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A new model to study visual attention in zebrafish

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ABSTRACT

The major part of cognitive tasks applied to zebrafish has not fully assessed their attentional ability, a process by which the nervous system learns, organizes sensory input and generates coordinated behaviour. In an attempt to maximize the value of zebrafish as an animal model of cognition, we tested the possibility to apply a modified version of novel object recognition test named virtual object recognition test (VORT) using 2D geometrical shapes (square, triangle, circle, cross, etc.) on two iPod 3.5-inch widescreen displays, located on two opposite walls of the water tank. Each fish was subjected to a familiarization trial (T_1), and after different time delays (from 5 min to 96 h) to a novel shape recognition trial (T_2). A progressive decrease, across time, of memory performance, in terms of mean discrimination index and mean exploration time, was shown. The predictive validity was tested using cholinergic drugs. Nicotine (0.02 mg/kg intraperitoneally, IP) significantly increased, while scopolamine (0.025 mg/kg IP) and mecamylamine decreased, mean discrimination index. Zebrafish discriminated different movements (vertical, horizontal, oblique) and the discrimination index increased significantly when moving poorly discriminated shapes were presented, thus increasing visual attention. Taken together these findings demonstrate that VORT is a viable, fast and useful model to evaluate sustained attention in zebrafish and for predicting the efficacy of pharmacotherapies for cognitive disorders.

1. Introduction

Zebrafish are providing an attractive model for behavioural studies. Although these fish are relative newcomers to studies of learning and memory (Sison et al., 2006), a number of studies revealed that they are capable of performing well in a range of learning tasks such as avoidance learning (Blank et al., 2009; Morin et al., 2013), olfactory conditioning (Braubach et al., 2009), shuttle box alternation learning (Pather and Gerlai, 2009), place conditioning (Eddins et al., 2009), appetitive choice discrimination (Bilotta et al., 2005), visual discrimination learning (Colwill et al., 2005), associative conditioning task (Luchiari and Chacon, 2013), active avoidance conditioning (Xu et al., 2007), alternation based spatial memory task (Williams et al., 2002) and even automated learning paradigm (Hicks et al., 2006). Sison and Gerlai (2011) have designed an associative learning task that was deliberately made to resemble a classical radial arm maze in which the traditional cue (food reward) was replaced by the sight of conspecifics. Parker et al. (2012) have developed a 3-choice serial reaction time task (3CSRTT) in which zebrafish could learn to perform a complex operant task, which requires more than 20 days, similar to that developed for rodents to test sustained attention and impulsivity. More recently, a new T-maze task has been set up (Braida et al., 2013a). Zebrafish were trained to reach a reservoir and stay for at least 20 s. The running time difference between the first and the second trial was calculated as a measure of memory of the spatial location of reward. In these conditions nicotine enhanced memory while scopolamine impaired it.

As recently reported by Echevarria et al. (2011), apart from the 3CSRTT, the majority of the above mentioned tasks have not fully assessed attentional ability, a process by which the organism learns and the nervous system receives or acquires sensory input and generates coordinated behaviour of animals (Bushnell, 1998).

Cognitive impairment is a core symptom of neuropsychiatric and neurologic disorders. Attentional deficit has been considered to be one of the most consistent areas of impairment associated with psychiatric and neurodegenerative diseases (Kelip et al., 2008; Perry et al., 2000) as Parkinson's disease, Alzheimer, attention deficit hyperactivity, stroke, epilepsy (Biederman et al., 2006; Rubia et al., 2005; Sahakian et al., 1988; Stretton and Thompson, 2012) and schizophrenia (Lyon et al., 2012). Attention includes a series of cognitive processes and disorders related to attention may underlie cognitive dysfunctions.

The novel object recognition (NOR) test evaluates an animal's attention that is elicited by the presentation of novel stimuli. Interest in NOR

Abbreviations: VORT, Virtual Object Recognition Test; 2D, bidimensional; T1, Familiarization trial; T2, Recognition Trial; IP, Intraperitoneally; 3D, Tridimensional; NOR, Novel Object Recognition Test; WT, Wild Type; NIC, Nicotine; SCOP, Scopolamine; MEC, Mecamylamine.

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is very recent and a large proportion of the literature on cognition has been dedicated to object and visual recognition in humans, pigeons and primates (Spetch et al., 2006; Wallis and Bülthoff, 1999). The principal advantage of the NOR test is the rapid testing sequence and no necessary training other than the initial exposure session. In a recent paper we created a modified version of NOR, named virtual object recognition test (VORT), in mice where the 3D objects were replaced with virtual stationary or moving geometrical 2D shapes presented on iPod screens (Braida et al., 2013b). This test, as that used in humans, is predominantly based on visual stimuli.

In an attempt to maximize the value of zebrafish as an animal model of cognition, we tested the possibility to apply VORT to fish either using stationary or moving 2D shapes.

It is known that cortical acetylcholine release has been implicated in novelty-induced arousal, attention, the encoding of novel stimuli and memory consolidation (Acquas et al., 1998; Sarter and Bruno, 2000). Thus, to test the task validity, different cholinergic drugs were injected intraperitoneally (IP) to fish.

2. Materials and methods

2.1. Animals

Adult short-finned wild-type (WT) zebrafish (*Danio rerio*) (0.4–1 g) of heterogeneous genetic background were obtained from a local aquarium supply store (Aquarium Center, Milan, Italy). Zebrafish were 6–12 months of age and were 3–4 cm long. In all experiments, the sex ratio of zebrafish was approximately 50–50%. Males and females were identified as previously reported (Braida et al., 2012). Fish were kept at approximately 28.5 °C on a 14:10-h light/dark cycle. Behavioural testing took place during the light phase between 09:00 and 14:00 h. Tank water consisted of deionized water and sea salts (0.6 g/10 L of water; Instant Ocean, Aquarium Systems, Sarrebourg, France). Approximately 30 adult fish were maintained in 96 L home tanks (75 cm long, 32 cm wide and 40 cm high) provided with constant filtration and aeration. Animals were acclimated for at least 2 weeks before the experiments. Fish were fed daily with brine shrimp and flake fish food (tropical fish food, Consorzio G5, Italy). The experimental protocol was approved by the Italian Governmental Decree No. 18/2013. All efforts were made to minimize the number of animals used and their discomfort.

2.2. Virtual object recognition test (VORT)

A transparent Plexiglas tank (filled with tank water at a level of 10 cm) was used in VORT. The apparatus (Fig. A) was characterized by a rectangular environment (70 cm × 30 cm × 10 cm). After a week habituation to the apparatus, each fish was restricted for 5 min in a 20 cm central area delimited by two opaque barriers to visually isolate the stimuli areas where two identical white geometrical shapes on a black background were shown on two iPod 3.5-inch widescreen displays located externally to the opposite 10 cm wide walls. Then, each fish was subjected to a familiarization trial (T_1), and after different time delays to a novel shape recognition trial (T_2). Both T_1 and T_2 consisted of a 10 min session during which two identical white geometrical shapes on a black background were shown on two iPod screens.

For static cue, shapes were simple geometrical shapes (square, triangle, circle, cross, etc.) with equal surface (2.5 cm^2). A complete list is given in Fig. 2C. For dynamic cue, the same above shapes moved horizontally, vertically or diagonally (distance 320 px) at a constant speed of 120 px/s. The videos were created in Adobe Flash, with a frame size of 320 pixels × 480 pixels, with a rate of 30 frames per second. The videos were encoded with a video encoder H264 (Apple), a software program that converts information written in one format or code to a different one. Usually, this is carried out in order to standardize information, improve speed and security, and reduce the file in size which then saves space.

The shapes were looped on a 3rd generation iPod Touch (Apple) through iTunes for the duration of the experiment (320 pixels horizontal axis and 480 pixels vertical axis). The luminosity of the screens was constant across the two screens and testing sessions.

After T_1 the fish returned to the home tank. During T_2 each fish was placed again in the central area after different time delays, from 5 min to 96 h, during which one of the two identical static familiar shapes was replaced with a novel one. The time delays and doses were chosen on the basis of our previous work, using the same task, in mice (Braida et al., 2013b). For dynamic cue the same or different shapes presented in T_1 moved in a different direction than in T_1. Attention was paid to counterbalance the choice of the shapes. Within every time delay, all the pairing discriminated shapes were randomly tested.

Shape recognition was manually scored with a stopwatch, by an experimenter blind to the treatment, in terms of exploration time whenever the zebrafish approached to the iPod area (10 cm) and directed its head toward the shape. All the fish were drug naive, and each fish was used only once. 9–11 fish per group were used.

2.3. Drug and treatment

Body weight was measured as previously described (Braida et al., 2007). Fish were removed from their home tank using a net and placed in a container, filled with tank water, positioned on a digital balance. Zebrafish weight was determined as the weight of the container plus the fish minus the weight of the container before the fish was added. The mean of three measurements was recorded. All the drugs were injected IP in a volume of 2 μl/g using a Hamilton syringe, according to Braida et al. (2013a). All drugs were dissolved in sterile saline (0.9%) and were prepared fresh daily. The drugs were: nicotine bi-tartrate salt (Sigma–Aldrich, St.Louis, MO, USA), (0.02 mg/kg) given 20 min before the test; scopolamine hydrobromide (Sigma–Aldrich, St. Louis, MO, USA) (0.025 mg/kg) given 20 min before the test; and mecamylamine hydrochloride (Sigma–Aldrich, St. Louis, MO, USA) (0.1 mg/kg) given 30 min before the test. Control groups received sterile saline solution (0.9%) which was given 20 or 30 min before T_1. The range of doses of nicotine and of antagonists were chosen on the basis of their activity on T-maze task and on the basis that they did not modify swimming behaviour (Braida et al., 2013a).

2.4. Statistical analysis

All data were expressed as mean ± S.E.M. Pair-wise comparisons were assessed with Student's *t*-test. Different groups were assessed by one-way analysis of variance (ANOVA) for multiple comparisons followed by Tukey's post-hoc test. Data were expressed as discrimination index [(time spent exploring novel shape − time exploring familiar shape) / (time spent exploring novel shape + time exploring familiar shape)], as previously described in mice (Braida et al., 2013b). Data from fish receiving saline 20 or 30 min before T_1 were pooled after making sure that there was no statistical difference between the two groups. The level of significance was taken as $p < 0.05$. All statistical analyses were done using software Prism, version 6 (GraphPad, San Diego, CA, USA).

3. Results

3.1. Different time delays progressively decrease memory performance

In Fig. 1 the effects of different time delays on VORT, in terms of discrimination index (Fig. 1A) and the exploration time respectively (Fig. 1B), are shown. One-way ANOVA showed a significant difference among groups in delay time (from 5 min to 96 h) ($F_{(3,36)} = 7.46$; $p < 0.0005$). Post-hoc analysis revealed that the mean discrimination index, evaluated at 96 h, was significantly lower than that evaluated at the remaining intervals. Concerning the exploration time, one-way ANOVA revealed a significant difference among the groups ($F_{(9,90)} =$