The social behaviour in zebrafish (*Danio rerio*) – the metabolic, neuropsychiatric and ecotoxicological relevance

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ABSTRACT

Zebrafish (*Danio rerio*), a teleost cyprinid, is a species that became an ideal model for aquatic ecotoxicology due to large spectrum of methodologies developed in early 1980. Zebrafish as a model organism has many advantages, such as: reduced costs (fish breeding is cheaper than mice), small size, ex utero development of the embryo and individuals grow at a rapid rate (mature adults develop in about three months) and embryos are transparent. In addition, zebrafish has a high degree of homology to the human genome, which is why it is used as a model organism for studying: genetics, metabolics, pharmacology, cancer, neuropsychiatric manifestations, ecological toxicology etc. Thus, in this mini-review we are summarizing the aforementioned described aspects, by focusing on the social behavior and how this is influenced by their metabolic, neuropsychiatric and ecotoxicological relevance.

KEYWORDS:

Zebrafish, social behavior, metabolism, ecotoxicology.

INTRODUCTION

The development and mechanisms underlying the social behavior of vertebrates are not fully understood at present. There are numerous human clinical conditions in which abnormal social behavior is the main symptom, through the main conditions are: alcoholism and fetal alcohol syndrome. To facilitate the understanding of the mechanisms of social behavior and the mechanisms underlying the abnormal social behavior, animal models, such as zebrafish or mice, are often used (Parichy, 2015).

Observations which are made on field of the zebrafish behavior are scarce and anecdotal, because of that, this assessment is analyzed in laboratory. Behavior that has received considerable attention is the formation of free social aggregation, which have been identified and studied in the laboratory field. (Engeszer et al., 2007b; Gerlai, 2014).

Also, experiences with different physical contexts during early development can have dramatic and prolonged effects on behavior, modeling neural systems and other underlying systems in ways that influence later adult behavior (Hensch, 2004; Fu et al., 2011).

THE SOCIAL BEHAVIOR IN ZEBRAFISH

Teleost fish is the most diverse and biggest taxon existing vertebrate, having until now thousands of species described, having a wide variety of social behaviors. For example, different teleost species of can use a variety of strategies for feeding and mating, parenting behaviors, and social hierarchies. They may differ in their territoriality and degree of aggression, using visual, olfactory and sonorous stimuli. As with other species, the social behavior of fish also depends on their internal state, especially at hormonal levels (Fernald, 2012; Hofmann, 2006).

The social behavior of zebrafish is influenced by the presence or actions of other specific elements (Al-Imari et al., 2008)(8). Some individuals of zebrafish include scaling / schooling, courting, fighting / aggression, and breeding; it can also manifest itself in social recognition, approach / audacity (social investigation) and social preference.

The neuropeptide hormones here modulate the basic physiology that governate social interactions. A multitude of discoveries regarding neuroendocrine mechanisms of social behavior were extrapolated from animal models to humans. During evolution, hypothalamic neuropeptides and hormones which are released kept their structures and also their biological functions, including effects on behavior.

In nature (Engeszer et al., 2007; Spence et al., 2006) and in the laboratory (Gerlai et al., 2000; Miller et al., 2007; Miller et al., 2008), zebrafish aggregate and swim in groups, a behavior called bank. This behavior has an effect is in reducing the risk of predation, thus, the improvement in feeding and breeding was observed. For a detailed understanding of social behavior, internal and external influences leading to the variety of non-human phenotypes are examined. Due to the diversity of pigment patterns among Danio Rerio fish, visual signals may be particularly important in mediating social behaviors in this group. Understanding the development of the pigment model in zebrafish D. Rerio allows for integrative analyzes of how molecular variation leads to morphological variation among individuals and how it influences social interactions.

Social behavior in new contexts can be influenced by recent experiences, but also by the impact of the physical environment on the social behavior of adults, it can persist even

when moving to new habitats. It was observed that after sheltering in more complex physical environments, zebrafish formed aggregates, which suggests that the main persistent effect is a change in spacing patterns.

CURRENT STUDIES OF SUPERIOR BEHAVIORAL FUNCTIONS ON ZEBRAFISH

The main complex behaviors were studied zebra fish are: learning and memory, aggression, anxiety, reward dependence, mate choice, social preference and locomotion.

Regarding **locomotion** (swimming), Gerlai and co-workers (2000), described where and how fast the zebrafish swam on exposure in a new environment, as well as after getting used to the environment. Their purpose is to observe particular swimming patterns, to make analogies with anxiety states, as well as the influence of alcohol (Gerlai et al., 2000)

Reward is one of the areas where adult zebrafish have contributed to behavioral genetics. The reward behavior of animals is an instinctive action to seek resources and to reproduce. This behavior can be altered by the abuse of various drugs, such as: cocaine (Darland et al., 2001), amphetamine (Webb et al., 2009) and opiates (Lau et al., 2006). The reward behavior can be the first step toward addiction. The conditioned place preference test (CPP) is often used to measure reward behavior in zebrafish, which groups a primary clue (for example, a drug) with a secondary stimulus, such as a colored aquarium compartment. The main neurotransmitter associated with reward behavior is dopamine (DA). Brennan and his colleagues observed that there is a robust change in place preference (PP) following nicotine treatment and also ethanol treatment (Killy et al., 2008). A comparison was made on the treated fish brain with those untreated, using microarray

analysis, identifying 1362 genes that were altered significantly following drug application, including 153 that responded to both the ethanol and the nicotine.

Learning and memory can be controlled by several circuits of the brain, each of them is distinctly euro-anatomical. Mammal studies include spatial learning (hippocampus), implicit learning (such as simple motor reflexes; cerebellum) and avoidance learning (amygdala). Currently, there are many paradigms for measuring learning and memory in zebrafish. Associative learning can be measured by associating two previously irrelevant stimuli, such as color, reward or aversion (repulsion to something). For example, Suboski and his collaborators associated morphine, which is a neutral stimulus, with the aversion effects of the alarm substance (Suboski et al., 1990). Avoidance learning can be evaluated using a transfer box, fish are able to quickly associate an unconditioned stimulus (for example: slight electric shock) with a conditioned stimulus (for example: light (Xu et al., 2008) or color (Blank et al., 2009). Spatial learning can be measured using either a transfer box (Pather et al., 2009; Williams et al., 2002), or a maze T (Al-Imari et al., 2008). The mechanism being a simple one, the fish must learn to get a reward either by alternating the part of the visited tank or swimming properly through a maze.

Aggression represents a variety of complex behaviors (such as: approaching, undulating body movement, flapping, biting, mouth opening behavior, loading, changing body color, elongation, and surrounding) directed at conspecifics (or other objects) in adults; it can occur in the context of resource protection (eg, women), territorial defense (territorial behavior) and dominance (Jesuthasan et al., 2008). In the laboratory, aggression can be

measured by mirror image stimulation (MIS), fish do not recognize their own image, so they become aggressive as if an intruder was present (Gerlai et al., 2000; Rowland et al., 1999). This method avoids damaging the fish and provides immediate feedback on the activity of the subjects (Marks et al., 2005), thus the MIS protocol is simple to establish and execute. Gerlai and his collaborators (2000), observed that zebrafish exhibit agonistic characteristic positions, including erection of the dorsal fins, tail, pectoral and anal coupled to bite, slamming of the tail and short quick swimming strokes against the mirror. Another method of measuring the behavior is presented by Marks and his collaborators (2005), who used the recording interactions of two free-swimming fish. Also, a positive correlation between aggression and boldness has been reported (Moretz et al., 2007), aggression being a very plastic behavior. The number of interactions can be influenced by habitat complexity and growth conditions (Marks et al., 2005; Basquill et al., 1997). In 1999, Miklosi and his collaborators observed a lateralization of the aggression behavior of zebrafish, using mainly the right eye to see the predators. It was observed that the main neurotransmitter 5-hydroxy-tryptamine (5-HT), which controls the aggressiveness has influence on the animals, those showing a high level tend to be shy, while those with a lower level are more impulsive and more aggressive (Popova et al., 2008). The agonistic behavior of zebrafish can be changed if they are exposed at the pharmacological compounds, including ethanol (Gerl et al., 2000) and 17 α -etinilestradiol (a synthetic estrogen) (Larson et al., 2006). Arginine vasotocin (AVT), which is a hormone produced by neurosecretory cells from the posterior pituitary gland of the brain is involved in social and sexual behavior, is found in the preoptic magnocellular area and it changes

size depending on the state of dominance of the fish. Therefore, the involvement is observed in the preoptic area of zebrafish in controlling the social hierarchy and agonistic behavior that is used to establish it (Larson et al., 2006).

Anxiety is a complex behavior evoked by the environment or by dangerous or potentially dangerous stimuli. This behavior is characterized by reduced exploration and is commonly manifested in thigmotaxis, geotaxis (diving), scototaxis, opercular movements, frost, body color change and zigzag movement (Blaser et al., 2010; Blaser et al., 2012). Anxiolytic drugs generally reduce this anxiety-like behavior, while anxiogenic agents potentiate these responses. Zebrafish anxiety-like behavior frequently overlaps with fear-related behavior (Maximino et al., 2010). Anxiety tests were determined for fish although it is not yet known whether anxiety or fear is measured or whether there are differences between these two states (Blaser et al., 2009). Protocols for measuring anxiety tend to evaluate one of the two variables. The first set of protocols records how adult zebra fish react to unusual environments, such as the time they spend on the edge of a tank. (Peitsaro et al., 2003; Lopez-Patino et al., 2008), at the bottom of a new tank (Levin et al., 2007; Egan et al., 2009) or on the dark side of a light / dark tank (Blank et al., 2009; Serra et al., 1999). The second set of protocols analyzes: freezing, long-term growth of basal locomotor activity (Gerlai et al., 2009; Blaser et al., 2009; Lopez-Patino et al., 2008), and clustering, considered solid measures for measuring anxiety. By comparison with other behaviors, the methods for measuring anxiety have been validated using pharmacological compounds that are used for human patients. Substances that have been shown to be anxiogenic: application of caffeine (Egan et al., 2009;

Wong et al., 2009), pentylenetetrazol (Wong et al., 2009), the alarm substance (Egan et al., 2009; Speedie et al., 2008) and cocaine withdrawal (Lopez-Patino et al., 2008). In contrast, many anxiolytic substances have been characterized including nicotine (Levin et al., 2006), diazepam (Lopez-Patino et al., 2008; Bencan et al., 2009), fluoxetine hydrochloride and ethanol (Egan et al., 2009). Finally, a link between anxiety and cortisol levels has been demonstrated, which is the main stress hormone of zebrafish (Egan et al., 2009).

THE ALCOHOL EFFECTS

One of the most widely used drugs in the world is alcohol. The effects of ethanol consumption on rodent neurotransmitters are, in small quantities, stimulated, and through chronic consumption of large quantities they are affected or suppressed (McBride et al., 1990).

Zebra fish is an ideal model for testing drugs, such as alcohol, because its absorption is simple. The alcohol mixed with the water in the reservoir is absorbed through the blood vessels of the gills and into the skin, reaching a balance between the blood alcohol and the alcohol of the reservoir.

In 2000, Gerlai and his collaborators, suggested that zebrafish as a tool for analyzing the effects of alcohol on adult brain function. The behavioral effects of exposure of zebrafish to alcohol, both acute and chronic, have been investigated (Gerlai, 2003; Gerlai et al. 2006).

In 2000, Gerlai and his collaborators, designed a series of simple, easily quantifiable tests to examine the effects of alcohol administration in different concentrations on locomotion, aggression, tendency to form groups, alarm response, light / dark preference and pigmentation.

Dlugos and Rabin (2003) compared the effects of acute alcohol administration and cornice for three zebrafish strains. The strains differed in terms of the amazing response and the cohesion of the shore, which suggests that there is a genetic basis for both the initial sensitivity and the development of tolerance to alcohol.

Larson and his collaborators (2006) showed that there are clear differences between dominant and subordinate fish in AVT expression, such as aggression, courtship and parental behavior in vertebrates, although the system varies depending on the taxa.

They noticed that in an open space the zebra fish as opposed to rodents, spend the first 5 minutes within a 30-minute interval to explore the area, and the rest as close to the bottom of the area (aquarium). As opposed to rodents, zebrafish have a thigmotactic behavior (movement of an organism in response to contact with a solid body), (Blaser et al., 2010; Buske et al., 2014).

The main effects of alcohol on zebrafish are shown in Table no. I.

Table I. The effects of alcohol

Study	Behaviour	Function/ Activity	Effect
Naumann et al., 2010	Agression	Receiver modulator GABA-A	Increases aggression
Gerlai et al., 2000	Anti-predatory		It affects in large doses
Egan et al., 2009	Anxiety		It reduces anxiety
Gerlai et al., 2000	Group preference		Reduced to high concentrations

Gerlai et al., 2000	Preference for light/ dark		Lowered at high concentrations
Gerlai et al., 2000	Locomotion		Reduced at high concentrations
Kily et al., 2008	Reward		Profit

*GABA- Acid gamma-aminobutyric

ECOTOXICOLOGICAL RELEVANCE

Ecotoxicology is a discipline of environment science which requires knowledge in several areas (eg chemistry, ecology, toxicology, climatology, microbiology, engineering, mathematics, public policy and legislation environment), to understand the possible dangers due to chemical substances which are found in pollutants released continuously in aquatic environments around the world. The most common spill cases are heavy metals, pesticides, PAHs (Polycyclic Aromatic Hydrocarb), but often are encountered pharmaceutical products. They present a concern from the toxicity point of view as their interactions with various mixtures emitted by industrial and municipal effluents from various sources of pollution (Blaise 2013).

Zebrafish is widely used to monitor pollute toxicity present in the environment, however, it has several disadvantages, such as: statistical experiments are difficult and low sensitivity of detection. Environmental pollution particularly that of water, is one of the common problems worldwide. Water

pollution affects the survival and reproduction of aquatic organisms, and in addition presents affections of human health by bioconcentrate. Zebrafish is susceptible to different pollutants, being considered an ideal model organism for environmental monitoring. The main indicators that were observed are: characteristic changes in morphology, behavior, and physiology and gene expression. The International Organization for Standardization (ISO) first published the zebrafish toxicity test in 1984 (International Organization for Standardization. 1996).

Detection of a specific pollutant may be indirect, on monitoring basis of different parameters. For example, heavy metals could be identified by monitoring the enzyme activity or gene expression of the biomarker (Xu et al., 2008).

The main pollutants which are used in environmental monitoring studies are: heavy metals, endocrine disrupters and organic pollutants, these conditions can be found in Table no .II.

Table II. Applications of zebrafish in environmental monitoring

Classes	Contaminants	Study	Diseases
Heavy metals	Cadmium acetate, lead acetate, mercury chloride, zinc chloride	Richetti et al., 2011	It inhibits the activity of enzymes
	Mercury chloride	Senger et al., 2010	
	Metilmercury	Samson <i>et al.</i> , 2001	Functional impairment and delayed mortality syndrome
	Tributyltin	McAllister and Kime, 2003	Reproductive toxicity
	Copper and zinc	Rougier <i>et al.</i> , 1996	Susceptibility to infection by listeria after exposure to copper and zinc

	Aluminium, cadmium, iron, Copper, nickel, mercury, cobalt, lead	Dave and Xiu, 1991 Dave, 1985	Toxicity: dose response, effects on hatching and survival
	Lead and uranium	Labrot <i>et al.</i> , 1999	Acute toxicity and toxicokinetics
	Cadmium	Wu <i>et al.</i> , 2012 Hen Chow and Cheng, 2003 Chan and Cheng, 2003	Affects gene expression Ectopic apoptosis induction Abnormal somite patterning and defects in axonogenesis
	Cadmium, zinc	Ling <i>et al.</i> , 2011	Indirect detection of the presence of heavy metals in the aquatic environment
	Copper, mercury, cadmium and zinc	Chan <i>et al.</i> , 2006	
	Arsenic	Li <i>et al.</i> , 2012	Dvr1 showed a decrease when zebrafish embryos were exposed to arsenic compounds.
	TiO ₂ , ZnO nano-scale	Xiong <i>et al.</i> , 2011	Sublethal effects, including respiratory toxicity, trace element disorders In tissues, inhibition of Na ⁺ , K ⁺
Endocrine disruptors	BDE-47	Chen <i>et al.</i> , 2012	Causes behavioral disruption and toxicity for future generations
	PCBs	Abnet <i>et al.</i> , 1999 Henry <i>et al.</i> , 2001 Orn <i>et al.</i> , 1998 Fox <i>et al.</i> , 1994 Andersson <i>et al.</i> , 2001	Transactivation activity of aryl hydrocarbon receptors in COS-7 CYP1A induction in a zebrafish liver cell line Reproduction Kinetics of bioconcentration clearance Bioaccumulation with different routes of exposure
	PAHs	Incardona <i>et al.</i> , 2004	Morphological abnormalities occurring after cardiac dysfunction
	PBDEs	Chen <i>et al.</i> , 2012	Disruption of transport, storage and metabolism of retinoid in zebrafish at concentrations relevant to the environment. It causes adverse effects on the neurodevelopment of the offspring.
	DE-71	Yu <i>et al.</i> , 2010	
	TCDD	Heiden <i>et al.</i> , 2008	It causes chloracne, cancer, hepatotoxicity, diabetes, immune changes of the gonadal, pulmonary tuberculosis and other complications, such as diabetes and infertility.
	Bisphenol A and triclocarban	Chung <i>et al.</i> , 2011 Saili <i>et al.</i> , 2012	

	Bisphenol A, endosulfan, heptachlor, methoxychlor, tetrabromobisphenol A	Chow et al. 2012	Caused severe malformations of the otic vesicle. In small doses, the larva shows hyperactivity and learning deficiencies in adult fish.
Organic pollutants	Chlorpyrifos and nickel chloride	Kienle et al., 2009	It affects behavior.
	chlorpyrifos	Richendrfer et al., 2012 Levin <i>et al.</i> , 2003	It affects behavior. Effects on survival, response latency and spatial discrimination
	PFNA	Zhang et al., 2012	It changes the expression of the protein gene that binds fatty acids and affects the level of liver triglycerides
	Methyl paration	Huang et al., 2012	It affects the activity of some enzymes

BDE-47 = brominated diphenyl ether; PCBs= Polychlorinated biphenyls; CYP1A = cytochrome P450 1A; PAHs= Polycyclic aromatic hydrocarbons; PBDEs = polybrominated diphenyl ether, DE-71 = commercial mixture of PBDEs, TCDD = 2, 3, 7, 8-tetrachloro-p-dioxin, FABP = fatty acid binding proteins, PFNA- = perfluorononanoic acid

CONCLUSIONS

Zebra fish are an increasingly common model organism used in human disease studies. During their growth, zebrafish are a good model in behavioral neuroscience, and can be used for a broader understanding of behavior make an interest for future research. Also, as a model body for environmental monitoring it has low costs, ease of reproduction and many other advantages, so over time the sensitivity of their monitoring has been continuously improved. In terms of social behavior, it can be influenced by recent experiences, but also by the impact of the physical environment on the social behavior of adults. The relevance in the brain functions and zebrafish behavior has a great interest, as central nervous system (CNS) disorders can be studied and modeled with this species. Alcohol abuse and alcoholism are among the most expensive and devastating diseases, but they are of interest because the mechanism is not known at all, so zebrafish are lately proposed to understand this mechanism.

ACKNOWLEDGE AND DISCLOSURE

C.A. is currently supported by a PN-III-P1-1.1-TE-2016-1210 grant offered by UEFISCDI.

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Submission: 22 feb 2020
Acceptance: 17 apr 2020