Effects of Environment Changes on Rat`s Learned Behavior in an Elevated Y-Maze

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***Abstract*—In this paper we investigate the Rat`s behavior in a novel Y-Maze experimental environment. The maze is a complex one, composed by three Y junctions (decision points) and is elevated 30cm from the floor. In each side of the experimental area are hanged four different signs. Rats are placed in the starting location and allowed to choose the target route of food location, one from 5 in total. In this work different environment setting are changed during and after the learning process, and the rat learning performance is evaluated. Some of the changes are: changing the rat position at the starting location, changing the sign position, removing all the signs, reducing the light intensity, experiment in dark environment, experimental environment half covered with and without signs.**

***Index Terms*—rat, behavior, spatial learning, Y-maze, memory**

I. Introduction

Rats have been used in experimental mazes since the early 20th century. Many studies have examined rat's behavior in different types of mazes: T-maze, plus maze, radial arm maze, water maze (Morris maze), etc. These maze set-ups are used to study spatial learning and memory in rats, which helped to uncover important principles about learning. These findings can shade lights about learning in other species including humans.

Now days, an important role of mazes is to determine whether different treatments or conditions affect learning and memory in rats. Most of the studies are focused on how drugs, illness and age of the rodents affect their behavior and its spatial recognition memory [1], [2].

Some research are focused and have used a variety of open field designs, placing free-standing corners, monitoring the rodents behavior and fear, and measuring the time spent at the exposed edge [3].

Algthough, many researchers are concentrated in mazes with open or closed arms, studying the rodents behavior. The aversiveness of the open arms is considered by many authors as a sufficient condition to produce the typical behavioral patterns rats exhibit in the elevated mazes [4].

In this study we are focused in Y–Maze, which is commonly used for assessing spatial working memory in rats and mice, especially for spontaneous alternation tasks. Also, Y-maze is used to find out the differences between rats with different age. Some of the researchers strongly support the idea that the ability to learn the Y-maze conditional task depends on the function of the glutamate –nitric oxide–cGMP pathway in the brain. The reduction in the function of the pathway in mature compared with young rats is responsible for the decrease in the ability to learn the task [5].

The rats spatial behavior relies on external and on internal cues. Landmarks are external cues placed in the environment, which help the animal to localize itself. On the other hand internal cues, such as vestibular and kinesthetic sensations, are generated during self-movement of the animal. Combination of these cues provide animals with means to find their way and organize the spatial recognition map. Hence, internal and external cues are utilized by animals in navigating and constructing spatial representation [6]. However, it is not clear how the rat's behavior and the spatial learning are affected by the changes in the external cues.

In this paper we represent a novel Y-maze, composed by three Y junctions (three decision points for the rat), which makes it more complex and difficult to learn and memorize the right route. Also, in each side of the maze are hanged four different sings. Firstly the rats are left to get familiar with the new environment and to overcome the fear and stress. Then they are trained to learn the task, to achieve the target food location. The rat learning performance is evaluated in terms of percentage of successful rate, time to reach the target food location, moving speed and adaption to the environment changes. The focus is on how the rat reacts to these changes and how the performance change.

The paper is organized as follows: The experimental set-up, procedure and the subjects are explained in section 2. The experimental results in different environment settings are presented in section 3, before concluding in section 4.

Manuscript received October 1, 2014; revised July 11, 2015.

doi: 10.18178/jomb.5.2.113-118

II. Methods and Materials

A. Subjects

The subjects were male rats (Witsar Rats), 11-12 weeks old, weighting 270-290 g in the beginning of the study [7]. All the rats were housed one per cage (25x40x20cm plastic cage) in a controlled conditions room: room temperature 23-24 ℃, light-dark cycle 12 hours (lit from 8:00 AM to 8:00 PM and dark the remaining hours). The cage was cleaned once a week and new wood excelsior is inserted. The transportation of each rat from the housing room to the experiment area was made by using another cage (20x30x15cm) covered by black curtain to avoid the rat's confusion.

The food was restricted to 85-95% of their free-feeding weight (around 15g per day) and the amount of water was not restricted. Around 3g of food were consumed during the experiment and at the end the rats are fed with the remaining amount of daily food. The rats were weighted twice a day, before starting the experiment and after it.

B. Experimental Environment

A 200x220cm area, aspirated and air conditioned at 25℃, was used during the experiment. The environment is surrounded by heavy black curtains to isolate Y-maze area. Curtains help to avoid any light leaking from the outer environment and to reduce in minimum the undesired noise. The environment was light up by two fluorescent bulbs, where we were able to control the light intensity.

Inside the experimental environment we build up a novel Y-maze, 167 x 197cm, as shown in Fig. 1. The maze where the rats were trained contains one starting point and 5 food locations. In the end of each route we put a metallic cup, where four of them are covered with a metallic net and one uncovered (the target food location or food location 3). We put food in each cup, so the food odor was equal for each route. Rats can eat the food only in the uncovered cup (F. L. 3, Fig. 1).

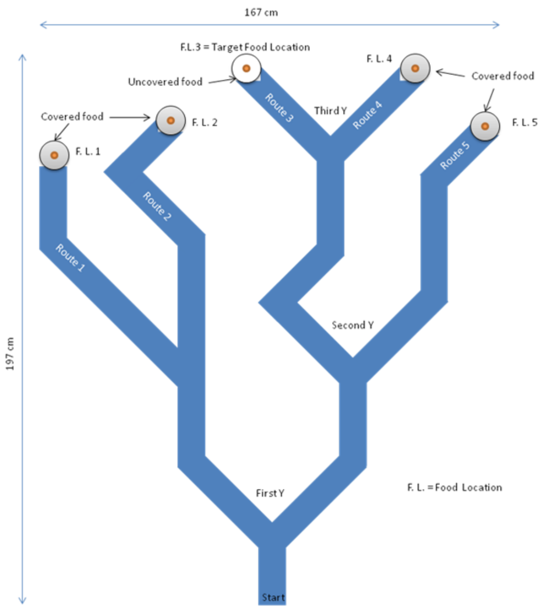

Figure 1. The novel Y-Maze.

The Y-maze is elevated 35 cm from the ground by wood columns (Fig. 2 and Fig. 3). We used polystyrene material (10cm width) to build the maze and covered it with a transparent PVC sheet. This is done to avoid the contact of the polystyrene material with organic acid (acetic acid), which we used to clean the routes after each trial of the experiment. All the routes were surrounded by 2 cm band in order to protect the rats from falling down.

Inside the experimental area we hanged four different signs: square-at home side, triangle-at the destination side, X -in the left and circle-in the right sides of the maze. Each one hanged 43 cm from the floor or around 8 cm above the maze. Sings are printed in black, in 42 x 42 cm white cardboards, as shown in Fig. 2 and Fig. 3.

Also, we used a tracking system composed by a camera, fixed in the center of the experimental area and 170cm up from the maze (Fig. 2), and an ASUS i5 notebook. The camera is KBRC-M05VU with frame rate 30 frames per second; infrared, self-adjustment of exposure and image adjustment functions. We build a Matlab based GUI in order to collect the camera data and to calculate the time spent by rat to reach the target food location. In addition, the route and moving speed are generated.

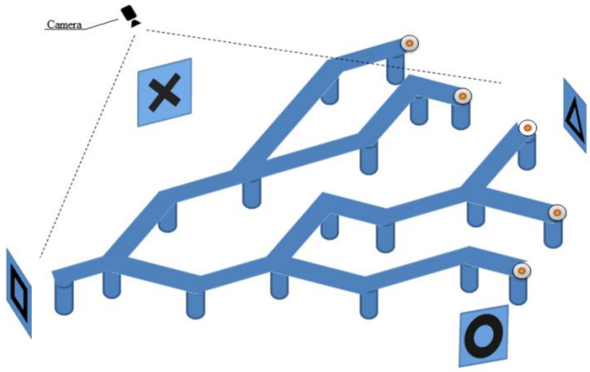

Figure 2. Experiment environment.

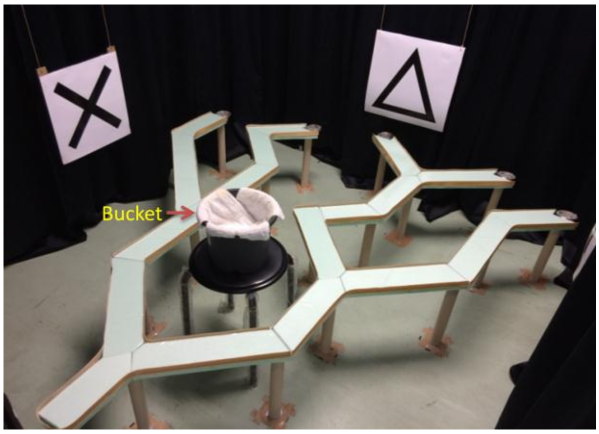

Figure 3. Real experiment environment.

C. Procedure

All the processes were approved by the Care and Use of Laboratory Animals in the University of Toyama, and are followed all the NIH guidelines for Care and Use of Laboratory Animals.

The whole experimental procedure lasted for around 80 days. Handling, training and recording session took place from 6:00 to 8:00 PM. Each day we did the handling procedure with two training rats, for around 5 minutes. Beside the fact that this process makes them more familiar with human's touches, we even changed the gloves for every rat, so they get used to the same smell. Before starting the maze experiment, we placed the rats for around 5 minutes in its bucket in the center of the maze, 65 cm from the floor or 30 cm above the maze (Fig. 3). This procedure is done to let the rat observe the maze and the signs, in order to be familiar with the environment and the maze. The bucket then is moved out of the maze. In the first two consecutive days, the rat explored the environment (maze), for around 15-20 minutes each day (without placing any food in any destination). Always the rat is handled by the experimenter and placed at the starting point (Fig. 1). The rat position was toward the food locations (or triangle sign, Fig. 2).

The rat task was to learn the route how to reach only the uncovered food location (target food location), as shown in Fig. 4. As it is a complex maze with three Y junctions, in first two days of training we put some pieces of food in the route from initial position to food location 3, to help the learning process.

Every day (including weekends), each rat made 20 trials. After the rat ate the reward, the experimenter handled the rat to the bucket, and cleaned the entire maze by paper towels and organic acid (acetic acid) in order to remove the remaining odors.

When the rat learned the task, we changed different environmental settings. For each setting changed we made the experiment for three consecutive days (20 trials per rat). After the rat finished the trials, it is send back to the housing room, and the other rat is taken to make the same training procedure.

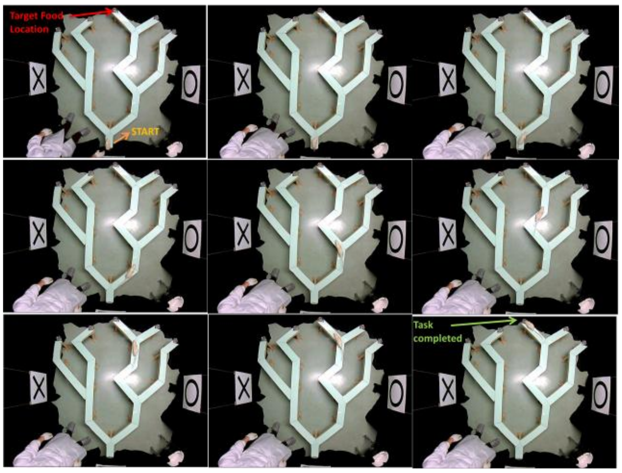

Figure 4. Rat reached the target food location.

D. Measures

All the trials were recorded and then analyzed. The rat learning performance is evaluated in terms of percentage of successful rate, time to reach the target food location, moving speed and adaption to the environment changes. We should mention here that in those trials where the rat reached the food locations 1, 2, 4 and 5 (not the goal ones), time is measured till the rat reached food location 3. Every day the successful and not successful trials were counted. Standard Deviation (SD) of time is also calculated. By analyzing the data, we found the rat speed in the first, second and third Y junctions, how many days the rat needed to learn the task, how useful were the signs and how much effect had the changes in the environmental settings in the rat behavior.

The changing's of the environment settings are as follows:

(a) Changing the rat position at the starting location (head of the rat directed toward the home sign - square sign),
(b) Changing the sign position,
(c) Removing all the signs,
(d) Reducing the light intensity,
(e) Experiment in dark environment,
(f) The experiment environment half covered (removing two sides of curtains).

Changes in the environmental settings were done after the learning process.

III. Results

Not surprisingly, at the beginning of the experiment, the rats move slowly and the unsuccessful rate percentage was high. After approximately 25 days of training (Fig. 5), they have learned to follow the route to the target food location with a success rate of 90 to 100%. During the task learning session, the rat's behavior (successful rate between rats) differs a lot. As presented in Fig. 5, Rat 1 has shown a non-stable learning performance, comparing with the Rat 2 performance. After 14 days their performance has become almost similar.

A. Time

The average time of each rat to reach the target food location is shown in Fig. 6.

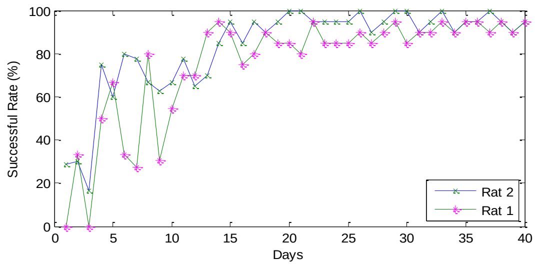

Figure 5. Successful rate for each rat.

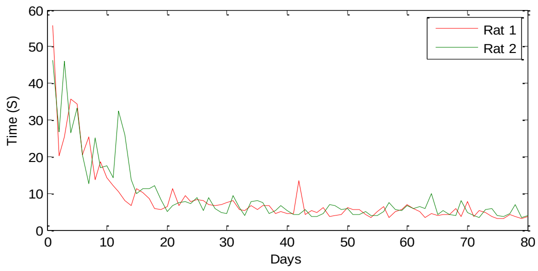

Figure 6. Average time for each rat.

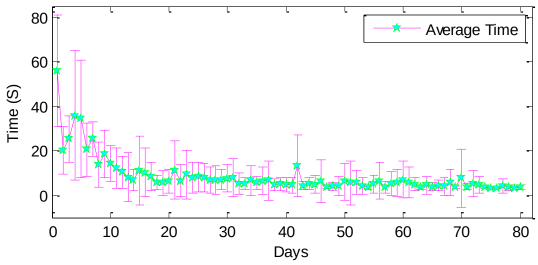

Figure 7. Average time and Standard Deviation - Rat 1.

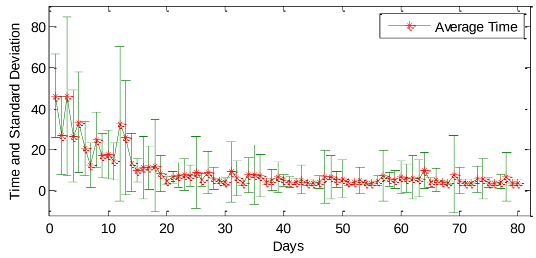

Figure 8. Average time and Standard Deviation - Rat 2.

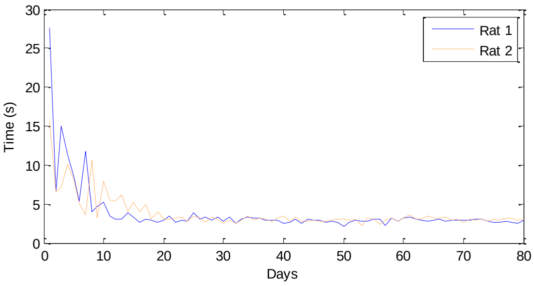

Figure 9. Fastest time for each rat

The average time is reduced by 83% for both rats during the course of training process. However, in some specific days the average time to the target food was increased due to spontaneous alternation behavior of the rat [4] (even when they learned the task, in one or two trials they tend to go in other food locations to explore and to mark the routes with their wee). This can be seen also from the standard deviation graphs shown in (Fig. 7, Fig. 8).

Fig. 9 shows the shortest time to the target food location for each rat. The time is reduced during the first days and after 25 days there is a little change.

By comparing the graphs, Rat 1 has shown a higher activity behavior (much faster) than Rat 2 during the learning session between 10th and 20th days. The average fastest time, after the learning sessions (after 25 days) is around 3 seconds.

The speed of the rats, which is related with time spent to reach the target food location, is increased a lot comparing with the learning session where the average speed for both rats was 33.2 cm/s. The highest speeds were 77 cm/s and 67 cm/s for rat 1 and rat 2, respectively.

In this study, the speed in each Y junctions is also analyzed. Almost in every day of the experiment, for each rat, the speed in the first Y junction was lower comparing with the speed in the second and third Y junctions. This is clearly distinguished during the learning session, where there is a clear distinction in the rats speed, between the first Y junction and other Y junctions. This difference can be related with the difficulties that rats have to make the decision which route has to follow to reach the target food location. Complexity of the maze and the fact that in the learning session they still did not create a cognitive map in the hippocampus, but used the information from the striatum region of the brain, led to spent more time to decide which route to follow.

When the rat has learned the task, this speed distinction is very small.

B. Changes in Rats Position

We investigated the effect of the rat initial direction on the success percentage rate. In 11th and 12th training days in half of the trials (10 trials) the rats head were directed to home sign (square), which makes the task more difficult for them. The rest 10 trials, the rat direction was toward to destination routes (triangle sign). The results showed that in the first 10 trials where the rats head were directed toward home sign the successful percentage was 60% (in the 11th day) and 50% (in the 12th day). The other 10 trials where the rats head was directed toward the destination sign, the successful percentage was 80% (in the 11th day) and 90% (in the 12th day). By analyzing the overall percentage in these days, for each rat, we can say that reaching the target food location success rate is decreased by nearly 40% (Fig. 10).

We did the same experiment when rat has learned the task (days 34th, 35th and 36th) and the successful percentage was above 85%.

The results show that the percentage of success rate was almost the same, which means that the position and orientation of the rat does not affect the rat's behavior when the rat has learned the task. This suggests that in the initial phase of learning, the rat place and direction is important for the rat's route following behavior.

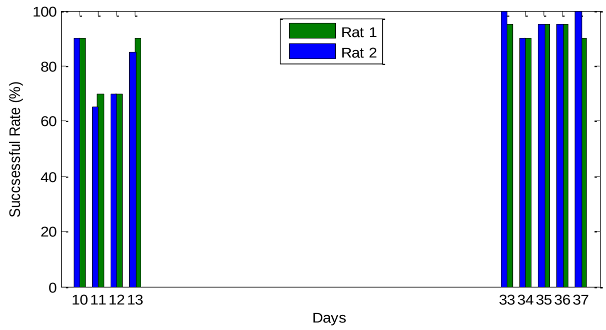

Figure 10. Successful Rate in rats position change

In addition, this happens because the environment map was not fully created or memorized in the rat's brain resulting in localization errors. These results suggest that the rats used striatum brain region and not the hippocampus for localization. When rats learned the task, it is easy for them to localize their position and does not matter in which direction they are placed in the starting point.

C. Changes in Signs Positions and Removing All the Sings

After the rats has learned the task we made two other experiments, 3 consecutive days per each, related with signs. In the first one the sign positions are changed: square sign from home side is placed in the destination side; triangle sign from destination side to home one; X and circle sings are also changed respectively, from left to right and vice versa.

The successful percentage for both of the rats was around 90 %, which means that the changes in the signs position did not affect too much the rat behavior to go in the target food location.

The second experiment, performed in a distance time from the first one, was removing all the signs from the experiment environment. We were impressed; the rat's performance was 100% successful, which confirms the fact that when the rat has learned the task, also a cognitive map is created in his brain, and he does not need anymore any external cues.

D. Light Intensity Reduced and Experiment in Dark Environment

Also, other experiments are performed with the rat. We changed the light condition. When the intensity of light is reduced (the routes of the maze still distinctive for the rat) the successful percentage and the time was different for each rat. The rat 1 has shown a lower percentage comparing with the rat 2, 85% and 95%, respectively. In the other hand, the average time spent to reach the target food location was 5.87s and 8.45s, for rat 1 and rat 2, respectively.

The other experiment was performed in a dark environment. As expected, the successful percentage was decreased till 60% and the average time spent by each rat to reach the target food location was increased till 13.33s. Despite the fact that rats has learned the task, when they do not see (without visibility), it is very difficult for them to find the right route and need more time to decide which route to follow.

E. Experimental Environment Half Covered

The last experiment we did is removing the curtains of two sides of the experiment environment. This experiment is done to understand better if the other environment will affect the rat's behavior after they have learned the task. At the end of the experiment (3 consecutive days) the results were same for both rats with a successful percentage almost 100%, which means that the rat's performance is not affected by other environment.

F. Food Location Preferences

Even when the success percentage rate was above 90%, in some of the training days the rat followed route to other (wrong) food locations. This behavior is known as spontaneous alternation. The results show that in most of the wrong routes the rat reached the food location 1 (Fig. 11). The results also show that after getting in the third Y junction, in 99.7 % of all experiments trials the rats reached the food location 3.

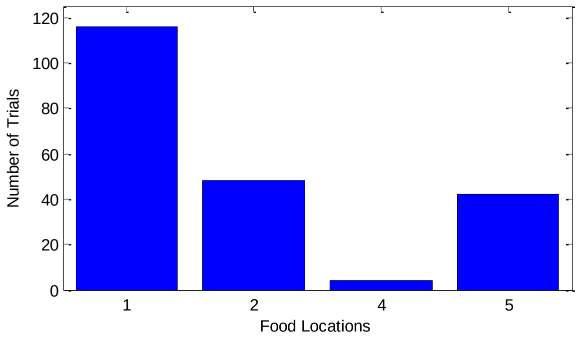

Figure 11. Food location preferences

IV. CONCLUSION

In this paper we presented the Rat`s behavior in a complex Y-maze environment. The results showed that the rats were able to learn the route to the target food location in around 25 days. Before and after the learning session, different experiments are performed. The initial heading orientation affected the rat's performance. The time is reduced when the rat has learned the task. In some of the experiments, the rats were able to adapt to the changes in the environment settings, which means that the successful percentage was high and the time spent to reach the goal was low.

In addition, light is very sensitive in rat's behavior. Experiments in dark environment led to a low successful percentage and a high time performance for all rats.

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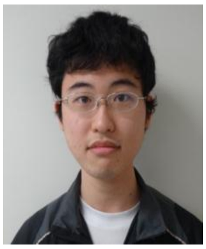

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