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1 Effects of Ischemia on Spatial Learning in Mice

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Introduction

Spatial memory of animals has been studied using several different paradigms, with the Morris water maze being one of the most common among them. This paradigm uses water-escape but we have proposed the dry-type that uses food reward as a more reliable way to study spatial memory in mice (Yoshida et al., 2001).

The role of the hippocampus in spatial memory has been well documented in many species including humans (Maguire et al., 2006), monkeys (Lavenex et al., 2006), birds (Watanabe, 2001; Watanabe & Bischof, 2004), and goldfish (Saito & Watanabe, 2004; 2006). Lesion studies are the classic method to demonstrate involvement of a particular brain region in a behavioral task. There are several methods researchers use to make lesions to the hippocampus. One of these is brain ischemia, in which the brain in deprived of blood supply by closing carotid arteries temporally. Because the hippocampal neurons are sensitive to a shortage of blood, a prolonged ischemia causes reliable damage to the hippocampus (Bendel et al., 2002; Kelly et al., 2001; Matsunaga et al., 2003; Nakamachi et al., 2005; Schmidt & Reymann, 2002; Tajiri et al., 2004). Using ischemia, one can avoid damage by insertion of electrodes, or cannulae into the brain. The ability to use this technique with mice is important given the wide range of genetically modified mice

available, which provides the possibility of evaluating genetic contributors (Goto et al., 2010). In the present study, we evaluated the effect of ischemia on spatial learning in mice.

Methods

Subjects

Ten male C57/BL6J mice were used. Mice were nine weeks old at the beginning of the experiment. Three to five mice were kept in each cage (29 cm long × 19 cm wide × 13 cm high) on a reversed 12:12 h light/dark cycle. Room temperature was kept at 24 c.

Apparatus

The training apparatus was a circular white polypropylene tank with internal dimensions of 90 cm (diameter) and 38 cm (depth). The tank was situated in a laboratory room (170 cm × 170 cm × 280 cm), elevated 50 cm above the floor. A circular acrylic plate was situated in the tank, 16.5 cm below the top edge of the tank. The plate had symmetrically arranged 61 holes (1 cm in diameter and 0.5 cm in depth) 10 cm apart from each other. The room contained a digital camera, computer equipment, and furniture in addition to two posters on the walls. These objects served as external cues; the arrangement of these cues remained unchanged throughout the period of experiment. A digital camera (Logitech QuickCam Fusion, Logitech), connected to a computer (Inspiron; Dell, Round Rock, TX) running video tracking software (ANY-maze; Stoelting Company, Wood Dale, IL), allowed observers to watch and record mice with minimal disturbance.

Surgery

Five mice received the ischemia procedure. These mice were injected with pentobarbital (40 mg/Kg, intramuscularly) and fixed on their back to a surgical table. Then, local anesthesia (Xylocaine) was injected into the incision site. Next, both common carotid arteries were exposed, the vagal nerve was separated carefully from the arteries under a surgical microscope, and the arteries were clipped with surgical clips (501786-G, World Precision Instru-

ments). Each artery was clipped with 2 clips. The duration of the clipping was 20, 25, or 35 min. Finally, the clips were removed and the incision site was sutured.

The remaining five mice (control group) received the anesthesia and their common carotid arteries were separated from the vagal nerves but, no clipping was done. After two days of recovery, the behavioral test was conducted.

Behavioral Procedure

The basic protocol for the dry maze experiment has been described previously in Yoshida et al (2001). Briefly, subjects first received two sessions of four habituation trials each. In the habituation trials, all holes on the acrylic plate were baited with a 25-mg food pellet (Obara Medical, Tokyo, Japan). Each trial was initiated by placing the mouse on the edge of an open field at one of six starting locations (northwest, west, southwest, south, southeast, and northeast). The trial lasted 120 s, or until the mouse reached one of the baited holes. Subjects that did not locate baited holes were guided to one of the nearest holes and fed with a pellet. During the habituation trials, each subject experienced all six starting locations. Following habituation, each subject underwent three sessions of five study trials.

During training, only the hole in the northeast location was baited with a pellet. One training session consisted of four trials and the training was continued for four sessions. Each trial was initiated by placing the mouse at one of four starting locations randomly chosen without replacement on a trial-by-trial basis. The trial lasted 120 s, or until the mouse located the baited hole. Subjects that did not find the baited hole were guided to it and fed with a pellet, and given a latency score of 120 s. At the end of each trial, each mouse was allowed up to 15 s to consume a pellet on the open field. After each trial, the open field was wiped with 70% ethanol. The inter-trial interval (ITI) was 20–25 min.

Each mouse received a single probe trial 20–25 min after the final training trial on the last day. In the probe trial, no food was baited and the exploratory behavior of each mouse was recorded for 60 s.

Histological Examination

After the behavioral test, mice were injected with over-dose pentobarbital

and perfused with saline followed by 4% paraformaldehyde solution. The brains were removed from the skull and kept in the fixative for 24 h, and subsequently transferred into a 30% sucrose solution. Next, the brains were frozen, cut at 20 micron with a cryostat, and stained with cresyl violet. Brain slices were examined under a microscope to detect cell death in the hippocampus.

Results and Discussion

Histology

Figure 1 presents examples of histology. Every ischemia mice showed cell loss in CA1, CA2 and CA4, but slight damages to CA3. These results agree with hippocampal damages after ischemia reported by Yang et al. (2005). Although there were some individual differences in damage size, we treated all mice that underwent the ischemia procedure as one group.

Behavioral Results

The left panel of Figure 2 presents mean distance traveled to reach the goal

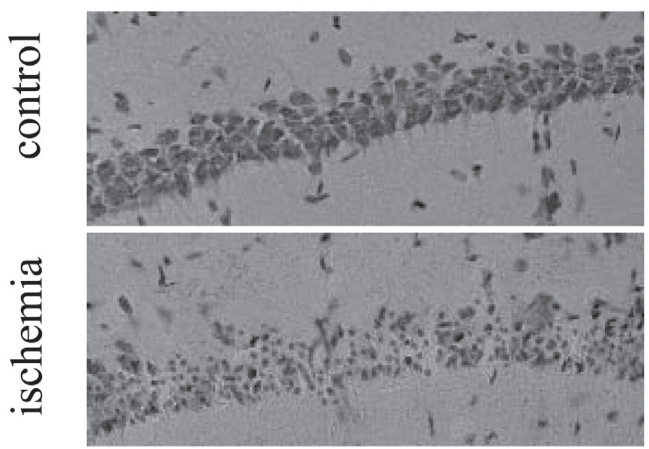

Figure 1. Example of CA1 layer for control mice (top) and mice in the ischemia group (bottom), which show greater cell loss in this layer.

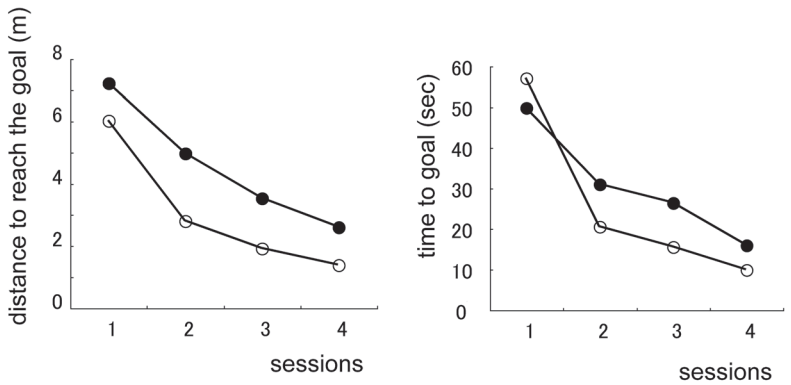

Figure 2. Behavioral results of the spatial learning task. The left panel shows distance travelled from the start point to the goal, and the right panel shows time to reach the goal.

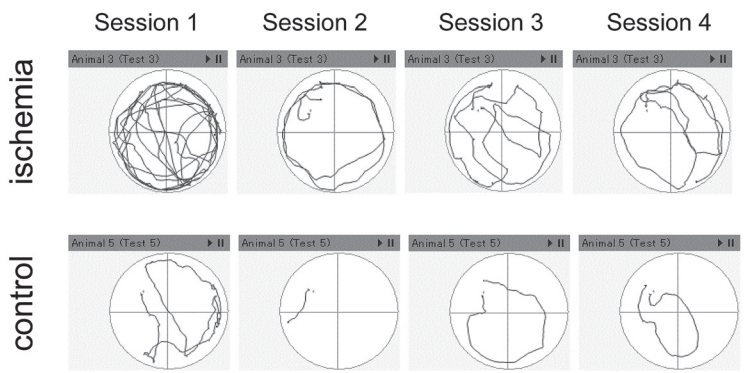

Figure 3. Examples of movement tracking of randomly selected mice. Data were obtained from the first trial of the Session 1 to Session 5. The top and bottom panels show the subjects with ischemia and control, respectively.

(i.e., baited hole). A two factor, group by session Analysis of Variance (ANOVA) revealed a significant effect of the group [$F(1,39)=6.04$, $p<0.05$] and the sessions [$F(3,39)=10.30$, $p<0.001$], but no interaction [$F(3,39)=0.13$, ns]. The right panel of the Figure presents mean time to reach the goal. A two factor, ANOVA (group x session) revealed a significant effect of the sessions [$F(3,39)=21.2$, $p<0.001$], but no significant effect of the groups [$F(1,39)=1.73$, ns]. Figure 3 shows tracking paths of representative subjects from each group. The subjects with ischemia traveled a longer distance to

reach the goal.

Figure 4 shows results of the probe test. The y axis indicates staying at the goal area, as measured by time and distance traveled within goal area. Both the time spent at the goal area and distance travelled within the goal area showed significant differences between the two groups [$t(8)=3.44$, $p<0.01$ and $t(8)=2.73$, $p<0.05$, respectively]. Examples of the movement tracking are presented in Figure 5, which show that mice with ischemia stayed within the goal area less than the controls.

These results show that mice with ischemia had deficits in spatial learn-

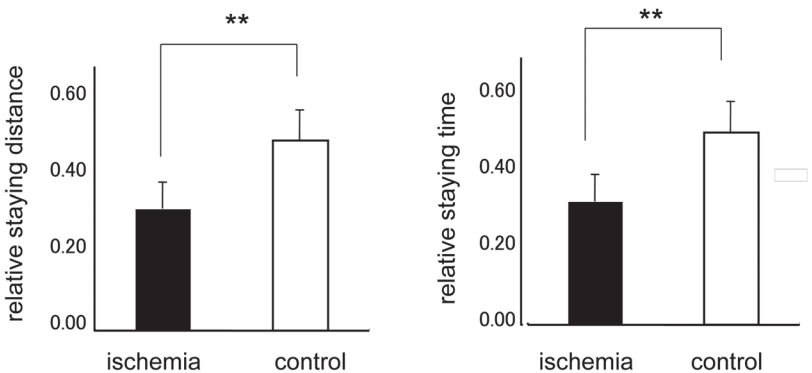

Figure 4. Results of the probe test. Data represent relative staying at the quadrant in which the goal was located. The left and the right panels present data based on the traveling distance and staying time, respectively.

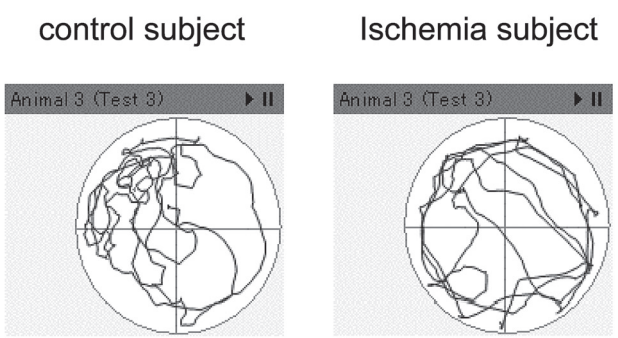

Figure 5. Examples of movement tracking of randomly selected mice. The control mouse stayed longer at the goal area, whereas the mouse with ischemia explored all areas almost randomly.

ing compared to controls. Because the hippocampus, particularly the cells in CA1, is highly sensitive to ischemic lesions, the present results suggest that hippocampal damages may underlie the deficits in spatial learning.

The present results showed that ischemia in C57/Bl mice caused hippocampal damage, which in turn impaired spatial learning in the dry Morris-maze. The method presented herein can serve as a reliable approach to produce hippocampal damages in mice, and provides a new tool for the study of hippocampal function.

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