small molecules, and cost-effectiveness.

Recently, the use of zebrafish larvae has gained great momentum in behavioral research, especially in the fields of neuropharmacology and circuit neuroscience, as technology improves [20] Using zebrafish larvae can analyze thousands of compounds per day [21] Similarly, it is also possible to see/monitor/monitor neuronal activity in zebrafish larvae when they engage in certain behaviors [19] Apart from that, zebrafish larvae β 3R (replace, reduce, improve) principle. [22]. New non-animal testing techniques for reproductive toxicology: Overall, the use of larvae has some limitations, but the above results make it an attractive model in biomedical research. Zebrafish larvae are known to be sensitive to a variety of stimuli, including touch, smell, chemosensory, auditory, vestibular input, heat, and vision [23] Among the behaviors of zebrafish larvae, movement or swimming has attracted the attention of neuropharmacology. The development of the larval structure is somewhat limited, i.e. lack of proper development and endocrine system [24], although zebrafish embryos develop as early as 27 hpf, zebrafish begin swimming 48-72 hours after fertilization (hpf) after hatching is decorionic and can float in response to touch [25] . After 4 dpf, zebrafish larvae develop a pattern of increased activity in the dark followed by rest in the light when exposed to varying light and dark stimuli [26]

In recent years, this behavior has been increasingly used to screen for many neuroactive drugs. This review focuses on light and dark stimulus-induced locomotor behavior in zebrafish larvae as a model to study neuroactive drugs. We briefly describe the behavior of zebrafish larvae and then discuss the utility and importance of light and dark stimulus-induced locomotor behavior in identifying neuroactive substances. Additionally, we discuss recent advances in behavior research in the context of the emergence of various high-performance methods to monitor larvae. Advances in behavior research in the context of the emergence of various high-performance methods to monitor larvae. [20]

BRAIN AND BEHAVIORAL RESPONSE

The organizational structure and cellular morphology of the zebrafish brain is similar to that of other vertebrates, such as chicken, mouse, and human [27, 28] neurotransmitter receptors, transporters, and enzymes for synthesis and metabolism. It also contains the same neurotransmitters as higher neurons such as GABA, glutamate, dopamine, norepinephrine, serotonin, histamine, and acetylcholine [28, 29] Also, many brain regions associated with human disease show molecular and structural homology in zebrafish [30, 31]. For example, the zebrafish's habenula and amygdala are involved in the regulation of emotions, as are humans and mice.

Habenula regulates the release of serotonin and dopamine and is evolutionarily conserved. Overactivation of habenula can cause similar conditions such as depression and anxiety-related behaviors in mammals and zebrafish [11] Recent studies have shown that these cells can recognize and function even without neocortex activity and even complex decision making of small animals such as zebrafish [32] Visualizing brain activity through imaging models is an important step in understanding how the brain drives normal and abnormal behavior in zebrafish. Capture, optokinetic responses and optokinetic responses in larval zebrafish [20]

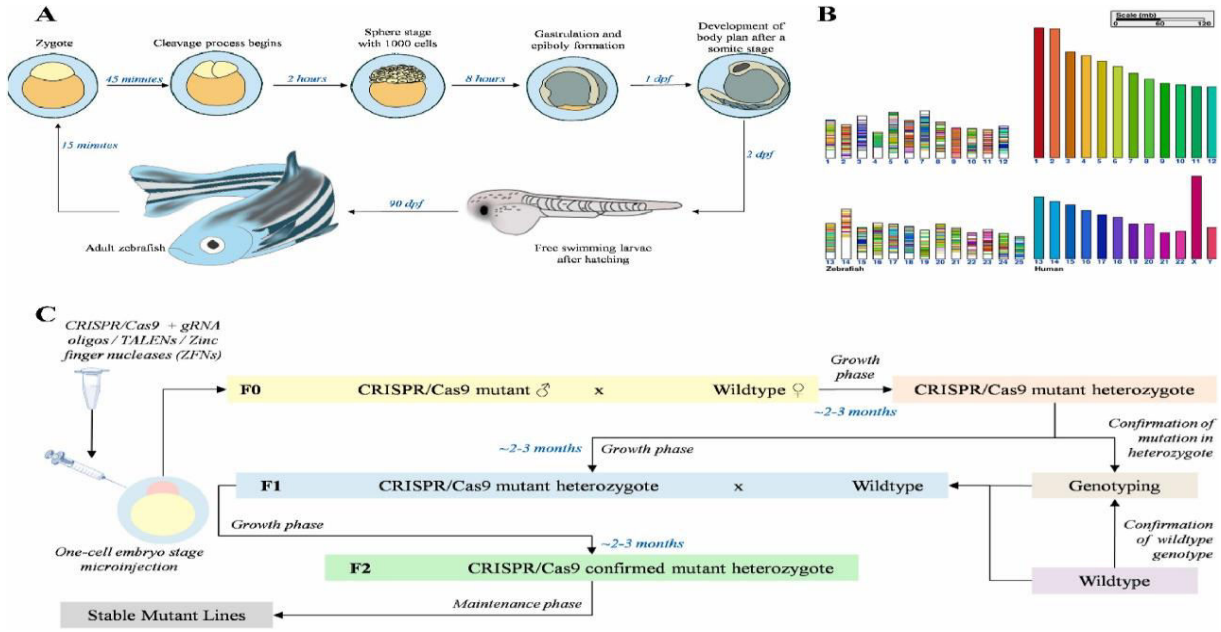

Figure 1. [**A]** Demonstration of zebrafish life cycle, one of the most useful models, **[B]** Comparison of human and zebrafish genomes with the help of Cinteny web server (http://). /cinteny.cchmc. This diagram shows a diagram of the [**A]** zebrafish life cycle, which makes it one of the most valuable organisms, with the help of the Cinteny web server (http://cinteny.cchmc.org/). and zebrafish genomes), where chromosomes are separated and arranged in a specific color code that encodes many syntenic regions of the two, and **[C]** mechanisms to generate stable zebrafish mutant lines in the laboratory.

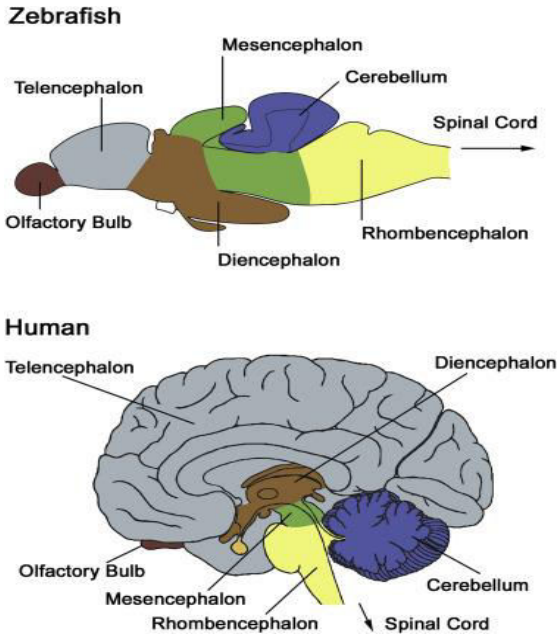

Figure 2. Schematic representation of sagittal sections of an adult human (bottom) and zebrafish brains (Top) at 5 days post-fertilization. Homologous regions they are shaded in the same colors to illustrate the conservation of basal vertebrates' brain organization from human to larval zebrafish, despitea dramatic difference in scale and complexity.

TYPES OF BEHAVIORAL ENDPOINTS

Behavior Repertoire in Larva

The behavioral characteristics of zebrafish larvae are compared with adult zebrafish and mice, revealing the effectiveness of zebrafish larvae in neuroscience [19] Zebrafish behavior has been used for many research purposes, such as behavior and toxicology. Pharmacology, Neuroscience. and Genetics [33, 34] . In neuropharmacology, the behavioral repertoire of zebrafish larvae has been used for many purposes - to identify new drugs with central nervous system (CNS) effects, to redefine drugs with CNS effects, to identify drugs for treatment of various mental disorders such as anxiety and depression [35] The commonly exploited behaviors of zebrafish larvae are described below.

***Sleep/Awake Behavior*:** Zebrafish sleep/wake behavior is similar to humans [36] Zebrafish larvae between 6 and 10 dpf were used in sleep studies. Similar to human infants, larval zebrafish at 6-10 dpf show higher skipping rates compared to adult zebrafish [37] Sleep/wake patterns in larval zebrafish have been used to study the effects of the drug on the nervous system [31]. The effect of modafinil, an anesthetic, on the sleep cycle of zebrafish larvae is described. The effect of modafinil, a drug used in the treatment of narcolepsy, on sleep/wakefulness, day and night shifts was evaluated with 24-hour behavioral monitoring. The effects of modafinil on zebrafish larvae are similar to those of sleepy/sleepy animals [38, 39, 31] showed that monitoring the behavior of zebrafish larvae using a video monitoring system could be used to determine the anti-stress effect. A detailed analysis of various behaviors was also performed using the rest/sleep behavior mechanism in zebrafish larvae to analyze 5648 compounds. This test was able to identify 547 compounds that significantly altered larval behavior compared to control [31] Thus, the zebrafish rest/sleep cycle can be used to detect or screen for neuroactive drugs.

Startle Response: The initial response is one that develops rapidly in all types of animals and is resistant to sudden, intense and sudden stimuli such as noise and unexpected touch. Zebrafish larvae exhibit rapid acceleration and deceleration in movement/swimming in response to visual, tactile, or auditory stimuli. The startle response is important because it provides an integrated state of sensory and motor stimuli. The tactile startle response occurs as early as 2 dpf and can be induced by touching the head or tail of zebrafish larvae [40] During the visual response, zebrafish larvae were placed in a cage with infrared light and video camera. After a short time, they were briefly exposed to white light and the results were recorded to measure the visual effects. The test that measures the visual response is also called the visuomotor response [41] Zebrafish larvae show the acoustic startle response after 5 dpf [42] they respond to noise greater than 200 Hz. The threshold response is important in the study of human neuropsychiatric diseases because an abnormal response is indicative of more serious neurological problems [43] Study of the startle response plays a major part in neuro pharmacological research as some drugs that alter prepulse inhibition could be used for the treatment of diseases such as schizophrenia [44].

Habituation: Habituation is a form of non-learning that repeats negative responses [45]. It is found in many species, from invertebrates (such as sea hares and fruit flies) to vertebrates (such as mice), and is a mechanism by which the brain filters out irrelevant stimuli. Zebrafish larvae were found to be viable at 6 dpf. Habituation deficits ae associated with many neuropsychiatric disorders such as schizophrenia and ADHD effects on nonparticipating subjects [46]

Thigmotaxis: The tendency of an animal to move in contact with a vertical surface is called haptotaxis. In haptotropism, animals avoid the space of the arena and move to the edge or periphery of the new environment (e.g., walls) [47]. For this reason, it is also called the character of the wall. It is an effective stress reliever [40] and has been mutated in many species such as fish, mice, and humans [48] Haptotropic zebrafish larvae prefer to live near the walls of multi-well plates or petri dishes. They exhibit tactility as early as 5 dpf [48] Anxiolytics such as diazepam and anxiolytics such as caffeine and pentylenetetrazole have been shown to reduce and increase tactility in zebrafish larvae [49] Thus, tactility is important for research on anxiogenic drugs.

Optomotor Response: When zebrafish larvae show moving stripes, they tend to swim in the same direction as the moving stripes. This visual-motor behavior in zebrafish is called the optokinetic response. Zebrafish larvae begin to exhibit this behavior at 6 dpf [50] This response can be used to detect activity in zebrafish larvae and to test compounds using small doses at the start of detection [51]

Locomotor Behavior: Movement is essential for the survival of animals. Zebrafish larvae can swim at 4-5 dpf [52] once they have a swim bladder. Zebrafish movement is a behavior created by the activity of many neurons (reticulospinal neurons of the brain and descending vestibulospinal or neuromodulatory projections). All of these pathways are mutated in vertebrates [53] In addition, the neurotransmitter system found in zebrafish is also found in zebrafish and other vertebrates [54] There are many behavioral tests used to identify neuroactive substances [55] One is the light-dark locomotor test, in which locomotor activity and movement patterns of zebrafish larvae are analysed by placing them in multi-well plates for high-throughput auto-sensing. In a closed room, after acclimatizing for a while, expose them to varying light and dark. Test the distance and movement patterns of zebrafish larvae under all conditions for neurobehavioral effects. When exposed to changing light and darkness, zebrafish larvae exhibit unique movement patterns [56] Switching from light to dark increased metabolic activity in zebrafish larvae, while switching from dark to light decreased the activity of bacteria in fish zebrafish larvae. The increase in locomotor activity during the light-dark transition has been attributed to increased anxiety in zebrafish larvae [57]These locomotor activities depend on the integrity of brain function, nervous system development, and visual pathways [58]

Optokinetic Response: The optokinetic response is a stereotypical eye movement in response to movement in the visual field. It helps to improve the image on the retina. It is also important for maintaining vision, which helps animals to spatial orientation, find prey, and escape from predators [59]. Zebrafish larvae produce optokinetic responses at 4 dpf, which can then be accurately measured [60] Optokinetic responses of zebrafish larvae are evoked by the movement of moving images or rotating black and white lines on a liquid crystal (LCD) monitor. [59]. Many drugs that act on the central nervous system, such as sedatives, antipsychotics and antidepressants, regulate eye movements. Therefore, the photokinetic response can be used as a test to clarify the CNS effects of drugs.

Prey Capture: When zebrafish embryos hatch, they begin swimming and catching potential food, also known as prey capture. This behavior has been observed in zebrafish larvae as early as 4 dpf [61] Prey capture is a complex locomotor behavior in which larvae use vision and fine axial motor control to locate and track prey [62] and this behavior can be elicited in zebrafish larvae by providing a small bubble in the test area. The zebrafish larva's responses to air bubbles include recognition, approach, decision making, and catching [61]. Prey capture is essential for the survival of zebrafish larvae after assimilation of the yolk sac. Since animal capture is directly related to cognitive decisions [61] it can be used to study drugs that affect cognition.

Colour preference**:** Environmental characteristics such as lighting (bright/open areas vs dark/protected areas), color and location (perimeter – center) can create messages that help people assess the resilience or safety of the environment [63]. Adult zebrafish have been reported to prefer black [64], red, yellow and green. However, little research has been done on zebrafish larval preferences. Zebrafish color preferences knowledge may be applicable to future color-based memory and learning paradigms. Colors can be useful in tests involving anxiety/fear and non-deterrent colors can be useful in tests of appetite.

Turning behavior: Turning behavior differs from swimming behavior due to its asymmetry. Bowing is often different from the next swimming habit. Behavioral changes in zebrafish larvae have been studied in detail [65]. Zebrafish larvae exhibit two types of rotation. The first type of rotation is continuous rotation, it has no triggering effect, it creates a 30° rotation when completed the first time. This turn differs from the traditional swimming competition, which starts with little or no head changes. These roads are characterized by a slow angular velocity and no reverse bends after the first turn. After hatching, these changes occur and continue throughout ontogenesis [66, 67] and assist in foraging and avoiding predators. The second type of rotation is more difficult and is characterized by a faster rotation; includes evasive behavior. In this case, zebrafish larvae C-bend very quickly. These changes often include a large reverse bend back to support. After this response had dissipated, the zebrafish larvae were subjected to daily swimming bursts that did not change.

Freezing: Freezing is an indicator of stress [68] and is defined as partial or complete movement outside the gills and eyes. Zebrafish larvae, like adults, exhibit freezing behavior when exposed to harsh or stressful environments (Ahmad & Richardson, unpublished data) [69, 68]. The larvae, for example, 3-4 dpf, spend longer in the frozen or resting state than those at 5, 6 or 7 dpf [40] The high freezing point of small fish may be due to swim bladder enlargement and continuous swimming starting at 5 dpf [70]. However, this freezing behavior is reduced if embryos are exposed to test equipment 24 hours prior to testing [40]. Thigmotaxis may be an important parameter in experiments with anxiolytics and anxiolytics that increase or decrease the duration of activity. [71].

***Erratic movements*:** Sudden sharp changes in direction and/or speed of movement indicate a rapid anxiety-like dart behavior called jerky foot. It has been well studied in adult zebrafish [72, 69] Sudden sharp changes in direction and/or speed of movement indicate a rapid anxiety-like dart behavior called jerky foot. It has been well studied in adult zebrafish [68, 73, 74] but this has not been studied in detail. Movement disorders usually occur at high speeds. These high speeds may serve as an indirect measure of impaired movement in complex zebrafish. Automated measurement with accelerometers could be a useful tool for monitoring movement disorders in zebrafish [68]

High-Throughput Behavior Profiling: Zebrafish larvae can be kept in individual multiwell plates for several days, ideal for behavioral studies for genetic, pharmacological and neurotoxicological studies. Observations include circadian changes in activity [75] as well as responses to changes in ambient light and acoustic/vibration stimuli, which can be transmitted unevenly across multiple wells with further measures of motivation and behavior [76, 77, 78] and can be used to identify potential treatments. An example of a promising endophenotype in zebrafish is prepulse suppression of the startle reflex, which has been implicated in schizophrenia in the aforementioned analyses [79, 31] showed that changes in swimming behavior during the light-dark cycle can provide a better understanding of the effects of different strokes, which could lead to the effects in different chemicals in the blood vessels. Recently, they described phenotypes in experiments using mutant fish to expand gene expression, which they used in this analysis to identify potential new treatments [80, 26, 81]

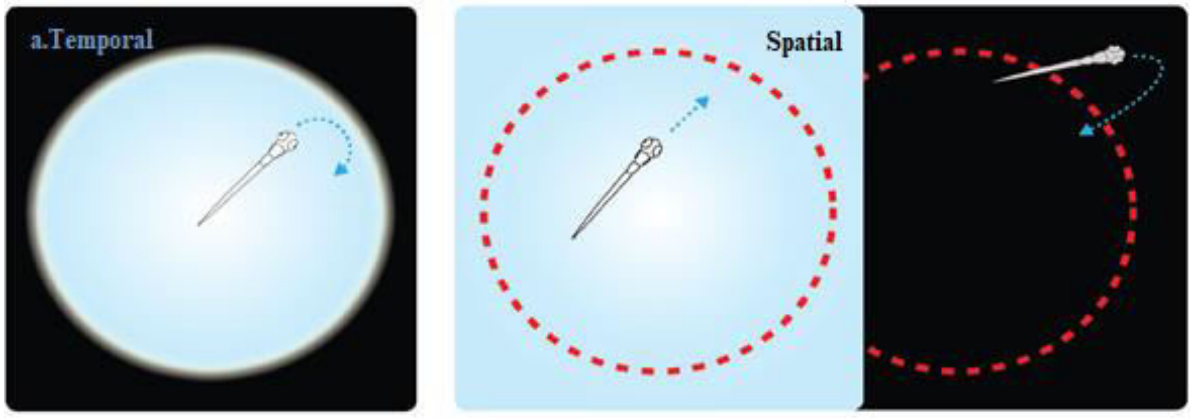

Figure 3 (a) Phototaxis using spatial and temporal cues. Larvae can live in a small area that can be seen moving away from the light-dark boundary (left). They can live in a virtual circle and when they cross the border all lighting is changed following simple sensorimotor rules. [82]

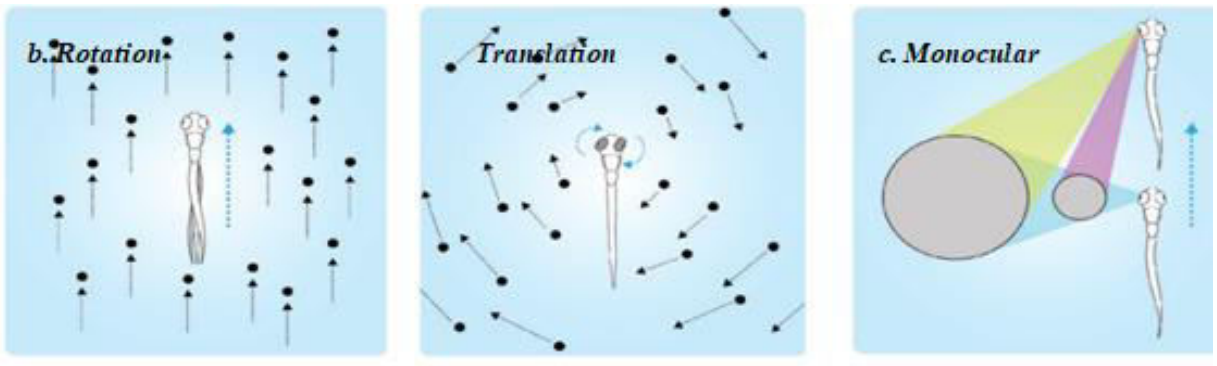

Figure 3, (b) The interpretation reveals optokinetic swimming behavior that opposes the perceived ego aspect in the opposite direction. Rotational motion elicits optokinetic responses. **(c)** For monocular vision, objects at different angles and distances correspond to the same viewing angle. However, as the fish swims forward, objects that are closer move faster on the retina. [82]

Ontogeny of Complex Behaviors: One potential issue with using the one-week-old zebrafish as a behavioral model is that the brain is still changing, so its behavior represents development during critical periods of the brain. Although children of this age [83] have a wide variety of innate reflex responses and exhibit different kinds of dissonance, their ability to demonstrate effective learning is controversial [84] and they display many relationships. Penetration attempts decrease with age, but recent research on the habenula and brain circuits has shown the potential for dysfunction in humans for at least three weeks. window to examine different behaviors. Careful research into the development of simple classical work and cold work has shown that these behaviors reach adults at three to six weeks of age [85] [86] Interestingly, it is also possible to say that there is still significant innate change and increase. visual reflexes. The development of learning during social interaction [87] Amusingly, this same time period may also see major alterations in innate reflexes and an increase in social interaction [88] The advance in learning may signify a maturation of neural circuits, but it may also outcome from a failure to recognise stimuli relevant to the larva, and some studies have designated classical conditioning assays applicable to seven-day-old larvae [89] and motor learning [90]

BEHAVIOR REPERTOIRE IN ADULTS

As zebrafish grow from larval to adult, their behavior is progressive, particularly in social areas, including herding and schooling, group shearing offence, aggressive encounters, and mating. Most studies of larval locomotor behavior have focused on velocity or changes in velocity. [91] However, neurotoxic effects can often be seen in children and adults when the body's body or swimming skills are changed. One of the best tests for larvae was described below (see Table 3). However, the behavior of adults is thought to be more complex than that of larvae, and changes in movement patterns are easier to observe due to their larger size. Little and Fingers show that the lowest concentration of toxicant causes changes in adult locomotor behavior between 0.1% and 5.0% of the lethal concentration [92] Details of the last character. Parameters obtained from the analysis include speed, rotation angle or frequency, and time spent in different types of swimming (normal swimming, macrokinetic swimming, microkinetic swimming, swimming breaks, etc.).), horizontal and vertical distribution of people, road bend and startle response [8]

Anxiety-Like Behavior: There are many ways to measure stress-like behavior in fish, most commonly zebrafish [93, 94] New tanks are used, and the light/dark preference test includes measurements of space-time distribution (time at the bottom of new tanks or dark. in light/dark tanks). images showing behavioral sensitivity to anxiolytic or anxiogenic treatment (freezing, non-freezing, continuous swimming, risk assessment, provocative) [95] In a recent meta-analysis, the light/dark test is more effective than the new test and both tests are important equally abolished the cortisol response [95]It has been detected that for standardized behavioral performs within the laboratory, such experimental conditions (test days and batches of fish) may have comparatively few experimental effects on the results of anxiety and locomotor activity [96]. Examples of chemicals shown to affect stress-like behavior in zebrafish light/dark and new tank experiments can be found at Stress-like changes in behavior are not only about protecting the predator, but also about feeding and seeking help: if the animal is "too cautious" due to the effects of the venom (for example, increased stress-like behavior), it will miss. important time for childbirth or feeding outside. Conversely, reducing stress-like behavior can lead to "negative behavior" and the emergence of predators, as in migratory salmon larvae [97] The standardization offered by these behavioral tests can support investigators recognise changes in these endpoints, which can lead to novel hypotheses on the ecotoxicological sub lethal effects of substances at complex behaviors.

Aggression and Mating: The behavior of the adult zebrafish can be very complex, but there are still stereotypical stages that help to value and model. An example is binary challenge in male zebrafish [98] , which can be divided into stages after evaluation and resolution. Each level has a different character. During the test, the fish was circled and exhibited biting behavior. During the solution, competitive behavior (biting, chasing, hitting) is always initiated by the winner. In addition, the concept of territorial behavior is level dependent [99] with dominant fish guarding the area where the food appears and chasing other fish, secondary fish rarely use aggression, but instead of entering when important fish are eating or chasing food. The nature of the setup may allow the emergence of satellite fish, which can be switched from a secondary role to protect a different area from that viewed by the main fish [100]. Events also affect subtle behavior such as texting. Pisces, who came only from the president after observing the fight, paid a lot of money to those who lost in the war. The result of winning or losing the battle is fixed at 85.7% and for every victory in the battle of the 5% of winners and losers against the ignorant results in a difference in production, which leads to a broadening of other attributes and has implications for molecular [98] and neuronal circuit analysis. Advanced methods of combat and behavior in the area require further analysis combining 3D tracking, automatic processing of

group behavior and modeling according to the initial Markov process. Similarly, rich behavior during speech [101] is amenable to quantitative study.

Shoaling: Zebrafish form flocks and polarized groups (called schools) not because of a reciprocity, but because they like each other. Living on campus has many advantages, including better discovery of animals, food and friends [102]. The zebrafish population is dynamic, the distance between animals increases over time after being placed in a new aquarium and decreases in flocks of fish. [103]. Research on zebrafish flocks has focused on what makes flocks attractive to humans. Zebrafish choose to congregate with certain fish on empty reefs. Detection of congeners requires a combination of movement and morphology with the head rather than the tail providing relevant clues [104] and fish of similar appearance [105]with activity level and sex composition also playing a role [106, 107] Lab experiments with shoals are often accomplished by employing an individual between two partitions and measuring the fraction of time that the animal is in the compartment with other conspecifics. The advantage of this test is that it is simple, but limits understanding, interferes with natural interactions, and offers walls that can lead to attractiveness as well as robust behavior. Application to ontogenetic analysis has shown that zebrafish are biased towards certain traits at three weeks of age [88] Analyzing ontogeny using more natural phenomena can reveal earlier, more complex relationships. Dissection of circuit mechanisms in social processing [108] should be fine in this mode, given that the imaging equipment was available at three weeks. The importance of the reward circuit in relationship formation [109] and the roles of dopamine oxytocin, and other peptides [110] remain challenging questions.

Sleep like behavior: Many studies have described sleep-like behavior in zebrafish. At night, the fish swim horizontally and make small pectoral fin movements, while adults have a period of inactivity of two to four minutes. Mouth and valve movements were simultaneously reduced, indicating a reduction in breathing [111] Sleep regression has been observed in zebrafish, indicating homeostatic regulation; Disruption of the normal night period (use of light, vibration, electric shock or forced exercise) deprives the fish of rest time and increases energy during sleep [111, 112] Finally, zebrafish also exhibit a circadian rhythm with more activity throughout the day. Research in other species has identified several important sleep-related neurotransmitters: increased dopamine levels in the brain reduce sleep duration. [113] GABA signaling promotes sleep, while GABAA receptor agonists are used to treat insomnia. Treatment with diazepam, pentobarbital, although this approach has not been directly studied in zebrafish [111]. alpha2 adrenoceptor agonists [114] and histamine H1 antagonists [115] All of these have been shown to induce sleep, suggesting that GABA, acetylcholine and histamine exert control over sleep. Several studies have also demonstrated a conserved role of hypocretin/orexin (HCRT) in sleep regulation. Zebrafish have the same HCRT receptor gene (hcrtr) expressed in a small number of glutamatergic neurons in the adult hypothalamus. [112] Loss of hcrtr function causes sleep fragmentation but not cataplexy or short-term wakefulness, suggesting that HCRT may help integrate sleep in fish. [112]. HCRT acts by stimulating the endogenous melatonin sleep-promoting system found in the pineal gland [116] Taken together, studies on zebrafish have confirmed that sleep regulation appears to be adaptive. Although zebrafish sleep research is still in its infancy, the high-throughput nature of the technology used to measure sleep makes zebrafish an ideal model for the analysis of novel hypnotic mutants.

Decision Making in Groups: Animal behavior is often studied by behavioral ecologists. [117, 118] Fish play an important role in these studies because cooperative behavior can be easily reproduced in the laboratory. [117]. Zebrafish may play an important role in laying the foundation for neuronal research. Zebrafish experiments develop models of collective decision making, in which animals combine private and social judgments to make decisions. [104] This experimental model demonstrates that the zebrafish stands out as a species with simple interactions, helping scientists understand various aspects of human behavior. [119]. Many other models have been used to model zebrafish coordination, including reinforcement learning or various models of sensorimotor control. Finally, we hope to understand the interaction of animals by combining simple models with accurate and detailed models of neuronal processes provided by research.

Reward behavior: Animals' instinctual drive to find resources and reproduce is fueled by reward behavior. However, drugs of addiction like cocaine, amphetamine, or opioids can potentially take over the brain's reward circuit. Thus, rewarding behaviors might represent the start of addiction. The conditioned place preference (CPP) test, which matches a main cue (such a drug) with a secondary stimulus like a colored aquarium compartment, can be used to quantify reward in zebrafish. The persistence of CPP after a period of abstinence can also be used to assess drug dependency. In keeping with research on other animals (ethanol has been demonstrated to be a rewarding stimulus for adult fish, for example), [72, 120],cocaine [121], amphetamine [122] , opiates [123], nicotine [120], food [123] and the presence of conspecifics [109]. Dopamine (DA) is a key neurotransmitter linked to rewarding behavior. Mammals

are motivated to apply the stimulus repeatedly by an increase in DAergic transmission from the ventral tegmental region to the nucleus accumbens (nAC). This important DAergic route in zebrafish most likely consists of projections from the ventral telencephalon to the diencephalic posterior tuberculum (subpallium, (Vv and Vd), see [124]. Reward-related behavior has also been linked to a number of other neurotransmitters. Given that morphine and food rewards both depend on the function of opioid receptors in zebrafish [123], Because of the separable reward behavior in tof, it is possible that different brain networks govern how the body reacts to morphine and food. These networks act downstream of opioid signalling. Alternately, several subgroups of dopaminergic nuclei in the forebrain may mediate the pleasurable effects of food and drug use [123]. In light of this, tof gives a fantastic chance to examine the neurological underpinnings of the brain's ability to distinguish between rewarding drugs, together with the research of hippocampal irradiation rats,

Learning and memory: Mammal studies have demonstrated that a variety of brain circuits, each of which is neuroanatomically different, are capable of controlling learning and memory. These include implicit learning (such as simple motor reflexes; cerebellum), avoidance learning (amygdala), and spatial learning (hippocampus). Several paradigms have already been established to test learning and memory in zebrafish, despite the fact that the neuronal basis of learning in zebrafish is not well understood. By associating two previously unrelated stimuli, such as colour, reward, or aversion, associative learning can be quantified. For instance, Suboski and colleagues combined the unpleasant effects of an alarm drug with a neutral stimulus (morpholine). [125]. Avoidance learning can be measured using the shuttle box; [126] or colour [127]) without the need (like little electricity). Spatial learning can be measured using the T-maze [121, 109] or a shuttle box [128]. Fish must learn to reap the benefits by skipping work or transferring them to one side of the aquarium. These behavioral tests are efficient and useful for screening for new genes that regulate learning and memory. Pharmacological studies have validated adult zebrafish as models of learning and memory, making it a promising area for future research.

Avoidance and attraction*:* When pollution causes the stimulus response, the result of the behavioral response (avoidance or preference) causes the body to change the exposure time. If the fish senses pollution, it responds by avoiding the chemical. Conversely, if the pollution causes a response, the fish will stay in the area, prolonging the exposure time. The avoidance-pleasure response (1) to the drug that activates the receptor; (2) the animal has sufficient history to effect the change or the body has sufficient experience to respond to the stimulus; (3) from adequate guidance for drug concentration gradients to accurate guidance from drug plums [129] Because many contaminants induce avoidance or attraction behaviors, avoidance and attraction behaviors in fish emerged as simple and realistic behavioral endpoints of exposure. The usefulness of avoidance behavior as an indicator of lethal toxic effects has been demonstrated over the past 50 years, and chemically induced avoidance or attraction can significantly alter the distribution and migration patterns of individuals and groups of fish. [130, 131] It has been shown that free-living fish in situ avoid oil-contaminated and gassy waters. A variety of freshwater fish have been documented to avoid heavy metals (e.g., cadmium, copper, cobalt, aluminum) at low ecologically important concentrations [132]

Swimming patterns*:* Avoidance behavior is a mixture of many behaviors that can lead to one endpoint, while motion analysis is a finer-scale method of examining the components of motion. Neurotoxicity often manifests itself in changes in the shape, frequency, or posture of swimming movements, and these changes often occur much earlier than death. [133, 2] Lethal exposures to metals and pesticides have demonstrated changes in swimming behavior and serve as models for additional stressors. [134]

Respiratory patterns*:* Respiration is a rhythmic neuromuscular sequence regulated by external environmental stimuli as well as endogenous biofeedback loops. Acute exposure to contaminants can induce reflex coughing and gill clearing responses to remove irritants from the eyeballs, and can also increase the frequency and amplitude of the respiratory cycle as fish regulate the amount of water in the respiratory stream. Continued exposure may lead to irregular breathing cycles, primarily due to reduced input, but also due to changes in the endogenous pacemaker. [135] It has been shown that the frequency and amplitude of the fish cough response and actuation rhythm change after exposure to various contaminants. For example, dieldrin, an organochlorine insecticide, increased respiratory rate at concentrations above 24 µg l-1 and induced coughing responses and erratic movements. Conversely, zinc at a dose of 300 µg/l reduced the amplitude of the respiratory response. At low environmental concentrations, it affects gill tissue and respiratory function. Respiratory rate, depth (volume), and cough frequency can be measured noninvasively using physiological cues from restrained sentinel fish. One such system is accomplished this with good repeatability has been described by [136].

EXPLORATORY-BASED MODELS

Behavioral tests in zebrafish are currently being used for high-throughput phenotyping and testing of various psychotropic drugs. [137, 138, 139]

T/Y Test: Maze Test discrimination test such as the T/Y maze has been used to demonstrate learning and memory in zebrafish, similar to mice. The T/Y search test uses positive emotions as a reward and can reward the zebrafish if it gives the right response when searching for a target. Spatial memory represented by percentage the, it's for the distance traveled. For example, a T-maze has a starting area, a long arm, two short arms, and two chambers. Food was used as a positive stimulus and given room for zebrafish training. Leave for a day or two before putting the fish in the starting place for testing. This behavior is recorded by the camera and the time taken to find the object is evaluated. Developed a simple apparatus and method as a T-maze [140] . The tank is separated by a central white divider that leaves enough space at the bottom for the zebrafish to swim freely between the two sections. A red card is attached to one end, allowing the fish to distinguish between the two parts of the tank. After 5 seconds of tapping on the location of the bowl, the food is served. The appearance of the zebrafish on the bait within 5 seconds was considered the correct response.

Novel tank test: A widely used behavioral analysis method in zebrafish research is the novel aquarium test (Figure 3a), which is conceptually similar to the open field test used for rodents exhibiting restless behavior while staying close to walls (thigmotaxis), but increases the study. getting used to the new environment [141] Likewise, exposure to a novel environment triggers a strong alert response in zebrafish. [137] When submerging to the bottom until you feel safe to swim in the upper part of the tank (geotaxis). Typical endpoints for this test include peak entry latency, number of peak transitions, time spent at peak, peak-to-floor time ratio, number of irregular movements such as fear/flight, freeze frequency and duration. [142, 138] Until recently, zebrafish behavioral quantification was mostly performed manually, making it susceptible to human error and data misinterpretation. However, automated video tracking techniques are increasingly being used to analyze animal behavior to provide standardized and objective observations of behavioral endpoints. [69] Another benefit of using a video tracking approach is the ability to store, replay, and reanalyze the video. Finally, the video tracking tool can calculate additional motion endpoints not available from manual observation, such as up/down travel distance, velocity, twist, and angular velocity. Comparing the data obtained from the video tracking system with the manually recorded data, it can be seen that there is a high correlation between these two factors. [69] We validate the video tracking approach as a reliable analytical method in zebrafish neurobehavioral studies.

The light/dark box: It is traditionally used in the neurobiology of rodent behavior and is based on an innate aversion to opening illuminated areas (scotophilia, scototaxis). [143]. Previous studies have shown that anti-anxiety compounds can promote exploratory activity (i.e., increase the number and duration of items in light segments), whereas anxiolytic drugs have the opposite effect. [143]. Importantly, this test is now being applied to zebrafish, in which they exhibit a natural preference for the dark side [64] (Fig. 3b). There are several modifications to the light and dark fish box test. [144, 64], Consistently demonstrates the usefulness of light/dark situations for modeling zebrafish restlessness. Our observations also support this notion, showing that the "more repulsive" behavior of zebrafish in light can be modulated by anxiety-inducing and anxiolytic drugs, which are strikingly similar to the behavior of mice in light and dark.

***The open field test*:** Other common materials used in rat experimental biopsychology [145, 146] A promising new aspect of zebrafish research is also provided. For example, some studies have used the open test for larval samples. ([147] The benefits of outdoor testing for adult zebrafish studies also seem plausible. Like mice, zebrafish prefer to stay close to walls, especially corners. When zebrafish acclimatized to the new environment, they entered the open central area as expected, indicating increased exploration (Figure 3c). Overall, this brief overview of zebrafish-based research leads to some important observations. First, zebrafish discovery appears to be driven by the same evolutionary mechanisms as mouse behavior that is better studied and understood [141]. These factors include the balance of exploration (finding new things, curiosity) and avoidance of confrontation.

Inhibitory Avoidance Test: The anti-tolerance test (IAT) is one of the most common cognitive tests to measure learning and working memory in zebrafish. The test can be set up using two tanks (Fig. 1A) and pipelines to explore various shapes (Figs. 1B-D). In a dual chamber tank, both sides of the tank/maze are alerted by a colored sheath or a dark area when a side is selected (such as ES) with a negative emotion. [148] , dropping weight [149] or mix. In IAT,

a positive side (usually a dark room) is chosen for CS-US coupling. During training, zebrafish were placed in a light chamber and allowed to pass through the dark chamber. ES will be applied when the fish enter the dark room. The training session can be repeated several times until a response is achieved. Probe testing was performed 24 hours after the last training (short term), allowing the fish to explore the area without ES. Late entry to the dark room during the training and probe sessions was noted as an indicator of memorability [141]. Other parameters such as the number of darkrooms and the distance traveled in the darkroom can be measured using software.

Appetitive Conditioning Test: Similar to IAT, use a rectangular tank or probe to create CS-US pairs under repetition. Chen et al. Cover the left and right arms of the T-maze with the green and red sheath as the light source [150]. One of the arms was used as a support center with brine shrimp as a health stimulant. The tests include the environment, education and research phase. The authors measured memory performance in terms of latency and time taken to reach the target area. Other health stimuli may be the image of a special, beautiful place made of artificial grass and stones.

***Novel Object Recognition Test*:** Similar to the Y-maze, which is based on the fish's response to new objects, the New Object Recognition Test is used to examine memory. In an experiment by [151] was performed as a zebrafish was placed in a tank containing two familiar objects (red cubes) and allowed to explore the tank for 10 minutes. An hour later, a red cube (familiar object) was replaced with a green cube (new object) and the discovery of the fish was recorded. A distance of less than 2.5 cm from an object is considered object detection [141]. Recognition memory scored based on search time and preference for new items.

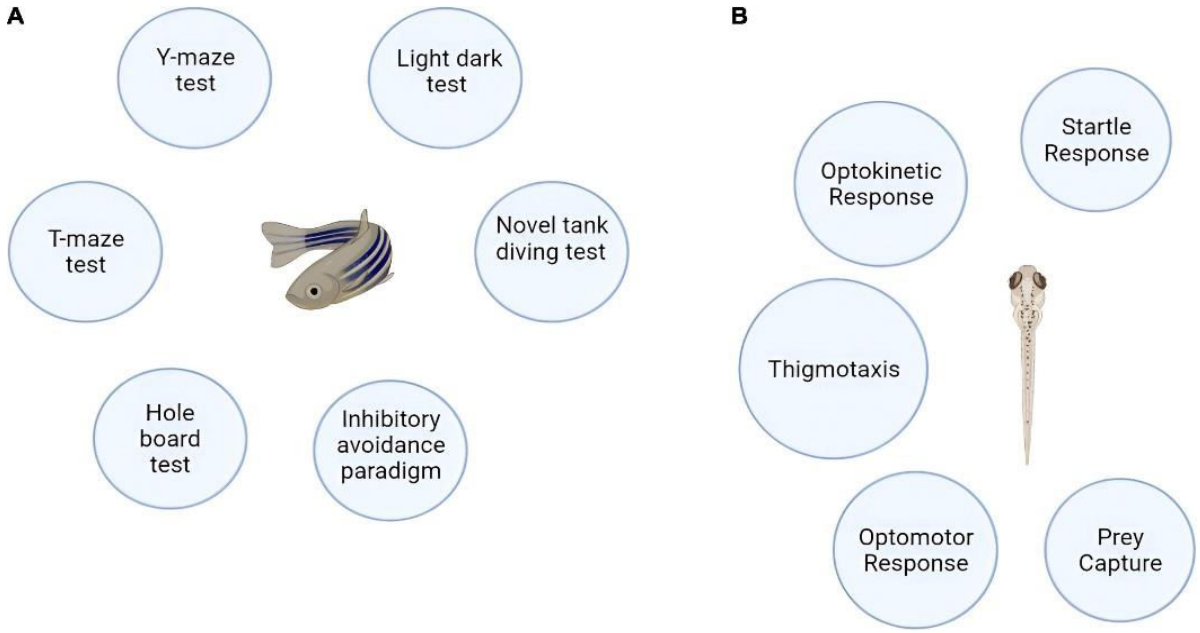

Figure 4.1 (A) Behavioral models of adult zebrafish. **(B)** Behavioral models of zebrafish larvae. [152]

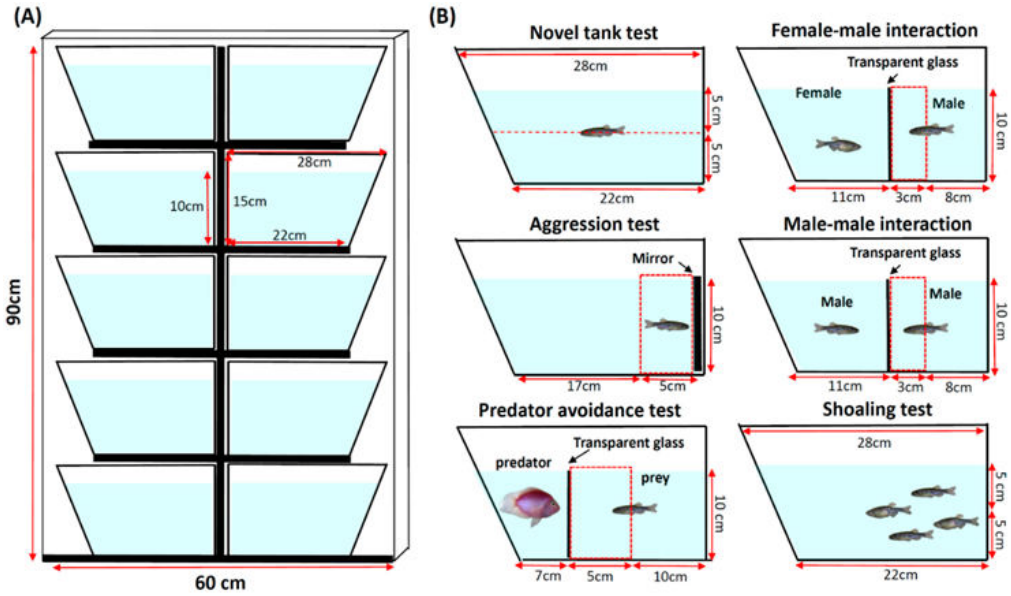

Figure 4.2 (A) The schematic picture for zebrafish tower setup. (**B**) The experimental setting of zebrafish tower for multiple behavior endpoints tests (Upper left corner: novel tank test, center left corner: aggression test, bottom left corner: predator avoidance test, upper right corner: female-male interaction, center right corner: male-male interaction, bottom right corner: shoaling test

Table 1. Protocols to measure behavior in zebrafish Larva

Stage	Behavior	Paradigm	Reference
Larva	Thigmotaxis	Percentage Of Distance Moved in Outer Zone	[153]
Larva	Thigmotaxis	Entries In Outer Area	[154]
Larva	Thigmotaxis	Distance Traveled/Time Spent in Each Zone	[155]
Larva	Thigmotaxis	Percentage Of Distance Moved in Outer Zone	[48]
Larva	Thigmotaxis	Distance Traveled in Outer Area	[156]
Larva	Light dark test	Total Distance Traveled	[157]
Larva	Visual motor response	Velocity, Total Distance Moved, And Mobility Time	[158]
Larva	Locomotor activity	Total Distance Traveled	[159]
Larva	Locomotor activity	Velocity, Total Distance Moved, And Mobility Time	[40]
Larva	Locomotor activity	Average Distance Traveled	[153]
Larva	Locomotor activity	Total Distance Traveled, Mean Speed, Turn Angle	[154]
Larva	Acoustic startle response	Head Angle	[155]
Larva	Visual motor response	Total Distance Traveled	[153]
Larva	Visual motor response	Total Distance Traveled	[160]
Larva	Visual motor response	Burst Swim	[161]
Larva	Visual motor response	Total Distance Traveled	[162]
Larva	Visual motor response	Average Distance Traveled	[73]
Larva	Visual motor response	Total Distance Traveled	[163]

Larva	Vibrational startle response	Total Distance Traveled	[160]

Table 2. Effect of different compounds on locomotor activity of larval zebrafish

Compound	Concentration	Age of zebrafish (dpf)	Reference
Sedatives			
Ethanol	4%	6	[147]
Fluoxetine	4.6 mM	3–6	[164]
Clozapine	12.5–50 mM	7	[165]
Diazepam	10 nM–100 mM	7–14	[166]
Pentobarbital	10 nM–100 mM	7–14	[166]
Stimulants			
Pentylenetetrazole	10 mM	5	[167]
4-Aminopyridine	0.6 mM	5	[167]
Ethanol	4%	6	[147]
Bisphenol A	0.01–1 µM	5	[168]
Aconitine	2.5–25 µM	5	[167]

Table 3. Effect of different compounds on locomotor activity of larval zebrafish.

Drug	Concentration	Tested Time/ Test	Effect	Reference
Response: Touch				
Dieldrin	20 µM	48 hpf,	Response Decreased	[169]
Sodium benzoate	100 ppm	3 dpf,	Response Decreased	[170]
DDT	10 µM	4 dpf	Response Increased	[169]
Diazinon	2 mg/l	8 dpf	Response Decreased	[171]
Cadmium	0.25, 0.5 mg/l	3 dpf	Response Decreased	[172]
Response: Startle				
Ethanol 4–6	30 000µM	4–6 dpf	Response decreased	[173]
Nicotine	20µM	1–7 dpf	Response decreased	[174]
Donepezil	3-10µM	6–7 dpf	Response Increased	[42]
Memantine	30µM	6–7 dpf	Response Increased	[42]
Rolipram	3µM	6–7 dpf	Response Increased	[42]
Response: Optokinetic				
Ethanol	1.5%	2-5 dpf	Higher visual threshold	[175]
Bisoprolol	30–300 µM	5-8 dpf	No significant effect	[176]
Response: Optomotor				
Atropine	100 µM	3-8 dpf	No effect	[177]
Chloropromazine	10 µM	3-8 dpf	Inhibition	[177]
Aspirin Exposed	500 µM	3-8 dpf	No effect	[177]
Diazepam	10 µM	3-8 dpf	Inhibition	[177]
Nicotine	6.2 and 62 µM	3-8 dpf	Inhibition	[177]

Response: Anxiolytics and Anxiogenics (Test)				
Buspirone	59.24 µM	Light/dark box	Total time spent in dark zone increased, latency to visit dark zone decreased, number of entries in the dark increased	[74]
Caffeine	100 mg/l	12-well plate assay	Increased thigmotaxis	[178]
Diazepam	2.5 µM	Light/dark box	Total time spent in dark zone increased, latency to visit dark zone decreased,	[74]
Ethanol	0.5–4%	Motor activity	Hyperactivity with 0.5-2% ethanol and hypoactivity with 4% ethanol treatment	[179]
Propranolol	10–100 µM	96-well plate	Decreased activity in the photomotor response assay	[180]

Table 4. Protocols to measure behavior in adult zebrafish

Stage	Behavior	Paradigm	Reference
Adult	Reward	Conditioned place preference	[72]
Adult	Reward	Presence of Conspecific	[109]
Adult	Social	Group preference	[72]
Adult	Social	preference Shoaling	[10]
Adult	Social	Area occupied	[181]
Adult	Social	Social preference Nearest neighbor distance	[181]
Adult	Anxiety	Group preference	[72]
Adult	Anxiety	Light/Dark preference	[182]
Adult	Anxiety	Time in enriched T-maze chamber	[183]
Adult	Anxiety	Place preference / Thigmotaxis	[184]
Adult	Anxiety	Anxiety Exit latency test	[64]
Adult	Anxiety	Anxiety Tank diving test	[69]
Adult	Anxiety	Locomotory activity	[72]
Adult	Learning / memory	Delayed spatial alternation	[185]
Adult	Learning / memory	Spatial alternation, learning and memory	[128]
Adult	Learning / memory	Active avoidance conditioning	[186]
Adult	Learning / memory	Learned alarm reactions	[69]
Adult	Learning / memory	T-maze	[184]
Adult	Learning / memory	Visual discrimination learning	[40]
Adult	Aggression	Mirror image test	[72]
Adult	Aggression	Pigment response	[72]
Adult	Aggression	Live observation of two fish	[187, 188]
Adult	Aggression	Startle reaction	[181]
Adult	Sleep	Pigment response	[189]
Adult	Sleep	Monitoring sleep postures	[36, 112]

Adult	Sleep	Locomotor inhibition	[189]
Adult	Locomotion	Turning angle	[190]
Adult	Locomotion	Total distance moved / Video tracking	[190]
Adult	Locomotion	Number of lines crossed	[191]
Adult	Locomotion	Mean velocity	[190]
Adult	Courtship	Observation of courtship postures	[101]
Adult	Mate choice	Video-stimulus technique	[192]
Adult	Olfaction	Response to amino acids	[193]
Adult	Alarm reaction	Response to alarm substance	[64]
Adult	Antipredation	Predator stimulation	[191]
Adult	Lateralization	Interaction with object	[194]
Adult	Audition	Response to startling noise	[195]
Adult	Vision	Optokinetic response	[196]

Table 5. Pharmacological treatments with known behavioral effects on adult zebrafish

Behavior	Modulating agent	Function / Activity	Effect	Reference
Reward	Cocaine	Psychostimulant	Rewarding	[121]
Reward	Food	Nourishment	Rewarding	[123]
Reward	Ethanol	GABA-A receptor modulator	Rewarding	[120]
Reward	Morphine	Opiate	Rewarding	[123]
Reward	Acetylcholine	Cholinergic agonist	Non-rewarding	[184]
Reward	Nicotine	NachR agonist	Rewarding	[120]
Reward	Amphetamine	Psychostimulant	Rewarding	[122]
Anxiety	Alarm substance	Hypoxanthine-3N-oxide	Increases anxiety	[69]
Anxiety	Pentylenetetrazole	GABA antagonist	Increases anxiety	[197]
Anxiety	Ethanol	GABA-A receptor modulator	Reduces anxiety	[69]
Anxiety	FG-7142	Benzodiazepine inv. agonist	Increases anxiety	[139]
Anxiety	Nicotine	NachR agonist	Reduces anxiety	[138]
Anxiety	Methyl lycaconitine	Nicotinic antagonist	Anxiolytic	[198]
Anxiety	Mecamylamine	Nicotinic antagonist	Anxiolytic	[138]
Anxiety	Buspirone Htr1	A partial agonist	Reduces anxiety	[198]
Anxiety	Dihydro-b-erythroidine	Nicotinic antagonist	Anxiolytic	[198]
Anxiety	Fluoxetine	5-HT reuptake inhibitor	Reduces anxiety	[69]
Anxiety	Morphine	Opiate	Reduces anxiety	[197]
Anxiety	Cocaine (withdrawal)	Psychostimulant	Increases anxiety	[139]
Anxiety	Diazepam	Benzodiazepine	Reduces anxiety	[139]
Anxiety	Caffeine	Xanthine alkaloid	Increases anxiety	[69]
Sleep	Dexmedetomidine alpha2	adrenoceptor agonist	Sedative	[199]

Sleep	Pentobarbital	Barbiturate	Hypnotic	[36]
Sleep	Diazepam	Benzodiazepine	Hypnotic	[36]
Aggression	Ethanol	GABA-A receptor modulator	Increases aggression	[181]
Aggression	17a-ethinylestradiol	Synthetic estrogen	Reduces aggression	[200]
Light/Dark preference	GABA	A receptor modulator	Decreased at high conc.	[72]
Learning	a FMH	HDAC inhibitor	Impairs long term memory	[201]
Learning	MK-801	NMDA antagonist	Impairs memory	[186]
Learning]	Nicotine	NachR agonist	Improves learning	[138]
Learning	L-NAME	NO synthase inhibitor	Impairs memory retention	[186]
Locomotion	Ethanol	GABA-A receptor modulator	Reduced at high conc.	[72]
Antipredation	Ethanol	GABA-A receptor modulator	Impaired by high doses	[72]
Group preference	Ethanol	GABA-A receptor modulator	Reduced at high conc.	[72]

TYPES OF MAZES USED IN ZEBRAFISH BEHAVIOR TESTING

All photos and their details are taken from the web page of **Conduct Science** ***(Zebrafish Mazes)***
https://conductscience.com

Different mazes are used in the following fields:

- Social Development and Behavioral Research
- Stress and Anxiety Research
- Learning and Memory Research
- Pharmacology and Toxicology Research
- Vision and Retina Research

Some of the tests below are similar to the mouse model, but zebrafish are inherently more demanding as they require an aquatic environment. This may seem obvious, but if working with fish is a new experience, it's easy to overlook simple things like water temperature or pH. Zebrafish are usually kept in tanks at 26 and 28.5 °C and pH values of 6.8 and 7.5 [202, 203]. The behavior of the aquarium should not deviate from these instructions because sudden temperature and pH changes can affect the fish. As with rats, some behavioral testing can be stressful for zebrafish, so it's important to know if (or not) your setup is stressful. Zebrafish in a new environment will often jump to the bottom of the aquarium in search of darkness or a hiding place, so it's important to pay attention to the size and depth of the aquarium and whether the setup requires some protection (such as a barrel enclosure) analysis is described below).

In conclusion, the tests listed below are designed to measure a subject's behavior. However, zebrafish are more commonly used to study social behavior because of their tendency to behave in groups. When shoals of fish swim together; It differs from training based on the structure of the player group. In studies involving different zebrafish species, it is important to understand that the behavior of the zebrafish flock will vary depending on many factors, including the fish present, the sex of the fish presenting it, and the new environment. [203]

GENERALLY, THE FOLLOWING TYPES OF MAZES ARE USED IN BEHAVIOURAL ASSAYS

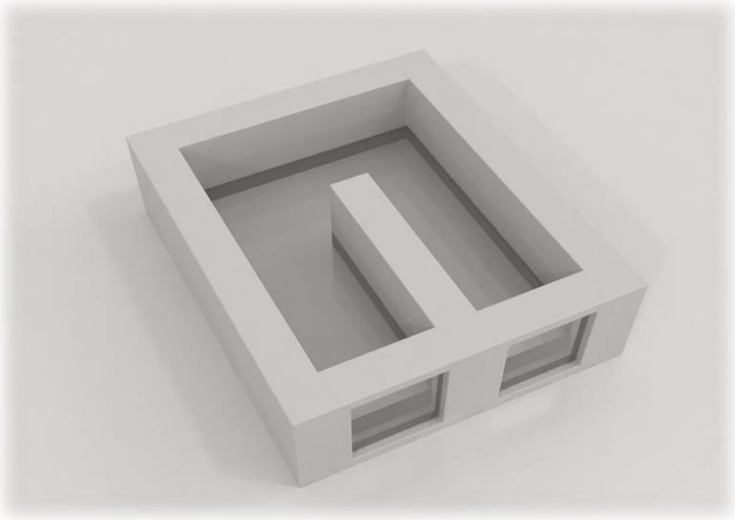

Figure 5*, The* ***Zebrafish 3 Chamber Social Behavior*** was used by Elena Dreosti *et al.* (2015), to assess the development of social behavior in young zebrafish. Experiments were performed in a custom-built behavioral setup that was assembled from structural framing and optomechanics. Fish were imaged in a custom-built behavioral arena that was fabricated with a laser-cut thick opaque white acrylic, sealed with silicone, with transparent and opaque dividers.

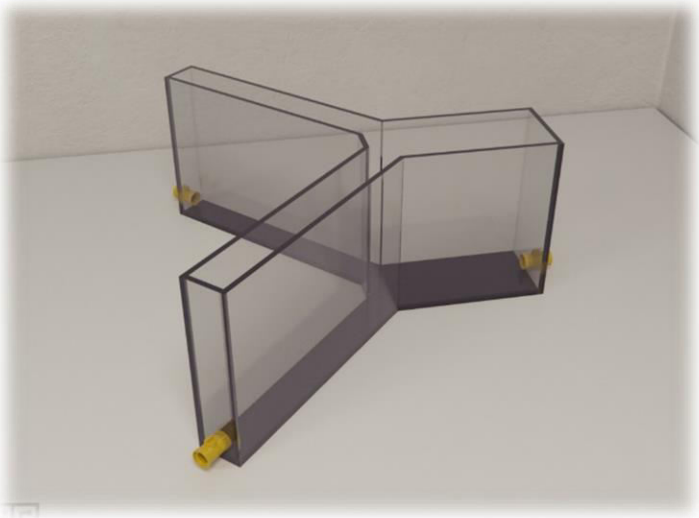

Figure 6**,** *The* ***Automated Avoidance Zebrafish Y-Maze*** is an operant conditioning assay used by Aoki, Tsuboi, and Okamoto (2015) to study avoidance behaviors in zebrafish when presented with an aversive stimulus. It consists of a Y-Maze with an LCD screen placed below the maze that displays visual cues throughout all regions of the maze. The Automated Avoidance Zebrafish Y-maze eliminates the need to control multiple stimuli, which reduces human error and also it is time-saving.

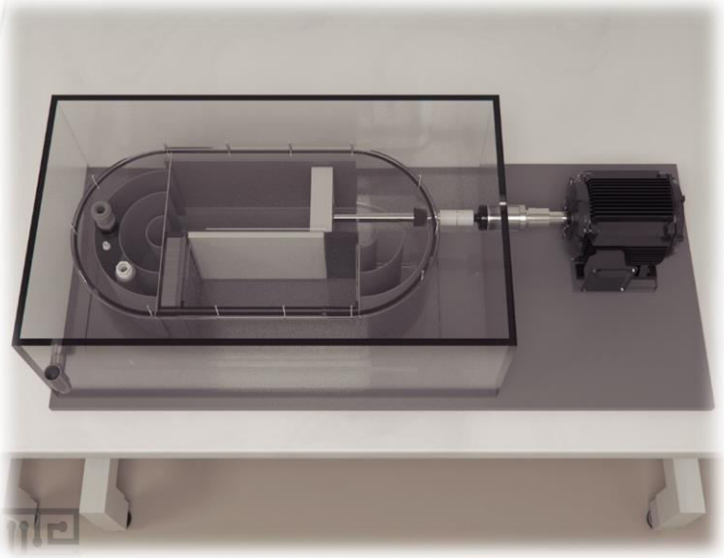

Figure 7. The ***Zebrafish Swim Tunnel*** consists of a temperature buffer tank, propeller, test section and an Ac motor. For the test, fish were placed in a swim tunnel with an adjustable flow that forced fish to swim to maintain their position. The test section was large enough to allow zebrafish to perform various swimming gaits. During the test, water temperature was maintained at acclimation temperature (28 °C). Zebrafish were tested under infrared light to reduce any visual disturbances that could affect swimming. An infrared camera and high-speed cameras were mounted above the swim tunnel to record the swimming tests. (Peinado et al., 2011, Ni & Li, 2010, Brandt & Vilcinskas, 2013)

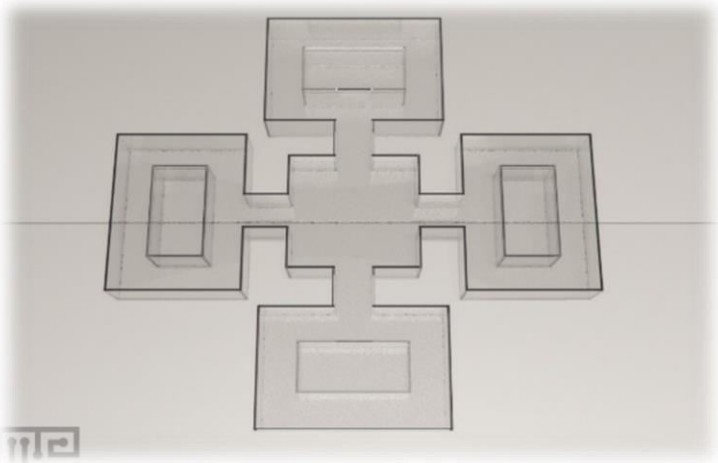

Figure 8, The ***Zebrafish Plus Maze*** is a "+" shaped maze that contains four end compartments and one central compartment. It is used to analyze associative learning behavior in zebrafish. which is used for rodents. The subjects are tested for their ability to associate the visual cue with the rewarding unconditioned stimuli while swimming in the maze. Sison & Gerlai (2011)

Figure 9. The ***Zebrafish Shuttle Box*** presents an automated method for high throughput learning paradigm testing. In this protocol, two screens are used from an automated computer screen to create computer animated zebrafish images, or any image in which the researcher specifies. This simple paradigm between binary choices makes this a useful high throughput assay for zebrafish. (Al-Imari, & Gerlai, 2008)

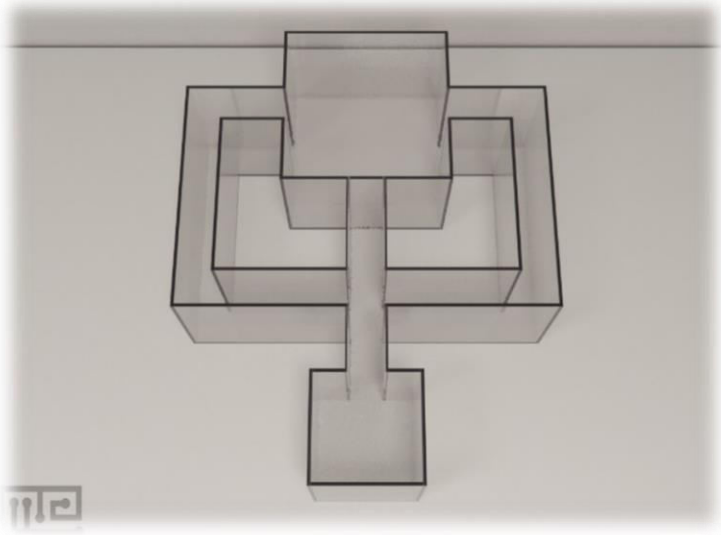

Figure 10. The ***Latent Learning Apparatus*** is used to analyze the learning and memory function in zebrafish. The apparatus contains a start box and a goal box connected via tunnels. The goal box is provided with stimuli to attract the subjects. The movement of the fish in and out of the goal box is controlled by modifiable guillotine doors. The walls of the maze are made of acrylic to ensure that subject can have clear visual access to the goal box from all locations. Jensen (2006)

Figure 11. The ***Light Dark Tank for Zebrafish*** is an acrylic tank (15 cm × 10 cm × 45 cm height × width × length) that is divided equally into one-half black and one-half white. Walls and bottom are either black or white, so as to create a similar experimental paradigm to the rodent light dark box.The tank contains central sliding doors, colored with the same color of the aquarium side, thereby defining an uncolored central compartment measuring 15 cm × 10 cm × 10 cm. Bourin, M., & Hascoet, M (2003)

Figure 12 The Zebrafish Sociability Chamber is used for studying the exploration and socialization behaviors in zebrafish. The concept of zebrafish sociability chamber derives from the 3-chamber sociability device used for socialization testing in rodents. The 5-chamber sociability tank analyzes the socialization behavior in fish by allowing them to interact with the social stimuli and explore the tank. Araujo-Silva, Pinheiro-da-Silva, Silva, and Luchiari (2018

Figure 13. The ***Black white preference tank*** assesses for wall color stimuli on diving, and the effects of depth stimuli on scototaxis. The Black White preference tank allows for three separate configurations. The split-depth tank configuration is composed of one side of the tank that is set to a depth of 10cm using a partition while the other side is set to a depth of 15cm.In the shallow configuration, both sides can be set to a depth of 5cm.In the deep configuration, both sides of the tank can be set to 15cm.Gravel substrate is placed on a floor 5 cm below the plexiglas partition on each side.The sides of the tank are either left uncovered (transparent), covered in black paper (black), covered in white paper (white), or covered in black on one side, and white on the other. Rachel E. Blaser, R.E., & Rosemberg, D.B. (2012)

Figure 14. The Mirror Biting Cattelan Tests are a popular method used in studies of agonistic interaction, especially in fish aggression studies as they require fewer participants and avoid pseudo-replication. Mirrors also provoke a strong, aggressive response in the subject without endangering them. Cattelan S, Lucon-Xiccato T, Pilastro A, Griggio M (2017).

Figure 15. Mirror Biting Pham tests are a popular method used in studies of agonistic interaction, especially in fish aggression studies as they require fewer participants and avoid pseudo-replication. Mirrors also provoke a strong, aggressive response in the subject without endangering them, Cachat J, Kyzar EJ, Collins C, Gaikwad S, Green J, Roth A, El-Ounsi M, Davis A, Pham M, Landsman S, Stewart AM, Kalueff AV (2013)

Figure 16. Zebrafish Y Maze Flow Modification: This Flow-Through Y-Maze is a modification of the Zebrafish Y-Maze. The flow-through Y-Maze apparatus consists of an aquatic tank shaped like a capital 'Y.' Two of the arms serve as the goal arms. The modification of the conventional aquatic Y Maze involves the addition of the pressure controlling flow meters at each intake valve of the goal arms to control and measure the rate of flow of the fluids into the arms. The Zebrafish Y-Maze is an adaptation of the rodent Y-Maze (also see T-Maze). The 'Y' design is preferred over the 'T' design due to its natural turns., Grella, S. L, Kapur, N., & Gerlai, R. (2010)

Figure 17. Zebrafish Environmental Enrichment Chamber: this is a widely used task to evaluate spatial and non-spatial learning as well as memory in zebrafish. The watertight apparatus is outlined by a dark panel; covering one side of each compartment as a visual cue to provide an axis of orientation for right/left discrimination. The apparatus is) primarily used for learning and memory but can also be used for various toxicity experiments. Bergendahl I.A, Salvanes A.G.V, Victoria A. Braithwaitec V.A (2015

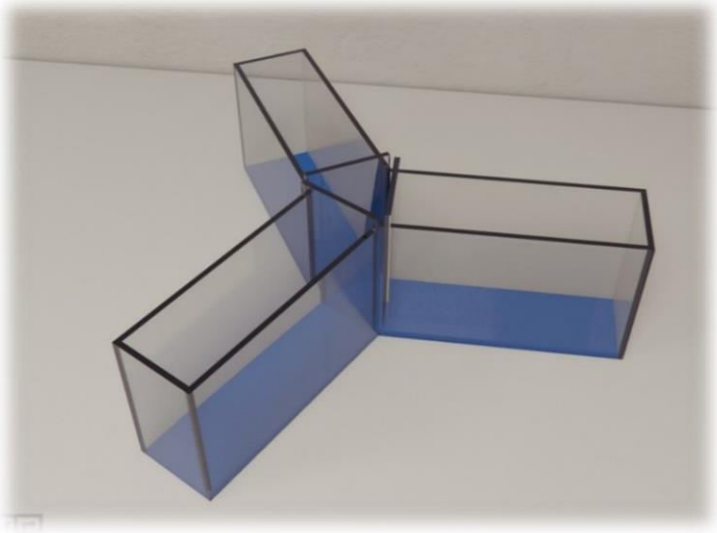

Figure 18. The Zebrafish Y Maze was originally validated in the literature by Cognato et al (2012). The Y-Maze memory task uses a simple and rapid training session for novelty exploration. Zebrafish spend more time in the novel arm than in the other arms of the Y Maze, both in response to novelty and spatial memory training-test intervals

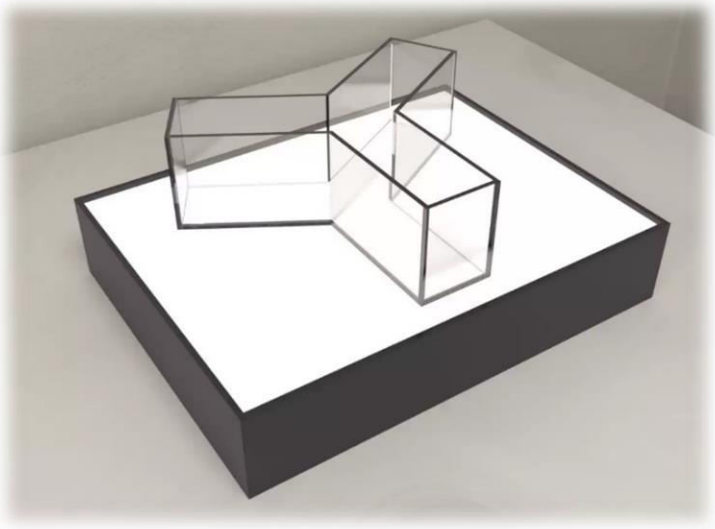

Figure 19. Zebrafish Y Maze Avoidance*:* The Zebrafish Y maze combines an LCD screen underneath the main Y maze apparatus as described by Aoki et al (2014).The original apparatus combined a new method by which zebrafish can be trained to avoid one arm of a Y-shaped tank by presenting a specific color on the floor paired with an electric shock.It includes the LCD screen and Zebrafish apparatus.

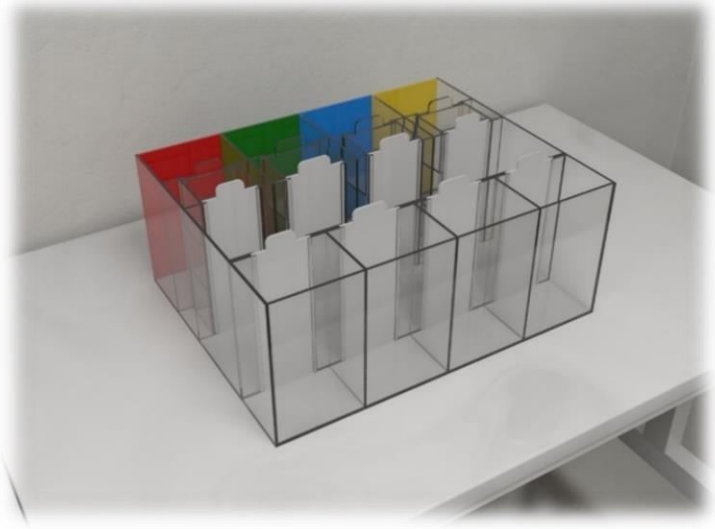

Figure 20. The ***Zebrafish Associative Learning Chamber*** is a simple test for evaluating visual discrimination with an associative learning task. The test allows for simple insertion of color cues for zebrafish to navigate between the Start chamber to the target chamber, and includes the entire package including insertable doors and color cues for experiment. Braida, D., Ponzoni, L., Martucci, R., Sparatore, F., Gotti, C., &Sala, M. (2014

Figure 21. The ***Place Preference Chamber*** is created to allow for maximum flexibility for your experiments. The chamber comes with 2 divider slots that allow the zebrafish to choose a chamber or retain the zebrafish in the center area. Floor is clear to allow for location preference. Darland, T. & Dowling, J.E. (2001)

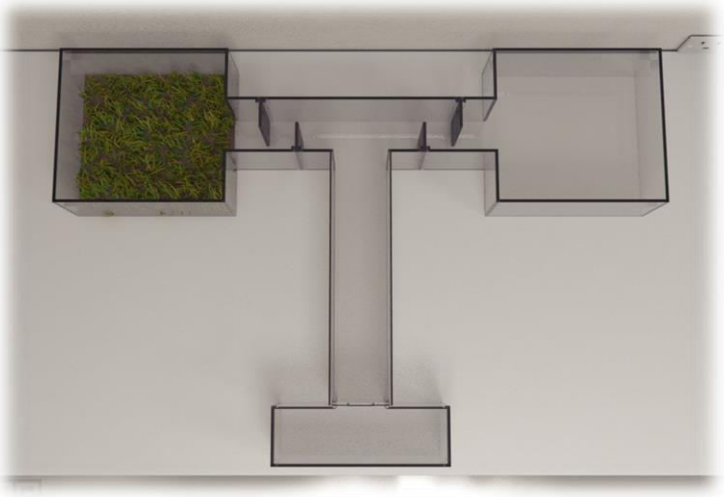

Figure 22. The ***Zebrafish Bifurcating T Maze*** has been described in the literature as a screening test for the role of nicotinic acetylcholine receptors in Zebrafish. It has been traditionally used as a screening maze, but can also be used for choice and learning experiments. Al-Imari, L., & Gerlai, R. (2008) be provided by the user. (Cognato, G.,Bortolotto, J., Blazina, A., Christoff, R., Lara, D., Vianna, M., and Bonan, C., (2012)

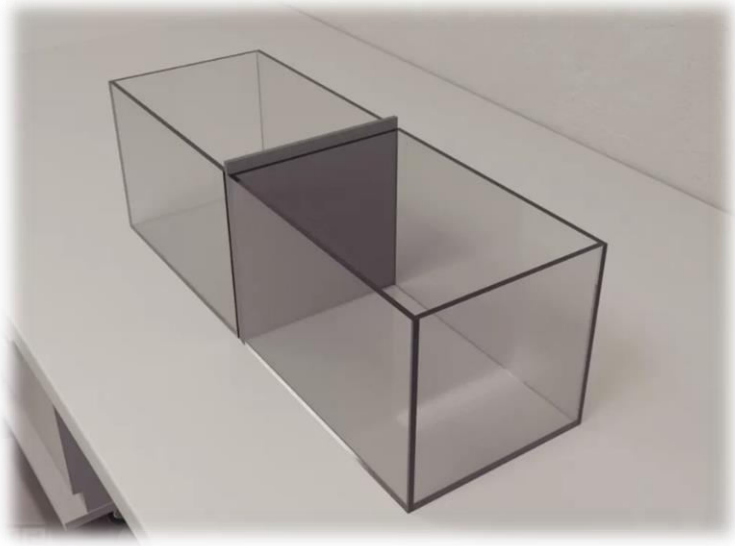

Figure:23 Mirror Biting Elwood tests are a popular method used in studies of agonistic interaction, especially in fish aggression studies as they require fewer participants and avoid pseudo-replication. Mirrors also provoke a strong, aggressive response in the subject without endangering them. Robert W. Elwood, Velizara Stoilova, Amy McDonnell, Ryan L. Earley, Gareth Arnott. (2014)

Figure 24. The ***Vertical Tank Array*** is used for anxiety experiments for Zebrafish. Vertical diving behavior is used to assess anxiety in zebrafish. Narrow tanks minimize horizontal movement to maximize the behavior assessment. Each tank holds 1.5L in a trapezoid pattern. Set of 6 arrays come together with a housing apparatus for easy video grading. Cachat, J.M. et al. and Kalueff, A.V. & Cachat, 2010).

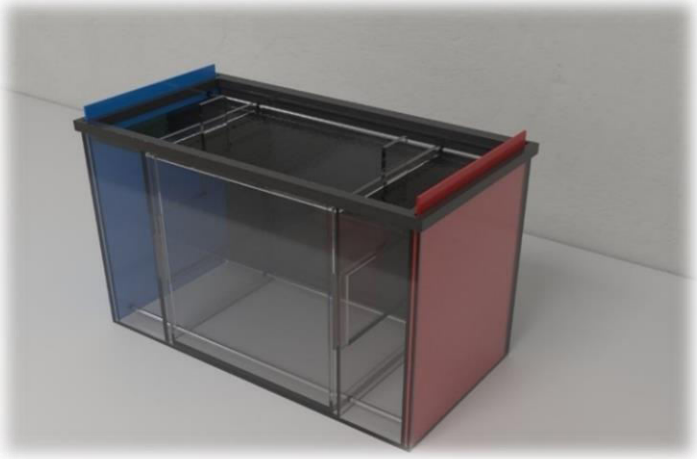

Figure 25. The ***Three-Chamber Paradigm*** is a widely used task to evaluate spatial and non-spatial learning as well as memory in zebrafish. The watertight apparatus from is outlined by a dark panel; covering one side of each compartment as a visual cue to provide an axis of orientation for right/left discrimination. The apparatus is primarily used for learning and memory but can also be used for various toxicity experiments. Arthur, E.D. Levin (2001)

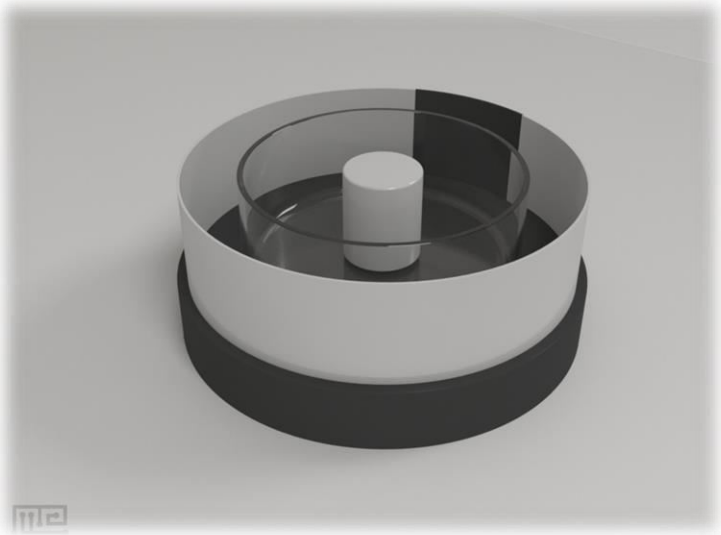

Figure 26. The ***Zebrafish Rotation Test Apparatus*** is a circular container with transparent walls surrounded by a rotating acrylic drum. This drum can be tagged with cues for zebrafish retinal degeneration experiments. Typically, a black segment is marked on the acrylic. A central post is placed to prevent the zebrafish from swimming across the midline of the innter chamber. Optional add ons include:●Backlight underneath the apparatus●Multi colored outer chamber. Lei Li, Dowling JE. (1997)

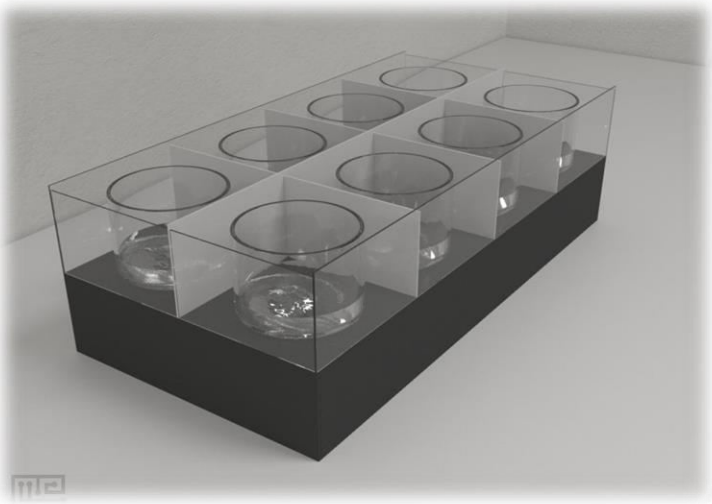

Figure 27. The ***Tap-Elicited Swim Test*** is used in free swimming zebrafish to evaluate the effects of EtOH, drugs and toxins on the learning process (non associative). Frequently measured behaviors include the "C-start" response, which increases in latency in early alcohol exposure. The Tap Swim apparatus comes with 8 easy to use arrays that attaches to an automated tap array underneath. This array is controlled with the Conductor software (free of charge) to control interval taps and timing between interval taps. Bailey JM, Oliveri AN, Levin ED (2015)

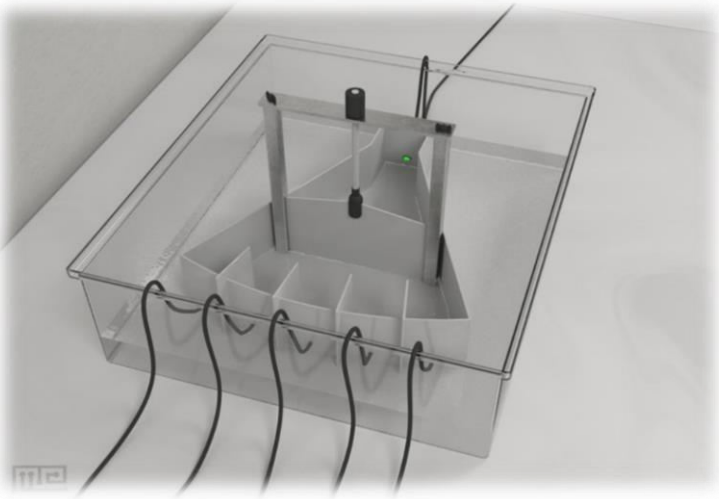

Figure 28. The ***Zebrafish Choice Chamber*** allows for experiments similar to the commonly used five-choice serial reaction time task (5-CSRTT). During testing, lights are illuminated and the gate is raised. A food delivery apparatus delivers rewards at a fixed time schedule. The proper light is illuminated in the interval or until the correct entry is chosen. The number of trials, accuracy, omissions, and latency are usually recorded. The choice chamber includes usually recorded. Parker MO, Millington ME, Combe FJ, Brennan CH (2012).

Figure 29. Mirror Biting Balzarini: Mirror tests are a popular method used in studies of agonistic interaction, especially in fish aggression studies as they require fewer participants and avoid pseudo-replication. Mirrors also provoke a strong, aggressive response in the subject without endangering them. Valentina Balzarini, Michael Taborsky, Sandro Wanner, Felizia Koch, Joachim G. Frommen. (2014)

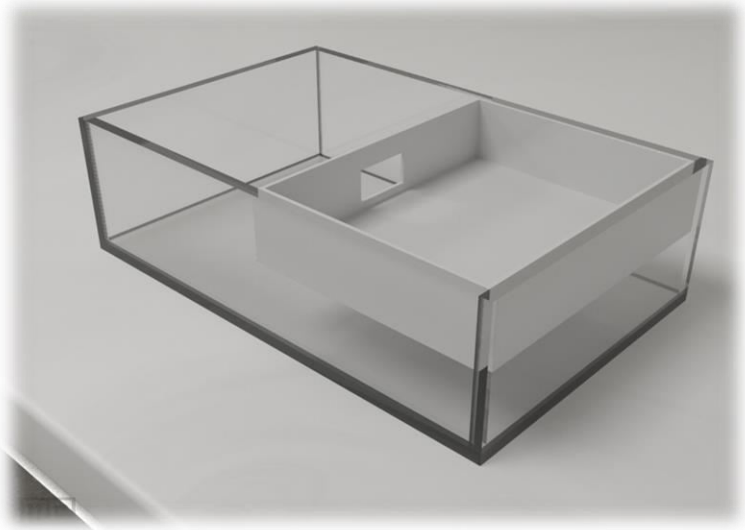

Figure 30. The ***Bite Test*** comes with a plastic apparatus that can be placed inside of the home tank, or a built-in apparatus that is continuous with the home cage. λThe Bite test apparatus (15×12×3.5 cm) is placed inside the home tank, so that it was filled with water to a depth of 3 cm It is connected to the main part of the tank by a small opening (3 cm wide) through which the fish can enter the box λThe bite apparatus can be removed for easy cleaning. [Miklosi A, Andrew RJ.]

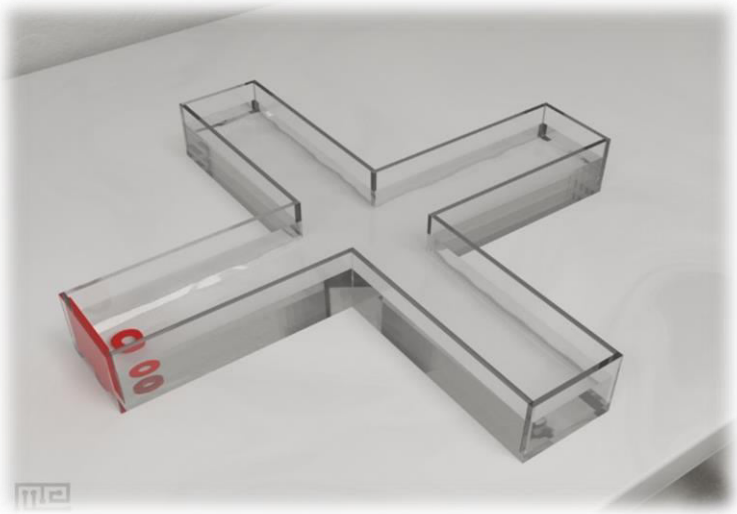

Figure 31. The Zebrafish T Maze allows for choice experiments. Arms are baited with colors or cues and zebrafish are allowed to make choices in experiments. Enrichment boxes can be added to allow for stimuli to drive these choices. Multiple sizing and shapes available. Drain provided upon request alacarte and can be lifted from the ground or table to allow for easy drainage. (Avdesh A, MartinIverson MT, Mondal A, Chen M, Askraba S, Morgan N, Lardelli M, Groth DM, Verdile G, Martins RN (2012)

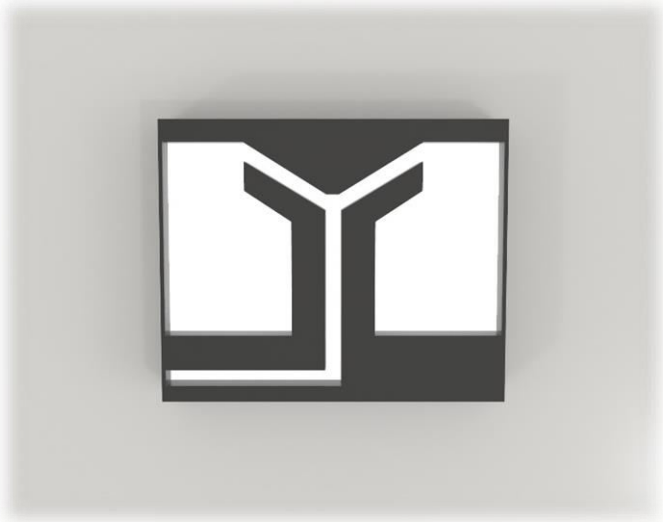

Figure 32. The ***Zebrafish larvae Y Maze*** takes advantage of a unique backlighting set-up to allow for fine behavior task assessment in larvae. Similar to the Zebrafish Larvae T apparatus and Drosophila mazes, this apparatus comes with a start lane, bidirectional swimming pools, and a unique backlight for easy video tracking. An easy-to-use cover seals the pools and watertight chambers ensures that you'll be able to use this apparatus for years to come. The apparatus comes with: Lid, Chambers, Backlight (Best, J. D., Berghmans, S., Hunt, J. J., Clarke, S. C., Fleming, A., Goldsmith, P. and Roach, A. G. (2008)

ZEBRAFISH AUTOMATIC MONITORING SYSTEM

Behavioral analysis with computer analysis in zebrafish research has advantages such as high productivity, efficiency, and the ability to generate detailed and precise data [204]. These technologies help us explore and improve our understanding of many biological processes, diseases and drug responses. Computer vision can monitor and analyze zebrafish behavior in real time. Software algorithms can detect and evaluate certain behaviors such as motion, shallowing, tapping (such as tank edges), and search patterns. A computer program for the analysis of zebrafish behavior is versatile. The main components often found in these systems are: [204]

Setup: This consists of one or more cameras located at the top of the zebrafish tank to capture the behavior of the fish. The camera must be able to record high quality video at a suitable frame for proper monitoring and analysis. (*See Figure 31)*

Image and recording software: Software required to control the camera, capture video and store it in a suitable format for subsequent analysis. The software should provide options for adjusting camera settings such as exposure, focus, and frame rate.

Tracking software: Use special tracking software to make video clips and monitor zebrafish movements. The software uses algorithms that identify and track fish in the video frame, thus eliminating various behaviors.

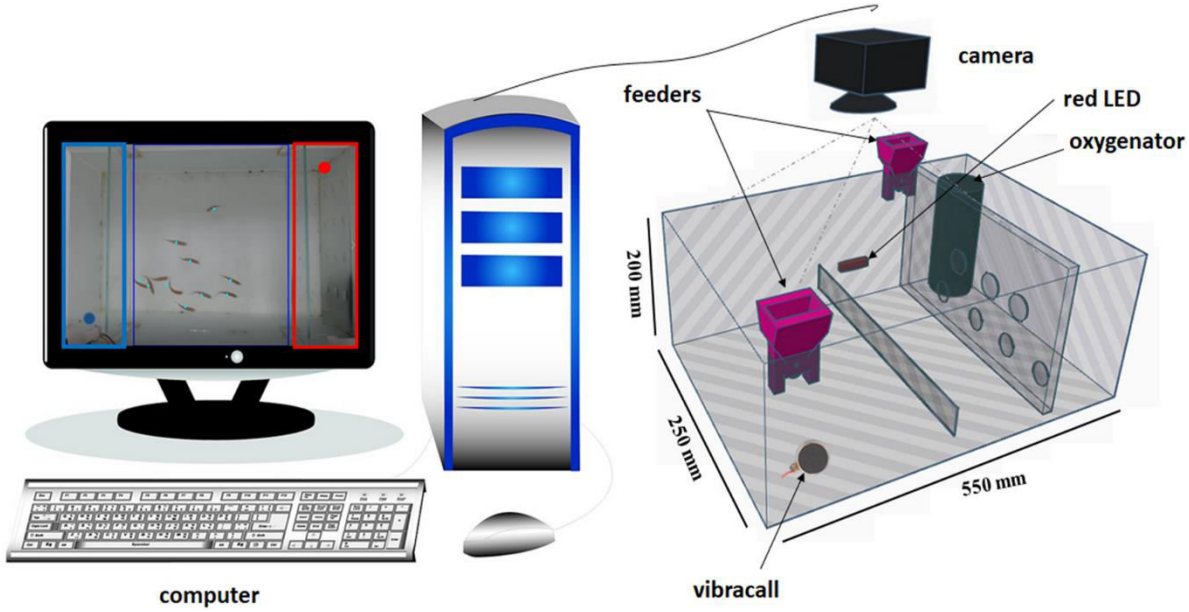

Figure 31. Experimental configuration to measure zebrafish learning. [204]The group of zebrafish swims in a sandblasted glass tank, divided into two feeding arenas. A red LED strip is attached to the external area on the right side of the tank as a light stimulus. And a vibracall was attached to the external area on the left side of the tank. A camera is mounted above the tank, approximately 40 cm. [204]

Behavior Analysis algorithms: These algorithms are an essential part of monitoring software and are responsible for identifying behavioral differences. Examples of behavior that can be analyzed include swimming speed, travel distance, angle of rotation, time spent in different areas of the aquarium, and correct interaction.

Data visualization and analysis tools: After the behavior data has been extracted, special software or programming libraries can be used to visualize and analyze the data. These tools allow researchers to interpret and compare results, perform statistical analysis, and create research visualizations.

Experiment design and management software: Sometimes computer systems are included with software to help design and manage experiments. The software allows researchers to set up specific experiments, control stimuli or environmental variables, and automate the data collection process.

Data Storage and Management: It is important to have a system to store and manage the large amount of data generated by behavioral analysis. This may include data storage, data storage or cloud solutions to secure data storage and provide easy access for future use. Working together, these components automatically monitor and analyze zebrafish behavior, providing researchers with a wealth of data that can be used to study many aspects of zebrafish biology, neurobiology, drug interactions, and insect pain pattern.

ISSUES WITH BEHAVIORAL ENDPOINTS

- A Due to the rapid development of zebrafish larvae, tests need to be carried out when the growth rate, that is, the necessary physical and functional conditions are met. [205] Some characters only appear in certain stages. Certain behaviors are only observed at certain stages [206] found that the time of day had an effect on the locomotor activity of zebrafish larvae.

- A major factor affecting the behavior of zebrafish larvae is the acclimatization time before testing. In the visual-motor response test, the acclimatization time and lighting conditions prior to the test will have a significant impact on the results. [206, 207]

- A recent study [208] found that large spatial sizes (24-well plates compared to 48- or 96-well plates) resulted in improved locomotor performance of experimental zebrafish larvae for play. Finally, when examining the locomotor activity of zebrafish, developmental disorders that may affect their power should be considered. [208]

- The rate at which zebrafish embryos are reared can also affect their behavior. [207] The study found that larvae in groups were more active in the dark than larvae living alone. Ambient light in the laboratory is important for zebrafish larvae, which can reduce or increase their locomotor activity depending on the intensity of the light. [208].

- Changes in responses/properties/indicators increase with increasing tissue levels, thus being the most variable behavior in tissue response. In addition, fish, like other animals, have states that affect consciousness and motivation and do not have a personality. [209]They are stronger in the morning than in the afternoon. Zebrafish larvae also exhibited circadian rhythmic locomotor activity at 5 dpf; locomotor activity was higher during the day than at night [210]

- The temperature of the cultured and tested embryos is another factor that can alter the behavior of the virus and the development of zebrafish larvae. [211, 171]. Typically, researchers use a water temperature of 28°C. The containing vessel used for experiments varies widely between labs.

- Urban water is often rich in neurotoxic pollutants, their concentrations can change even within a short period of time. The water should be carbon filtered and tested to eliminate/calculate the effects of chemical mixing. [209]

- Individual differences can be explained by categorizing behavioral phenotypes such as low- or high-functioning groups. Doing so will increase the power of the experiment; however, this may not be true for all aspects of behavior. For example, swimming in juvenile trout is not associated with true swimming (coordination). [212]
- Sometime issues are available; that is, regular food consumption leads to food preferences and a willingness to take risks. [213, 212].

- Fish dominance can be difficult to define if social context is used. Social status can also affect stress levels, which in turn affect activity and response to stimuli. [212].

TECHNICAL CHALLENGES AND FUTURE PROSPECTS

Microfluidic assays: Many of the issues discussed above can be addressed by growing zebrafish embryos in microfluidic systems. [214, 215] These can provide low volumes of continuous flow (a system in our lab has a static volume of 8 µl per well), eliminating the problem of evaporation as the microfluidic chip can be sealed. The obvious disadvantage of these systems is the miniaturization of the medium, which limits the ability to record distance measurements such as tactility. Therefore, parameters such as inhibitory responses that are not dependent on large pools of water may be more appropriate for screening stress and anxiolytic drugs in small biochips

Limitations of microtitre plates: Current testing on zebrafish embryos/larvae is usually done in small tanks, petri dishes or microtiter plates [216] Problems with these tests include: combining multiple embryos in one well; [216] absence of evaporation affecting the sides and corners of the water well and which could affect the image on video search equipment; and poor quality and high quality equipment. [217].

Automation and high-throughput screening: A recent study [218] presented an automated system for high-throughput screening that uses two different assays to identify cardiotoxic agents and angiogenesis inhibitors. They validated both studies with known positive and negative compounds and screened for unknown anxiolytic compounds [219, 220] used Zebra Lab (Viewpoint, France, available online at http://viewpoint.fr/zerbalab) to screen 4000 small molecules for their effects on chronic sleep/wake behavior using zebrafish larvae. [220] About 14,000 neuroactive small molecules were tested to find changes in the larva's response to high light. Both studies used the behavior of zebrafish larvae using automated video tracking technology in microtiter plates. Another scanning platform is under development that could open up new opportunities for high-quality scanning. [221]

CONCLUSIONS

Behavioral responses, such as changes in movement and locomotion in zebrafish larvae, are driven by neural processes associated with visual-motor activity and perception, cognition, and decision-making, and can be reduced or enhanced by different and different methods. Advances in stimulus delivery, behavior monitoring, and measuring and controlling brain activity have allowed for greater control. The behavioral gene library can be used to screen and discover neurological drug candidates in preclinical development and can be used for clinical translation in a variety of neurological diseases. By modeling between names (e.g., drug withdrawal/anxiety), new diseases (such as autism spectrum disorder (ASD), schizophrenia) and new methods (such as brain genes, central and cytokines) may lead to the use of larval and adult zebrafish as well as related procedures. Creating new interpretation models of drug responses, adding new behavioral data, and using new analysis methods such as automated video to increase the utility of zebrafish in neurobehavioral research. Finally, aligning zebrafish physiology with behavioral data using biomolecular markers such as gene expression or endocrine measures has become another important area of research**.**

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Conflicts of Interest: The authors declare no conflict of interest.

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