A neural mechanism of observational learning in rats using Barnes maze

DOCTORAL DISSERTATION

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By:

Motoki Yamada

Supervisor:

Yoshio Sakurai

Graduate School of Brain Science

Doshisha University

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Abstract

In many animals, observing behaviors of other conspecifics serves as an effective means for an adaptive learning about novel environments or tasks. This observational learning has been demonstrated by numerous behavioral studies in humans and experimental animals ever since Bandura, a famous social psychologist, advocated it in his book, *Social Learning Theory* (1977). Observational learning can be defined as that an animal observing behaviors of other conspecifics, which are performing a task, before engaging in the same task, can learn the task "more efficiently" than one learning the task following conventional learning procedures of the task.

However, the neural mechanisms underlying observational learning are unclear. Some studies attempted to investigate it by using electroencephalogram (EEG) or functional magnetic resonance imaging (fMRI) and found that neural activity increased within prefrontal cortices (PFCs) or inferior parietal cortices (IPCs) when participants observed another conspecific's motor actions while learning motor tasks. These studies also found that the brain regions above showed higher activities not when the participants passively observed but when they observed with intension to learn the motor tasks. However, these studies were not sufficient to elucidate the neural mechanisms of observational learning in detail, because they are correlational studies between brain activity and observational learning behaviors, and because brain activities detected by EEG and fMRI only reflect broad electrical signals of which origins are not clearly defined and

blood oxygen-level-dependent (BOLD) signals in relatively broad areas of the brain, respectively.

This study employed the method of electrical stimulation that can examine causal relationship between activity of certain brain areas and observational learning behaviors, by temporally and locally disturbing the brain activity. The very merit of electrical stimulation is that, not like irreversible destruction studies, the disturbing can be applied to specific periods, in particular observing periods, in observational learning process. The aim of the present study using the electrical stimulation method is to examine which brain regions, in particular prefrontal cortex and hippocampus which are thought to be the major learning-related brain regions, are deeply involved in observational learning in rodents.

In the first experiment, we aimed to develop an appropriate observational learning task that could be used not for humans or monkeys (employed in the previous experiments), but for rodents as subjects. This is because rodents, such as rats and mice, have been used in many invasive neuroscience experiments, e.g., administration of activity inhibitors into targeted brain regions and/or electrophysiological recordings of their neural activity. Some previous experiments in rats indicated that they learned the task efficiently by observing other rats' learning behaviors. There are, however, some controversies associated with these experiments using operant conditioning tasks, i.e., the tasks had multiple cues other than observation for learning and the rats sometimes needed training for a long time to elicit observational behaviors of the rats.

Therefore, the present study developed an observational learning task using the Barnes maze for rats. We considered this maze to be appropriate for testing the ability of rats for observational learning because it utilized the innate behavior of rats and required no artificial training for a long time. We compared the escape latencies between the rats which were trained of escape behavior using the conventional procedure (model rats) and the rats which observed the behavior of the model rats before training of the same escape behavior (observer rats). The results showed shorter escape latencies, i.e., quicker learning of escape behavior, of the observer rats than those of the model rats. Moreover, we found that observer rats did not exhibit this quicker escape behavior in an additional control experiment where the observer rats could not observe the escape behavior of the model rats. Thus, we concluded that rats possessed the ability of observational learning and that observing the learning behavior of the model rats was essential for observational learning in the observer rats.

In the second experiment, we investigated the brain regions involved in observational learning. We electrically stimulated the medial prefrontal cortex (mPFC) or dorsal hippocampus (dHPC) of rats and disturbed their neural activities during the observation periods in the observational learning task we had developed in the first experiment. The comparison of the escape latencies of the observer and model rats revealed that the observer rats with mPFC stimulation did not exhibit any observational learning, whereas both of the observer rats with dHPC stimulation and with no stimulation (control)

exhibited observational learning. The present results confirmed that mPFC is an important brain region for observational learning in rats.

The present study successfully discovered one of several brain regions involved in observational learning and suggests an appropriate method to study its neural mechanisms in detail. In future studies, we should investigate the projection of mPFC to other brain regions using optogenetics or electrophysiological recordings of neural activities and subsequently elucidate the neural circuits for the observational learning.

1. General introduction

1.1 Behavioral experiments of observational learning

In many animals, including human beings, observing behaviors of other conspecifics is useful for the adaptive processing of a novel environment. This notion was theoretically described for the first time in the famous book "*Social Learning Theory*" (Bandura, 1977). In this book, Bandura stated that "In the social learning system, new patterns of behavior can be acquired through direct experience or by observing the behavior of others", and suggests that observation enables us to learn, irrespective of direct experiences. He actually demonstrated that children who observed an aggressive behavior of adults against a doll showed a tendency to attack the doll later.

Before the works by Bandura, some behavioral experiments indicated the possibility of observational learning (or imitative learning) in different animal species. Most importantly, observational learning demonstrated in animals has been defined as "more efficient" learning compared to conventional learning, whereas observational learning in humans is considered to be quickly and completely acquired one through observation alone. For example, cats which had observed the avoidance responses of other conspecifics showed the avoidance response relatively earlier than those which learned the avoidance behavior through the conventional procedures (John et al, 1968). The observer cats also committed fewer errors than the cats underwent conventional learning. In addition, almost all of the

rhesus monkeys joining in an object-quality discrimination task, and had observed the other monkeys performance in the task, showed not only an increase in the discriminatory ability but also decreased the number of errors in the first trial of the task (Darby & Riopelle, 1959). Thus, individuals observing behaviors of the other conspecifics improved their ability to perform the tasks and committed fewer mistakes during their execution.

However, it is debatable whether the effective learnings, indicated in those studies, were actually the results of the observation of the behavior of the other conspecifics. For example, an experiment in young children in the "ghost" condition, in which the effective operation of the means apparatus was seen to occur without human agency, probably encouraged observational learning as in the model condition, where children observed the means actions of other individuals (Thompson & Russell, 2004). However, it is not necessarily the case that contradicts the fact of observational learning, because it is obvious for children to learn from observation whatever they see.

Another controversial issue is known as "local enhancement" (Thorpe, 1956). It is an explanation for observational learning that the presence of the demonstrator itself may increase the attention of the observer toward a task and may encourage its execution, just as pointed out in the drive theory of social facilitation (Zajonc, 1965).

Similarly, the manner by which other individuals affect observational learning is unclear. However, the fact is that it is produced by a learning

method that differs from the conventional one, in which an individual learns alone. Moreover, it is meaningful that a variety of learning methods has been experimentally demonstrated.

1.2 Neuroscientific researches on observational learning

Although many behavioral experiments have demonstrated observational learning, its neural mechanisms remain unclear. Most importantly, there are few experimental methods or tasks for investigating observational learning, which is considerably higher order function of the brain.

Over the past few decades, however, some neuroscientific studies attempted to clarify the mechanisms underlying observational learning using electroencephalogram (EEG), positron emission tomography (PET) or functional magnetic resonance imaging (fMRI). A majority of these studies measured brain conditions such as electrical brain waves or changes of cerebral blood flow during the period for acquiring new motor skills by observation. For example, one study revealed that passive action observation increased the activities of some brain regions, such as the premotor and inferior parietal regions (Grafton et al, 1996; Johnson-Frey et al, 2003). Another study found that these areas showed higher activities relative to passive observation when participants observed with the intention to subsequently reproduce a component action (Frey & Gerry, 2006).

The results of these studies lead to a knowledge of “mirror neuron” (Gallese et al, 1996; Rizzolatti et al, 1996). This neuron, which was discovered in the ventral premotor cortex (F5 area) of macaques by Rizzolatti

et al. (1996), responded not only when the subject performed an action but also while observing the performance of the same action by other individuals. It is thought that each mirror neuron responds automatically, not selectively, to the particular behavior corresponding to the neuron. It is hypothesized in macaques that the neurons in the superior temporal sulcus (STS), known to be active during observation but unresponsive while performing an action, the PFG area, which shows different responses according to the goals of behaviors, and the F5 area connect with each other and form the neural circuit (Rizzolatti et al, 1998). From a wider perspective, the neural circuit consists of the neurons in the inferior frontal gyrus (IFG), inferior parietal lobe (IPL), and STS (Inui, 2012). The mirror neuron system was at first thought to be associated with imitation learning, although there was little evidence to confirm that monkeys are capable of imitation learning. At present, the hypothesis is supported which the mirror neurons are involved in mentally simulating the movements of other conspecifics or assuming the aim of the behaviors (Decety et al, 1997; Iacoboni et al, 2005).

Although the neuroscientific studies, including those on the mirror neuron system, have tried to elucidate the neural mechanisms underlying observational learning, almost all the results from such studies in humans depended on fMRI analysis. It was, of course, meaningful that fMRI visualized the brain regions involved in observational learning, but it also had some disadvantages. For example, fMRI can only measure the changes in the blood oxygen-level-dependent (BOLD) signals mainly on the brain surface and cannot record the neural activities of the neurons or those in the

neural circuits. In the strictest sense, it analyzes the second-order changes, i.e., increase in blood flow rate following the spike firing of neurons. fMRI lack a sufficiently high temporal and spatial resolution to record neural and/or circuitry activities. Thus, it is difficult to conclude whether fMRI studies can contribute to the detailed clarification of the neural mechanisms. Above all, the subjects of most of the previous studies were humans, which made it difficult to employ invasive methods, such as destruction of brain areas or pharmacological inhibition, owing to the restrictions imposed by research ethics.

1.3 Strategy for elucidating neural mechanisms of observational learning

Suitable experimental methods are necessary to clarify the neural mechanisms of observational learning in detail. We aimed to develop a new observational learning task using rodents, not humans or monkeys.

There were two reasons for using rodents. First, many invasive neurological methods for rodents have been developed compared to other species, e.g., administration of activity inhibitors into target brain regions, electrical or chemical stimulation, and/or electrophysiological recordings of their neural activity. Second, rodents have fewer limitations on research ethics than those for humans or monkeys. Also, it is easy to add many populations to the data.

Actually, over the past few decades, some experiments demonstrated that rodents also showed observational learning, similar to humans or monkeys. Zentall & Levine (1972) reported that the rats, which observed other

conspecifics which presented response-relevant cues, improved their learning, while they were prevented from learning when they observed other conspecifics which presented no cues. In another experiment in rats, Heyes & Dawson (1990) performed an observational learning task using a bidirectional control procedure to reduce the possibility of local enhancement (see 1.1) and tested whether rats could actually learned through observation. In the task, the model and observer rats faced each other at the beginning of the trial. When the model rats pushed a joy stick to the right, the observer rats perceived it as moving to the left. In the condition where the observer rats had to push the joy stick to the same egocentric direction as the joy stick pushed by the model rats, the observer rats had to push it in a direction opposite to that of the visual cues (pushing of the joy stick by the model rats). That was thought to solve the problem like "auto shaping", seen such an experiment by Biederman & Vanayan (1988), describing "… in which pigeons were pre-trained to make a key-pecking response before being given observational discrimination training ..... birds that were allowed to observe a partially trained demonstrator performing an erect vs inverted triangle discrimination acquired the same discrimination faster than birds that had observed an over-trained demonstrator". In the result of Heyes & Dawson (1990), observer rats showed the tendency to push the joy stick in the same egocentric direction as the model rats. Similar results were also observed in mice (Collins, 1988).

Thus, numerous studies have demonstrated the presence of observational learning in rodents. There is, however, a controversial issue that almost all

the experiments were based on operant conditioning tasks in small chambers, where the subjects were trained to get a reward as reinforcement. As Mitchel et al. (1999) pointed out, a variety of cues, like odor of food as a reward or saliva secreted by other conspecifics, was distributed in the chambers and the observer rats sometime showed no observational learning during the training periods.

In order to overcome the above-mentioned issues, the present study developed an observational learning experiment in rats using Barnes maze task, not an operant learning task. This spatial maze task is based on the nature of rats: they dislike bright lights and tend to escape to dark places. This task is generally employed in behavioral or pharmacological experiments (Gawel et al, 2016; Hongying et al, 2014; Morel et al, 2015; Paul et al, 2009). We consider the Barnes maze task to be more advantageous for investigating observational learning in rats compared to the operant learning tasks, because the former utilizes the innate behavior of rats, does not require long training periods for shaping operant behaviors, and does not need rewards such as food, i.e., it is easy to eliminate cues, other than the behaviors of other conspecifics.

In our observational learning task, the observer rats at the center of the maze were able to see the escape behavior of the model rats, who were trained for the Barnes maze task according to the conventional procedures. Then, the observer rats performed the same task. We found that the observer rats escaped earlier than the model rats in the first session of training. This

result confirmed that rats could also learn through observation, corroborating the findings of the previous studies.

Following this study, we conducted another experiment using electrical stimulation in the same task, to detect the brain regions involved in observational learning. Electrical stimulation is generally considered to be a simple neuroscientific method that is appropriate for investigating the relationship between specific functions and certain brain areas. Though EEG or fMRI studies in human have contributed to elucidation of relatively broad brain areas related to observational learning, as described above, they are correlational studies between brain activity in specific brain areas and observational learning behaviors. On the other hand, the method of electrical stimulation can examine causal relationship between activity of certain brain areas and observational learning behaviors by temporally and locally disturbing the brain activity. The very merit of the electrical stimulation is that, not like irreversible destruction studies, the disturbing can be temporally controlled and applied to specific periods, in particular observing periods, in observational learning process.

However, few studies have used such invasive neurological methods and clarified neural mechanisms of observational learning in rodents. One previous experiment in mice tested the effects of electrical stimulation of some brain regions on observational learning (Jurado-Parras et al, 2012). The results indicated that electrical stimulation of medial prefrontal cortex (mPFC) prevented observational learning, while that of dorsal hippocampus (dHPC) did not affect it. The present study mostly referred to the method of

electrical stimulation used by Jurado-Parras et al. (2012) in determining the experimental conditions, such as the targeted brain regions and parameters of electrical stimulation. However, the experiment by Jurado-Parras et al. (2012) was also based on an operant conditioning tasks and was associated with the disadvantages described above (1.3). Thus, the present study aimed to make clear the brain regions related to observational learning occurring in a more suitable experimental protocol, i.e., our Barnes maze.

2 Experiment 1 - An observational learning task using Barnes maze in rats -

2.1 Introduction

The neural mechanisms of social interaction are unclear, although uncovering them is important for understanding the biological bases of communication, development, learning, and some mental disorders, e.g., autism and schizophrenia (Fernandez 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 behaviors of conspecifics 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 acquire the avoidance responses 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-reinforcement contingency (Denny et al, 1983; Heyes and Dawson, 1990; Saggerson and Honey, 2006) and a fear response (Jeon 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 & 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. In addition, the Barnes maze task requires no long-term and artificial training or reinforcement such as food, for shaping of operant behaviors because it utilizes innate behavior of rodents, i.e., escaping from bright lights. 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.

2.2 Materials & Methods

2.2.1 Study approval

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.

2.2.2 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 ℃, 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.

2.2.3 Apparatus

The apparatus for learning was the Barnes maze which consisted of a black, acrylic circular platform of 108 cm in diameter with 3 mm thickness, located 70 cm above the floor (Fig. 1-1). It had 18 holes (10 cm in diameter) and one of the holes had a detachable acrylic black box (12 cm × 23 cm × 12 cm), which was the goal rats escaped from the aversive stimulus of bright light from the ceiling. 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 in height) to cover the rats before starting trials in the Barnes maze (Fig. 1-2). The behaviors of the rats were recorded with a web camera (DC-NCR13U, Digital Cowboy, Hanwha Japan) located in the ceiling. Behavioral trajectories of the rats on the Barnes maze were analyzed with a video tracking software

(ANY-maze, MUROMACHI KIKAI Co., LTD). The platform was brightened in every trial by the ceiling LED light. All apparatuses were located in a dark space surrounded by thick soundproof curtains in the experimental room.

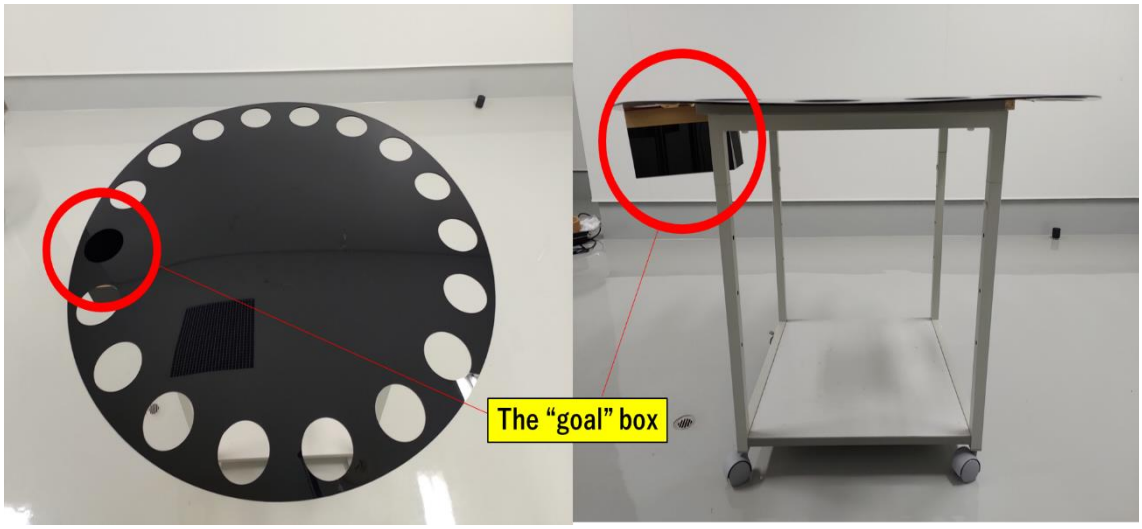


Fig. 1-1 The Barnes maze viewed from the top (left) and the side (right)
The box surrounded by the red circle is the goal into which rats enter to escape from aversive bright ceiling light. The other holes are hollows and rats can not enter into them.

Fig. 1-2 The metal wire mesh cylinder (left) and the gray translucent cylinder (right)

This metal wire mesh cylinder shown above was used in Experiment 2. We used a smaller one with the ceiling in Experiment 1. The rats were covered with the metal wire mesh cylinder could observe their surrounding experiment from the center of the Barnes maze, whereas rats covered with gray translucent cylinder could not see that.

2.2.4 Experimental procedures

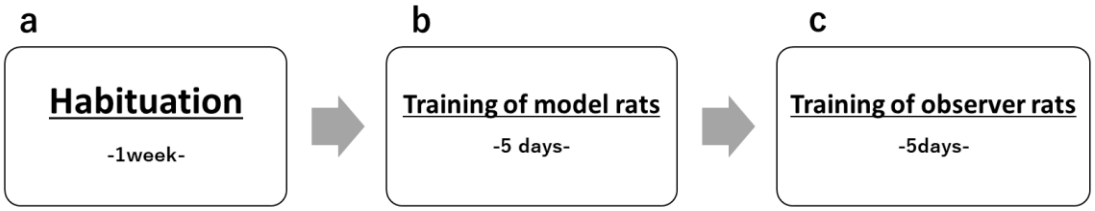


Fig. 1-3 The successive total procedures during 17 days

(a) Rats were housed in pairs and accustomed to the experimental environment for a week. **(b)** The model rats were trained of spatial learning to escape into the goal box in the Barnes maze for 5 sessions (days). **(c)** The observer rats were trained of spatial learning to escape into the goal box after observing the behaviors of the model rats for 5 sessions.

The total procedures are summarized in Fig. 1-3.

(i) Habituation

Before starting the experiment, rats were housed in pairs to be accustomed to each other and the experimenter handled them for 1 week. This habituation period was so important because, as one previous study indicated, rats which rose in social community tended to imitate a bidirectional control behavior they observed, whereas rats which rose in isolation tended to fail to use a conspecific as a reference point (Reed et al. 1996).

(ii) Training of model rats

Following the last session of habituation, the model rats were trained of spatial learning to escape from the aversive lights into by entering the goal box for 5 sessions. 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 and was kept waiting for 3 min (Fig. 1-4a). This was because, in training sessions of observer rats, they were given the same time to observe the behaviors of model rats. After that, the rat was returned to its home cage and the platform was cleaned with water so that no olfactory cues or no footprints remained. Then the rat was again placed at the center of the platform and covered with the gray translucent cylinder and was kept waiting for 1 min until the cylinder was removed (Fig. 1-4b). The reason was to almost randomly change the direction of the rat's head in every trial. Subsequently, the rat was allowed to run and escape into the goal box (Fig. 1-4c). 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 box, it was taken back to its home cage. When a rat fell from the maze, the experimenter quickly retrieved it, returned it to home cage and restarted the procedure after an hour. The position of the goal box was consistent during all sessions for each pair (model and observer) of rats.

The reason for setting upper limits on escape latency is that escaping behaviors in the Barnes maze task vary extremely among rats and there often are some rats which do not escape into the goal for a long time (Ogren

& Stiedl, 2013). This is why the latencies were usually analyzed by nonparametric rank tests, such as H test or Mann-Whitney U test.

(iii) Training of observer rats

After the last session of training of the model rat, the observer rats were given the observational learning task for the same period as the model rats. The procedure was almost same as for training of the model rats, except that the observer rat was able to observe not only the surrounding situation but also the behavior of the model rat. 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. 1-4d). While the gray translucent cylinder was removed and the model rat was able to escape into the goal box, the observer rat could see the model rat's behavior (Fig. 1-4e). The observer rat was kept waiting for 3 min even when the model rat entered the goal box in that time. As far as we analyzed the video recordings of rats' behavior, the model rats which jumped into the goal box in 3 min repeated entering into and getting out of the box twice on average. 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 (Figs. 1-4f, g).

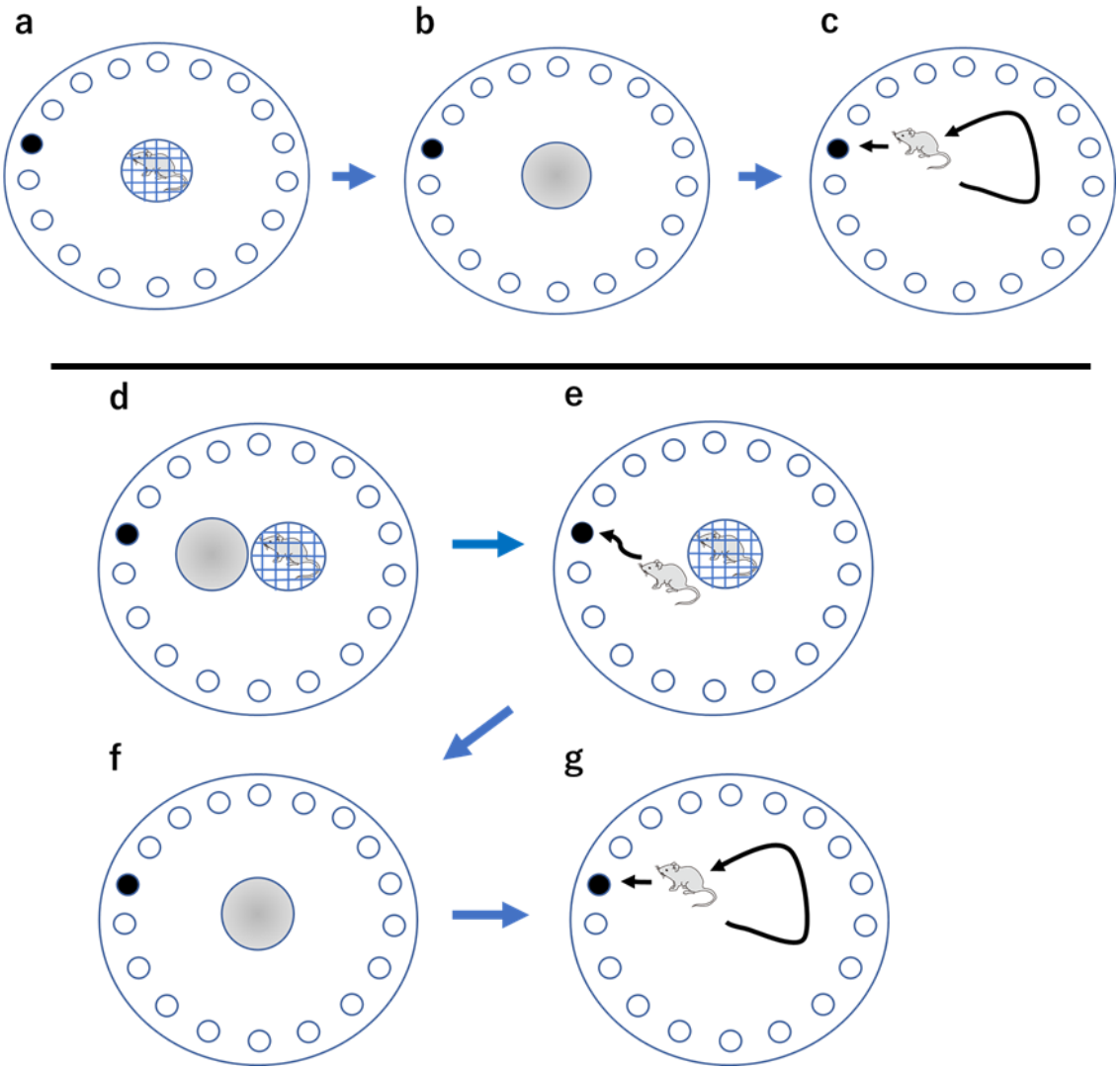

a
b
c
d
e
f
g

Fig. 1-4 Training procedures for the model rats (upper) and the observer rats (lower)

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 cylinder and the gray circles represent the gray translucent cylinder. The goal box position was consistent during all sessions for each pair of rats. **(a)** The model rat was placed in the center of the platform and then covered with the metal wire mesh cylinder. Three minutes later, it was returned to its home cage and the platform was cleaned with water so that no olfactory cues or no footprints remained. **(b)** The model rat was again placed at the center of the platform, covered with the grey translucent cylinder and then kept waiting for 1 min. **(c)** After the cylinder was removed, it was allowed to run and escape into the goal box. **(d)** 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. **(e)** The observer rat was kept waiting for 3 min and could see the escape behavior of the model rat during the waiting periods. Following that, both the model and observer rat were returned to their home cage and the platform was cleaned with water. **(f) and (g)** According to the same procedures as (b) and (c), the observer rat alone was again taken to the center of the maze, covered with the gray translucent cylinder, kept waiting for 1 min and then was allowed to escape to the goal box after the cylinder was removed.

2.3 Results

We compared the escape latencies between the model (n = 7) and observer (n = 7) rats in each session (day) using Mann-Whitney U test (Fig. 1-5). In order to see whether the observation period facilitated escaping behaviors in the observer rats, i.e., shorter latencies than the model rats, we mainly focused on the comparison in the first session (session 1). This is because the escape latencies of the observer rats in the following 4 sessions might be affected by learning by their own experiences in the former sessions. The result showed that the difference in session 1 was significant, i.e., the observer rats entered the goal box significantly faster than the model rats (U = 50.00, $p < 0.05$). For the comparison in sessions 2-5, Bonferroni correction was applied to avoid errors of significant levels caused by repeated use of U test. The results showed no significant differences in sessions 2-4 (session 2: U = 96.00, $p > 0.9$; session 3: U = 64.00, $p > 0.1$, session 4: U = 87.50, $p > 0.6$) except in session 5 (U = 33.00, $p < 0.01$). The significant difference in the last session might mean that the learning of the maze by the observer rats resulted in better than the model rats after the preceding 4 days of training.

Furthermore, we used paired Friedman's test for analyzing the learning curve across sessions in latencies of the model and observer rats. The results indicated that the model rats had almost completely learned the escape behavior in session 1 (sessions 1-2: G = 16.75, $p < 0.01$; session 2-5: G = 14.07, $p > 0.05$) while the observer rats continued the learning until session 3 (sessions 1-2: G = 11.00, $p < 0.05$; session 2-3: G = 9.54, $p < 0.05$). However, the U tests described above showed that the difference of latencies between

the model and observer rats were not found in sessions 2 and 3, indicating that the learning by the observer rats was not facilitated with observation of the model rats after the first session.

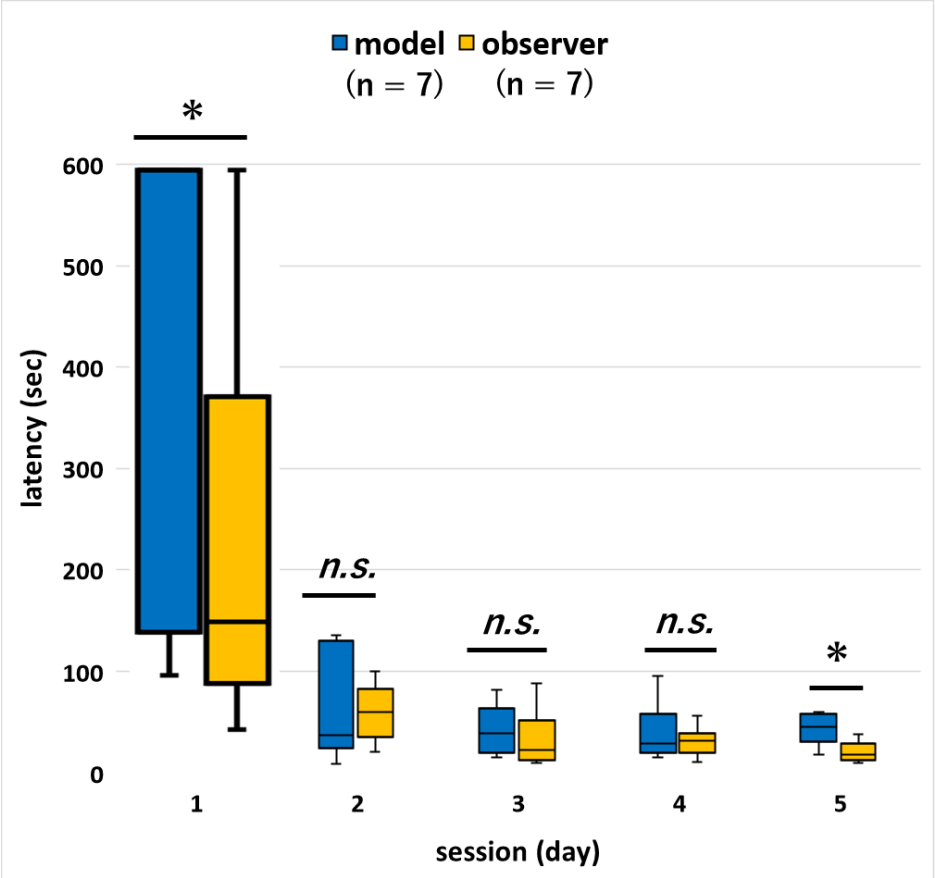


Fig. 1-5 Median latencies of the escape behaviors in the model and observer rats

In each session, the blue and the yellow box-and-whisker plots show the latencies of model and observer rats, respectively. The cross bar and vertical bar in each box are the median value and error bar, respectively. ＊represents significant difference (p < .05 or 0.01, U test) between the model and observer rats. Bonferroni correction was applied to avoid errors of significant levels caused by repeated use of U test.

We also made an additional control experiment using three pairs of rats (n = 6) 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 2.2.4) except that the observer rat was first covered with not the metal wire mesh but the gray translucent cylinder so that they could not see the behavior of the model rat. The result showed that no significant difference was found between the model rats and the observer rats in the first session ($U = 17.50$, $p > 0.1$) (Fig. 1-6).

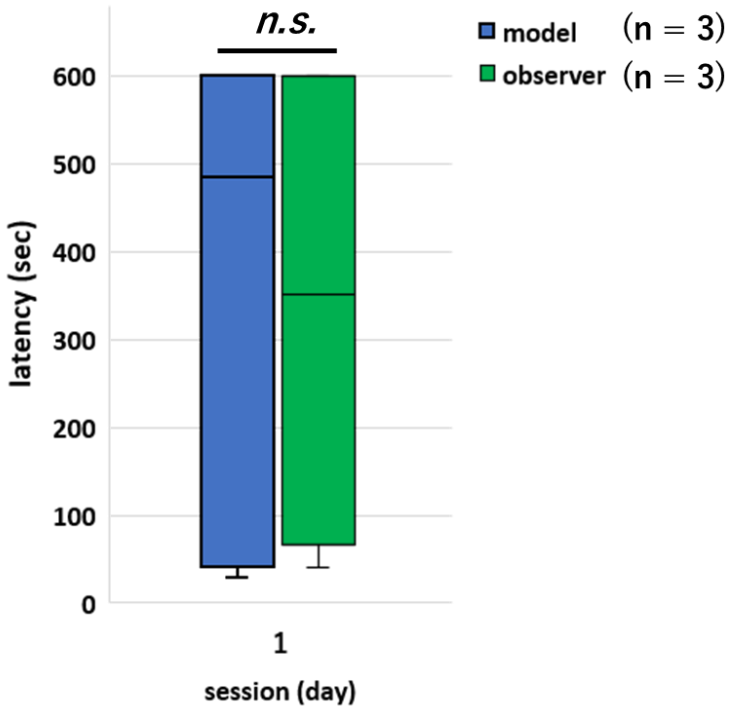


Fig. 1-6 Median latencies of the escape behaviors in the model and observer rats in the additional control experiment

In each session, the blue and the green box-and-whisker plots show the latencies of model and observer rats, respectively. The cross bar and vertical bar in each box are the median value and the error bar, respectively. The rats are different from ones in Fig. 1-5.

An example of raw data representing difference in trajectory length between the model and observer rats during the Barnes maze task is shown in Fig. 1-7.

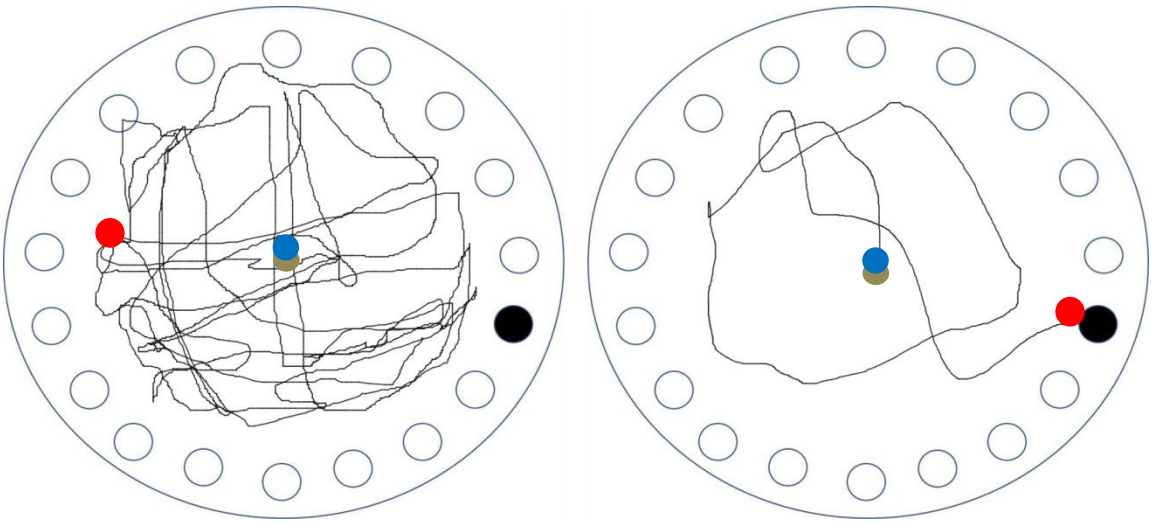
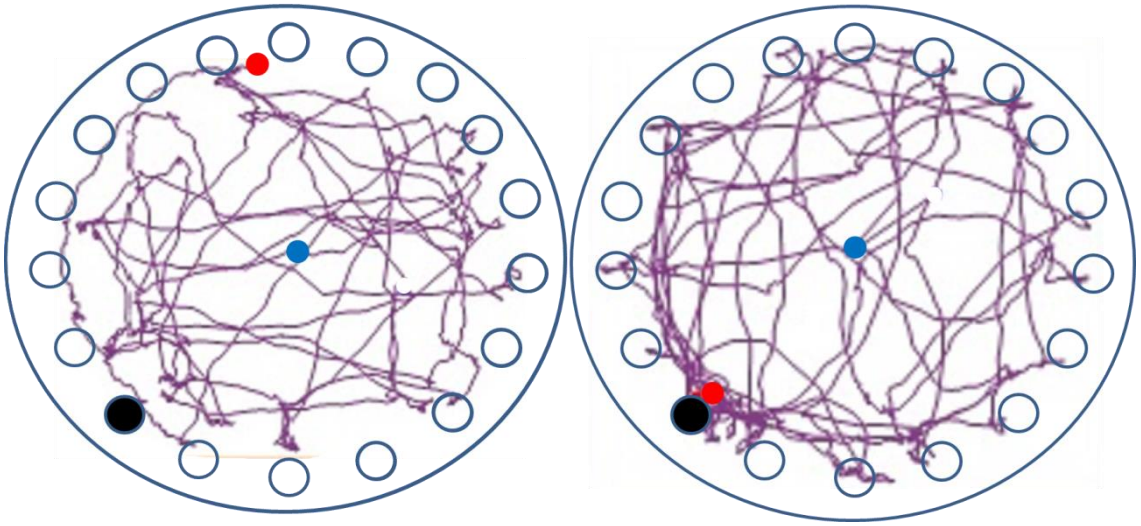


Fig. 1-7 An example of raw data of escape behavior trajectories of the model (left) and observer (right) rats

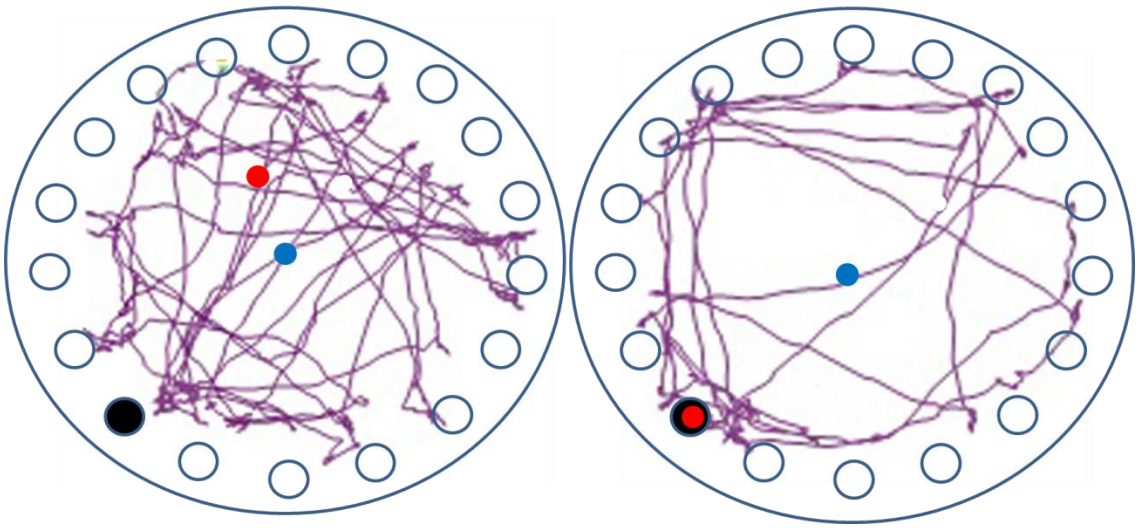
The small black circle is a goal box into which rats can escape. The all other white circles are empty holes. The blue small circle in the center represents the start position and the red one shows the last position of the trial. The black lines represent running paths of the model and observer rats in session 1 in the Barnes maze task.

Fig. 1-8 shows behavior trajectories in all pairs in the first session. The model is in left and the observer is in right in each pair. Though the models showed shorter trajectories than the observers in some of the pairs, there is no consistent difference in trajectory length between the model and observer rats. The trajectory lengths are not necessarily correlated to escape latencies in the Barnes maze because the rats often stopped and kept still on the maze.

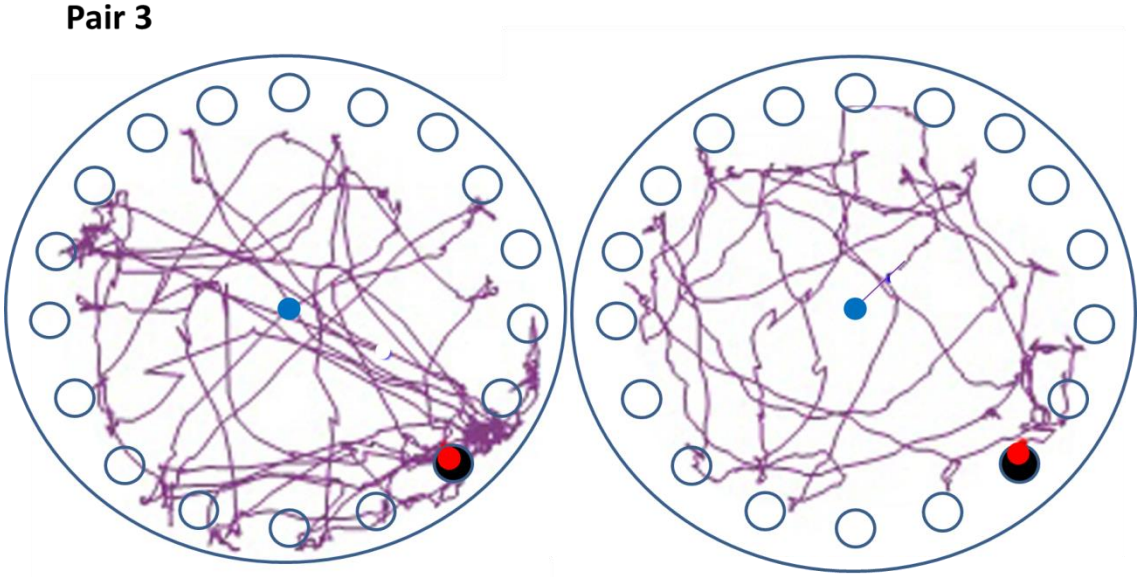
Pair 1



Pair 2



Pair 3



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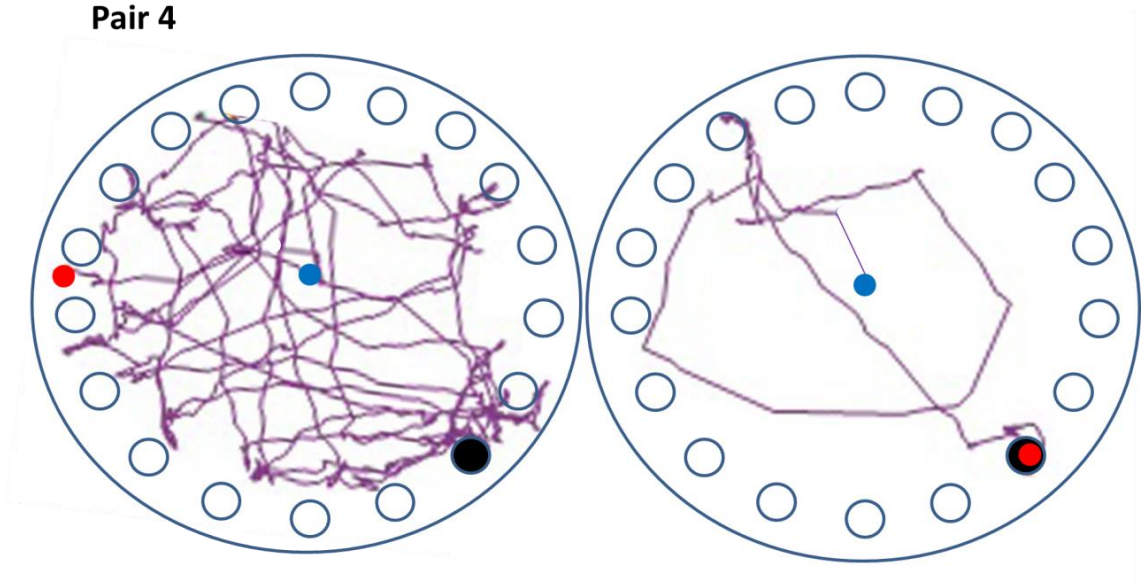

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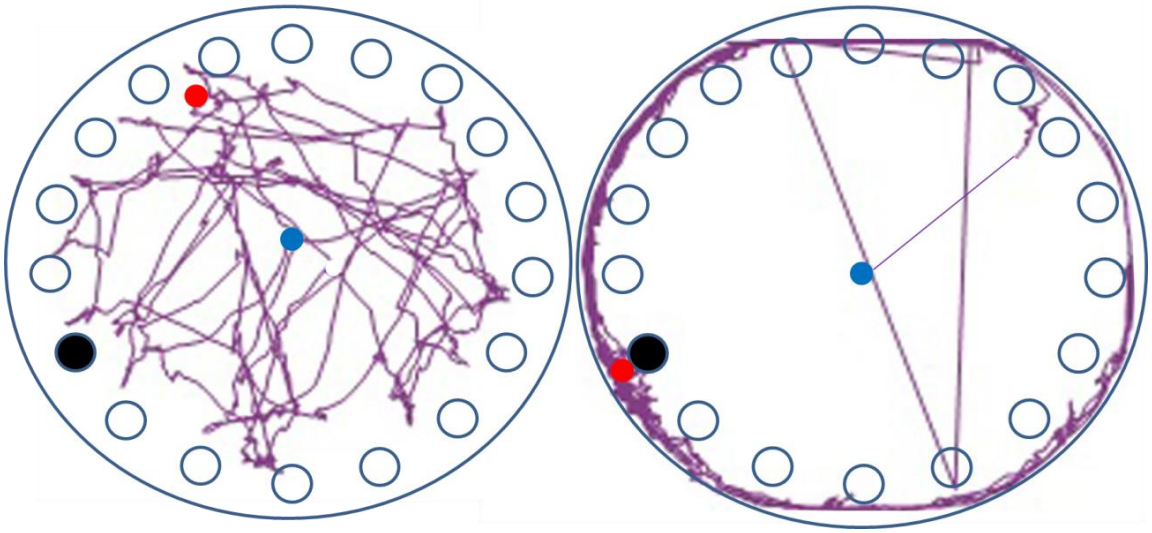

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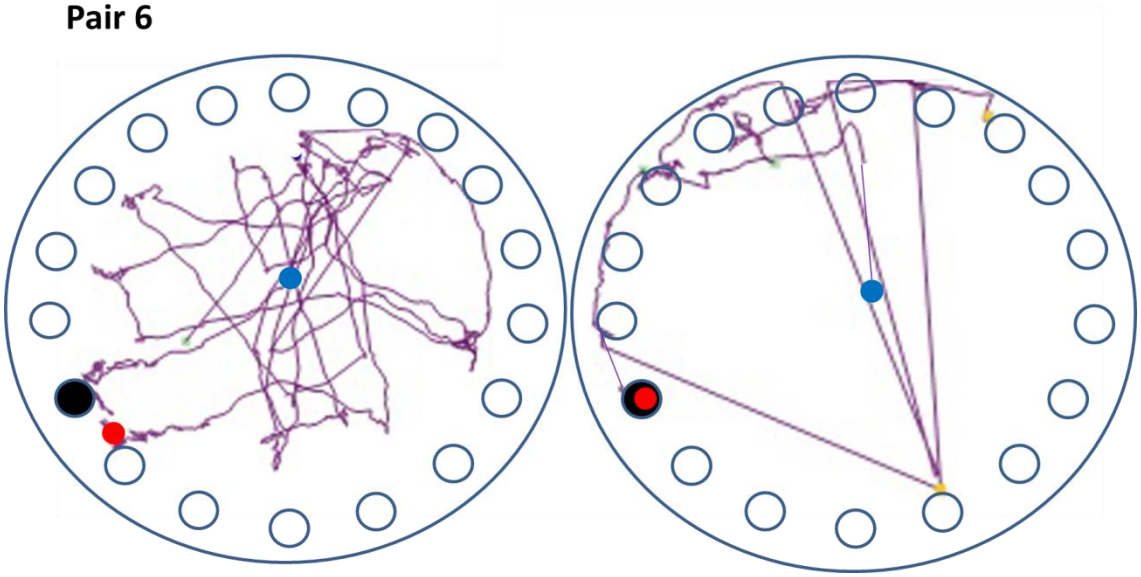


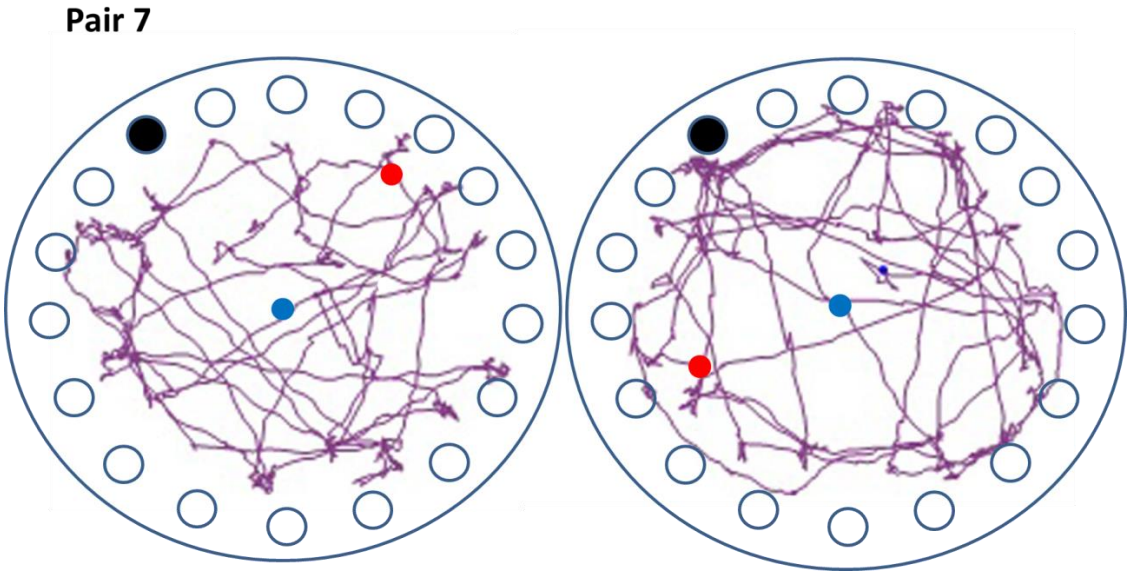
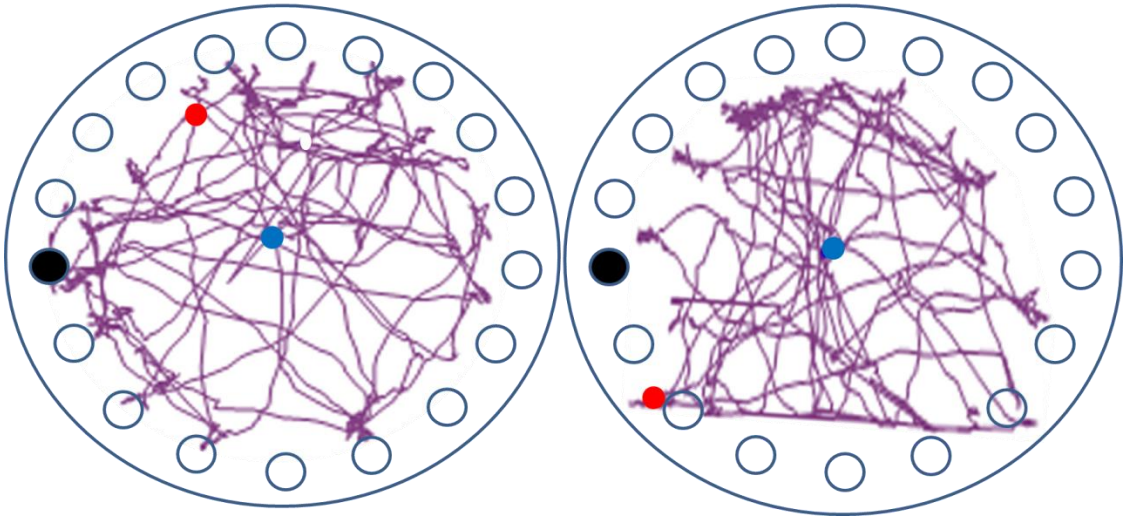


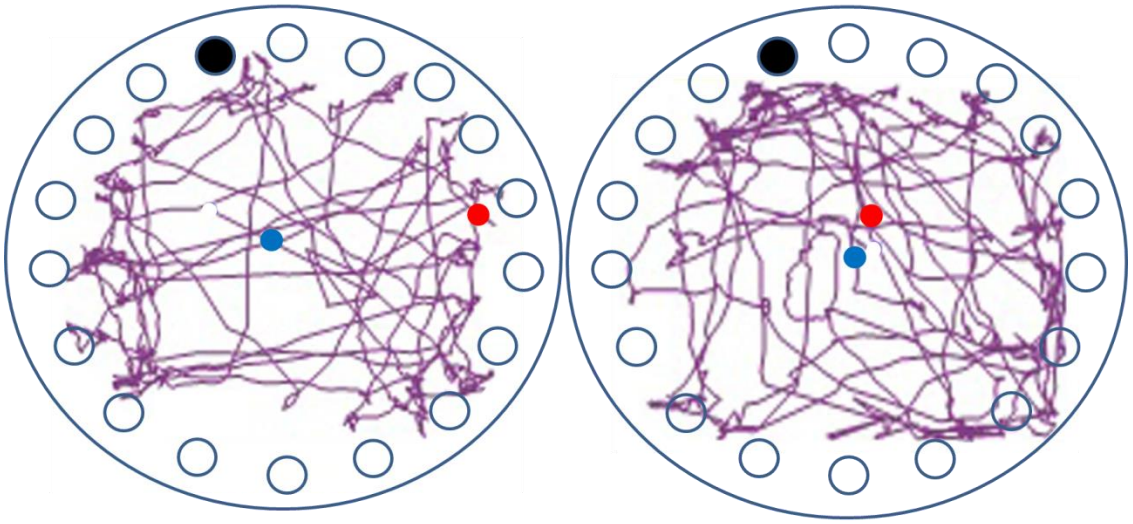
Fig. 1-8 Raw data of escape behavior trajectories of the model (left) and observer (right) rats in all pairs in the first session
Details are the same as in Fig. 1-7.

Fig. 1-9 shows behavior trajectories in all pairs in the first session during the additional control experiment. There is no consistent difference in trajectory length between the model and observer rats.

Pair 1



Pair 2



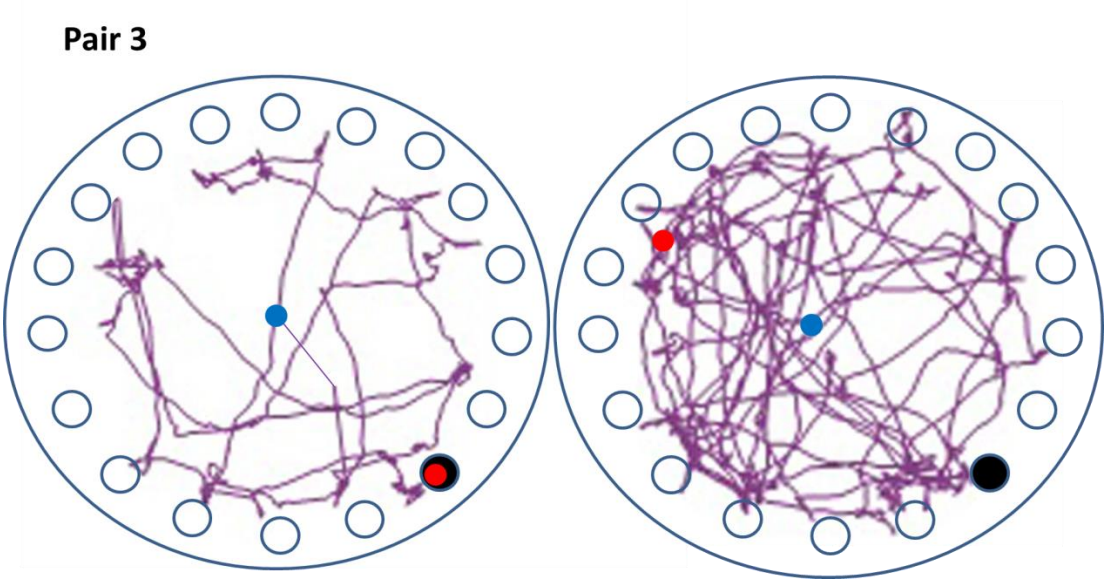


Fig. 1-9 Raw data of escape behavior trajectories of the model (left) and observer (right) rats in all pairs in the first session during the additional control experiment

Details are the same as in Fig. 1-7.

2.4 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 rats 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.

We have no clear answer to why the difference became significant again in session 5. However, we could at least conclude that both observers and models had fully learned the escape behavior by session 4 and some factors caused the difference in session 5. We consider the possibility of "latent learning" in the observational learning, i.e., the observer rats learned the location of the goal box more precisely due to the observation through the four successive sessions (sessions 1-4) and then showed even shorter escape latencies in session 5. Such latent learning can not explain the significant difference of latencies between the model and observer rats in session 1

because both the model and observer rats had no experience of the Barnes maze before the session 1.

We compared behavior trajectories of all rats besides their escape latencies in the Barnes maze in the first session (Fig. 1-8). Though the models showed shorter trajectories than the observers in some of the pairs, there was not always clear difference in trajectory length between the models and observers even in the first session, when the observer rats showed significantly shorter escape latencies than the model rats. This is because the rats, not like ones in the Morris water maze, often stopped and kept still on the maze and the trajectory lengths are not necessarily correlated to escape latencies in the Barnes maze. Therefore, behavioral trajectory is not so accurate to detect learning in the Barnes maze as escape latency. This is the reason why almost all studies using the Barnes maze employed escape latency to examine learning of the maze. In addition, it might be natural that the observer rats did not show the shortest trajectories to the goal box after the observation of the model rats escaping behaviors, because rats, unlike humans, are not considered to completely learn the best adaptive strategy by observation alone. They just learn the tasks “more efficiently" by the observation than the other conspecifics. This also means that the observer rats used information not only from the observation but also from their direct experiences (trial and error) in the maze.

In summary, we found the clear difference in the latency of escape behaviors 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. These results indicated that rats also showed “more efficient” learning through observing the behaviors of other conspecifics, that is to say, observational learning.

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 the escape 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. Another condition where the goal position for both the model and observer rats is changed every session might be meaningful to test whether the significant difference of escape latencies between the model and observer rats, found in the first session of the present study, is consistent.

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 is 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.

3 Experiment 2 - A disruptive effect of electrical stimulation on observational learning -

3.1 Introduction

Observational learning, defined as the ability to acquire new information by observing the behavior of others (Bandura, 1977), is crucial for behaving efficiently in social communities. Many different behavioral experiments have demonstrated that a variety of species can learn discrimination tasks more efficiently through observation than the other conspecifics following the conventional learning procedures of the same task (Darby and Riopelle, 1959; John, 1968; Vanayan et al, 1985). However, the neural mechanisms involved in observational learning remain unclear. A few studies have attempted to investigate them by functional magnetic resonance imaging (fMRI) in human participants, and analyzed their brain activities while they were learning visual information (Burke et al, 2010; Frey and Gerry, 2006). Though their results revealed rough brain maps related to the learning ability, the problem was that fMRI did not have time and space resolution high enough to detect neural and/or circuitry activities.

To clarify the neural mechanisms involved in observational learning in detail, it is necessary to develop animal models for experiments that directly control brain activities and quantitatively estimate the effects of such control on observational learning. We consider that rodents are appropriate as the subjects because more invasive neurological methods have developed for them than those for other species, e.g., administration of activity inhibitors

into targeted brain regions, electrical or chemical stimulation of them, and/or electrophysiological recordings of their neural activity.

We have developed an observational learning task for rats using Barnes maze and confirmed that the rats (observers) showed faster escape behavior after they observed behavior of the other rats (models) escaping into the box in the maze (Yamada and Sakurai, 2018). Using this observational learning task, the present study investigated brain regions that were necessary for observational learning in rats. A recent study suggests that electrical stimulation is a useful tool, by showing that stimulation of medial prefrontal cortex (mPFC) canceled out the benefit of observation in mice (Jurado-Parras et al, 2012). However, it remained uncertain whether the results can be generalized to observational learning in other situations, in particular in spatial tasks rodents are usually good at. Therefore, we employed the method of electrical stimulation as Jurado-Parras et al. (2012) did and tested whether mPFC was really important for observational learning in the spatial task we had developed.

3.2 Material & Methods

3.2.1 Animals

We used thirty male Long Evans hooded rats, weighing about 350 g (range, 300-400 g) and aged 10 weeks at the beginning of the experiment. They were housed in pairs in cages, in a temperature-controlled room (26 ± 2 ℃,

about 55 % humidity). All rats were given ad libitum access to food and water.

3.2.2 Apparatus

The experimental apparatuses were almost same as that used in the Experiment 1. We changed the circular, metal wire mesh cylinder used in Experiment 1 to a larger one (30 cm × 30 cm × 30 cm) with no ceiling, which allowed the cable connected to the rats heads to move freely.

For electrical stimulation, we employed the stimulus isolator (A365R, World Precision Instruments, Inc.) to generate electrical current and the Train/Delay Generator (model DG2A) to control the current parameters (Fig. 2-1). The stimulus isolator was connected to the two-pin socket on the heads of rats. The general view of the apparatus was showed in Fig. 2-2.

All apparatuses were located in a dark experimental room with ceiling fluorescent lights. The room and ceiling light were different from ones used in Experiment 1.

Fig. 2-1 The stimulus isolator (upper) and the train/delay generator (lower)

The stimulus isolator was used to generate electrical current which stimulated the target brains (mPFC and dHPC) of the rats. It was connected to the two-pin socket on the heads of the rats. The train/delay generator, connected to the stimulus isolator, was applied for controlling the current parameters.

Fig. 2-2 An overview of the whole of apparatus in the room used for Experiment 2

When the rat was kept waiting in the metal wire mesh cylinder (located in the center of the platform) for 3 min during training procedures, the rat was stimulated by electrical current delivered from the isolator. The behaviors of the rats were recorded by the camera located above the platform.

3.2.3 Surgical procedure

Before the experiment, the animals received surgery to be implanted stimulation electrodes in medial prefrontal cortex (mPFC) or dorsal hippocampus (dHPC) of their left hemispheres. The animals were anesthetized with 2.5 - 3.0 % isoflurane, supplied by an Anesthetic Vaporizer (MK-AT200, MUROMACHI KIKAI Co., LTD) at a flow late of 1.5 L/min oxygen. The electrodes were bipolar, made by bundling two tungsten microwires (0.2 mm in diameter) which were spaced about 0.5 mm between the microwires (Fig. 2-3) and connected to a two-pin socket through a flexible cable. Some of them were implanted in the left prelimbic area of mPFC (3.0 mm anterior, 0.3-0.7 mm lateral to bregma and 3.0 mm from the brain surface, Fig. 2-4a) and others in the left dHPC (3.0 mm posterior, 2.4 mm lateral to bregma and 2.0-2.2 mm from the brain surface, Fig. 2-4b) according to the atlas of Paxinos and Watson (2007). The implanted electrodes were fixed to the skull with implanted small metal screws and dental cement. The electrode implantation to one hemisphere was usual in almost stimulation or recording experiments. In particular, lesions in mPFC of both hemispheres by the electrode implantation should be avoided because such both sides lesions might cause changes in motivation and ingesting behavior.

The rats were divided into three groups: mPFC stimulation group (n = 14), dHPC stimulation group (n = 8) and control group (n = 8). In the rats in the control group, electrodes were implanted in mPFC, but they received no stimulation during the experiment.

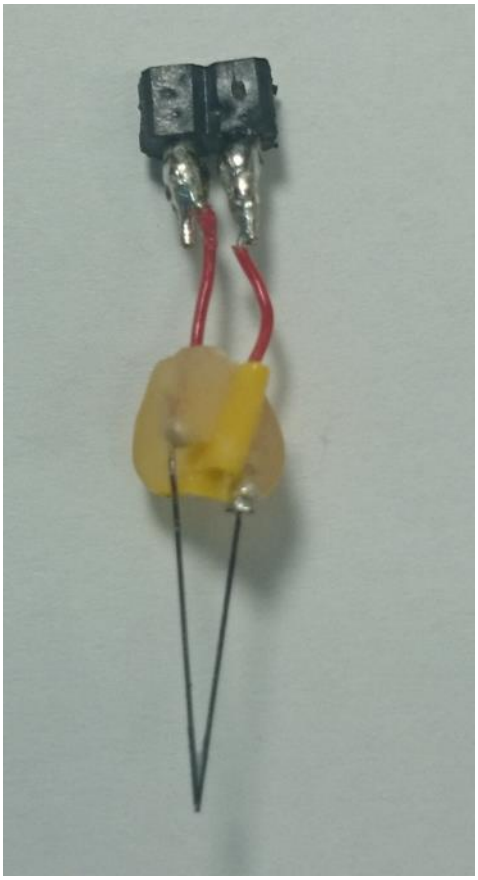


Fig. 2-3 The bipolar tungsten electrode implanted in rats brain

The electrode was made by bundling two tungsten microwires (0.2 mm in diameter). The microwires were spaced about 0.5 mm and connected to a two-pin socket through a flexible cable. The electrode was implanted in the left prelimbic area of mPFC or in the left dHPC. The implanted electrode was fixed to the skull with implanted small metal screws and dental cement.

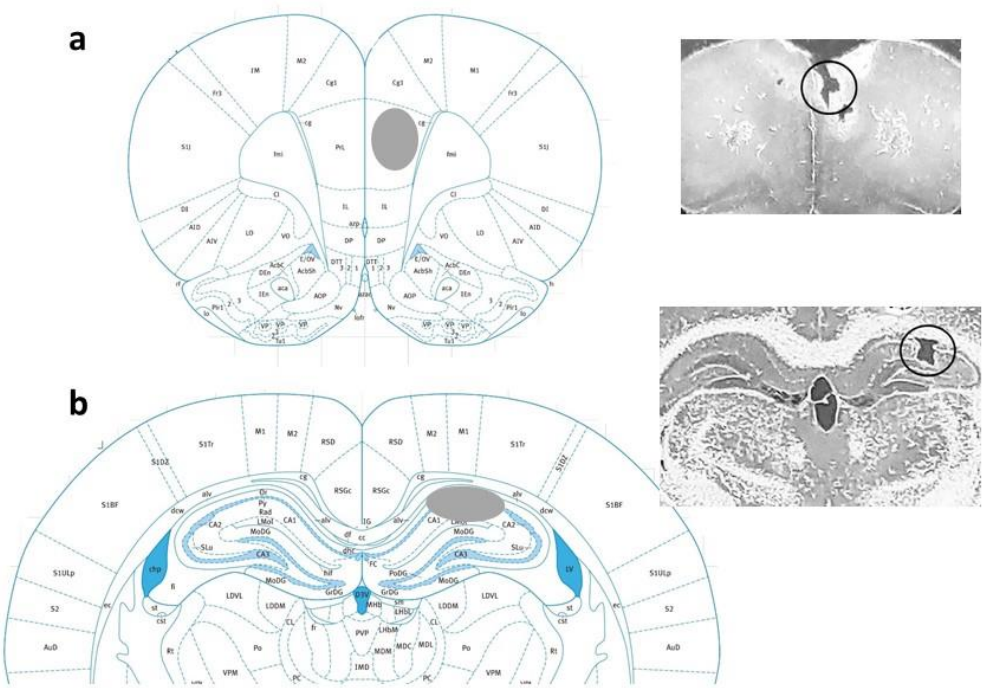


Fig. 2-4 The areas of electrical stimulation by implanted electrodes

(a) The gray circle represents the area of the tips of implanted bipolar electrodes in the rats of mPFC stimulation and control groups. A photo example of lesion made by electrodes is shown at the right. **(b)** The gray circle represents the area of the tips of implanted bipolar electrodes in the rats of dHPC stimulation group. A photo example of lesion made by electrodes is shown at the right.

3.2.4 The stimulation parameters

In the experiment, rats received current at 100 μA (frequency = 100 Hz, duration = 0.1 s, delay = 10 ms) delivered from the stimulus isolator. The stimulation parameters were determined according to the previous reports which indicated some behavioral effects of electrical stimulation in rats (Quirk et al, 2003; Mehdipur et al, 2015; Shimizu et al, 2017).

3.2.5 Experimental procedures

The total schedule is shown in Fig. 2-5. Except for the surgery and electrical stimulation, all rats followed the same schedule and training procedures as used in Experiment (1). We shortened, however, each of the training sessions for model and observer rats from 5 days employed in Experiment (1) to 2 days because the rats had shown successful observational learning in 2 days.

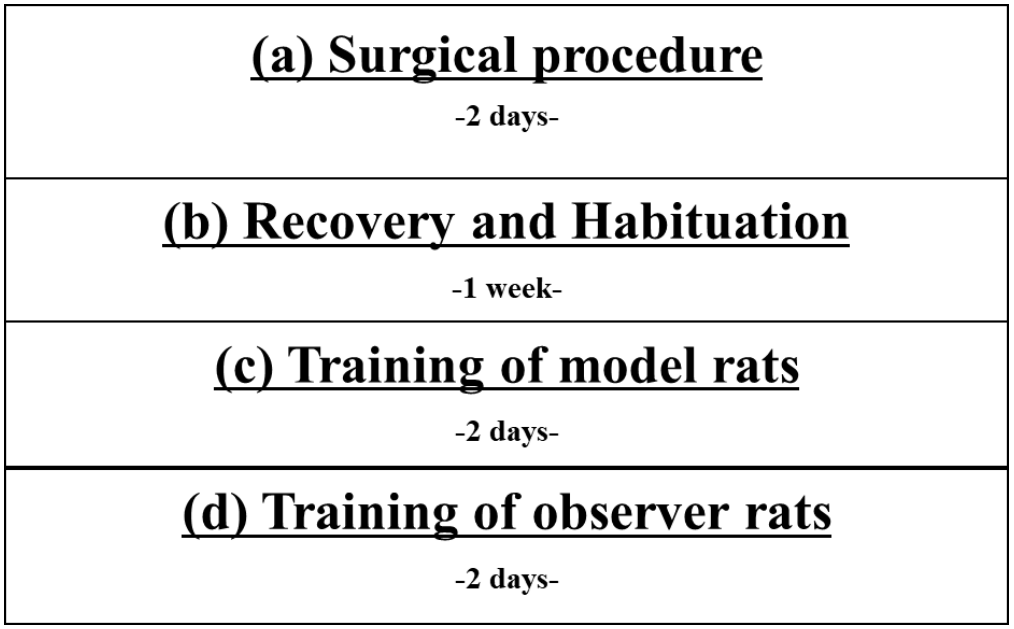


Fig. 2-5 The four successive procedures

(a) The rats received surgery to be implanted electrodes in mPFC or dHPC. (b) The rats were allowed to recover for a week after surgery and were housed in pairs. At the last session of recovery week, they were habituated to electrical stimulation by receiving current with the same stimulation parameters as used in the experiment for 1 min in a cage to confirm that the current did not cause any body movements. (c) The model rats were trained of spatial learning to escape into the goal box in the Barnes maze for 2 sessions. While the model rats were first kept waiting in the center of the maze, they were stimulated twice at random intervals. (d) The observer rats were trained according to almost the same procedures as those used for observer rats in Experiment 1, except that they were stimulated just the time when the model rats were entering into the goal box.

(i) Surgery, Recovery and Habituation

The rats received surgery to be implanted electrodes in mPFC or dHPC in 2 days. They were allowed to recover from surgery for a week before the start of the experiment. They were housed in pairs and then accustomed to the experimental environment. Also, they were habituated to electrical stimulation on the last session of the recovery week by receiving current with the same stimulation parameters as used in the experiment (see 3.2.4) for 1 min in a cage by the stimulus isolator, confirming that the current did not cause any body movements.

(ii) Training of model rats

After the last day of the recovery period, the model rats were trained of spatial learning task in the Barnes maze to escape from the aversive bright lights from ceiling. In every trial, the model was first taken from their home cage, placed in the center of the platform, and then covered with the metal wire mesh cylinder. The rat was then kept waiting for 3 min. During this period, the rat was stimulated by an electrical current identical to that received on the last day of the recovery period after the surgery, as described above. The model rats received the electrical current twice at random intervals within 3 min. This was based on the observation of behaviors of the model rats in Experiment (1), which showed that the model rats repeated entering into and getting out the goal box twice on average for 3 min when the observer rats were waiting. Following that, we trained the model rats in the same manner as used in Experiment (1).

(iii) Training of observer rats

Subsequent to the model rat's training, the observer rat was given the observational learning task in the same Barnes maze. The position of the goal box was consistent during all sessions for each pair of model and observer rats. The observer rat was also kept waiting in the metal wire mesh cylinder for 3 min, during which the model rat was running on the platform to escape into the goal box. The observer rat was able to see the model rat's escape behavior in the metal wire mesh cylinder and was electrically stimulated just when the model rat was entering into the goal (Fig. 2-6). The stimulation was given twice for each observer rat on average, and its parameters were identical to those for the model rats. Then both model and observer rats were taken back to its home cage and the platform was cleaned with water so that no olfactory traces remained on it. The observer rat alone was subsequently trained according to the same procedure as used for the model rat.

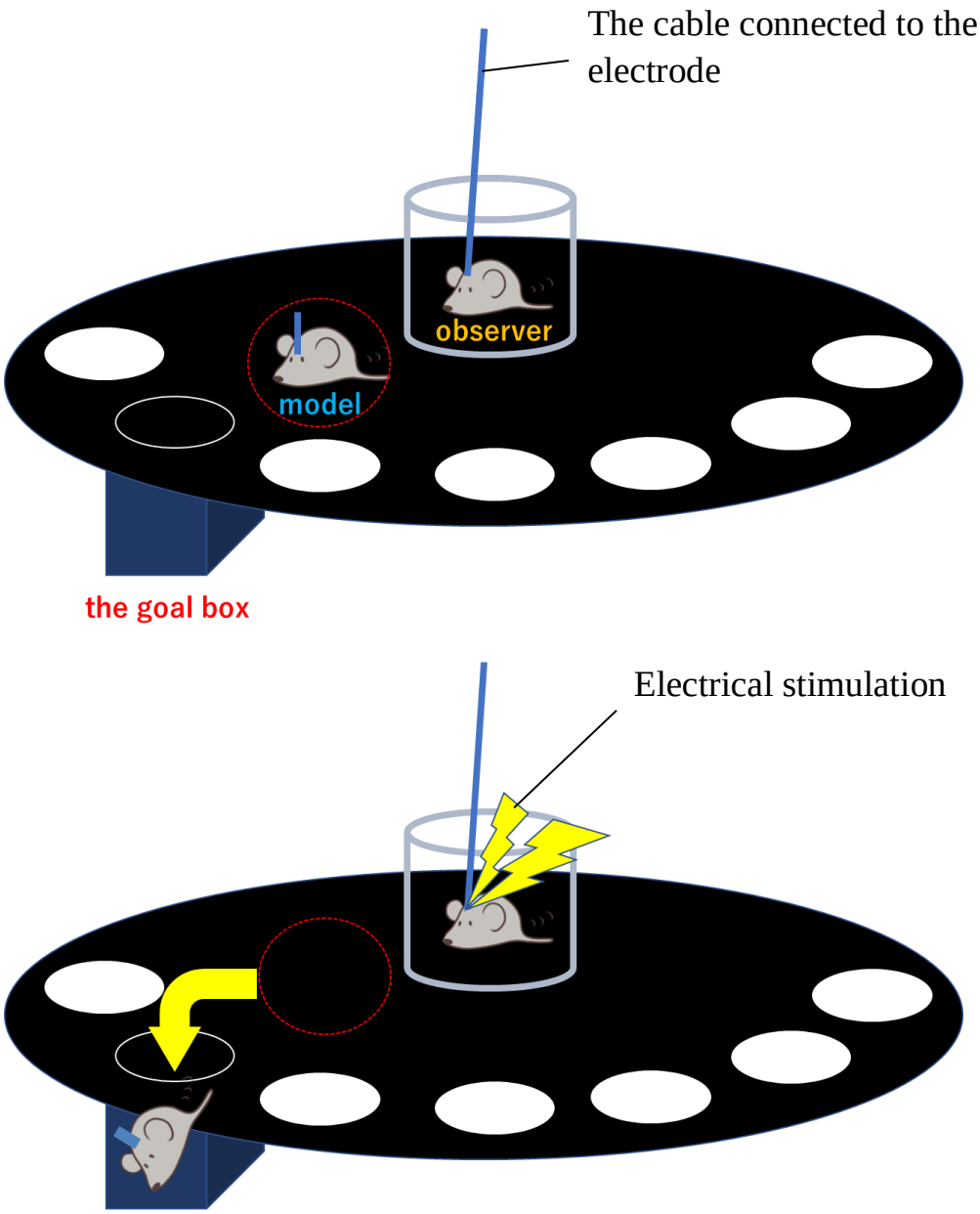


Fig. 2-6 Schematic illustration representing the period of electrical stimulation for the observer rats during the task

The upper panel represents an observing period and the lower panel represents the time when the electrical stimulation was applied to the observer rat, when the model rat was entering into the goal box.

3.3 Results

We compared the escape latencies of the model and observer rats in each session using Mann-Whitney U test (Fig. 2-7). In order to see whether the observer rats showed shorter latencies than the model rats, we mainly focused on the comparison in the first session (session 1) as in Experiment 1. This is because the escape latencies of the observer rats in the second session might be affected by learning by their own experiences in the first sessions. The results showed no significant differences in session 1 between the model and observer rats in the mPFC stimulation group (session 1: $U = 110$, $p > 0.1$). In contrast, the dHPC stimulation group showed the significant difference in session 1 ($U = 57.00$, $p < 0.05$), just as in Experiment 1, and the observer rats escaped faster than the model rats. The same difference was observed in the control group ($U = 53.00$, $p < 0.05$).

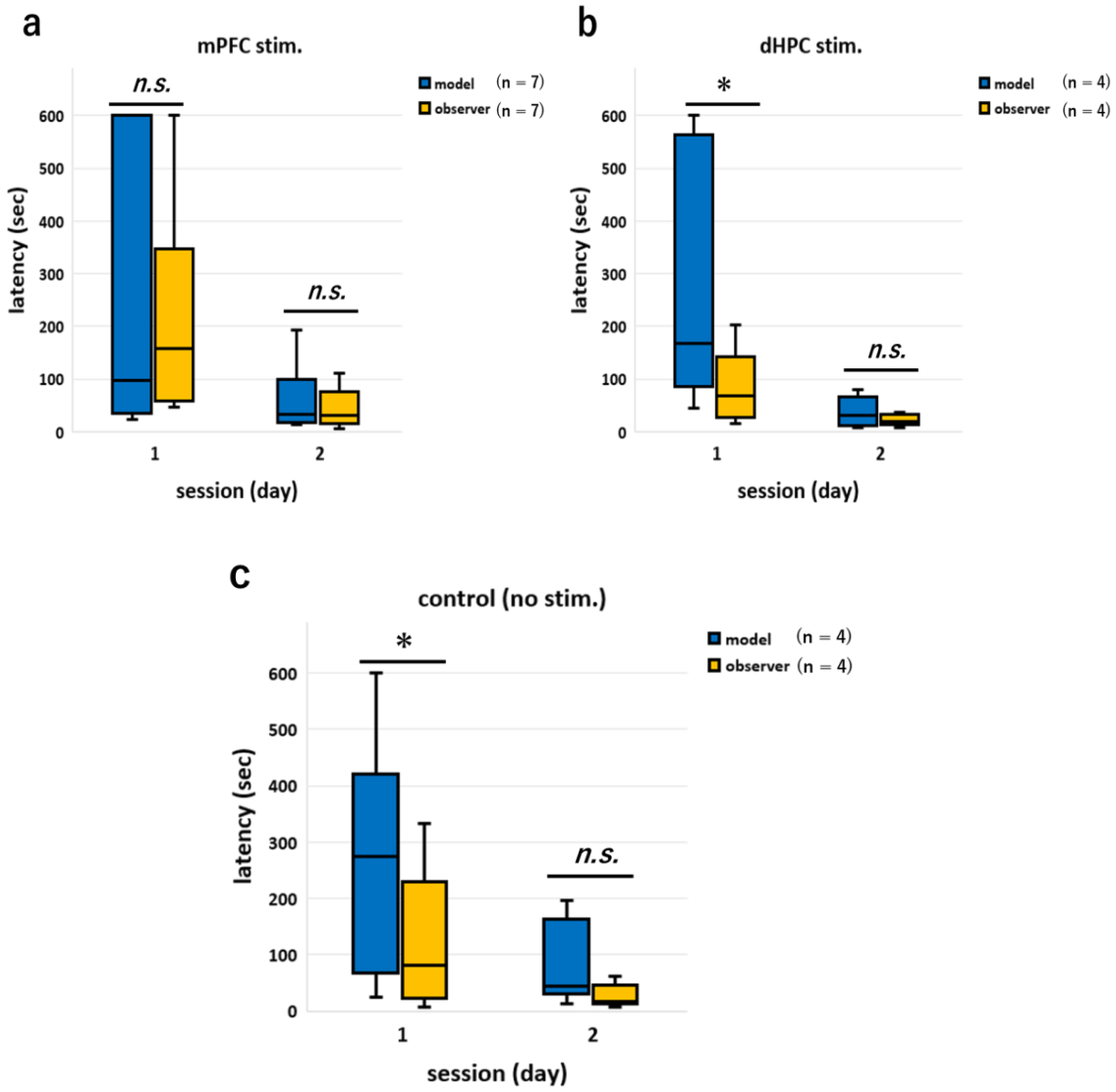


Fig. 2-7 Median latencies of the escape behaviors in the mPFC stimulation (a), dHPC stimulation (b) and control (c) groups

In each group, the blue and the yellow box-and-whisker plots show the latencies of model and observer rats, respectively. The cross bar in each box is the median value. * represents significant difference ($p < .05$ or 0.01, U test) between the model and observer rats. Bonferroni correction was applied to avoid errors of significant levels caused by repeated use of U test. The mPFC stimulation, dHPC stimulation, and control groups included 14, 8, and 8 rats, respectively.

We also compared the latencies of the observer rats in session 1 among the three stimulation groups (Fig. 2-8). The result of H test showed the significant difference among the groups ($H = 7.765$, $p < 0.05$). Furthermore, using Mann-Whitney U test, we found the significant difference between the mPFC stimulation group vs the dHPC stimulation group ($U = 87.00$, $p < 0.05$) and the significant difference between the mPFC stimulation group vs the control group ($U = 99.00$, $p < 0.01$), while no significant difference was found between the dHPC stimulation group vs the control group ($U = 36.50$, $p > 0.1$). In the model rats, no significant difference was found among the three stimulation groups ($H = 2.431$, $p > 0.1$).

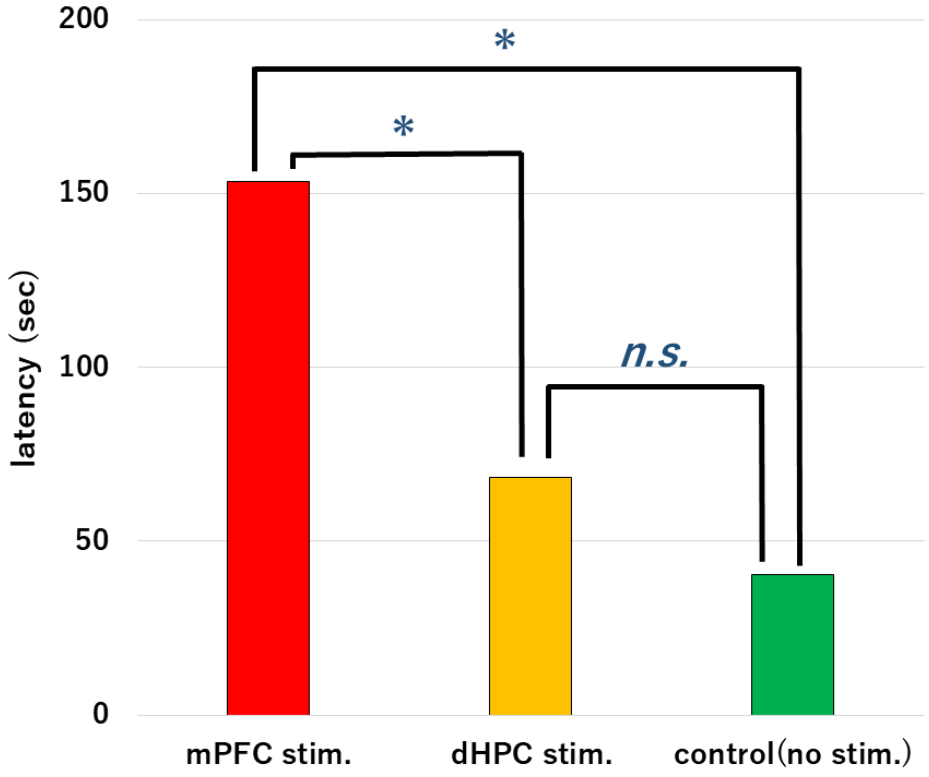


Fig. 2-8 Comparison of median values of the latencies in session 1 in the observer rats among the three stimulation groups

The red column represents the median value of the mPFC stimulation group, the yellow represents the dHPC stimulation group and the green represents the control group, respectively. H test revealed significant difference among the 3 groups. ＊represents significant difference (p < .05 or 0.01, U test) between mPFC and dHPC stimulation groups and between mPFC stimulation and control groups.

An example of raw data representing difference in trajectory length between the model and observer rats in the mPFC stimulation group during the Barnes maze task is shown in Fig. 2-9.

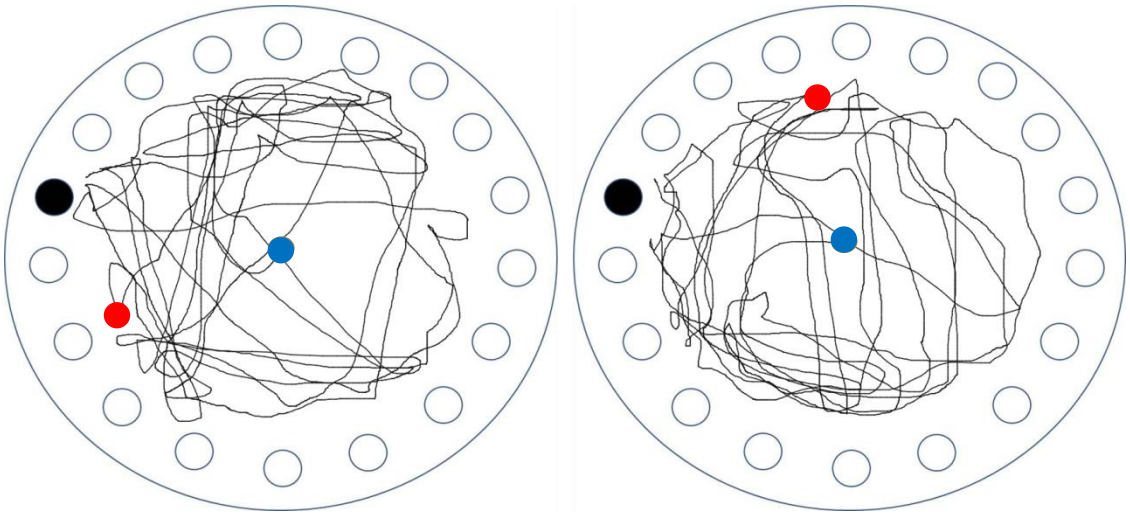
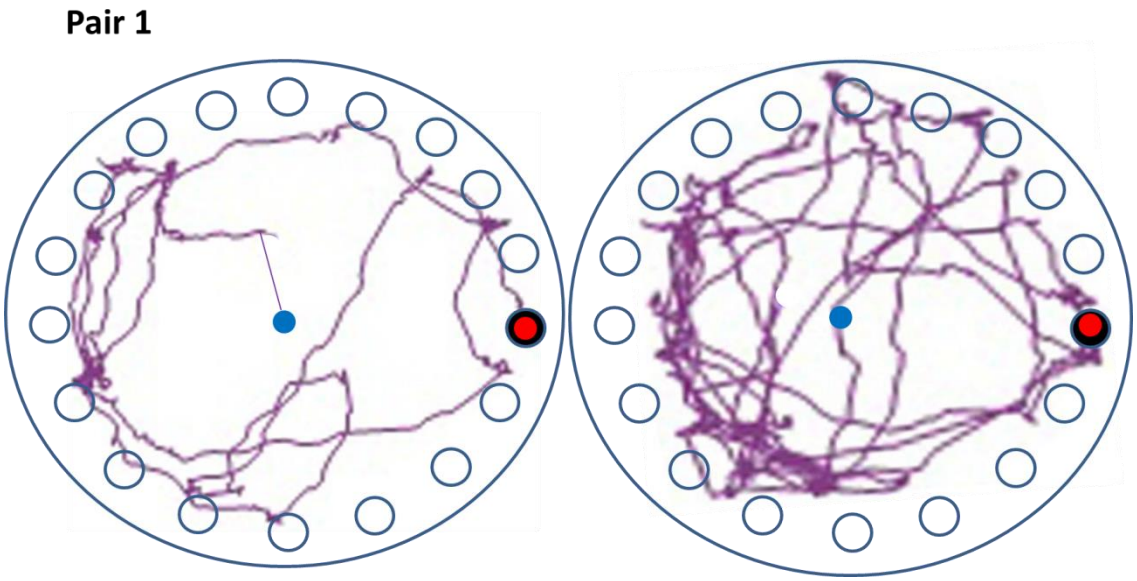


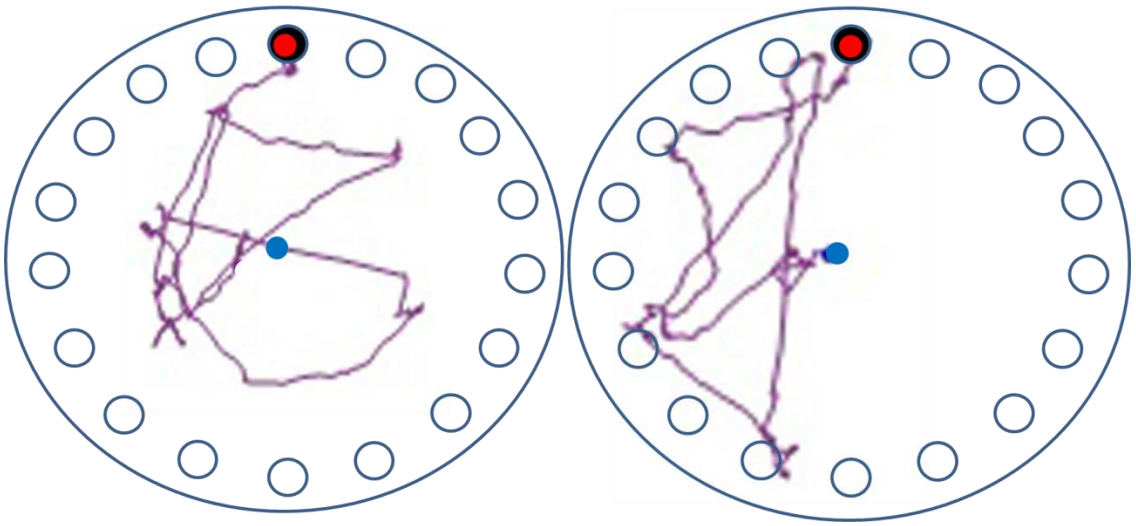
Fig. 2-9 An example of raw data of the escape behaviors of the model (left) and observer (right) rats in the mPFC stimulation group

The details are the same as in Fig.1-7.

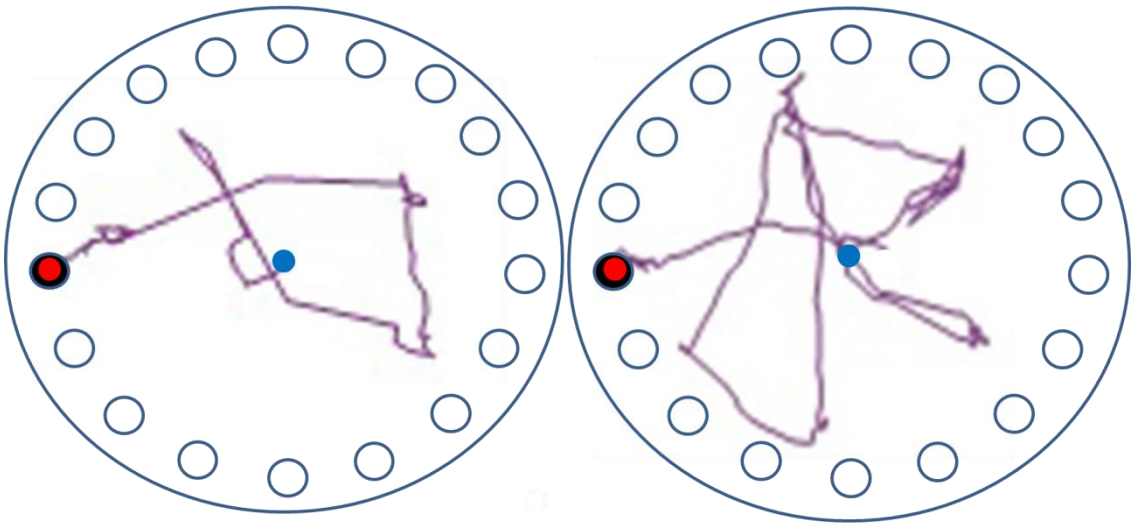
Figs. 2-10, 2-11 and 2-12 shows behavior trajectories in all pairs of the mPFC stimulation group, the dHPC stimulation group and the control group in the first session, respectively. The model is in left and the observer is in right in each pair. There is not consistent difference in trajectory length between the model and observer rats. As in Experiment 1, the trajectory lengths are not necessarily correlated to escape latencies in the Barnes maze because the rats often stopped and kept still on the maze.



Pair 2



Pair 3



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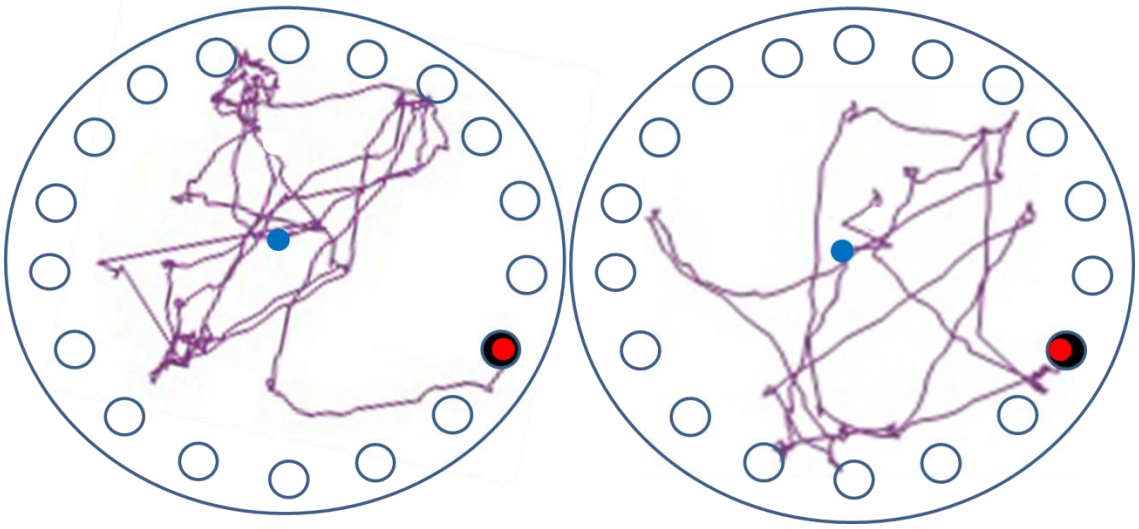

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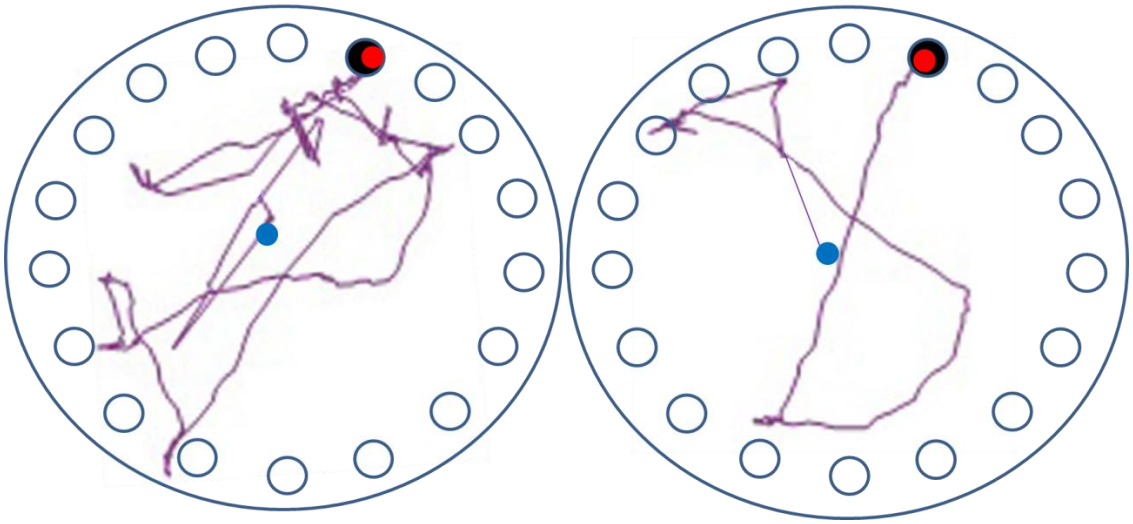


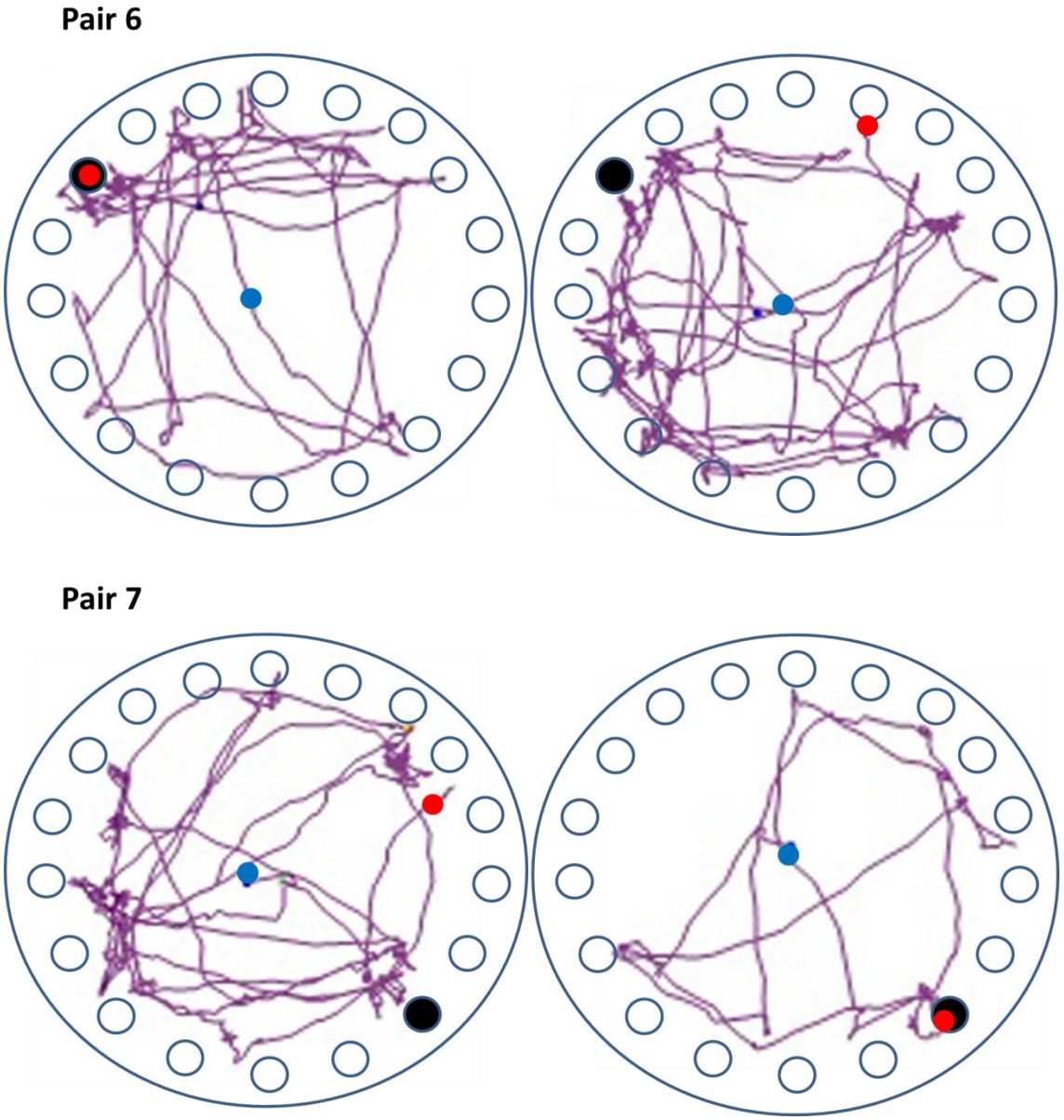
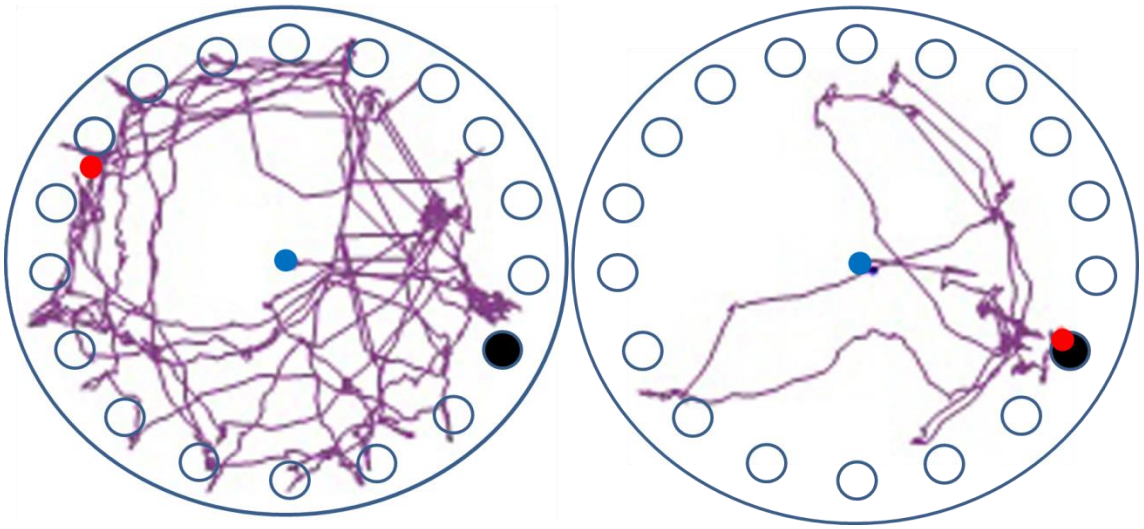


Fig. 2-10 Raw data of escape behavior trajectories of the model (left) and observer (right) rats in all pairs in the mPFC stimulation group in the first session

Details are the same as in Fig. 1-7.

Pair 1



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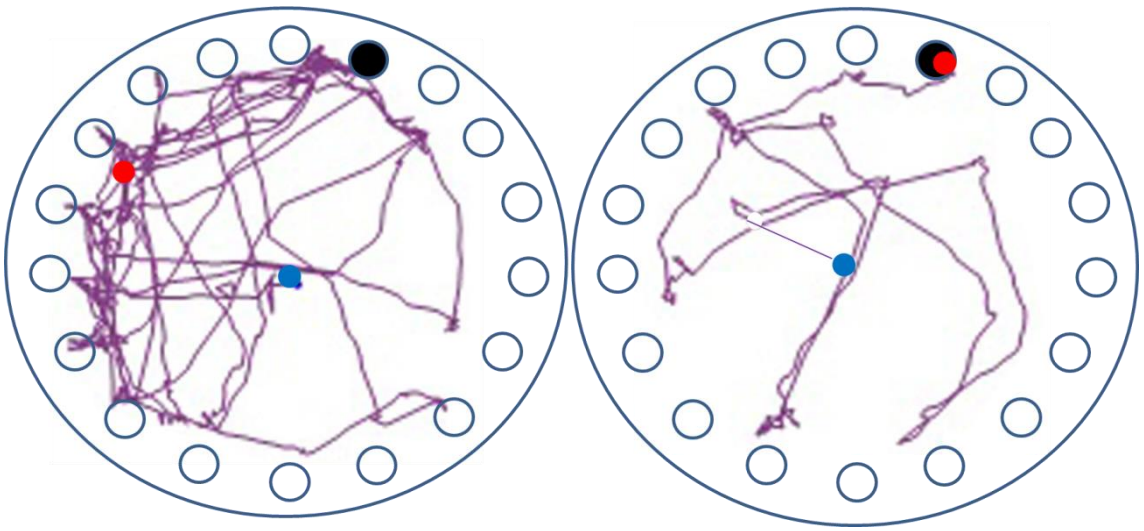


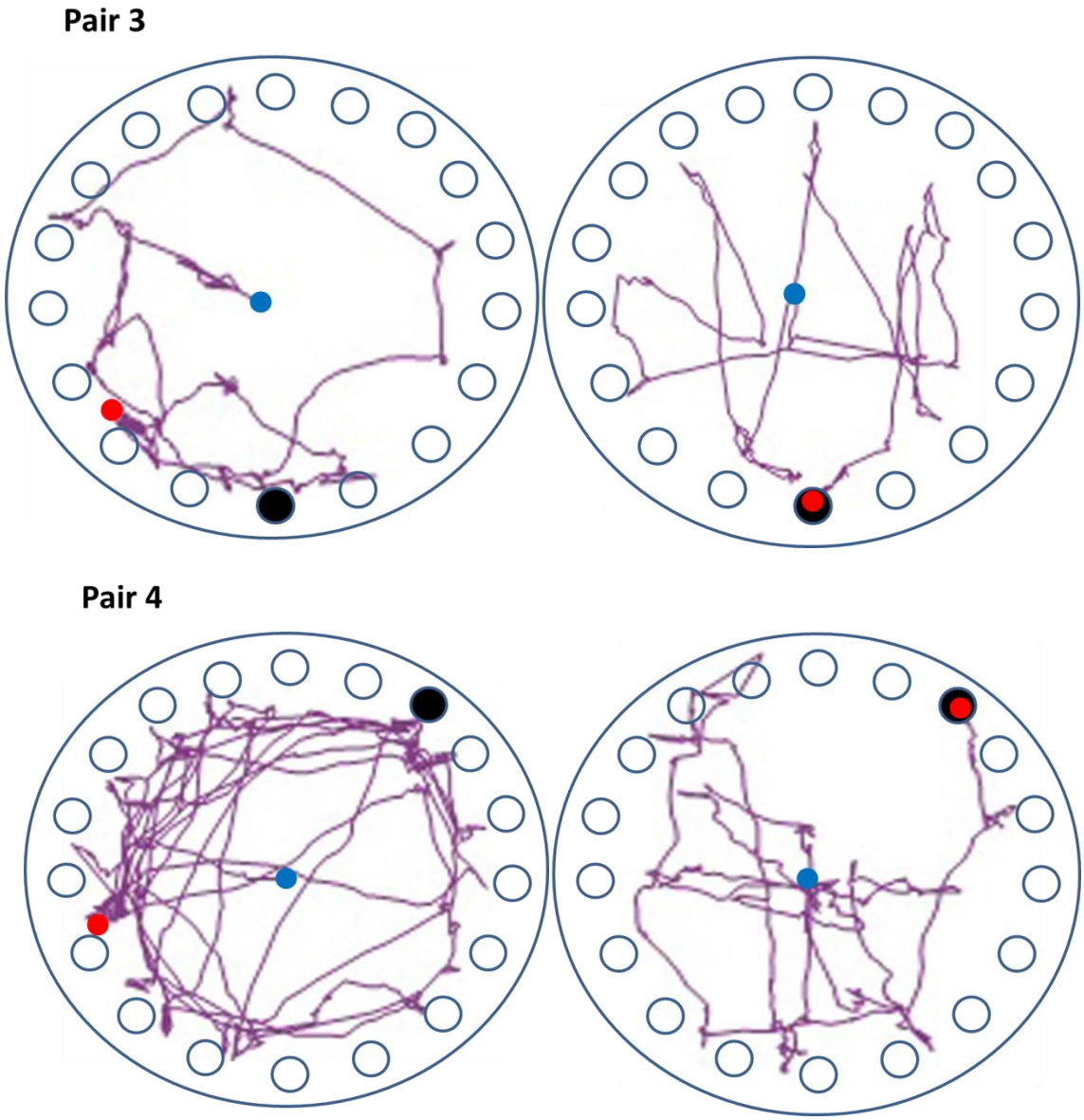
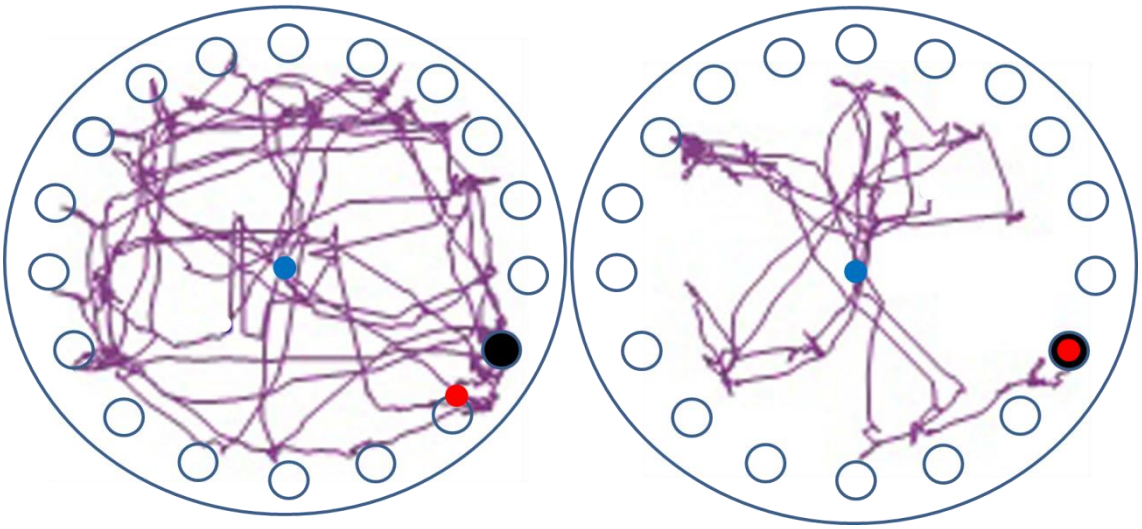


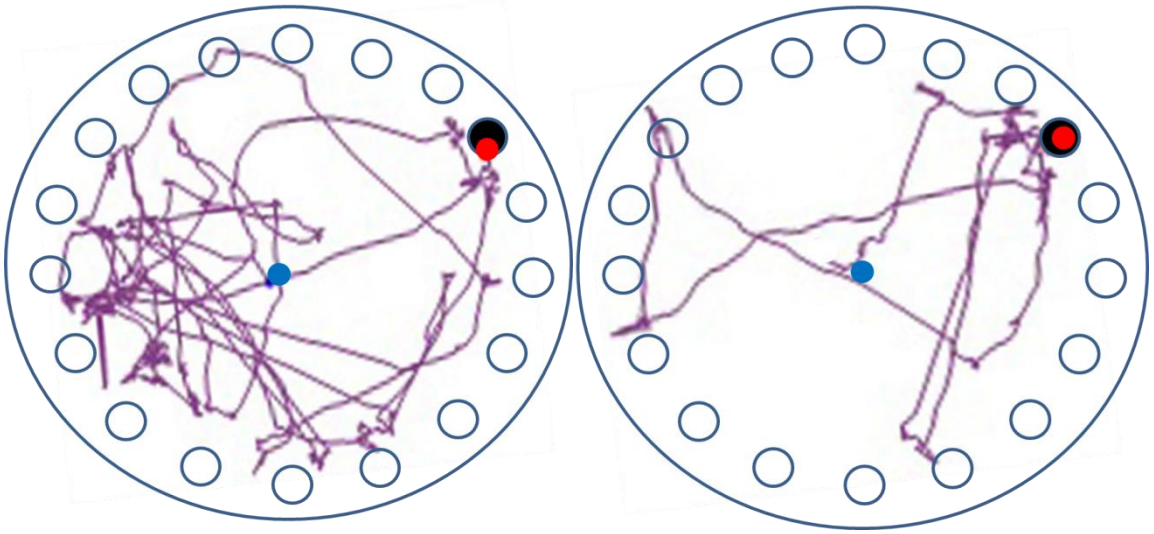
Fig. 2-11 Raw data of escape behavior trajectories of the model (left) and observer (right) rats in all pairs in the dHPC stimulation group in the first session

Details are the same as in Fig. 1-7.

Pair 1



Pair 2



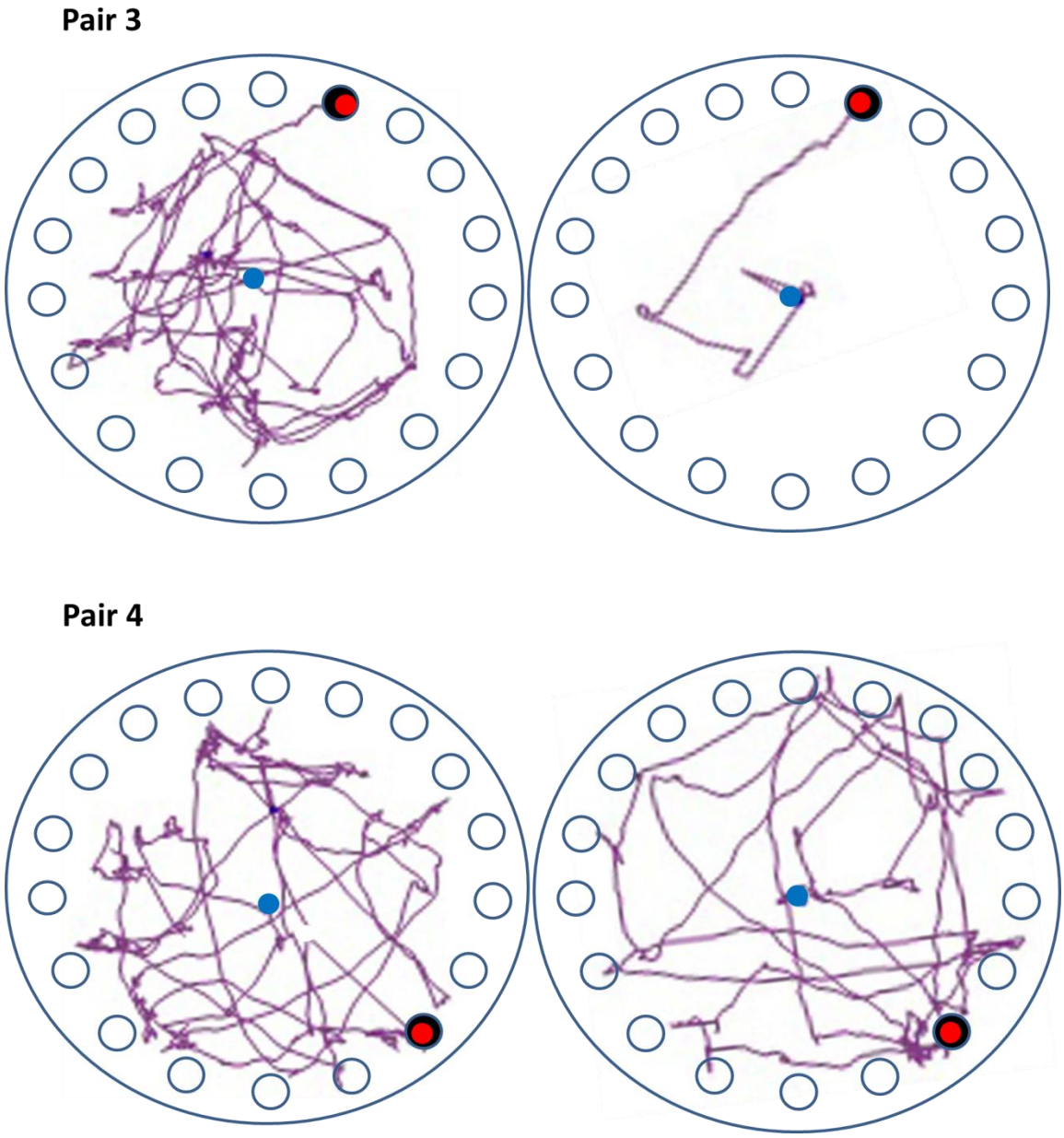


Fig. 2-12 Raw data of escape behavior trajectories of the model (left) and observer (right) rats in all pairs in the control group in the first session
Details are the same as in Fig. 1-7.

3.4 Discussion

In the present study, the observer rats with electrical stimulation of dHPC and just electrode implantation to mPFC showed the shorter escape latencies than the model rats as the intact observer rats in Experiment 1, whereas the observers with electrical stimulation of mPFC showed no shorter escape latencies. Furthermore, in the comparison of the latencies of the observer rats among three stimulation groups in the first session, we found that mPFC stimulation alone had the disruptive effect on the latencies of the observer rats. These results suggest that electrical stimulation of mPFC during the observation periods prevented the observers from observing the learned behavior of the models and resulted in no efficient learning by the observation. This observational learning is based on visual observation of the model's behavior, because the platform was cleaned with water prior to trials of the observers in order to remove olfactory traces of the models on it. In addition, we also confirmed it in a supplementary experiment in which the observer rats were kept waiting in an opaque acrylic cylinder when the model rats were escaping into the goal. Those observer rats, which had been unable to see the model rat's escape behavior, did not show any shorter escape latencies than the model rats. This means that the observational learning in the present Barnes maze was based on visual observation and olfactory and other trajectory traces of the models, even if there were, did not affect escape behavior of the following observer rats.

The present study conducted the electrical stimulation to both the model and the observer rats in the mPFC stimulation and dHPC stimulation

groups. This is because the all experimental parameters including the maze, the room, the ceiling light and the electrical stimulation should be identical between the model and observer rats except the chance for the observer rats to observe the model rat's behavior during the task. Therefore, shorter escape latencies of the observer rats, if any, could be attributed to observational learning.

Before the training of the task, we confirmed that the current of electrical stimulation did not cause any body movements (see 3.2.5). If the current of electrical stimulation to mPFC had disturbing effects on motor movements and/or visual perception, both the model and the observer rats in the mPFC stimulation group might had difficulty to escape to the goal. However, this was not the case in the present study because the escape latency of the model rats in the mPFC stimulation group was not different from those of the model rats in the dHPC stimulation and no stimulation (control) groups. This means that the electrical stimulation to mPFC had no specific disturbing effect on motor and/or visual functions compared to the stimulation to dHPC and no stimulation.

There remain several issues in the present study. First, we have no clear answer to what caused the disruption of the observational learning of the mPFC stimulation group. We assume that neural activities of mPFC of the observer rats might have been disturbed during higher cognitive processing, i.e., paying attention to the escape behavior of the model rat and/or associating the model rat's behaviors with the goal position. Second, we need to confirm that the disruptive effect on observational learning in the

observer rats could be seen by other stimulation parameters or by receiving stimulation at other timings.

On the other hand, the present study surely supports the notion by Jurado-Parras et al. (2012), in which electrical stimulation of mPFC during the behavioral demonstration by the models negatively affected the observational learning by the observers. In addition to Jurado-Parras et al. (2012) that reported observational learning in mice using a lever-pressing task in an operant chamber, our present study has revealed observational learning in rats in the spatial task. The observational learning by rodents using operant chambers sometime had difficulty, e.g., multiple cues in the experimental situations and complex trainings for a long time sometime confounded the observer rats and they did not show observational learning (Mitchel et al, 1999). On the other hand, the Barnes maze task is a simple spatial task utilizing innate behavior of rodents, i.e., escaping from bright lights, and requires no long-term and artificial training for shaping of operant behaviors. This might have an advantage in testing the ability of observational learning in rodents.

The present study is the first step that only pointed out the mPFC as one of the important brain regions involved in observational learning in rodents. In future studies, we will investigate the function of neural circuits including mPFC and relevant brain regions (e.g., Taber & Fibiger, 1993) in observational learning using our behavioral task. For such studies, investigating the activities of neurons in mPFC and anterior cingulate cortex (ACC) is meaningful as they play an important role in processing

information about others in a social context (Apps et al. 2016). In addition, several neuroanatomical studies have pointed out that ACC is connected not only to mPFC but also to temporo-parietal junction (TPJ), which is thought to be involved in guessing mental states of others (Frith & Frith, 2006; Hampton et al, 2008). Recent studies suggest that the ACC of rodents is a homologous region to the human ACC that is related to cost-benefit decision making and learning through observation of others (Hillman & Bilkey, 2012), and inactivation of ACC impairs observational fear conditioning in mice (Jeon et al, 2010). Although it has remained unclear whether mPFC and ACC neurons and their circuitry are directly related to observational learning, we assume that they have a crucial role in directing the attention of observer rats to behaviors of other conspecifics, and we aim to reveal the neural correlation and interaction of mPFC and ACC for observational learning in the Barnes maze in future studies.

4 General discussion

Consistent with our previous study findings, the present study confirmed that rodents are appropriate as experimental animals for investigating observational learning and that mPFC is an important brain region involved in such learning in rodents. There remains, however, a problem in examining neural mechanisms of observational learning using rodents. The cerebral cortex areas of rodents, which are thought to be homologous to those of humans or monkeys, have not been exactly identified neuroanatomically (except for the anterior cingulate cortex gyrus, ACCg; Apps et al, 2016), though it is said that the subcortical areas of rodents are almost homologous to those of macaques (Bowen and Martin, 1997). More comparative studies of neuroanatomy and neurophysiology to identify the homology should be done in the future.

In recent years, some experiments have taken notice of social transmission of threat avoidance in rodents in a classical fear conditioning task (Oisson & Phelps, 2007; Jeon et al, 2010; Allsop et al, 2018). For example, Sterley et al. (2018) found that mice experiencing foot shocks emit a pheromone which was sufficient to induce in the receiver mice increased levels of circulating cortisol and meta-plasticity of excitatory synapses on corticotropin-releasing hormone neurons in the hypothalamus. similarly, when the stressed prairie voles froze in response to the conditioned stimulus, their partners also froze and raised their cortisol levels (Burkett et al, 2016). These studies also suggest that information from ACC is transmitted to the basolateral

amygdala (BLA), known to encode fear information during classical conditioning, and that the activities in ACC and BLA are synchronized in the theta range during observational fear learning (Fig. 3-1). Considering the anatomical homology as described above, it is reasonable to analyze the neural activities of the subcortical areas related to observational fear learning in rodents, because the results of such studies will allow us to consider those structures almost identical with those in macaques regarded as sub-human species. On the other hand, there have been few studies in rodents which look into activity of the cerebral cortex neurons in processing of higher order functions of cognition during observational learning independent of emotions such as fear.

Consequently, it is essential to discover the neural circuits composed of neurons in several brain areas in rodents, including the cerebral cortical areas, during their observation periods for learning. It could be a cue for starting to reconsider the results of the fMRI studies in humans. This is because most of them showed that the distribution of the activating brain regions, found during action observation, largely overlapped with those during direct experience (Cross et al, 2009; Frey & Gerry, 2006; Mattar & Gribble, 2005; Grezes et al, 2003). If this is also the case in rodents, it is meaningful to compare neural circuits working when rats observe behaviors of other conspecifics in a task with those working when they learn alone according to a conventional procedure in the same task.

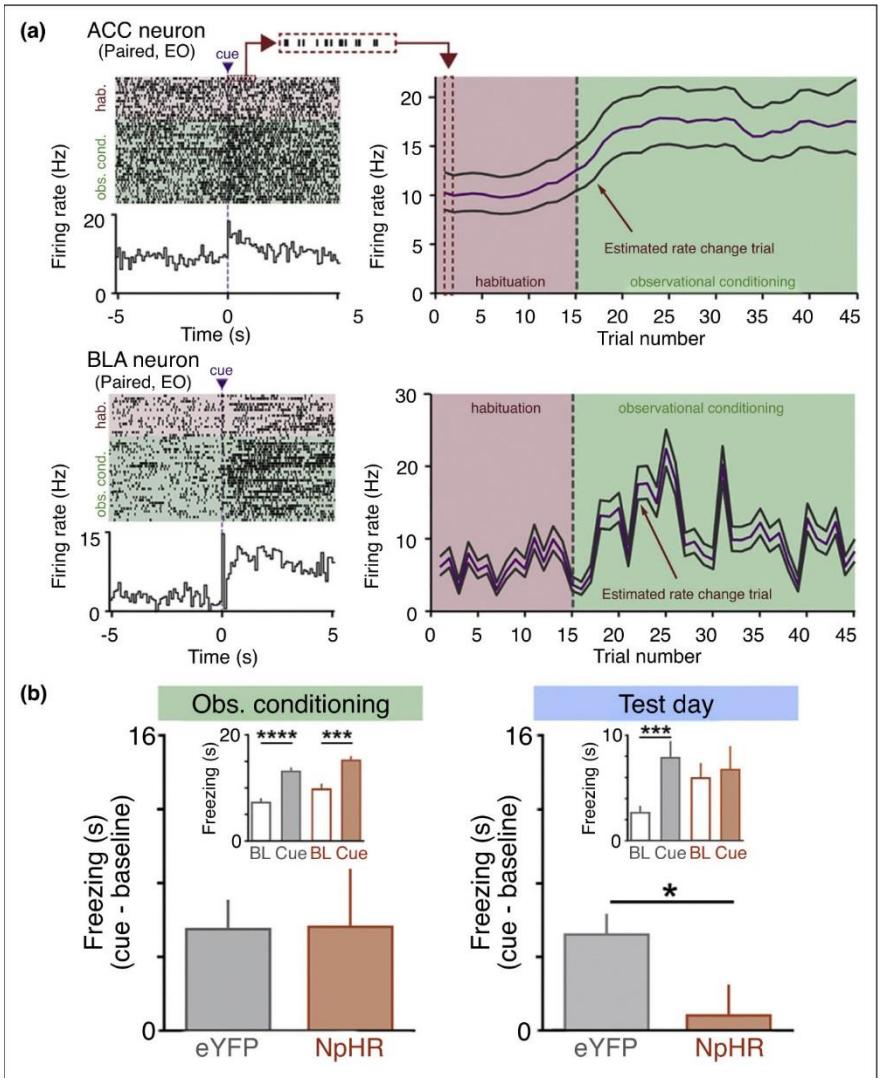


Fig. 3-1 Social transmission of threat avoidance in rodents

(a) During observational learning, ACC plasticity precedes plasticity in BLA neurons. (b) Activity of ACC → BLA projection neurons is necessary for observational learning of fear responses. The graphs are taken from Carcea & Froemke (2019).

In order to proceed toward such studies, it is necessary to identify the related neural circuits, to make projections of neurons involved in processes for observational learning clearer. According to our present study, it may be very important to investigate the neural activities and the projections of mPFC neurons, identified as indispensable for observational learning, using electrophysiological recording and optogenetics to voluntarily control neural activities. In future studies, we will reveal whether or not the neural circuits involved in observational learning are in common with those for other types of learning. If they are different, the former is regarded as an original system and may be a part of mirror neuron systems in rodents. Also, such future studies will provide more evidence for the different learning systems including observational learning.

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