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Research Article

A study on behaviour of zebra fish

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Abstract: In neurobehavioral research, animal model have played a crucial role in yielding experimental data as well as, in the development of new insights and theories of brain pathogenesis. Researchers are always trying to develop novel animal models to understand fundamental features of physiological, behavioural and psychological disorders. Zebra fish (Danio rerio) is now considered globally as a new and useful model in biomedical research. Zebra fish is an excellent alternative to mammalian animal model because it does not possess the complex behavioural phenotype exhibited by many other animal models and yields robust responses analogous to humans. In this study, behaviour of zebra fish was evaluated by using various behavioural paradigms.
Keywords: Zebra fish, alternative animal model, biomedical research.

INTRODUCTION

Behaviour is the most complex function of the nervous system. Adverse psychosocial factors, diseases, drugs, stress and environmental changes affect the neuronal system of an organism which leads to behavioural disturbances. Neurotransmitters like acetylcholine, dopamine, epinephrine, norepinephrine, serotonin, gammabutyric acid, glycine and glutamic acid play an important role in behaviour regulation. Any alteration or impairment in the neurotransmitter life cycle such as hypo/hyperactivity may change the behavioural responses leading to psychiatric illness. Several mental and neurological disorders are associated with behavioural disturbances. Behavioural alterations may also be accompanied by endocrine dysfunctions such as hypo/hypergonadism, precocious puberty and hyperthyroidism etc. Since brain and behavioural processes are both extraordinarily complex, viable animal models (rodents and primates) which display both intensive behaviour and have easily accessible neural process are being increasingly utilized in behavioural neuroscience experiments. A battery of behavioural paradigm of animals has been extensively used as a model system [1, 2, 3, 4, 5] to infer about the fundamental features of human behaviour and physiology. Rats and mice are unquestionably the most successful models in behavioural neuroscience because they have striking similarities with human genetic and physiologic systems. They have been found useful in identifying the mammalian brain regions and neurotransmitter systems involved in cognition, learning and other varieties of behavioural functions. But several aspects of murine biology have limited its use in large scale genetic and therapeutic screening [6, 7, 8, 9]. The maintenance of rodents for experimental purposes is difficult and expensive and besides this, in many cases rodent models are also not satisfactory in various CNS disorder. Scientific communities are continually seeking to obtain basic biological information and to understand fundamental features of physiological, behavioural and psychological disorders that can be applied to human diseases. The zebra fish provides an inexpensive, potentially high-throughput test subject that could prove useful in gaining a greater understanding of neuro pharmacological mechanisms in mammals, facilitate behaviour based drug discovery [10] and in studying in complex neuropsychiatric illness [11, 12]. The relevance of the complex behavioural functions of zebra fish in modelling of anxiety and fear has been shown in many recent studies [13, 14, 15, 16, 17]. The aim of the study was to observe normal behaviour of zebra fish by using various test models to develop baseline data.

MATERIAL AND METHOD

Zebra fish (3-5 cm in length) were collected from Ben River (tributary of Ganges) in Pnatnagar, Uttarakhand. Animals were maintained in the department animal unit in groups of 20-25, in 10-15 litre aquarium tanks for two months prior to setup the experiment. The animals were provided with a clean environment in a proper functioning aquarium under controlled condition of alkalinity, pH (6.8-7.5), temperature (26-28.5 °C), light condition 12:12 (light: dark) hardness (75–200 mg/L CaCO3), ammonia, dissolved oxygen (7.8 mg/L at 28.0 °C), salinity (0.25–

0.75 ppt) and conductivity was monitored on regular basis to ensure good water quality for housing zebra fish [18]. Animals were fed 1-2 times daily of fish food. All fishes were naive, and were allowed ten days to adapt the laboratory environment before experimentation. The standard drug used in the study, benzodiazepine known anxiolytic (diazepam) was purchased from Ranbaxy, India.

Behavioural tests were conducted between 10:00 AM to 3:00 PM. Before experimentation, zebra fish were transferred in their home cages from the animal unit to the experimental room one hour before each test session. After the habituation period in the laboratory the zebra fish were subjected to the test. In each study the zebra fish were randomly selected (n=8) and divided into groups; Group-I placebo control and group-II drug (diazepam) treated. The drug was prepared immediately before use and administered (0. 5, 1.0, 2.0 and 4.0 mg/litre) through dissolving in water. Individual zebra fish was transferred from its home tank to a 250 ml beaker filled with 200 ml de-chlorinated water (control) or 200 ml de-chlorinated drug treated water. Each subject was randomly assigned to a treatment group. One animal at a time from particular group was immersed in a solution containing the drug/vehicle for 30 min prior to behavioural observation. Experiments were conducted 30 minutes after vehicle/drug administration to the respective group. All the apparatus were cleaned thoroughly before and after each trial to remove any trace of odour. The experiment was done in a sound attenuated room and each six minute test sessions was recorded via an overhead video camera which was used to analyze the behaviour later. After six minutes the zebra fish was removed from the test tank. A number of tests session were conducted to observe the behaviour of zebra fish. All behavioural recordings were carried out with an observer not aware of the treatment and behavioural endpoints of the zebra fish.

EXPERIMENTAL PROCEDURE

Novel tank diving test

The novel tank test is most commonly used to assess zebra fish behaviour and anxiety paradigms [15, 19, 20, 21]. This test exploits the natural tendency of zebra fish to seek refuge when exposed to a novel environment [22].The size of the novel tank used was (15 height X 28 top X 23 bottom and 7 cm width. The behaviour was recorded by a side view camera. In this test, the fish initially dives to the bottom of the tank and remains there until it feels safe enough to explore the rest of the tank and gradually explores the upper zone of the tank [19]. The time spent in bottom dwelling, the time elapsed in the upper part of the tank, and erratic movements have been interpreted as indices of anxiety [19]. Reduced exploration (i.e. more number of frequent freezing decreases to reach the top) and elevated erratic movements show anxiety in this test [15, 22].

Aquatic light/dark transition test (Scoto taxis)

A Scoto taxi is an alternative to novel tank and open field tests. It is very similar to murine light/dark box [23], which exploits the tendency of zebra fish to explore a novel environment when confronted with the aversive properties of a brightly lit area (scotophilia, scototaxis) [23]. Analogous to rodent model, zebra fish exhibit a natural preference for the dark side when given a free choice between a dark and a light chamber [24, 25, 26]. This test has been used to investigate the anxiolytic or anxiogenic properties of a variety of drugs [27]. Studies showed that anxiolytic drugs increase the exploratory behaviour and time spent in white compartment while anxiogenic drugs cause the opposite effect [27, 28, 29].

Open field test

Open field exploration task is one of the popular tests of anxiety in rats [30]. This test is readily adaptable to zebra fish and therefore is widely used [19]. In rodents, a suppression of exploratory behaviour includes freezing, thigmotaxis and a reduction in locomotor activity are the behavioural measures of anxiety but freezing and thigmotaxis have been considered as indicators of anxiety in fishes [31, 32]. In open field exploration task, zebra fish initially exhibit fear [32, 33, 34] including thigmotaxis/centrophobic behaviour as observed in rodents.

RESULTS AND DISCUSSION

Behavioural studies on aquatic animal are gradually increasing exponentially. Several assays are being used specifically to test anxiety in the zebra fish [19,25]. One stimulus that causes anxiety in zebra fish is novelty and in novel tank assay, anxiety is indicated by the presence of the fish at the bottom of the tank [27]. Another index of anxiety in zebra fish is the preference for dark over light environments, or scototaxis [25]. When anxious, fish displays a preference for dark surroundings and they freeze when forced into light surroundings [16]. Cortisol levels also increase upon exposure to a stressor [19]. As in mammals, stresses modify fear response and increase anxiety in zebra fish [26]. Additionally, bottom-dwelling (or diving), leaping, hyperactivity and erratic movement have also been suggested as species-specific indicators of anxiety in the ‘open tank’ paradigm [22, 35, 36].

The effect of diazepam was studied in zebra fish at the dose of 0.5, 1.0, 2.0 and 4.0 mg/litre in open field, light and dark transition and novel tank test and behavioural assessment were done by using various parameters. Diazepam showed enhanced time spent by each subject in centre of the tank than time spent in the periphery of the tank in open field test, whereas increased time spent in black compartment than white in light and dark transition test and enhanced time spent in upper half of the tank compared to the time spent on the bottom of the tank in Novel tank test. The effect of

diazepam was compared with control (Table-1), and the effect of diazepam was significant.

Table-1

Drug/Dose	Open field test		Novel tank test		Light/Dark transition test	
	Time spent in the centre of the tank (seconds)	Time spent in the periphery of the tank (seconds)	Time spent in upper half of the tank (seconds)	Time spent in the bottom of the tank (seconds)	Time spent in the black half of the tank (seconds)	Time spent in the white half of the tank (seconds)
Control	66.25 ± 4.04	288.625 ± 1.05	10 ± 1.399	183.625 ± 5.99	241.375 ± 5.56	101.875 ± 3.37
Diazepam 0.5 mg/l	245.25 ± 7.80	96 ± 2.075	14.375 ± 1.24	156.625 ± 5.80	228.375 ± 3.95	124.25 ± 2.28
Diazepam 1.0 mg/l	331.25 ± 4.238	28.625 ± 4.26	34.75 ± 2.15	133.5 ± 3.28	181.125 ± 7.38	143 ± 3.93
Diazepam 2.0 mg/l	320.75 ± 6.49	39.875 ± 4.876	36.125 ± 1.95	144.25 ± 2.15	155.5 ± 2.34	199.875 ± 3.99
Diazepam 4.0 mg/l	296 ± 5.291	54.625 ± 1.97	33.375 ± 1.52	173.25 ± 3.21	179.375 ± 3.53	180.625 ± 3.53

Values are mean ± SEM; n = 8 per group

Our results confirm that when a zebra fish is presented to an unfamiliar environment it shows robust anxiety-like behavioural responses, which were measured by using the novel tank diving test, open field test and light/dark transition. Psychoactive drugs like diazepam reverted anxiety-like behavioural responses, due to high sensitivity, the behavioural and physiological endpoints of the zebra fish can be manipulated. Due to this high sensitivity and manipulation, the novel tank paradigm possesses a great potential for use in the screening of novel compounds of possible therapeutic value.

Behavioural endpoints such as thigmotaxis (staying closed to the walls) (Heisler et al., 1998), decreased exploration and freezing found in mice or rats which indicate anxiogenic behaviour is now applied to zebra fish model of anxiety [14,19,21,22]. Centre and periphery ratio of rodents in open field test and top: bottom ratio in novel tank test [5] is similar kind of behavioural endpoints. Studies have indicated that similar environmental conditions cause anxiety like behaviour both in rodents as well as in zebra fish [26, 37]. Fish can also form special memories [38,39] just like the rodents [40,41] and use them to guide themselves and establish safe zones in novel environment. [42]. A similarity has also been found between the hyper arousal behaviour found in rodents in dangerous situations and erratic movements shown by Zebrafish in novel tank [19]. Thus, zebra fish could be considered as useful animal model for the study of anxiety and screening of new drugs on the basis of comparative study of behavioural endpoints of zebra fish. The light dark preference test used for rodents is also a useful paradigm for investigating anxiety like behaviour in Zebrafish. [27]. Studies show that anxiolytic drugs increase the exploratory behaviour and time spent in white compartment while anxiogenic drugs cause the opposite effect [27,28,29. The anxiolytic effect of diazepam was observed in the present study too. Open field activity test in zebra fish is extensively used in pharmacological studies. Swimming in the centre of an open field suggests an anxiolytic like behaviour. α- Fluoro methyl histidine shows an anxiolytic effect by increasing swimming time in the centre [44] and similar effect was observed in the present study. Exposure to chronic fluoxetine and acute ethanol reduces the erratic movements in open field [19].The result of the present study is critical for the validation of Zebrafish as a model of anxiety.

There are several characteristics which make Zebrafish an important test subject which could prove useful in gaining a greater understanding of neuropharmacological mechanisms in mammals and facilitate behaviour based drug discovery [10]. Since zebra fish have robust physiological responses and quantifiable behavioural and neuropathological phenotypes analogous to humans [43]. Several beneficial properties make zebra fish a promising alternative to mammalian model. Low maintenance cost and rapid life cycle of zebra fish makes easy to maintain in large number of fish in small area which is important for large scale behavioural studies. Zebra fish readily acclimatizes to new environments, is constantly active and very little disturbed by the presence of observers. These qualities make zebra fish an excellent species choice for behavioural study.

Zebra fish has similarity in basic organisation of brain components to that of humans which make it useful in the study of brain disorders [45, 46, 47]. Zebra fish brain aminergic system has many structural properties common to the mammalian systems. The noradrenergic, serotonergic, aminergic and histaminergic systems of zebra fish are highly similar to the mammalian system [45, 46, 47]. The dopaminergic systems are also almost similar but with a major difference of a lack of dopaminergic neurons in zebra fish mesencephalon [46]. The basic and complex brain

phenomena as well as endocrine mechanisms of zebra fish and mammals are substantially homologous [48]. Just like humans, zebra fish employs cortisol as the primary stress response hormone unlike corticosterone by rodents [49]. The hypothalamus pituitary inter renal (HPI) axis of zebra fish is homologous to the hypothalamus pituitary adrenal (HPA) axis of humans. Corisol is the primary stress hormone in both species [50]. Zebra fish model enables greater insight into neural mechanism associated with anxiety related disorders since it possess all the classical vertebrates' neurotransmitters and its neuroendocrine system yields robust cortisol responses to stress. Much like rodents, zebra fish has the ability to learn through classical conditioning. It also offers an alternative and efficient mode of drug delivery via the gills [22, 31, 36]. Zebra fish can serve, therefore, as an inexpensive and potentially high throughput model for behavioural phenotyping and psychopharmacological screening of medicinal plants for drug development [10, 19, 51].

Thus the zebra fish, Danio rerio (Hamilton) emerged as one of the most important vertebrate model organism which has opened new avenues in biomedical research [52, 53, 54]. They have become a useful addition to the existing rodent and primate model. Evidences suggest that zebra fish is widely applied as an animal model species to biological psychiatry [55], behaviour based drug discovery [10] and for the screening of therapeutic drugs [51, 53].

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