Extending the T-maze to Evaluate Socially Motivated Behavioral Profiles in Adult Zebrafish

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INTRODUCTION

Zebrafish are highly social and exhibit complex behaviors whose neural and genetic basis is not well understood. We designed a behavioral paradigm to simultaneously test multiple behavioral characteristics, namely, associative learning, decision making, social motivation, anxiety and short-term memory as well as exploratory tendencies. We used visual social reward for motivating directional turns in freely-swimming adult zebrafish, *Danio rerio*. Our goal was to minimize handling stress, while allowing animals to continuously learn. To enable this, we extended the T-maze by adding a return arm with a 90° turn at each end of the "T". This required the test subject to make four successive turns to return to its original release location. Gates placed at strategic locations closed behind the fish to facilitate forward swimming. Exploration allowed the fish a glimpse of conspecifics placed within an inner tank. This prompted the test subject to make a second turn and approach conspecifics via the return arm. Finally, preference for a dark environment (safety) lured the test subject to make two more turns before reaching the original release location. For each successive test, recall of social reward (memory) motivated the fish to re-explore the maze and make either right- or left-turn decisions. Three to five successive reward-driven turns to the rewarded side ended the trial and generated a logistic learning curve. Significant learning occurred within 30 minutes (n=15). In addition, relative-time spent within each section of the maze generated a socially-motivated behavioral profile with "decision-making" and "frustration" phases exhibiting the highest variation across decision cycles for most individuals. Only males were tested in the current study. Our paradigm can be adapted to study behavioral characteristics in many species.

BACKGROUND

- Zebrafish are an excellent model organism for neurological studies.
  - Commonly used in genetics, oncology, and developmental biology
  - Genome completely sequenced
  - Rich expression of innate behavior (e.g. dominant and submissive behavior, food searching behavior, shoaling)
  - Easily maintained and bred under laboratory conditions.
- **Hypothesis:** Test the possibility of rapid behavioral profiling in zebrafish learning to navigate an extended T-maze.

METHODS: Materials

- **Zebrafish maintenance**
  - Rearing in 14:10 light:dark cycle
  - Fed daily with brine & dried flake food
  - Building the extended T-maze with Plexiglass material.

METHODS: Setup, Training and Tracking

1 Experimental Design

- Examine zebrafish social behavior to create a baseline for normal social interactions within a group.
- Create a learning maze using social interaction as a reward.
- Collect and analyze data for rapid learning in adult wildtype adult zebrafish.
- Collect and analyze data for behavioral profiling of adult wildtype zebrafish learning.

2 Social behavior study

- Dominant fish display aggressive behavior towards subordinates - chasing and biting.
- Dominant fish remain primarily isolated while subordinates remain in groups.
- Proportions of wins and losses can be used to determine social hierarchies.

3 Video-based Analysis

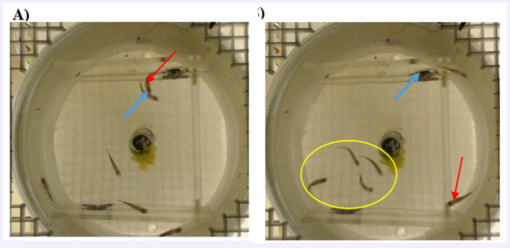
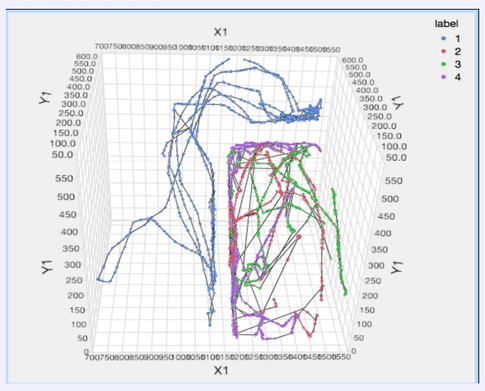


Fig. 1. **Development of socially motivated dominance hierarchies**. Placement of fish within a tank leads to the identification of a dominant individual (red arrow) that tends to observe conspecifics (within yellow oval) from its "personal" space (B). This is preceded by "dashes" (high speed swimming) and chases towards conspecifics (A).

Fig. 2 (below). **3D scatterplot generated form tracking data using iDTracker software** (Pérez-Escudero et. al., 2014)**.** Tracks show attraction of a test fish (blue trace) to 3 target fish (conspecifics) placed within a subenclosure (red, green & purple tracks). Social behavior studies provided motivation to develop a socially-motivated navigational training paradigm for zebrafish.



4 Extended T-maze setup

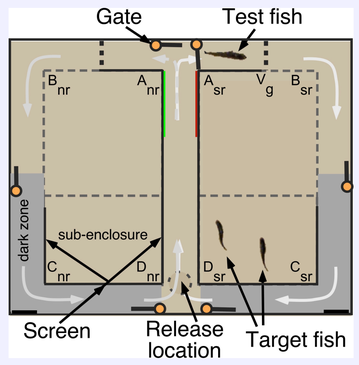


Fig. 3. **Diagrammatic representation of the social reward-driven extended T-maze.** Fish can be continuously and rapidly trained via a social reward-based paradigm involving navigation and swimming.

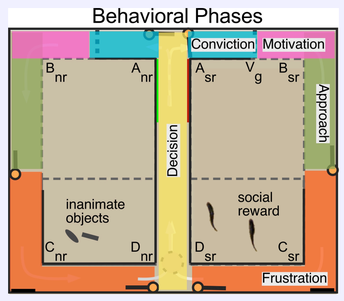


Fig. 4. **Behavioral profiling zones labeled within the extended T-maze setup**. To complete one "run" or cycle of training, fish have to navigate (swimming forward only) to reach the initial release location. Gates at key decision-making landmarks (see fig. 3) are sequentially closed after a fish voluntarily passes that location.

RESULTS

5 Socially-motivated navigation

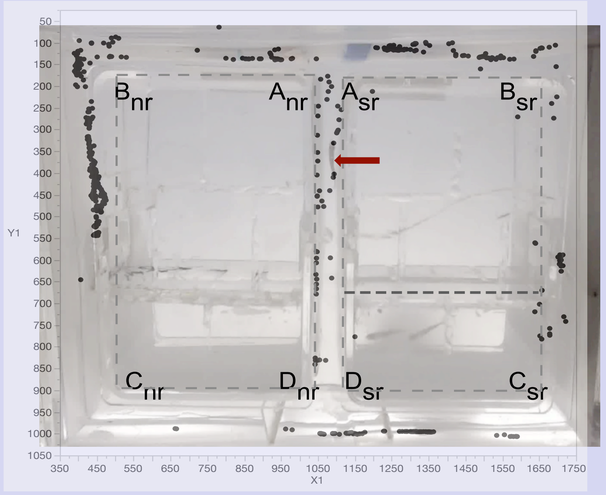


Fig. 5. **Screen capture of a single video frame.** Photograph shows the Test fish (arrow) swimming up the stem of the "T"-maze. Target fish (conspeciics) are placed in the sub-enclosure within the main tank. The test fish has to make a first turn in the correct direction to observe conspecifics and then a second turn to approach them. Once the fish gets close to conspecifics, gates close to facilitate unidirectional swimming, leading to repeated turns to continuously observe and approach conspecifics. iDTracker software (Pérez-Escudero et. al., 2014) shows fish locations prior to training.

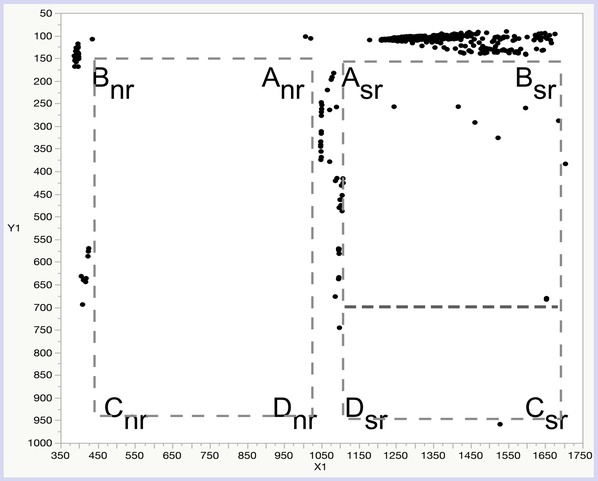


Fig. 6. **Scatterplot generated form tracking data post-training.** Downsampled data to show fish locations (recorded at 30 fps) post-training. Fish turn towards the social reward side (right) almost exclusively after training.

6 Socially-motivated learning & memory

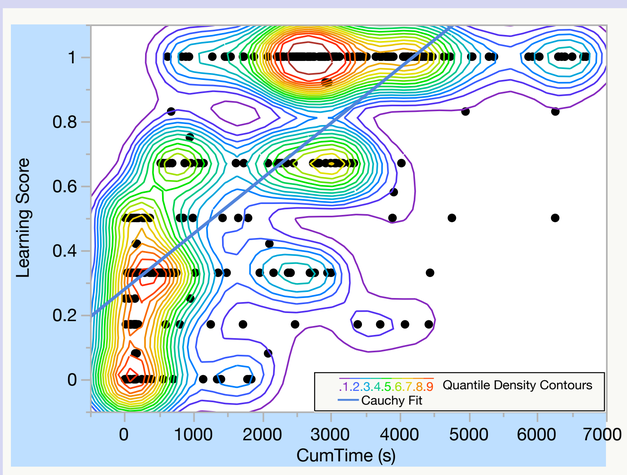


Fig. 7. **Nonparametric density plots indicating learning in wildtype zebrafish.** A Learning Score was generated from successive turns in the "correct" direction. Straight line represents cauchy fit (JMP software), indicating, on average (n = 11) max Learning Score at ~ 50 min.

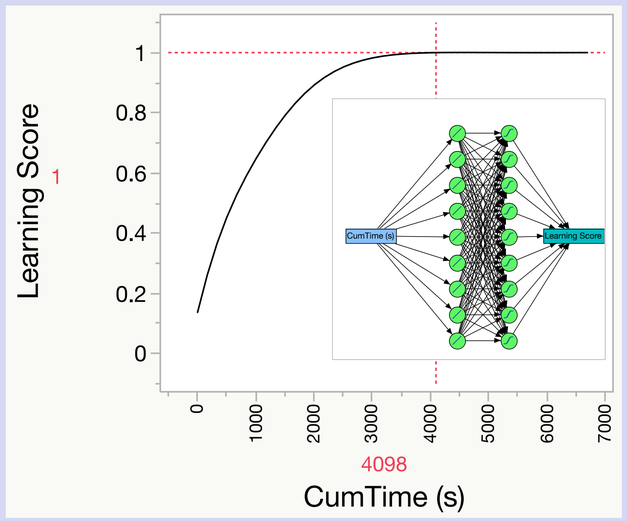


Fig. 8. **Learning curve with best fit generated using a neural network demonstrating rapid learning in wildtype adult zebrafish.** A mixture of TanH and Sigmoid distributions were used to train a 2-layered network (see inset) to fit the behavior of zebrafish during training (n = 11 fish). Fish were pseudorandomly rewarded either on the left or the right side of the T-maze during any one training session. On average, learned behavior is consistently observed after 70 minutes and significant learning (Learning Score of 0.75) in ~30 min.

RESULTS

7 Social reward-driven behavioral profiling

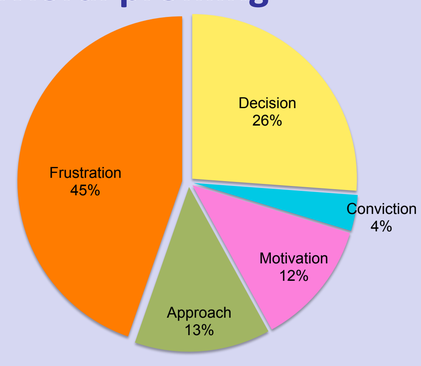


Fig. 9. **Pie chart showing the relative duration in which the fish was situated in a particular zone while negotiating the maze**. A large portion of time was spent in the "Frustration " zone as the fish tried to find the best way to get another glimpse of the conspecifics. A disproportionately long time was also spent in the decision-making zone.

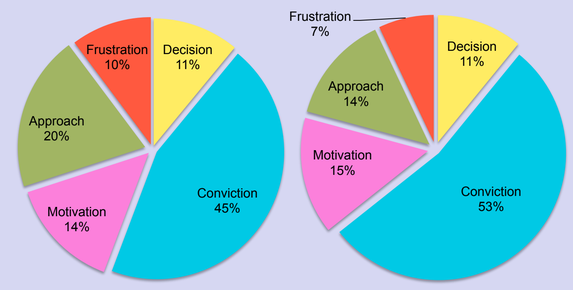


Fig. 10. **A pair of pie charts comparing relative swim speeds in different zones of the setup prior to- and post-learning.**

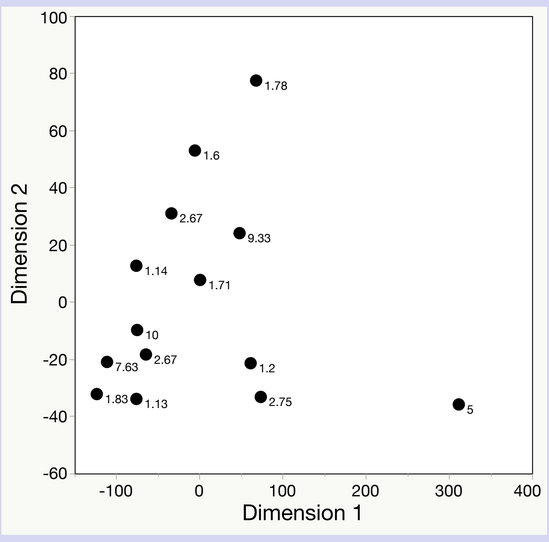


Fig. 11. **MDS plot to visualize dissimilarities in behavioral profiles of fish with different Learning Indices** (LI's from 1.13 to 10). Fish with similar LI's can be placed far apart and those with different LI's in close proximity. Configuration explains 99% of variation.

SUMMARY

1. We demonstrate the feasibility of an extended T-maze to test learning and memory in zebrafish using social motivation as reward.
2. Our navigational learning paradigm provides a quick method to train and test learning deficiencies in adult zebrafish.
3. Our training paradigm also provides a quick method for behavioral profiling of adult zebrafish.

REFERENCES & SUPPORT

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