Can robotic fish help zebrafish learn to open doors?

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ABSTRACT

Zebrafish is a widely used animal model in behavioral neuroscience. However, zebrafish learning capabilities are not completely understood. Technological advancements in robotics promise fine behavioral control of artificial conspecifics to study complex aspects of social behavior. In this work, we developed a training system aimed at investigating individual and social learning of zebrafish. The system consists of a shallow water tank, a 2-dimensional robotic platform, and a real-time tracking software. In the tank, a focal individual is separated from a shoal of conspecifics by a one-way glass and a transparent partition, allowing the focal fish to see the shoal. In the transparent partition are two doors, one that automatically opens when the focal individual spends a predetermined amount of time in front of it and another that remains closed regardless of the fish behavior. We tested the system by training one naïve fish in individual learning and one fish in social learning over 20 sessions. Test results show that the fish can learn to open the door and also validated the effectiveness of the developed system applying on individual and social learning.

Keywords: Zebrafish, social learning, biorobotics, live tracking

1. INTRODUCTION

Zebrafish (*Danio rerio*) is an increasingly popular animal model due to its small size, high reproduction rate, and ease of maintenance.[1] It has been widely used for the study of social behavior,[2] brain functions,[3] pharmacology,[4] and genetics.[3] Studying learning in zebrafish can help clarify the genetic underlying mechanisms of learning and memory. Previous work has demonstrated the learning ability of zebrafish for a range of learning tasks. For example, zebrafish can remember their environment after being allowed to explore it.[5] Also, zebrafish performed very well in a plus maze experiment, showing significant acquisition of the association between cue and reward, as well as between location and reward.[6]

Although individual learning in zebrafish has been widely studied, the mechanisms underlying social learning are still unclear. Specifically, observational learning of zebrafish has not been fully explored and is still poorly understood. Some authors considered observational learning as synonymous to imitation, which is defined as the acquisition of a topographically novel response through observation of a demonstrator making that response.[7,8] Recent work on social learning of escape routes in zebrafish demonstrated that zebrafish can socially learn

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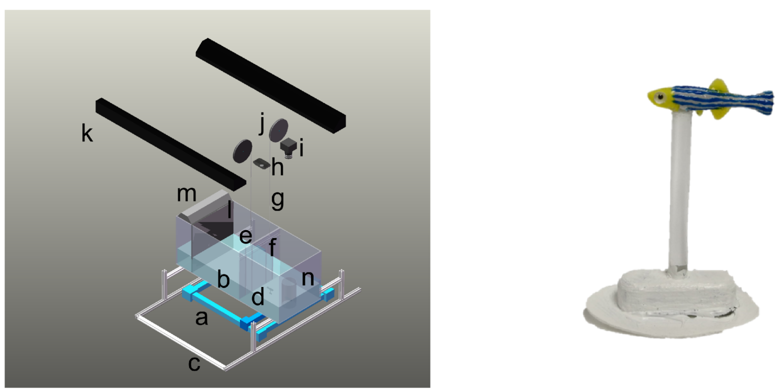
particular escape routes and learn to escape faster from an approaching threat based on the behavior of a demonstrator.[9] When studying observational learning, one important challenge is how to control the information demonstrated to the focal subject. These studies are generally performed using live conspecifics as demonstrators. Although using live animals as demonstrators can help understand social interactions and information transfer, it is difficult to fully control the behaviors of animals and clearly identify how information is transmitted. The need for control and standardization could be met through animal-robot interactions.[10–13]

In this work, we developed a training system for the investigation of individual and observational learning of zebrafish in a spatial discrimination experiment. Specifically, across 20 training sessions, focal fish separated from a shoal of conspecifics by a transparent partition and a one-way glass are trained to get closer to the shoal by opening a door. With respect to individual learning, the live fish was alone and allowed to explore the experimental arena. Through exploration, the fish would trigger a door to open by staying inside a predefined triggering region for a chosen amount of time. With respect to social learning, a 2-dimensional platform with a bioinspired conspecific replica was used to demonstrate the correct door and the wrong door. Our pilots results showed that zebrafish were able to learn to open the doors in both cases and that developed system could support research on both individual and social learning.

2. EXPERIMENTAL APPROACH

2.1 Robotic platform

To perform social learning experiments, a two-dimensional platform under the experimental tank was used for controlling a robot mimicking the motion of a live fish, as shown in Fig. 1. The platform was based on a Cartesian plotter (XY Plotter Robot Kit, Makeblock Co., Ltd, Shenzhen, China), with its moving part carrying a step motor, on which a 3D printed base holding two magnets was attached. The plotter could be remotely controlled to move and rotate the replica.



(a) (b)

Figure 1. **(a)** Experimental apparatus: **a** 2-dimensional platform; **b** Experimental tank; **c** Aluminum frame made by T-slot bars; **d** Replica robot; **e** and **f** Doors made by acrylic sheet; **g** Fishing line; **h** Logitech Webcam; **i** Flea3 camera; **j** Pulley system; **k** 36-inch light; **l** One-way glass; **m** 12-inch light; **n** Cylinder and live fish; **(b)** The flexible zebrafish replica.

To fabricate the replica for the current work, a mold based on a live fish was first designed in Solidworks (Dassault Systèmes SolidWorks Corp., Waltham, Massachusetts, USA) and built using a 3D printer (Ultimaker 2+, Ultimaker B.V., Geldermalsen, The Netherlands). Then, the mixture of two silicone parts (Smooth-On, Inc., Macungie, PA, USA) was poured into the mold to generate the flexible and transparent replica. The replica was painted by silicone-based paint (Smooth-On, Inc., Macungie, PA,USA) to make its coloration more realistic, as shown in Fig. 1.

2.2 Experimental apparatus

A transparent glass tank (74 × 30× 30 cm; length, width, and depth) with its walls and bottom covered with contact paper was supported by a frame built with T-slot bars (McMaster, Robbinsville, NJ, USA), directly above the platform, and 29 cm above the floor. Two partitions were fixed in the tank along the lengths of 30 cm and 64 cm to separate the tank in three chambers, as shown in Fig. 1. One partition contained two doors, each 1.5 body lengths (BLs) wide, to ensure the smooth passage of both the robot and the fish. The two doors were mounted on the transparent partition (McMaster, Robbinsville, NJ, USA) along the width of 1/4 and 3/4 and two acrylic pieces were glued on each side to limit the movement of the doors to the vertical axis. The second partition was made of a 5.9 mm thick one-way glass, separating the fish shoal from the focal subject, in order to motivate the focal subject to come closer without interference from the shoal members.

Each door was remotely lifted and released by a pulley via a transparent fishing line (Berkley Trilene XT Extra Tough, Pure Fishing, Inc., Columbia, SC, USA) tethered to a servo motor (HS-5086 WP, Hitec RCD USA, Inc., Poway, CA, USA). The motors were controlled by an Arduino Uno microcontroller (Arduino Srl, Italy). Three fluorescent strip lights (All-Glass Aquarium Co., Inc, Franklin, Wisconsin, USA) were used to provide the illumination condition for the experiments: the 12-inch light with a power of 8 W was mounted on the top of the chamber for the fish shoal to ensure subject see the fish shoal while two 36-inch lights with a power of 30 W along the sides of the tank were used to illuminate the whole setup. Live tracking of the position for the focal subject was realized by a Logitech C920 (Newark, CA, USA) webcam with a resolution of 640 × 480 pixels. Simultaneously, a Flea3 FL3-U3-13E4C USB camera (FLIR Integrated Imaging Solutions Inc., Richmond, BC, Canada), with a higher resolution of 1280 × 1024 pixels, captured the overview of the setup. The whole setup was surrounded with black curtains to avoid the external visual stimuli to the live fish.

2.3 The tracking and control system

A real-time tracking and control system was developed in Matlab 2018a (The MathWorks, Inc., Natick, MA, USA) to autonomously control the experimental variables, as illustrated in Fig. 2. The motion-based live tracking algorithm was based on the Matlab computer vision toolbox. Specifically, at each time step, an initial image mask was first obtained by the subtraction of two continuous grey-scale frames, which were captured by the webcam with a frame rate of 20 fps and were cropped according to the predetermined region of interest. After filtering the noise and filling in the holes in the initial mask, the processed mask was used to locate the centroids of moving subject by blob analysis. Then, a rectangular region surrounding the tracked centroids was filtered with a predefined threshold and used to determine the precise position of the live fish by morphological operation and blob analysis. If the two steps failed to detect the position of the subject, a Kalman filter was used to predict the position based on the history of the trajectory. In addition, a region of 2 × 2 BL in front of the correct door was selected as a triggering region for the timing of the focal subject in front of the door so that the correct door can be opened autonomously according to the triggering threshold.

The motion control for the robot was based on the time series position of zebrafish generated by a stochastic mathematical model of zebrafish swimming, which we developed in Refs. 14 and 15. At each time step, a PC sent the movement command to the platform via an Arduino Uno microcontroller with a BaudRate of 115200 baud and monitored the presence of the fish. When the presence of fish reached the triggering threshold of the door (3 out of 5 s), the PC would send the commands to doors via another Arduino Uno with a BaudRate of 9600 baud.

2.4 The mathematical model of zebrafish

To include social interaction between robot and the live fish, the motion of the robot was generated based on a mathematical model developed in our previous work.[14,15] Specifically, the turn rate ω_t with a jump characteristic and the forward speed U_t of zebrafish can be described by two stochastic differential equations

$$\mathrm{d}\omega_t = \theta_\omega(\omega_t - F_t^\omega - I_t^\omega)\mathrm{d}t + C_U^\omega \mathrm{d}W_t + \mathrm{d}J_t, \tag{1}$$

$$\mathrm{d}U_t = -\theta_U(U_t - m_U - I_t^U)\mathrm{d}t + N_U \mathrm{d}W_t^*, \tag{2}$$

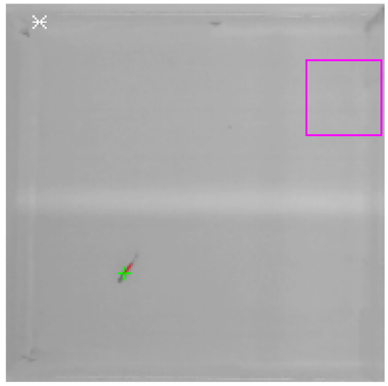


Figure 2. A screen shot of the live tracking system. The magenta square shows the triggering region. The indicator on the left corner was used to show the presence state of fish in the triggering region. The green cross represents the current centroids of the fish. The red line shows the trajectories of fish in the last five time steps.

where θ_ω and θ_U are mean reversion coefficients; $\theta_\omega F_t^\omega \mathrm{d}t$ describes wall avoidance; I_t^ω and I_t^U reflect the feedback from the sought social interaction with the live fish; C_U^ω is a function of the speed U_t, which is used to capture the interaction of turning motion and forward speed; W_t is a standard Wiener process; J_t represents the jump process; m_U is the nominal speed; and N_U weights the noise of another standard Wiener process W_t^*. Details of the model can be found in Refs. 14 and 15.

2.5 Experimental procedure

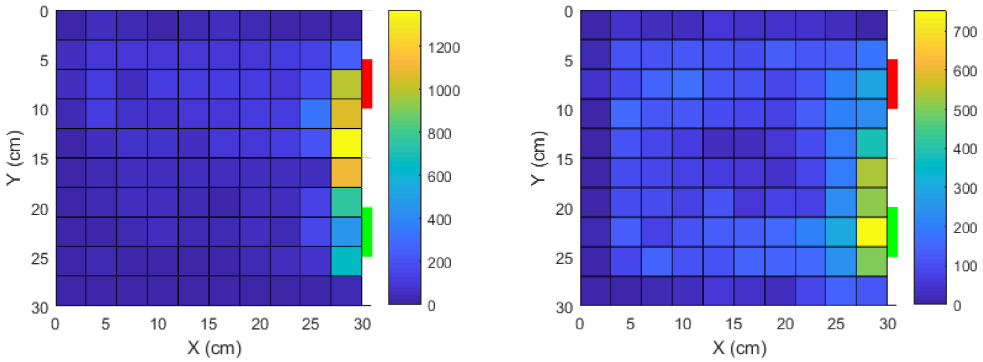
Adults zebrafish were acquired from Carolina Biological Supply Co. (Burlington, NC, USA). The experimental procedure were approved by the University Animal Welfare Committee of New York University under protocol number 13-1424.

Both conditions started as follows: the focal fish was placed in a transparent cylinder (8 cm diameter) for habituation; after 10 minutes, the cylinder was lifted, releasing the focal subject and allowing it to explore the arena. In the individual learning condition, the focal subject was alone during the whole trial. In the social condition, an expert robot would demonstrate the correct and wrong doors to the focal subject during the habituation time following this sequence: the robot would interact with the focal fish in the cylinder for 30 s. Then the robot would swim towards the correct door. After the door was opened, the robot would go through the door and do tail-beating motion at a far point for 5 s. After the robot went back to the focal chamber, it would start interacting with the subject for 30 s, then swim towards the wrong door, which would not open. After repeating the procedure 6 times, the robot would stay at a farthest position along the central line of the middle chamber, and the focal fish would be released from the cylinder.

Following the successful opening of the door, the fish was rewarded by letting it interact for 2 minutes with the fish shoal after going through the correct door. Each focal subject was trained twice per day over 20 sessions. To study the spatial preference of zebrafish, three tests were performed: one before any training session, a second after 10 training sessions, and a third after 20 training sessions. During a test, the fish was alone and allowed to explore the focal chamber for 10 minutes, while the door were kept closed.

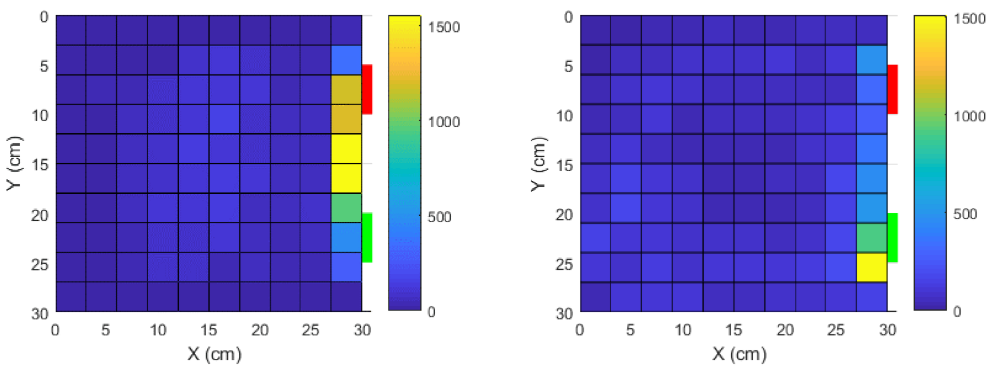
3. RESULTS

Test results for two pilot fish, one for individual learning, and one for social learning, are shown in Figs. 3 and 4 respectively. Before any training, fish spent most of the time in the regions near the partition with doors, which indicates that the fish shoal was attractive to the focal subject. Namely, the focal fish was motivated to open the door, validating the experiment design and apparatus. We also found that, before the training procedure, the fish spent most of the time in the middle region between the two doors. Both fish preferred the regions in front of the correct doors, showing that the fish learned to identify spatial position of the correct door following the training procedure.



(a) (b)

Figure 3. Evolution of position distribution for a single fish in individual learning condition, separating (a) the initial test before training, and (b) the final test after 20 training sessions. The grid size is 1 × 1 BL. The color shows the number of time steps for which the fish appears in each grid. The red and green markers are the correct and wrong doors, respectively.



(a) (b)

Figure 4. Evolution of position distribution for a fish in the social learning condition, separating (a) the initial test before training, and (b) the final test after 20 training sessions. The grid size is 1 × 1 BL. The color shows the number of time steps for which the fish appears in each grid. The red and green markers are the correct and wrong doors, respectively.

4. CONCLUSIONS

The present study explores the use of a robotic replica as an alternative to a live conspecific to serve as a demonstrator teaching a complex task to zebrafish. We developed a robotic platform that, coupled with live video-tracking, allows a remote-controlled replica to interact with live zebrafish and show them how to open a door in order to get closer access to a reward such as a shoal of conspecific.

Our pilot trials suggest that the fish shoal can provide enough motivation for the focal fish. At the same time, zebrafish are able to learn to open a door and identify the spatial position of the correct door, measured by their time distribution spent in the focal region. Our study indicates the feasibility of using interactive robots to study complex behaviors such as social learning. A comprehensive experimental study with a large fish population is ongoing, and we hope to report soon on it in a journal publication.

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