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Successive and discrete spaced conditioning in active avoidance learning in young and aged zebrafish

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ABSTRACT

We designed an automated device to study active avoidance learning abilities of zebrafish. Open source tools were used for the device control, statistical computing, and graphic outputs of data. Using the system, we developed active avoidance tests to examine the effects of trial spacing and aging on learning. Seven-month-old fish showed stronger avoidance behavior as measured by color preference index with discrete spaced training as compared to successive spaced training. Fifteen-month-old fish showed a similar trend, but with reduced cognitive abilities compared with 7-month-old fish. Further, in 7-month-old fish, an increase in learning ability during trials was observed with discrete, but not successive, spaced training. In contrast, 15-month-old fish did not show increase in learning ability during trials. Therefore, these data suggest that discrete spacing is more effective for learning than successive spacing, with the zebrafish active avoidance paradigm, and that the time course analysis of active avoidance using discrete spaced training is useful to detect age-related learning impairment.

1. Introduction

Zebrafish (*Danio rerio*), a tropical freshwater fish, is used as a model organism for genetic studies of vertebrate developmental mechanisms. It is particularly notable for its prolific reproductive capacity and easy maintenance, and has been used by researchers to produce numerous transgenic strains. A comparison with the human reference genome shows that approximately 70% of human genes have at least one obvious zebrafish orthologue, and 84% of the genes known to be associated with human diseases have a counterpart in the zebrafish genome (Howe et al., 2013). Therefore, zebrafish can be an efficient vertebrate model system for the study of biological mechanisms of the human diseases.

In recent years, interest in cognition and memory has increased significantly, and the zebrafish has proved to be a useful model for its study (Kalueff et al., 2014; Meshalkina et al., 2016; Oliveira, 2013). Typically, experimental settings for cognitive evaluations often require an accurate control of multiple input and output signals. A low-cost I/O board (Arduino family) is a useful microcontroller for high throughput automation of cognitive evaluation systems (D'Ausilio, 2012), which can be improved further by creating refined protocols with automatic controls. Active avoidance tests are widely used for the analysis of learning in zebrafish. For this test, zebrafish are placed in a two-compartment shuttle box and learn the association on between a conditioned stimulus (CS, e.g., light) and an unconditioned stimulus (US, e.g., electric shock). However, learning efficiency can be misinterpreted if a dark chamber is used as the non-shock compartment, due to its natural preference by zebrafish (Avdesh et al., 2010); therefore, learning tests need further refinement for accuracy and efficacy. It has been shown that learning and memory are enhanced when the information is presented separately over time compared to that presented massed together (Smolen et al., 2016). In zebrafish, spacing effects on learning and memory formation are not well understood. Cognitive ability is acquired during brain development, reaching a peak in the adult stage. Aging is believed to contribute to a change in cognition. A two-year-old zebrafish shows cognitive impairment compared to a one-year-old fish; however, a reduction of the baseline locomotor activity level in the two-year-old zebrafish may lead to misinterpretation of results in active avoidance tests (Yu et al., 2006). Further, individual differences in age-related cognitive decline of zebrafish have not been well studied. Thus, recognizing early symptoms of learning defects using improved tests, without the contributing locomotor ability change, may accelerate research on dementia.

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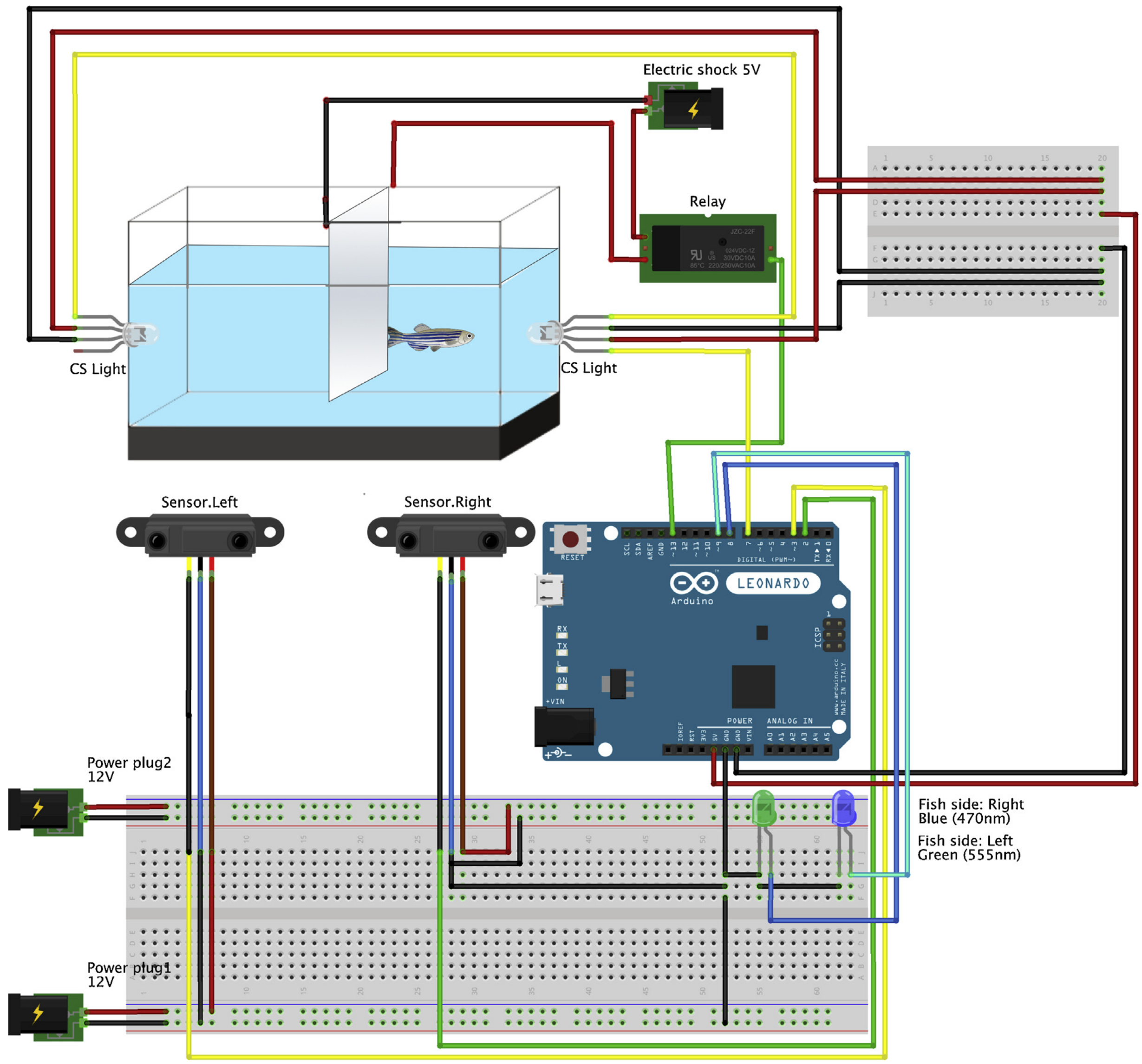

Fig. 1. Schematic drawing of the experimental setup.

2. Method

2.1. Animals and housing conditions

AB strain wild type zebrafish (*Danio rerio*) were raised and maintained under standard conditions for experiments approved by the Institutional Animal Care and Use Committee at Chiba University. Adult (7-month-old and 15-month-old) zebrafish were kept in separate tanks at 28 ± 1 °C pH 7.0, and with a 14–10 h light/dark photoperiod (9:00–23:00 light). All experiments were conducted during the light cycle.

2.2. Apparatus

The zebrafish were trained and tested, individually, in four active avoidance tanks controlled by Arduino Leonardo microcontrollers (D'Ausilio, 2012). As shown in Fig. 1, the active avoidance tank was white in color, with an outside length of 180 mm, width of 125 mm, and depth of 135 mm; the tank was divided, with a white barrier of length 125 mm and width 125 mm, into two equal chambers. The barrier was raised, 1 cm above the bottom of the 5-cm-level of water filled in the tank, to allow the zebrafish to move between the chambers in order to avoid electric shocks. The chamber-change movement of the zebrafish was detected by a light beam sensor (FS-N11CN and FU-L54Z, Keyence) and communicated with the Arduino Leonardo. A red, green, and blue (RGB) light-emitting diode (LED) (PL9823-F8, Shenzhen Rita Lighting Ltd) on each short-side, and two stainless steel woven wire mesh (30 mesh Type 304, q-ho.com) as electrode plates (5 V DC) on each long-side of the tank, were installed. The Arduino Leonardo devices of all the tanks were connected to a personal computer to monitor the progress and output results of the experiments.

2.3. Baseline locomotor activity

Zebrafish were housed and tested separately in active avoidance tanks. We used the Noldus DanioVision system to record baseline locomotor activity, with white background light, for 1 h. Baseline locomotor activity was measured as the mean of distance covered per minute.

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