Spatial Localization Ability of Planarians Identified Through the Light Maze Paradigm

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Author Contributions: Renzhi Qian and Yuan yan contributed equally to this work. Kaiyuan Huang, Renzhi Qian and Yuan yan designed research. Renzhi Qian, Yuan yan, Yixuan Zhang, Yuanwei Chi , Yuxuan Chen, Kun Hao, Zhen Xu performed research. Guang Yang, Zilun Shao, Yuhao Wang, Xinran Li, Chenxu Lu and Yu Pei analyzed data. Kehan Chen draw blueprint of the maze. Wenqiang Zhang, Baoqing Wang and Zhengxin Ying gave suggestions to the research. Kaiyuan Huang wrote the manuscript.

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Abstract

Spatial localization ability is crucial for free-living animals to fit the environment. As shown by previous studies, planarians can be conditioned to discriminate a direction. However, due to their simplicity and primitiveness, they had never been considered to have true spatial localization ability to retrieve locations of objects and places in the environment. Here, we introduce a light maze training paradigm to demonstrate that a planarian worm can navigate to a former recognized place from the start point, even if the worm is transferred into a newly produced maze. This finding identifies the spatial localization ability of planarians for the first time, which provides clues for the evolution of spatial learning. Since the planarians have a primitive brain with simple structures, this paradigm can also provide a simplified model for a detailed investigation of spatial learning.

Main Text

Introduction

Spatial localization, the ability to learn and remember locations and routes, is crucial to survival for most animals. The ability can be used to forage for food, return to sites of storage or safety, and avoid sites of danger, thus helping animals survive and fit the environment. Thus this ability is considered to have evolved in almost all species.(1)

Planarians are free-living flatworms that usually live and stick under rocks, debris and water plants in streams, ponds, and springs(2). Although it is considered one of the first class of animals to have a centralized brain structure, it had evolved various sensory abilities, including sensitivity to light(3), temperature(4), water currents(5), chemical gradients(6), vibration(7), magnetic fields(8) and electric fields(9). For freely living in the environment, it is easy to think that planarians have evolved spatial localization ability. The study of planarian behavior mainly focused on simple classical conditioning and proved that planarians can learn simple associative tasks. Moreover, planarians are concluded to have abilities to discriminate directions in a Y or T maze(10). However, due to the primitiveness and simplicity of planarians, they are not considered to have the ability of true, complex spatial localization ability(11).

To prove the hypothesis that planarians have evolved spatial localization ability, we designed a light maze paradigm inspired by the Morris water maze paradigm(12). The Morris water maze designed by Morris in 1981, is widely used to evaluate the rats' spatial localization ability through distal localization rather than proximal localization(12). Proximal localization is when the goal object is visible, audible or detectable by smell, and distal localization is when the goal object is invisible, inaudible and cannot be detected by smell. The Morris water maze uses water as the aversive stimuli to prompt the rats to swim to a platform. Since planarians have innate behavior to avoid light(3), similar to the Morris water maze, we chose light as the aversive stimuli to prompt planarians to move to the dark chamber we designed in the maze. The planarians' primitive eyes can only sense the light rather than imaging; thus, they cannot see the dark chamber. To avoid any chemical information that a worm might leave in the dark chamber, on trial 4, all of the mazes are changed to newly produced ones. Thus, the information that a worm use to navigate to the dark chamber can only be distal.

As illustrated above, in this finding, we use the light maze paradigm to demonstrate that planarians are able to use distal information to localize a dark chamber in the light maze. This finding proves that the planarian worms have spatial localization ability, which is not yet discovered, indicating that planarians might have higher cognition ability that we had never considered. As a primitive forerunner of later animals, the study of planarian cognition can be evolutionarily instructive to the study of later animals. Also, since the planarians' nervous system has simple structures, it can provide a model for spatial learning studies.

Results

To investigate the spatial localization ability of the planarians, we designed the light maze. It is a square maze with a dark chamber near one corner (Fig1.A). The dark chamber has only one entrance. In the light maze paradigm, each worm went through a training trial each day from 8:00 p.m. to 9:00 p.m. for a total of 6 training trials. In each training trial, a planarian is put in the start point (opposite the dark chamber corner) and prompted by the light to find the dark chamber. In trial 4, all of the mazes are changed to newly produced ones to prevent chemicals priorly secreted by the worms that might lead the worms to the dark chamber. The worms are fed from 8:00 a.m. to 10:00 a.m. on day 1 and day 4 to control worms' satiety.

We totally trained 35 worms (1 worm is excluded due to self-fission in the training process) using the process above and measured the time for each worm to get into the dark chamber (Fig2.A). During the training process each day, only partial worms showed learning effects and get to the dark chamber fast. Other worms cannot get to the dark chamber in a short time and choose to rest outside under the light very soon. Therefore, to evaluate the worms' learning effect, we set the maximum time at 40 mins for the worms that did not enter the dark chamber in the trial. If a worm stopped moving outside the dark chamber for more than 20 mins, its data from that single trial is excluded. We also counted the worms that used less than 20 mins to get to the dark chamber and did not come out in 30 mins, considering them as worms that showed learning effects. From trial 3 to trial 6, there are 10-12 worms that showed learning effects in each trial (Fig2.B). Comparing trial 4 to trial 2, 3, 5 and 6, there is no significant difference, showing that the worms might not use chemical cues in this case (Fig2.A).

To further understand the worms' learning effect, we drew the typical routes of trial 1 and trial 4 of 2 worms which showed the learning effect in trial 4 (Fig2. D-G). In trial 4, the mazes are newly manufactured, and no chemicals or mucus can affect the navigation of the worms. As the worms did not know the dark chamber priorly, the routes of trial 1 were mainly distributed on the walls of the maze. In trial 4, the worms that showed learning effects quickly secede from the wall of the maze and find the entrance of the dark chamber, demonstrating that worms can use distal information to navigate to their destinations.

Discussion

From the 1950s to the 1960s, numerous studies had been done to understand planarian learning and behavior(13). The most commonly used procedure is a classical conditioning protocol to make worms associate a light stimulus with an electrical stimulus(14). Some studies showed that planarians start eating faster in a familiar environment than in an unfamiliar one. Other studies showed that worms can learn more complicated tasks such as discriminating directions in a Y or T maze(10, 15). However, due to the manual practical difficulties and the control of variables, a large number of research results failed to be reproduced in lots of cases(13). Consequently, this field became largely abandoned, and even the ability of planarians to form long-term memory was questioned(16).

Some classical conditioning paradigms of the planarians can be attributed to pseudoconditioning or sensitization instead of true learning and memory encoded by the brain(17). While spatial learning is a more complex behavior that requires the integration of different neural circuits in higher animals(18). Therefore, spatial learning can be a great point to investigate the memory and learning of planarians. As in our paradigm, we applied the light maze paradigm to identify the spatial localization ability of the planarians, which can be regarded as true learning and memory.

The control of variables and manipulations of the experimenters can also largely affect the worms' behavior. In previous studies, including light intensity(19), water temperature(20), water existence(10), time of day(20), time of year(21), chemical components of water(21), chemical components of food(22), worm's appetite level(23), slime trails(24), worm fatigue state(21), magnetic fields(25), training conditions and manipulation of the experimenter(21) were considered to affect the worms' behavior. Our light maze paradigm was designed to decrease the number of variables as much as possible to make the experiment easy to reproduce. In our paradigm, the light intensity, water components, water temperature, appetite level and training time of day are needed to be controlled, and we strictly controlled these variables in the experiment.

The results demonstrated that partial worms showed learning effects in each trial. A worm might show learning effects in some trials but not in other trials. In each trial except for trial 1, more than one-third of the worms that did not enter dark chamber quickly stopped moving. We think that this phenomenon might be caused by multiple factors. The habituation phenomenon might be one factor. In trial 1, When the worms habituate to the environment and light stimulation, it might have ignored the light and choose to rest under the light. Also, the navigation ability of planarians might be primitive, and cannot accurately solve every task. So, many worms cannot solve the task in some trials.

Although we endeavored to exclude proximal cues that worms might use to navigate, we still do not understand which kind of distal cues the worms might have utilized. We speculate that the worms used the light intensity gradient combined with the geometric information of the maze to navigate to the dark chamber. The detailed mechanisms of the worms' navigation still wait to be investigated.

In conclusion, we identified the spatial localization ability of planarians by presenting a new paradigm using the light maze we designed. The paradigm can also be a new tool for the analysis of spatial learning in planarians. In this work, planarian worms were found to use distal cues instead of proximal cues to navigate to the dark chamber. Identifying the spatial localization ability of such a primitive invertebrate might provide insights into the evolution of spatial learning.

Materials and Methods

Planarians A laboratory strain of *D. japonica*, originating from wild collected *D. japonica* (identified by cytochrome c oxidase subunit 1 gene) from the Cherry-Valley in Beijing Botanical Garden, Haidian district, Beijing, China in 2019. Worms are maintained in Montjuic Water(26) in the dark and fed with chicken liver twice a week on Monday night and Thursday night. The length of planarians used in the experiment varies from 1 cm to 1.5 cm.

Experimental Setup 3D-printed mazes are used for the whole training process. The material used for 3D printing is photosensitive resin and PLA (Polylactic acid), the main body of the mazes is printed in white using photosensitive resin, and the dark chamber lid is printed in black using PLA (The dark chamber lid can be detached from the main body of maze). The inner side of the mazes is coated with Parylene film (thickness of 12μm) to block the toxicity from the resin(27). The structure and parameters of the maze are shown in (Fig1.C). During training, the mazes are put in a line on a black acrylic board to avoid light reflections, and each maze is filled with 15 mL of Montjuic Water. A LED lighting tube is set above the entrance of the dark chamber(Fig1.B) to make the entrance lighted and avoid. The distance between the bottom of the LED lighting tube to the acrylic board is 125 mm. The light intensity distribution in the maze is measured and shown in (Fig). Worms are carefully manipulated through a smooth woolen brush.

Training Procedure To save cost, we used 6 mazes to train the total 24 worms in the first 3 trials. In trial 4, all mazes used to train the worms are newly manufactured. The training procedure started at 8:00 p.m. and ended at 9:00 p.m. each day for a total of 6 trials in 6 days. 1 day before the training, worms are taken out from the home well to a petri dish. Worms are fed at 10:00 a.m. on day 1 and day 4 in a 12-well plate, each worm in a single well. Before training, worms are taken out to a 12-well plate, then the water in the mazes is changed(add to 15 mL) and the mazes are set up as described above. Then the worms are put in the starting point of their own maze. The room temperature during training is controlled at 20±1 ℃. After the training, the maze along with the acrylic board is put in an incubator to control the environment temperature.

Statistical Analysis All data were analyzed using PRISM (GraphPad Prism 9.0.0(121)). Routes are drawn by hand with Photoleap(2.13).

References

1. Vorhees CV & Williams MT (2014) Assessing Spatial Learning and Memory in Rodents. *ILAR Journal* 55(2):310-332.
2. Vila-Farré M & J CR (2018) The Ecology of Freshwater Planarians. *Methods in molecular biology (Clifton, N.J.)* 1774:173-205.
3. Shettigar N*, et al.* (2017) Hierarchies in light sensing and dynamic interactions between ocular and extraocular sensory networks in a flatworm. *Science advances* 3(7):e1603025.
4. Inoue T, Hoshino H, Yamashita T, Shimoyama S, & Agata K (2015) Planarian shows decision-making behavior in response to multiple stimuli by integrative brain function. *Zoological Letters* 1(1):7.
5. Allen GD (1915) Reversibility of the Reactions of Planaria Dorotocephala to a Current of Water. *Biological Bulletin* 29(2):111-128.
6. Mason PR (1975) Chemo-klino-kinesis in planarian food location. *Animal behaviour* 23(2):460-469.
7. Anonymous (!!! INVALID CITATION !!! {}).
8. Brown FA & Chow CS (1975) Differentiation between Clockwise and Counterclockwise Magnetic Rotation by the Planarian, Dugesia dorotacephala. *Physiological Zoology* 48(2):168-176.
9. Brown HM & Ogden TE (1968) The electrical response of the planarian ocellus. *The Journal of general physiology* 51(2):237-253.
10. Corning WC (1964) Evidence of Right-Left Discrimination in Planarians. *The Journal of Psychology* 58(1):131-139.
11. Perry CJ, Barron AB, & Cheng K (2013) Invertebrate learning and cognition: relating phenomena to neural substrate. 4(5):561-582.
12. Morris RGM (1981) Spatial localization does not require the presence of local cues. *Learning and Motivation* 12(2):239-260.
13. McConnell JV (1966) Comparative Physiology: Learning in Invertebrates. 28(1):107-136.
14. Thompson R & Mc CJ (1955) Classical conditioning in the planarian, Dugesia dorotocephala. *Journal of comparative and physiological psychology* 48(1):65-68.
15. Best JB & Rubinstein I (1962) Maze learning and associated behavior in planaria. *Journal of comparative and physiological psychology* 55:560-566.
16. Travis GDL (1981) Replicating Replication? Aspects of the Social Construction of Learning in Planarian Worms. *Social Studies of Science* 11(1):11-32.
17. Kartry AL, Keith-Lee P, & Morton WD (1964) PLANARIA: MEMORY TRANSFER THROUGH CANNIBALISM REEXAMINED. *Science (New York, N.Y.)* 146(3641):274-275.
18. Lin X*, et al.* (2021) Noncanonical projections to the hippocampal CA3 regulate spatial learning and memory by modulating the feedforward hippocampal trisynaptic pathway. *PLoS biology* 19(12):e3001127.

19. Harden Jones FR (1971) The response of the planarian Dendrocoelum lacteum to an increase in light intensity. *Animal behaviour* 19(2):269-276.
20. Vandeventer JM & Ratner SC (1964) VARIABLES AFFECTING THE FREQUENCY OF RESPONSE OF PLANARIA TO LIGHT. *Journal of comparative and physiological psychology* 57:407-411.
21. McConnell JV (1967) Specific Factors Influencing Planarian Behavior. *Chemistry of Learning: Invertebrate Research*, eds Corning WC & Ratner SC (Springer US, Boston, MA), pp 217-233.
22. Mori M*, et al.* (2019) Calcium ions in the aquatic environment drive planarians to food. *Zoological Letters* 5(1):31.
23. Shomrat T & Levin M (2013) An automated training paradigm reveals long-term memory in planarians and its persistence through head regeneration. *Journal of Experimental Biology* 216(20):3799-3810.
24. Riccio D & Corning WC (1969) Slime and Planarian Behavior. *The Psychological Record* 19(3):507-513.
25. Miller & Kellman J (2022) The influence of geomagnetism on the maze behavior of Dugesia Tigrina.
26. Merryman S, Sánchez Alvarado A, & Jenkin J (2018) Culturing Planarians in the Laboratory.), Vol 1774, pp 241-258.
27. O'Grady BJ*, et al.* (2021) Rapid prototyping of cell culture microdevices using parylene-coated 3D prints. *Lab on a chip* 21(24):4814-4822.

Figures

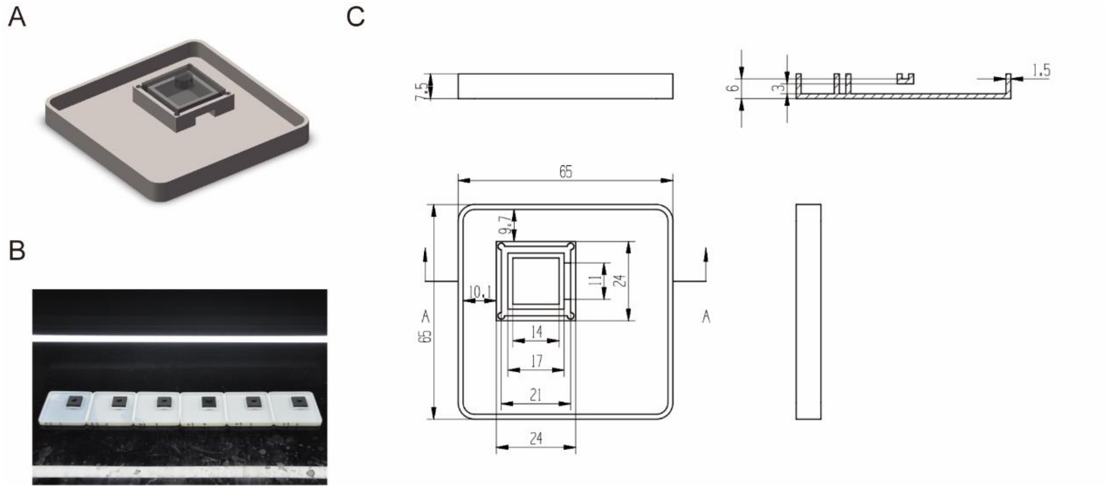

Figure 1. The light maze and its parameters. A. The conceptual drawing of the light maze. B. The experimental set up of the light maze paradigm. C. The blueprint of the light maze. The upper right shows the lateral view of section A. Length unit: mm.

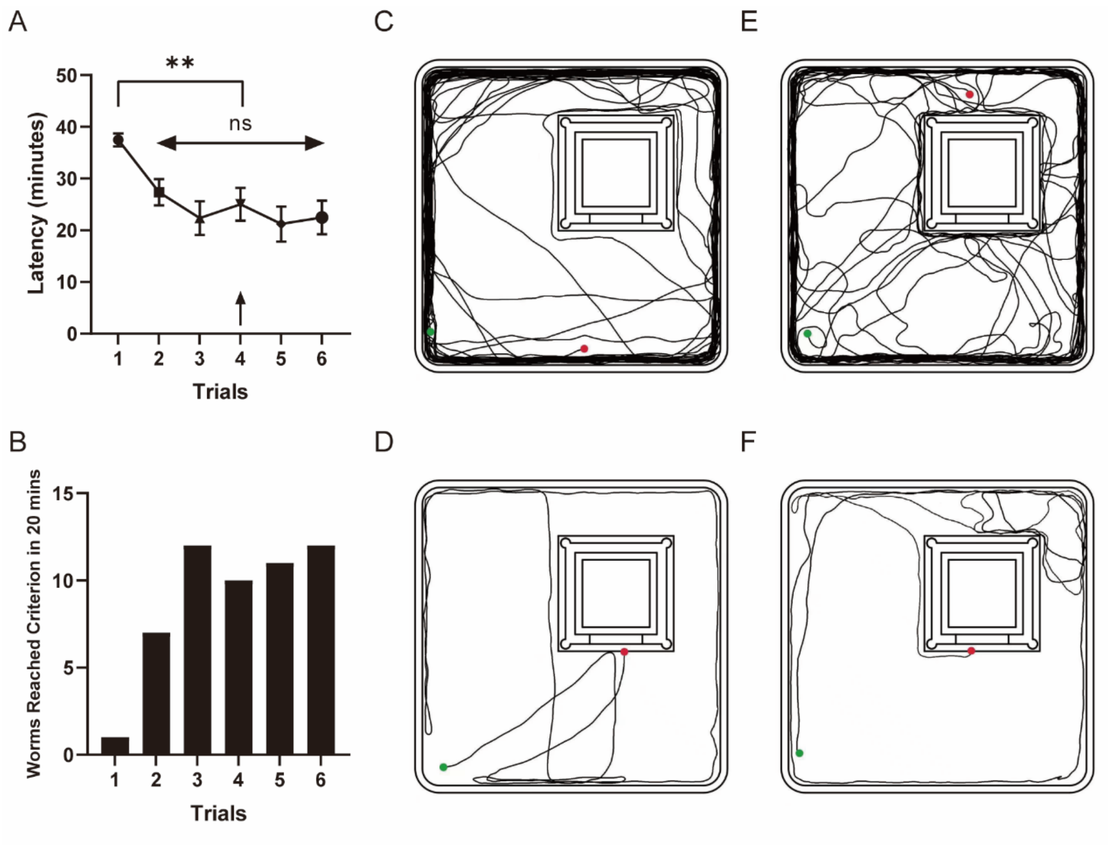

Figure 2. Results of the light maze experiment. A. Latency of each trial. B. Worms reached criterion in each trial. C-G Representative routes of learned worms. C, E shows routes of trial 1. D, F shows routes of trial 4. C, D belong to 1 worm and E, F belong to 1 worm. The green spot is the start point; the red spot is the end point.