Cognitive Neurodynamics
https://doi.org/10.1007/s11571-018-9493-1

BRIEF COMMUNICATION

An observational learning task using Barnes maze in rats

Motoki Yamada[1] · Yoshio Sakurai[1]

Received: 16 February 2018 / Revised: 15 May 2018 / Accepted: 21 June 2018

Abstract

Observational learning, which modulates one's own behavior by observing the adaptive behavior of others, is crucial for behaving efficiently in social communities. Although many behavioral experiments have reported observational learning in monkeys and humans, its neural mechanisms are still unknown. In order to conduct neuroscientific researches with recording neural activities, we developed an observational learning task for rats. We designed the task using Barnes circular maze and then tested whether rats (observers) could actually improve their learning by observing the behavior of other rats (models) that had already acquired the task. The result showed that the observer rats, which were located in a metal wire mesh cylinder at the center of the maze and allowed to observe model rats escaping to the goal in the maze, demonstrated significantly faster escape behavior than the model rats. Thus, the present study confirmed that rats can efficiently learn the behavioral task by observing the behavior of other rats; this shows that it is conceivable to elucidate the neural mechanisms of social interaction by analyzing neural activity in observer rats performing the observational learning task.

Keywords Rat · Observational learning · Barnes maze · Social interaction · Behavior

Introduction

The neural mechanisms of social interaction are still unclear, although uncovering them is important for understanding the biological bases of communication, development, learning, and some mental disorders, e.g., autism and schizophrenia (Marta et al. 2017). Observational learning is one of the main components of social interaction and need to be investigated in neuroscientific studies with animal experiments. In some animals, including humans, observing conspecific's behaviors is crucial for behaving adaptively in social communities. An earlier study on observational learning confirmed that rhesus macaques could learn to accurately choose dishes with a reward without trial and error by observing other individuals choosing between two dishes, only one of which contained food hidden by an object (Darby and Riopelle 1959; Riopelle 1960). Another early study reported that cats could learn to get rewards more effectively after observing the behavior of other cats than by being trained in the normal way for the same task (John et al. 1968). In the last few decades, some studies reported that rodents also might be able to learn a response–reinforcer contingency (Denny et al. 1983; Heyes and Dawson 1990; Saggerson and Honey 2006) and a fear response (Daejong et al. 2010) by observing behaviors of other individuals.

The aim of the present study is to confirm that rats can indeed learn by observation. We constructed an observational learning task using Barnes maze, which is generally used as the conventional learning apparatus for rats (Paul et al. 2009; Rosenfeld and Ferguson 2014; Hongying et al. 2014; Morel et al. 2015; Gawel et al. 2016). It is advantageous to use the maze as an observational learning task, for a rat located at the center of the maze platform can easily observe another conspecific in the same maze trying to escape to a goal. The result could suggest the next experiment to understand the neural mechanisms of social interaction by analyzing the neural activities of rats participating in the observational learning task.

✉ Motoki Yamada
kdq1007@mail4.doshisha.ac.jp

[1] Laboratory of Neural Information, Graduate School of Brain Science, Doshisha University, Kyotanabe 610-0394, Japan

Published online: 26 June 2018

Materials and methods

Animals

Fourteen male Long Evans hooded rats, weighing about 300 g (range, 270–330 g) and aged 8 weeks at the beginning of the experiment, were housed in cages (25 cm × 30 cm × 25 cm) in pairs. One rat in each pair was randomly assigned to be the "model" and the other was designated the "observer". Throughout the experimental sessions, the subjects were housed in a temperature-controlled room (26 ± 2 °C, about 55% humidity) on a 12–12 h light–dark cycle. All rats were given ad libitum access to food and water. A single experimenter handled them for 5 min per day for a week before the experiment. All experiments were performed in accordance with the Guidelines for Animal Experiments at Doshisha University, with the approval of the Animal Research Committee of Doshisha University.

Apparatus

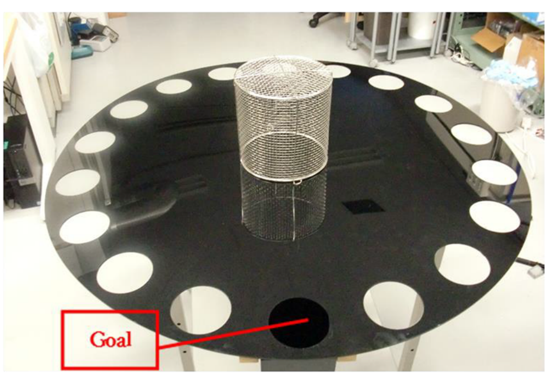
The Barnes maze consisted of a black, acrylic circular platform of 108 cm in diameter with 3 mm thickness, located 70 cm above the floor. It had 18 holes (10 cm in diameters) equally spaced in the periphery (Fig. 1a). One of the holes had a detachable acrylic black box (12 cm × 23 cm × 12 cm) just under the hole entrance. The box enabled the rats to escape from an aversive stimulus of bright light from the ceiling. The other holes were not covered so that the rats could not escape. The platform was brightened in every trial by three light bulbs (100 V, 6.4 W) positioned 120 cm above the maze, which were aversive for rats. We used a circular, metal wire mesh cylinder (20 cm in diameter, 20 cm in height) in which the observer rats were placed and a circular, gray translucent cylinder (24 cm in diameter, 28 cm height) to cover the rats before starting trials in the Barnes maze. The experiment was conducted in a darkroom with some visual cues on the walls. The behaviors of the rats were recorded with a web camera (DC-NCR13U, Digital Cowboy, Hanwha, Japan) located in the ceiling of the darkroom.

Experiment procedures

Training of model rats

The total procedures are summarized in Fig. 1b. After the last session of habituation, the model rats were trained of spatial learning to escape from the aversive lights by entering the goal box. In every trial, the model rat was first taken from its home cage and placed in the center of the platform; it was then covered with the metal wire mesh cylinder (Fig. 2a). Then the ceiling lights of the darkroom were turned on and the rat was kept waiting for 3 min. Subsequently, the rat was returned to its home cage and the platform was cleaned with water so that no olfactory traces remained. Then the rat was again placed at the center of the platform and covered with the gray translucent cylinder (Fig. 2b). The rat was kept waiting for a minute, then the cylinder was removed and it was able to run and escape to the goal box (Fig. 2c). The direction of the rat's head

a



b

Habituation
Being housed in pairs and accustomed to the experimental environment
-1week-

→

Training of model rats
Spatial learning to escape into the goal box in the Barnes maze
-5 days-

→

Training of observer rats
Spatial learning to escape into the goal box after "observing" the behaviors of model rats
-5days-

Fig. 1 **a** The Barnes maze, **b** the three successive procedures

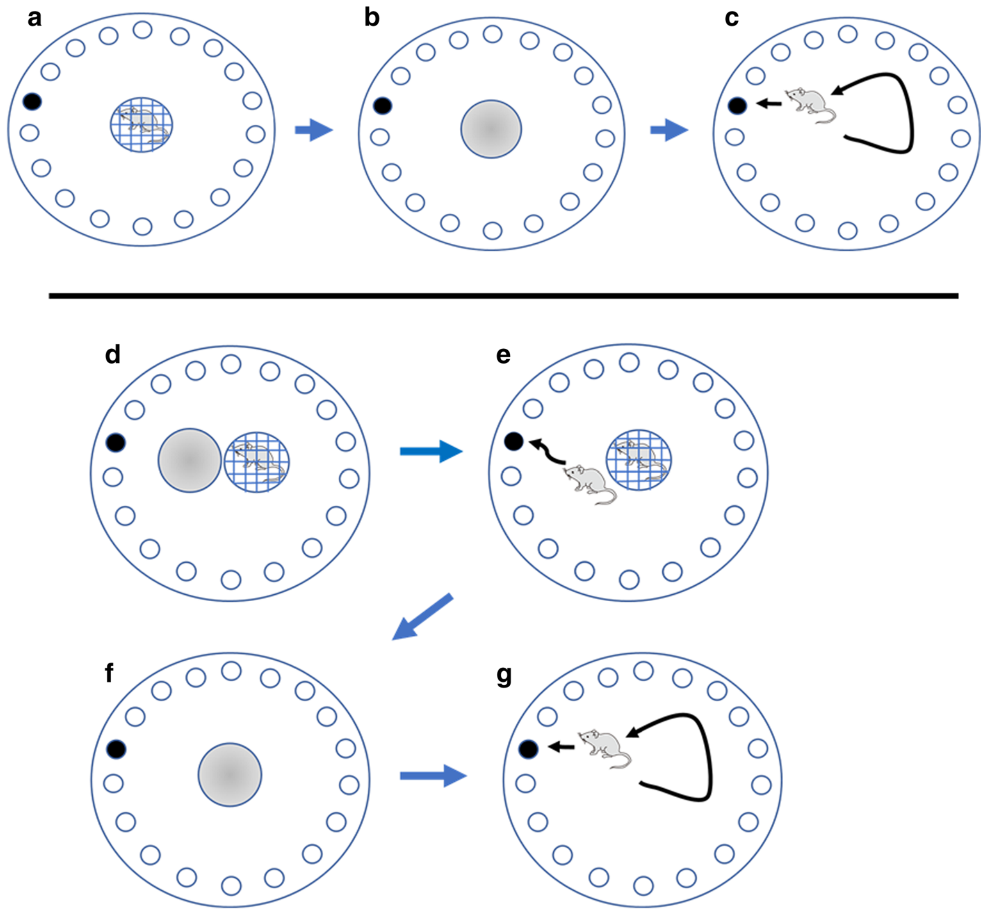


Fig. 2 Training procedures for model (upper) and observer (lower) rats

changed almost randomly in every trial. The position of the goal box was consistent during all sessions for each pair (model and observer) of rats.

We measured the latency until the whole body and tail of model rat entered into the goal box from removal of the gray translucent cylinder. When the rat did not enter the goal box within 10 min, the experimenter gently guided the rat to the goal box. When 2 min had passed since the rat entered into the goal box, it was taken back to its home cage. When a rat fell from the maze, the experimenter quickly retrieved it, returned it to its home cage and restarted the procedure after an hour. Training of the model rats was carried out in two trials in each session for five successive sessions (days). The interval between the trials was about 30 min.

Training of observer rats

Subsequent to the model rat's training, the observer rat was given the observational learning task. The procedure was almost the same as for the model rat's training except that the observer rat first was given the opportunity to observe the model rat's behavior. The observer rat was located in the metal wire mesh cylinder at the center of the platform, while the model rat was in the gray translucent cylinder located adjacent to the observer rat (Fig. 2d). The lights in the ceiling were turned on, the gray translucent cylinder was removed and the model rat could escape to the goal box (Fig. 2e). The observer rat was able to see the model rat's escape behavior. When the model rat entered the goal box within 2 min, both the model and observer rats were taken back to their home cage after 2 min passed. When the model rat entered the goal box in 2–3 min, they were taken back to their home cage soon after that. No model rats failed to escape into the goal box within 3 min. Following that, the platform was cleaned with water and the observer rat alone was subsequently trained according to the same procedure as used for the model rat (Fig. 2f, g).

The small black circle is one example of the goal box position. The other white circles are not covered so that rats cannot enter them. The cross-striped circles are the metal wire mesh cylinders and the gray circles represent the gray translucent cylinders. The procedures are described in detail in the text.

Results

We compared the latencies of the model and observer rats in each session using Mann–Whitney U test. The results showed that the differences in session 1 and 5 were significant, i.e., the observer rats entered the goal box significantly faster than the model rats (session 1: $U = 50.00$, $p < 0.05$; session 5: $U = 33.00$, $p < 0.01$) (Fig. 3). In the other sessions, no significant differences were found (session 2: $U = 96.00$, $p > 0.9$; session 3: $U = 64.00$, $p > 0.1$; session 4: $U = 87.50$, $p > 0.6$). Furthermore, we used Friedman's test for analyzing the trend across sessions in latencies of the model and the observer rats. The result indicated that the model rats had almost completely learned the escape behavior in session 1 (sessions 1–2: $G = 16.75$, $p < 0.01$, sessions 2–5: $G = 14.07$, $p > 0.05$) while the observer rats continued the learning until session 3 (sessions 1–2: $G = 11.00$, $p < 0.05$, sessions 2–3: $G = 9.54$, $p < 0.05$).

We also made an additional control experiment using two pairs of rat (n = 4) to confirm that observing the behavior of the model rats contributed to the shorter latencies of the observer rats. The training procedure was identical with the previous one (see "Experiment procedures" section) except that the observer rats were first covered with the gray translucent cylinders so that they could not see the model rat's behavior. The result showed that no significant difference was found between the model rat and the observer rat in the first session ($U = 7.00$, $p > 0.7$) (Fig. 4).

In each session, the blue and the yellow box-and-whisker plots show the latencies of model and observer rats, respectively. The crossbar in each box is the median value.

The blue and the green box-and-whisker plots show the latencies of model and observer rats in the control experiment, respectively. The crossbar in each box is the median value.

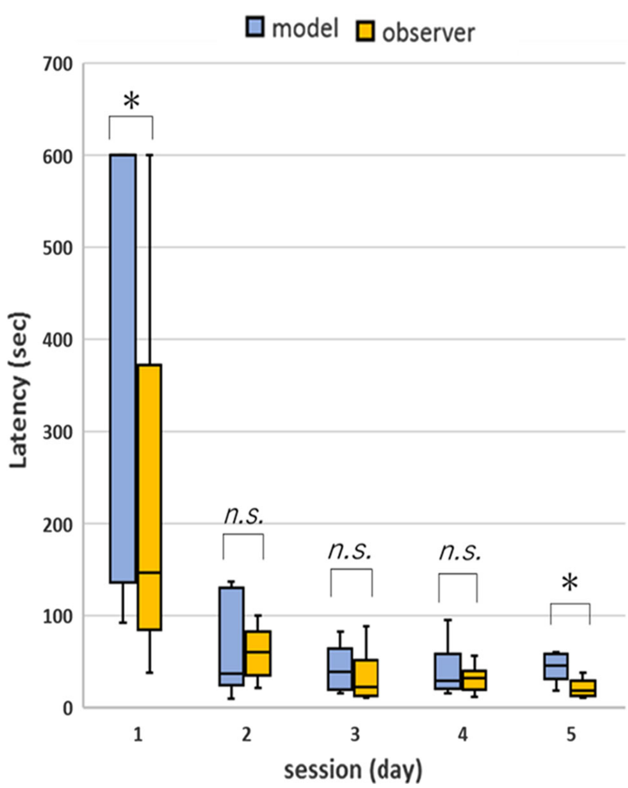


Fig. 3 Median latencies of the escape behaviors in the model and observer rats

Discussion

The purpose of the present experiment is to develop a convenient and reliable behavioral task for studying observational learning in rodents. We examined whether the observer rats, which observed the escape behavior of the model rats, displayed the escape behavior faster than the model rats in the Barnes maze. The results showed clearly that the observer rat could find and enter the goal box significantly faster than the model rats. Thus, this task is appropriate for studying observational learning in rats.

In session 1, the significant difference in latency was found between the models and the observers, whereas no significant differences were found in the other sessions except session 5. According to the analysis of the trend across sessions in latency, on the other hand, the observer rats continued learning of escape behavior until session 3 while the model rats had already learned in session 1.

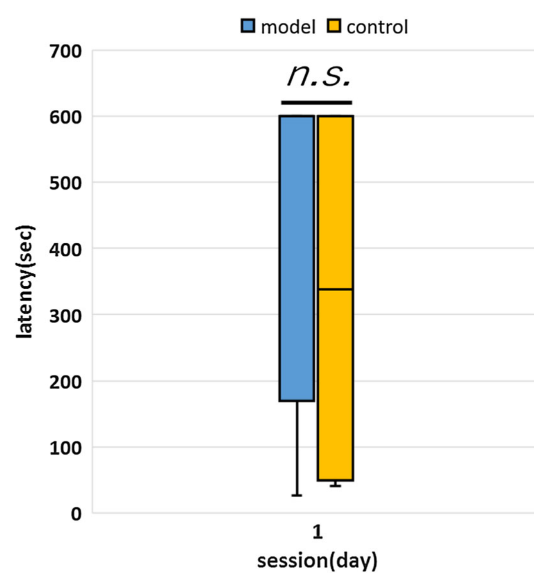


Fig. 4 Median latencies of the escape behaviors in the model and observer rats in the control experiment

We have no clear answer to why the difference became significant again in session 5. We can at least conclude that both observers and models had fully learned the escape behavior in session 5 and assume that some small additional behaviors, e.g., exploring behavior toward the other holes, might have increased the latency and caused the difference between observers and models. Such additional behaviors can easily appear in free-moving tasks using mazes and should be controlled more in the future study. It can be said, however, that the present task is simple, easily-done and appropriate for studying observational learning in rats. In the present study, at least, we found the clear difference in the latency of escape behavior between the observers and models in the first session. Furthermore, through the control experiment, we confirmed that observing the outside by the observer rats certainly had affected their shorter latencies of escape behavior.

There is, however, a controversial issue on the present task, i.e., what the observer rats actually observed and the contents the observer rats actually learned are unclear. It could be said, for example, that the shorter latencies of them, compared to the model rats, resulted not only from observing behaviors of other conspecifics, but also from enhancement of stimulus and/or retention of the enhanced stimulus, i.e., the location enhanced by escaping behavior of the model rats. In order to examine the effect of stimulus enhancement, a control experiment in which external cues, not the model rats, signal the location of the escape box is necessary. The implication of the present study for observational spatial learning is that Barnes maze is a conventional and useful tool for neuroscience research of it, as described above. However, our study also exhibits its limitation, i.e., external stimuli and environments possibly affect behaviors and learning of observer rats are sometime unclear. Therefore, planning and conducting adequate control experiments will hold the key to success of future neuroscience research of observational learning in rodents.

After a follow-up study with control experiments, we will clarify the neural mechanisms of social interaction and learning in rats by recording their neuronal activity while they perform the present task. Moreover, using rats enables us to conduct an experiment that stimulates the neurons by the method of optogenetics.

Acknowledgements We would like to thank Dr. Yuji Takano for his advice on the procedures for the Barnes maze task. This study was supported by JSPS KAKENHI Grant Nos. 16H01283, 16H02061 and 18H05088.

Funding This study was funded by JSPS KAKENHI (Grant Nos. 16H01283 and 16H02061).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Daejong J et al (2010) Observational fear learning involves affective pain system and $Ca_v1.2$ Ca^{2+} channels in ACC. Nat Neurosci 13:482–488. https://doi.org/10.1038/nn.2504

Darby CL, Riopelle AJ (1959) Observational learning in the rhesus monkey. J Comp Physiol Psychol 52:94–98. https://doi.org/10.1037/h0046068

Denny MR et al (1983) Two-choice, observational learning and reversal in the rat: S–S versus S–R effects. Anim Learn Behav 11:223–228. https://doi.org/10.3758/BF03199652

Gawel K et al (2016) Cholinesterase inhibitors, donepezil and rivastigmine, attenuate spatial memory and cognitive flexibility impairment induced by acute ethanol in the Barnes maze task in rats. Naunyn Schmiedebergs Arch Pharmacol 389:1059–1071. https://doi.org/10.1007/s00210-016-1269-8

Heyes CM, Dawson GR (1990) A demonstration of observational learning in rats using a bidirectional control. Q J Exp Psychol B 42:59–71. https://doi.org/10.1080/14640749008401871

Hongying T et al (2014) Critical role of inflammatory cytokines in impairing biochemical processes for learning and memory after surgery in rats. J Neuroinflamm 11:93. https://doi.org/10.1186/1742-2094-11-93

John ER et al (1968) Observation learning in cats. Science 159:1489–1491. https://doi.org/10.1126/science.159.3822.1489

Marta F et al (2017) Neural circuits for social cognition: implications for autism. Neuroscience 17:30483–30489. https://doi.org/10.1016/j.neuroscience.2017.07.013

Morel GR et al (2015) Cognitive impairment and morphological changes in the dorsal hippocampus of very old female rats. Neuroscience 303:189–199. https://doi.org/10.1016/j.neuroscience.2015.06.050

Paul CM et al (2009) Spatial memory: theoretical basis and comparative review on experimental methods in rodents. Behav Brain Res 203:151–164. https://doi.org/10.1016/j.bbr.2009.05.022

Riopelle AJ (1960) Observational learning of a position habit by monkeys. J Comp Physiol Psychol 53:426–428

Rosenfeld CS, Ferguson SA (2014) Barnes maze testing strategies with small and large rodent models. J Vis Exp 84:1. https://doi.org/10.3791/51194

Saggerson AL, Honey RC (2006) Observational learning of instrumental discriminations in the rat: the role of demonstrator type. Q J Exp Psychol 59:1909–1920. https://doi.org/10.1080/17470210600705032