Behavioural Brain Research 289 (2015) 48–54

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Acute caffeine administration affects zebrafish response to a robotic stimulus

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HIGHLIGHTS

- A robotic stimulus is used to study the effect of caffeine on zebrafish behavior.
- Information theory is used to quantify the influence of the stimulus on zebrafish.
- Caffeine administration modulates the average speed of zebrafish.
- The interaction with the stimulus is regulated by caffeine administration.
- Zebrafish exposed to elevated caffeine doses are more sensitive to the stimulus.

ARTICLE INFO

Article history:
Received 7 March 2015
Received in revised form 8 April 2015
Accepted 11 April 2015
Available online 20 April 2015

Keywords:
Anxiety
Caffeine
Fish replica
Robotics
Transfer entropy
Zebrafish

ABSTRACT

Zebrafish has been recently proposed as a valid animal model to investigate the fundamental mechanisms regulating emotional behavior and evaluate the modulatory effects exerted by psychoactive compounds. In this study, we propose a novel methodological framework based on robotics and information theory to investigate the behavioral response of zebrafish exposed to acute caffeine treatment. In a binary preference test, we studied the response of caffeine-treated zebrafish to a replica of a shoal of conspecifics moving in the tank. A purely data-driven information theoretic approach was used to infer the influence of the replica on zebrafish behavior as a function of caffeine concentration. Our results demonstrate that acute caffeine administration modulates both the average speed and the interaction with the replica. Specifically, zebrafish exposed to elevated doses of caffeine show reduced locomotion and increased sensitivity to the motion of the replica. The methodology developed in this study may complement traditional experimental paradigms developed in the field of behavioral pharmacology.

1. Introduction

Anxiety-related disorders represent a widespread emotional disturbance affecting a large proportion of individuals [1]. Pharmacological studies on animal models may greatly aid our understanding of these disorders, by affording a tool upon which contributing variables can be identified, adjusted, and dissected. Among such models, zebrafish (*Danio rerio*) is becoming a species of choice [2–5], due to its short inter-generation time, fast reproduction rate, high stocking density, availability of genetic sequencing, complex social behavior, and elevated homology of structure and function with human nervous system [4,6–11]. Among the different emotional domains addressed in zebrafish, anxiety-related behaviors have been analyzed in detail through different methodologies [12–19]. Beside the development of these methodologies, several authors also addressed the extent to which pharmacological treatments – known to modulate anxiety in humans and other mammals – affect zebrafish behavior [20].

Among different psychoactive compounds, acute caffeine treatment has been utilized to evoke anxiogenic behavioral responses in novel tank diving test [20–22] and light/dark box [23]. These studies have demonstrated that caffeine elicits characteristic diving behavior, increased thigmotaxis, reduced exploration, and increased erratic behavior in individual zebrafish. Such behavioral responses are, in turn, accompanied by elevated whole-body cortisol and brain c-fos [20,21,24]. While these efforts have contributed to the identification of the principal effects of caffeine on individual response, social behavior has yet to be fully examined [12,25].

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http://dx.doi.org/10.1016/j.bbr.2015.04.020
0166-4328/

In this study, we propose the integration of robotics and information theory to investigate the effect of caffeine on zebrafish social behavior. To this aim, we addressed zebrafish response to a robotic stimulus resembling (in dimension and morphology) a shoal of four conspecifics. Individual behavior was studied under control conditions and in response to the acute administration of caffeine (5 mg/L, 25 mg/L, and 50 mg/L; 0.026 mM, 0.129 mM, and 0.257 mM respectively). Specifically, in a binary preference test, we have studied the behavioral response of zebrafish subjects to a replica externally controlled using a mechanical platform [26,27]. Based on experimental evidence indicating that anxiety-evoking stimuli result in increased shoaling tendency [12,28–30], we expected that increasing caffeine concentration would result in a stronger interaction between the fish and the replica of conspecifics.

The interaction between the fish and the replica was quantified in an information theoretic framework through the transfer entropy, a construct often used to measure the mutual information flow between two dynamical systems and only recently proposed for the analysis of social behavior [26]. Transfer entropy quantifies the predictive power of a time series to anticipate another, possibly coupled, time series [31,32]. Specifically, the transfer entropy from a dynamical system (e.g. the replica) to another (e.g. the fish) can be associated with a causal relationship between the two systems, manifested in the form of an uncertainty reduction computed directly from raw time series (e.g. the tracked motion of the replica and the fish in the tank) [32]. Differently from traditional measures of shoaling tendency based on physical proximity [12], our approach considers the swimming bouts that the live fish takes up from the prescribed motion of the replica. In other words, transfer entropy is used to approximate the degree of predictability of fish position in time from the prescribed motion of the replica. In addition to influencing the interaction with the replica, we also anticipated that acute caffeine administration would modulate fish speed and activity along the water column based on available literature [12,20,25,33].

The use of a robotic stimulus is grounded on the need to generate a controllable and repeatable visual stimulus [19,34] on which to test the effect of caffeine. Specifically, the platform was pre-programmed independently of the motion of the live subjects to facilitate the assessment of information transfer. Given the unidirectional nature of the interaction, we treated the transfer entropy from the fish to the replica as a theoretical lower bound on which to contrast the transfer entropy from the replica to the fish.

2. Materials and methods

This study was approved by the University Animal Welfare Committee of New York University under protocol number 13-1424.

2.1. Animals

A total of 40 wild-type zebrafish, approximately 3 cm in body length, were used in this experiment. All animals were purchased from an online aquarium source (LiveAquaria.com, Rhinelande, Wisconsin, USA) and were kept for a minimum of 12 days before experimentation. Fish were stocked at a housing density less than 1.06 fish/L in 37.8 L (10 gallons) tanks, in a room lit between 9 am and 11 pm by full spectrum fluorescent light. Fish were fed around 7 pm each day with commercial flake food (Hagen Corp./Nutrafin max, Mansfield, Massachusetts, USA) acquired from Petland Discount, Brooklyn, New York, USA. Temperature and acidity of the housing tanks were kept at $27 \pm 1\,^{\circ}$C and 7 ± 1 pH, respectively.

Fig. 1. Visual stimuli. On the left, the shoal of four zebrafish, and on the right the control stimulus.

2.2. Platform and replica

The replica of the zebrafish shoal was designed in SolidWorks (Dassault Systèmes SolidWorks Corp., Waltham, Massachusetts, USA) and printed on a 3D prototyping machine (Stratasys, Dimension SST, Eden Prairie, Minnesota, USA) in rigid acrylonitrile butadiene styrene (ABS) material. Each replica was 3 cm in length and painted using waterproof spray paints (Krylon, Krylon Products Group, Cleveland, Ohio, USA) to imitate the color pattern of live zebrafish. To balance the visual cues offered to live fish, we also considered a control stimulus, comprised of the ABS skeleton holding the shoal replica to the end effector of the platform (see Fig. 1).

The replica was actuated using an external platform (Fig. 2(a)). The platform design is similar to [26] and consisted of a robotic arm with three degrees of freedom, which enabled the rigid body motion of the fish shoal replica in 2D. The platform comprised three rigid links, each actuated by a separate servo-motor (Futaba Corporation of America, Schaumburg, Illinois, USA; and Hitec RCD USA Inc., Poway, California, USA). The servo-motors were controlled using a pre-programmed microcontroller (Arduino Uno, Arduino, Italy), which allowed for imposing the desired motion to the end effector, where the replica was attached.

In our study, we selected a linear motion of 3 cm/s superimposed to an oscillation with amplitude of 10° at 2 Hz to imitate fish thrashing against the walls (based on personal observations of zebrafish tail beat undulations). As the replica traversed the tank, its orientation was reversed by 90° every time the direction of motion was changed, so that the replica was always oriented at an average angle of 45° with respect to the x-axis (Fig. 2(b)).

2.3. Experimental set-up

The set-up consisted of a rectangular 76 cm × 30 cm × 30 cm tank (length, width, and height respectively). An overhead camera