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Review

Deciphering Anxiety with Zebrafish: A Versatile Model for Anxiolytic Studies

Neeru Singh*, Lakhan Singh, Kulsoom Hamid, Vikrant Singh

Faculty of Pharmacy, Swami Vivekanand Subharti University, Meerut

Corresponding Author:
Dr. Neeru Singh

Email:
neeru12345a@gmail.com

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Abstract:

Background - Studies on behavioral pharmacology are increasingly using zebrafish as model organisms. Numerous anxiety-related behaviors in zebrafish have been documented, yet little is known about how anxiolytic drugs impact these behaviors. Anxiety is currently one of the primary unmet medical needs. Despite the wide variety of anxiolytic drugs available, many patients either do not respond well to current pharmacotherapy or see a lessening of their reactivity with repeated treatment. Search for novel compounds and learn how anxiolytic drugs function. **Main body of the abstract** - In the first task, we concurrently looked at the adult zebrafish's motility, color, height in the tank, and cohesiveness of the shoal. We examine the effects of buspirone hydrochloride, ethanol, benzodiazepines, and a common anxiolytic drug used in medical facilities for humans. Anxiolysis's symptoms were not brought on by anxiolytic drugs, which work by agonisting GABA receptors. We search for anxiolytic drugs in two genetically distinct populations of zebrafish, and the results show that the light/dark preference test is a sensitive, practical, and cost-effective technique. Two important behavioral characteristics seem to be shoal cohesion and tank height among the various groups of these treatments. **Conclusion** - The findings show that measuring the effects of human anxiolytic medications may be done simply and sensitively using zebrafish behavior.

Keywords –zebrafish, anxiety, shoal cohesion

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BACKGROUND

Zebrafish are being used more and more to investigate the molecular mechanisms underlying neurobiology, with particular applications in neurotoxicity and neuropharmacology. Zebrafish anxiety and stress reactions are also starting to be studied. Zebrafish are useful for studying the brain mechanisms behind a wide range of behavioral patterns. to ascertain the parallels and discrepancies between the neurological underpinnings of zebrafish stress response and anxiety. [1] *Taxonomy of Zebrafish* Species D. Rierio Actinopterygii is the class Phylum: Chordata; Order: Cypriniformes; Family: Cyprinidae Kingdom: Animalia; Genus: Danio [12]. Zebrafish are an excellent model organism due to its many intrinsic benefits, which include low cost, ease of handling, and minimal maintenance. Despite zebrafish's growing appeal in the field of neuroscience. Such a behavioral screening technique could advance our knowledge of how drugs work and how the nervous system functions. However, for better utilization of zebrafish in brain function studies, more specialized behavioral task characterization is needed. In zebrafish, a number of measures of anxiety and fear have been proposed. [2] Some characteristics of the zebrafish have persisted during evolution; for example, the genes of the fish and mammals,

including human orthologs, have a high level of nucleotide sequence similarity (average of about 70%). By submerging the fish in the medication solution for the appropriate amount of time and at regulated concentrations, the medications may be given to them successfully. The increasing number of behavioral test paradigms created specifically for this species allows for the effective detection of the effects of medications and mutations.[3] Zebrafish have especially well-characterized anxiety and fear reactions. Some contend that in mammals, anxiety and fear are separate phenomena with possibly unique underlying processes. Buspirone hydrochloride is used as an anxiolytic drug in patients with general anxiety disorder, or GAD.[3] In addition to benzodiazepines like clorazepate, alprazolam, diazepam, and lorazepam, it has been demonstrated to be helpful for GAD.. [3] Buspirone acts on both the pre- and post-synaptic terminals of the 5HT1A receptor in animals with a high degree of affinity.. In this work, we will examine the effects of buspirone hydrochloride in two genetically diverse populations of juvenile zebrafish (10–13 days post-fertilization). This drug's effects on young zebrafish have not been studied. Utilizing zebrafish at this early stage of development has the benefit of saving space because the fish are tiny (approximately 7 mm long) and can be easily screened on large-scale high throughput screens utilizing conventional multi-well plates. [3[Zebrafish have been the subject of a considerable rise in articles, suggesting that they are now a widely used model organism in preclinical research. [4] It has been demonstrated that the zebrafish genome has 25 chromosomes and genes that are quite comparable to those of humans. Moreover, it has been demonstrated that the zebrafish genome has 25 chromosomes with genes that are quite comparable to human genes, and that Danio rerio has counterparts for around 70% of human genes. [4] In addition, it has been demonstrated that the zebrafish genome includes 25 chromosomes and shares many genes with the human genome. Danio reriot has been proven to have counterparts for over 70% of human genes. Moreover, research has revealed that the genes of Danio rerio are almost 70% similar to those of humans. [4] Zebrafish are an excellent study model due to their tiny size, transparent bodies, and inexpensive maintenance and reproduction. [4] Because they naturally reside in groups (shoals), zebrafish offer a wide variety of social interactions due to their well-defined and established behavior. Complicated social behaviors, such as schooling, shoaling, or geographic preference for conspecifics. [4] Two medication classes that are frequently used to treat anxiety-related illnesses are benzodiazepines and antidepressants. [5] The suggested medications include serotonin norepinephrine reuptake inhibitors (SNRIs) and selective serotonin reuptake inhibitors (SSRIs) because of their excellent benefit/risk ratio. However, utilizing them results in sexual dysfunction and a delayed anxiolytic effect. [5] Over the past 10 years, it has been clear that the zebrafsh, a cheap marine vertebrate species with a lot of genetic and physiological similarities to humans, is a useful model for a number of CNS problems in people. Furthermore, the completely sequenced zebrafsh genome has orthologues for about 82% of the genes connected to human illnesses. [5] . According to a recent study, the zebrafsh has been effectively used in a number of investigations to find natural remedies that could have anxiolytic benefits. [5] Zebrafish, or Danio rerio, have long been a useful tool for developmental biologists. But recently, it has gained popularity as a drug discovery model. [6] Zebrafish are cheap to keep and have a quick growth time, with practically all of their organs developed by 7 days after conception. Their fecundity makes it simple to collect large numbers of animals for testing. [6] They have the ability to absorb compounds from the water in their tanks, and genetic manipulation is now feasible because to the almost complete sequencing of their genome. Because of these characteristics, zebrafish are increasingly being utilized in medicinal chemistry to investigate the impact of different compounds on whole animals .[6] Zebrafish are a useful model animal to study because they exhibit a variety of social traits that make them an effective tool in behavioral pharmacology. [6] Associative reactions in zebrafish (Danio rerio) are valuable for behavioral genetics, pharmacological and toxicological screening, and the identification of brain processes underlying behavioral modulation. [7] Zebrafish are quickly becoming one of the most potent, high-throughput vertebrate models available for CNS medication development.[8]

Cholinergic signaling, which is partially mediated by muscarinic receptors, has the ability to lessen zebrafish anxiety.Zebrafish are quickly becoming a viable model organism for the research of stress- and anxiety-related disorders [8–9]. Among a range of behavioral tests and models, the novel tank test (NTT) and the light-dark test (LDT) are the two most often utilized experimental paradigms for researching zebrafish anxiety. [9] The Danio rerio, often known as zebrafish, has garnered attention as a promising animal model for a range of human brain disorders. [10] Zebrafish have been utilized for mutational screening of genes related to the visual system and have lately gained popularity as a model for vertebrate vision studies. As of right now, [11] zebrafish are considered to be a suitable model for researching behavior, physiology, immunology, genetics, and nutrition. [12] Zebrafish are thought to be an excellent model since many of its major organs, including as the kidney, pancreas, adipose tissue, and skeletal muscles, are quite comparable to those of humans. [12] Mice are more expensive to produce each year than zebrafish, but zebrafish are less expensive to employ. [12] Zebrafish were found while looking for fresh experimental models to lessen, enhance, and eventually replace the usage of animal models.Functional neuroscience is frequently used to establish these brain-behavior relationships by correlating neuronal activity with the production of a behavior [12]. Once a causal brain-behavior link has been established, these relationships are verified by altering neuronal signaling in this route and monitoring any impacts on the behavior of interest. [13] zebrafish serotonergic system, after which serotonin's function in zebrafish stress, aggressiveness, fear/anxiety, and the impact of psychedelic substances is examined. [14] The primary methodological challenge in determining if these alterations can cause zebrafish to exhibit depressive-like behavior is the paucity of behavioral research with sufficient construct validity. [14] Zebrafish contain two copies of different genes in their serotonergic system; This duality hasn't been investigated yet by using methods such as knockdowns to understand the role of each phenocopy (or "ohnologue") in the control of behavioral patterns. [14] The telencephalon and diencephalon make up the zebrafish forebrain, with the former being primarily in charge of higher brain processes, particularly those manifested through dopaminergic transmission .[15] The usage of zebrafish has not been the subject of many research at the very beginning of development to evaluate the long-term consequences of early exposure to this pro-convulsant drug and to define the behavioral aspects of exposure to kainic acid. [15]

MAIN TEXT

Experimental Design

Zebrafish (Danio rerio) were procured from Triangle Tropical Fish (Durham, NC) and housed in an automated flow-through continuously filtered water system by Aquatic Habitats at a temperature of around 28.5°C on a 12:12-h light/dark cycle [1].391 mature male and female Danio rerio zebrafish, often known as "wild type" fish, were acquired from a commercial provider (Red Fish, Porto Alegre, Brazil). The fish were kept in groups of 30 to 50 in a 50-liter thermostat tank (28± 2 °C) Using treated water with Tetra's AquaSafe® (pH 7.2, conductivity 501 µS, filtered with Tetra Whisper® PF10), and continuously aerated (7.2 mg O2/L) after they had spent at least two weeks acclimating to the laboratory environment. Fish were housed on a 14–10 h day/night cycle, fed commercial flakes three times a day, and given live brine shrimp as a supplement [2]. We used a 4 × 2 between subject experimental design where the genotype had 2 levels (WT or ABSK population origin) and the buspirone concentration had 4 levels (control, 5 mg/L, 20 mg/L, or 80 mg/L). Buspirone concentrations were determined based on previous zebrafish research as well as our own pilot investigations, which demonstrated no adverse effects, death, or morbidity even at the highest dose selected. Buspirone hydrochloride tablets were used in the laboratory to manufacture the medication solutions. Whole buspirone tablets were homogenized in system water after being crushed to a known drug quantity per tablet.. After that, any solid vehicle or binding agent utilized in the tablet was removed from the medication solution by filtering it through coffee paper. [3] Zebrafish, or Danio rerio, are tiny, translucent freshwater fish with an average length of 40 mm. Their body is transparent, with black lines running parallel to it [4]. Their eyes are positioned in the center of their fusiform, laterally flattened body. The dorsal fin of zebrafish features a

navy blue region, whereas its caudal fin is striped. Nowadays, while studying the development of vertebrates, zebrafish are frequently used as model organisms in genetic and embryonic studies. [4] Zebrafish have a quick development period, with nearly all of their organs formed by 7 days after conception. They are also inexpensive to keep and their fecundity makes it simple to get large numbers of fish for experiments. [6] The purpose of this paradigm is to determine how a single experimental fish reacts to its mirror counterpart. On the left side of the tank is a mirror that is angled at a distance of 22.5º from the rear wall. As a result, the fish's mirror image appears closer to the left side of the tank as it swims there [6] After a 30-second habituation period and a 10-minute habituation period, the experiment is video filmed for 60 seconds each time.[6] The tank is split into four equal halves. Accessing the left side suggests avoiding the "opponent," while accessing the right side suggests choosing to be close to them. [6] Another metric and analysis used to create an aggressive index is the length of time the fish spends engaging in aggressive display or attack activity. The act of a fish raising its anal, caudal, pectoral, and dorsal fins is also considered an aggressive show of body position. Biting and opening the lips while swimming quickly in the direction of the adversary is a hallmark of attack behavior [6] Furthermore, studies have employed startle response and pigment response to try to comprehend aggressive behavior in zebrafish. [6] In biomedical research, zebrafish (Danio rerio) have gained popularity as experimental animals. When one thinks of animals utilized in medical research, these animal models most likely aren't the first that spring to mind. One of the main reasons scientists from all over the globe are getting more and more interested in this little tropical freshwater fish is because the embryos of this species may develop outside the mother's body and are transparent. [12]

Behavioural Test

Drug effects were behaviorally tested between 8:00 a.m. and 5:00 p.m. during the light phase. To avoid mixing medication treatment with breeding or holding circumstances, fish were randomly assigned to treatment groups and vehicle-treated controls in each research. De-ionized water and sea salts (Instant Ocean, 1.2 g/20 liters of H2O) were combined to create the tank water. Constant filtration and aeration were used to sustain the groups of adult fish in the tanks. To assess the influence of home tank size on the innovative tank diving behavioral test, the fish housing tanks in experiment 1 were 1.5 liters and 3 liters in size. Every 1.5-liter tank of the same capacity was utilized for the diving test. Each concentration and population had twenty fish analyzed, for a total of 160 juvenile zebrafish analyzed. [1]. Fish from each of the eight groups were tested at roughly the same time on average because all fish were tested only once and in a way that was randomized across genotype and concentration [3]. Acute exposure to dissolved medications or solvents at one or more concentrations was followed by testing of zebrafish behavior in unfamiliar surroundings. [7].

Behavioral tests used to detect zebrafish behavior that resembles sadness or anxiety.

1. ***Novel tank test (NTT)***: comparable to the rodent OFT. When zebrafish are housed in a tank with two equal horizontal sections, the following parameters are measured: the length of time the fish spend in each section, how often they enter the higher section, 1 the time it takes them to do so, the freezing time, the irregular movements, the immobility, the swimming velocity and the length of distance covered. In addition, zebrafish exhibit variations in their NTT behavior according on strain, sex, age, and housing circumstances [4]. Fish that have been exposed to ethylene, for instance, exhibit modest excessive movement in the water in their home tanks but more anxiety-like behavior with unchanged locomotion in freshly replenished water [9]. Fish behavioral responses in this paradigm are also modulated by the size and form of the apparatus; for example, zebrafish kept in a narrow tank that is the same as the NTT throughout testing exhibit no NTT-specific characteristics, like a lack of diving reaction or variations in swimming speed. It is still unknown what factors primarily regulate zebrafish NTT diving reflexes. [9]
2. ***Open field test (OFT)***: comparable to the rodent OFT. The characteristics of thigmotaxis (peripheral swimming) and exploratory behavior are assessed in zebrafish kept in an innovative tank. [4] Light/dark test: This examination is similar to the rodent version of the same name. By measuring the amount of duration of time spent in the tank's white half, the

quantity of entries formed into the white half, as well as the delay in doing so, the test assesses scototaxis, or the inclination toward dim environments. [4]

3. Zebrafish tail immobilization examination (ZTI): Comparable toward the rodent tests for the despair-like phenotype, such as the test of forced swimming and tail suspension. The quantity of activity episodes, total activity, total mobility, & duration of stay moving about are the most crucial factors. [4] Shoaling test: This is comparable to the rodent social behavior test. The exam gauges the impact of anxiety on interpersonal relationships. The height of exploration and the separation between individuals are the two most crucial factors. [4]

4. The examination of social preferences is comparable to the rodent social behavior test. This test looks at how Danio rerio behaves with their conspecifics. The amount of time that the fish under observation spend close to the social stimulus is quantified. [4]

5. ***Novel object approaching -*** comparable to the rodent novelty exposure challenge. The following characteristics are examined in this test: thigmotaxis, the quantity and length of freezing episodes, the patient's latency to approach new objects, the amount of time they spend near new objects, and the placement of novel objects in tanks they are familiar with. [4]

6. ***Predator avoidance*** - Rats' avoidance of predators is somewhat comparable. In this experiment, a zebrafish's natural predator is placed in a tank that is well-known to the animal, and the following fear and avoidance behaviors are monitored: distance, geotaxis (drooping toward the bottom of the tank), activity (i.e., average speed), turn angle, quantity of freezing episodes, and duration of freezing for the predator and the tested subject. [4]

7. ***Light dark Test*** - A rectangular glass or acrylic tank with two equal vertical sections—black and white or black and clear chambers—is a classic LDT equipment. Animals tend to prefer the transparent compartment over the black one, therefore the color of the lighter half (white vs. transparent) might affect their choice [9]. Zebrafish exposed to the persistently erratic tension protocol over time showed a decrease in both the quantity of admissions into the light compartment and the amount of time they spent in it. However, by considerably lengthening the amount of time stressed zebrafish Invested in the light section and reducing latency toward the light region, PME (prevention medical examination) showed benefits akin to those of anxiolytics. [5] . Additionally, there was a notable rise in the number of entry into the light chamber [5]. The light-dark test results were subjected to statistical analysis using the Kruskal-Wallis test and Dunn's multiple comparison post-hoc test. Significant differences were defined as those with a p-value of less than 0.05. The program used for the analysis was GraphPad Prism 6. The mean plus standard deviation was used to express the results. [10]

8. Shoaling test- This is comparable to the rodent social behavior test. The exam gauges the impact of anxiety on interpersonal elationships. The height of exploration and the separation between individuals are the two most crucial factors. [4] Fish under stress showed observable changes in shoal cohesiveness, and the length of time that shoal cohesion persisted in stressed fish rose significantly. [5] In contrast to the indigenous population. Furthermore, a decrease in the duration required for shoal cohesiveness within the CUS zebrafish was noted, suggesting considering the CUS paradigm was causing anxiety.In order to evaluate complex behaviors including learning, attention, memory, aggressiveness, addiction, anxiety, and stress, behaviorl protocols have been established [5]. [6] Several procedures for evaluating complex behaviors, including memory and learning, social preference, aggressiveness, addiction, locomotor activity, and anxiety, are described in this article. [6]

CONCLUSION: Since rats' systems are comparable to ours with regard to genetics and physiology, they been employed as models of anxiety. These behavioral criteria might be helpful for zebrafish anxiety evaluation due to their simplicity and ability to identify unique and common behavioral changes with various anxiolytic medications. Reference [2]. These little fish may be useful in these kinds of investigations because of the ease of use and non-invasiveness of the bath immersion chemical delivery method, together with the behavioral analysis's efficacy. [3] Model of the zebrafish presents a fascinating substitute Within the realm of neuronal research, notwithstanding several drawbacks. Scientists can employ Danio rerio for high-volume

screening of new compounds; nonetheless, It ought to be considered an additional technique to complement the current experimental pharmacology approaches. [4] Benzodiazepines, antidepressants, and buspirone are examples of drugs having anxiolytic effects that reduce latency and promote Investigative conduct in the upper regions of the brain. Thus, the acute PME's anxiety-reducing properties (prevention medical examination) were assessed using the NTT. The serotonergic system in zebrafsh has also been connected to the therapeutic advantages of anxiolytic drugs .[5] Regardless of the new drug's biological target, a complex behavior related to drug administration appears to be an intriguing pharmacological tool for drug discovery, especially when used as a predictive tool for drug design. For example, if the drug acts via serotonin transporters, nicotinic receptors, or other targets, an anxiolytic behavior appears. When evaluating zebrafish's value as an instrument for study, there are several significant factors to take into account. [6] Research into associative behavioral reactions in these subjects, such as fear in unfamiliar situations, may significantly progress toxicology and translational medication development. [7] Thus, like clinical generalized anxiety- vs. fear/panic-like states and the corresponding rat comparable models that do discriminate these states, there may be subtypes of anxiety-like states in zebrafish. Simultaneously, it is plausible that the affective phenotypes (and corresponding circuits) of zebrafish are simply less complex than those of mammals, and thus may represent more generalized affective categories (e.g., anxiety-like), rather than targeting more focused AD subtypes, such as anxiety versus fear/panic. [9] It may be claimed that ferulic acid has anxiolytic function in zebrafish using the light-dark paradigm, and that this action is likely due to a GABAA receptor connection. [10] Zebrafish models are employed in biomedical research on a range of human illness situations, and they provide advantages over animal models in this regard.Zebrafish hold great potential as a prototype organism for research the physiological and behavioral functions of serotonin [12] .[14] The establishment of tolerance to repeated exposure to kainic acid at the juvenile and adult stages was facilitated by exposure to the acid at 7 days post-fertility (dpf). This suggests that the neuronal refinement that occurs during this early stage might have a significant impact on the likelihood of subsequent convulsions. [15]

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