Effects of separate lesions of the hippocampus and the perirhinal cortex on performance in spontaneous recognition task for object and place\textsuperscript{1)}.

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Abstract

Separate lesions of the hippocampus and the perirhinal cortex in rats led to distinctly different patterns of behavioral impairments in spontaneous object and place recognition tasks, in which preference to a novel object and location respectively, was tested. In both tasks, rats were tested for two delay conditions: 15 and 25 min. The lesion of the hippocampus had no effect on preference between novel and familiar objects in the object recognition task, but it impaired discrimination between objects set in novel and familiar locations in both delay conditions in the place recognition task. In contrast, the lesion of the perirhinal cortex only impaired preference in the 25-min delay condition in the object recognition task. These results suggest that the hippocampus and the perirhinal cortex are functionally differentiated from each other in recognition memory for objects and places. \textit{(Japanese Journal of Physiological Psychology and Psychophysiology, 22(3): 257-266, 2004.)}

Key words: hippocampus; perirhinal cortex; rat; recognition memory; spatial/object.

Damage to the hippocampus and its neighboring structures, the entorhinal, perirhinal, and parahippocampal cortices, brings about severe memory deficits in humans (Buffalo, Reber & Squire, 1998; Scoville & Milner, 1957). Similar findings were also reported in monkeys and rats (Meunier, Bachevalier, Mishkin & Murray, 1993; Mumby & Pinel, 1994; Suzuki, Zola-Morgan, Squire & Amaral, 1993). In particular, research with experimental animals has shown that the hippocampus might be the most important component for spatial and associative memory (Mishkin, 1978; Olton, Becker & Handelmann, 1979). However, more recent findings suggest that there are many types of memory besides spatial and associative memory and some of them might not be involved in the hippocampal function. For example, following lesion of the perirhinal cortex or antagonization of the muscarinic

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receptor or N-methyl-d-aspartate (NMDA) receptor in the perirhinal cortex, performances in the object recognition memory task (Abe, Ichitani & Iwasaki, 1999; Abe, Ishida & Iwasaki, 2004; Aggleton, Keen, Warburton & Bussey, 1997; Buffalo et al., 1998; Meunier et al., 1993; Mumby & Pinel, 1994; Yukie, Abe, Oosaki & Konagaya, 1998) and the object discrimination task (Abe & Iwasaki, 2001; Buckley & Gaffan, 1997, 1998; Eacott, 1998; Mumby & Glenn, 2000) were disrupted. On the other hand, lesions of the hippocampus or the fornix, which brought about the deterioration in performance in the spatial memory task, did not disrupt the memory for the object (Ennaceur, Neave & Aggleton, 1996). Therefore, the perirhinal cortex is considered to play the most important role in the memory for objects. Accordingly, the functions of the hippocampus and perirhinal cortex in memory might differ from each other. However, neuroanatomical findings showed that most of the cortical information was input to the hippocampus via the perirhinal cortex. It is possible that lesions of the perirhinal cortex could bring about similar deficits to hippocampal lesions by blocking this stream of information to the hippocampus.

Some research focused on whether the perirhinal cortex is related to recognition memory for places. Wiig & Bilkey (1994) reported that lesions of the perirhinal cortex in rats caused a deficit in the performance in delayed non-matching to places (DNMP) in a T-maze. They also reported deficits in DNMP performance in a radial arm maze following lesions of the perirhinal cortex produced by ibotenic acid (Liu & Bilkey, 1999, 2001). On the other hand, separate lesion of the perirhinal cortex (Ennaceur et al., 1996) or combined lesion of the perirhinal cortex, entorhinal cortex, and area TE by NMDA (Aggleton et al., 1997) did not deteriorate the DNMP performance in a T-maze. Furthermore, lesions of the perirhinal cortex by NMDA did not cause a deficit in DNMP with two levers in an operant box (Aggleton et al., 1997), and aspirational damage of the perirhinal cortex did not bring about a deficit in delayed matching to place (DMP) (Glenn & Mumby, 1998). As stated above, whether the perirhinal cortex plays some roles in the recognition memory for places is still controversial. This controversy might be based on the procedural differences in behavioral tasks and surgical methods in the various studies. In particular, though these findings tried to evaluate the recognition memory for places, the rule-learning needed for correct performance and the number of stimuli to be remembered were quite different each other.

Using a memory task that includes some rule-learning also causes problems when evaluating the relative participation of the perirhinal cortex in recognition memory for objects and places. That is, if performance deficits are revealed following lesion of the perirhinal cortex, it is difficult to specify whether they were caused by disruption of the rule to perform or recognition memory for the object or place.

Ennaceur & Delacour (1988) introduced a spontaneous object recognition memory task, in which no rule-learning was needed. In this task, rats explore objects spontaneously and normally spend more time exploring a novel object than a familiar one. Therefore, this task is thought not to require a (non-)matching rule or any other rules. A spontaneous place recognition memory task could be designed similar to the spontaneous object recognition memory task. Using these tasks, it might be possible to examine relatively how the perirhinal cortex participates in the recognition memory for objects and places, without considering the effect on the rule-learning following lesion of the perirhinal cortex.

Another factor that prevents us evaluating simply how the perirhinal cortex participates in the recognition memory for objects and places is the number of stimuli to be remembered. The recognition memory for objects and places has been evaluated using different apparatus and procedures, and the number of stimuli to be remembered has differed among studies (for example, Aggleton, et al., 1997, Ennaceur et al., 1996). In monkeys, the performance deficit for object recognition memory following lesion of the perirhinal
cortex was reported to be affected by the number of stimuli to be remembered (Eacott, Gaffan & Murray, 1994). To evaluate the participation of the perirhinal cortex and hippocampus in recognition memory for objects and places, the number of the stimuli to be remembered should be the same.

In this research, we evaluated the effect on recognition memory for objects and places following lesion of the hippocampus or perirhinal cortex using a spontaneous object or place recognition memory task, in which the number of stimuli to be remembered was kept constant.

**Method**

**Subjects:** Sixty-seven adult male Long-Evans rats (260 g on average) were housed individually in a temperature-controlled colony room on a 12:12 h light:dark cycle with free access to food and water. They were randomly assigned to perirhinal lesion (P), hippocampal lesion (H), or control (C) groups in the spontaneous object recognition task (N=10, 7, 15, respectively) and in the spontaneous place recognition task (N=10, 12, 13, respectively). All experiments were performed according to the Guidelines for Experimental Animal Care issued by the Japanese Prime Minister's Office.

**Apparatus and stimuli:** All behavioral experiments were carried out in a gray wooden box (90 x 90 x 30 cm³) with sawdust on the floor. The stimulus objects for the spontaneous object and place recognition tasks were made of glass, plastic, and metal: carafe, beer can, door knob and so on. In the object recognition task, three sets of four different objects were used as samples. Another four objects were prepared for discrimination. In the place recognition task, four different sets of four identical objects were used. Every object was too heavy for the rats to displace.

**Surgical procedure:** Prior to the behavioral testing, the lesions in the perirhinal cortex or the hippocampus were made according to the following procedures. The rats were placed on a stereotaxic instrument after pentobarbital anesthesia (40 mg/kg, i.p.). Additional anesthetic (ketamine hydrochloride) was administered during surgery when necessary. For bilateral lesions of the perirhinal cortex, the incisor-bar was set at +5.0 relative to the horizontal plane. A midline incision was made and six drill holes were made on the skull surface 2.0, 3.4, and 4.8 mm anterior to the interaural line and 5.0 mm lateral of the midline. Through these holes, an electrode (22 gauge) was inserted at an angle of 16.5° outward from the sagittal plane into the bilateral perirhinal cortex. They were set 7.5 mm from the skull surface and the heat was applied at 56°C for 45 sec to solidify the target area. For bilateral lesion of the dorsal hippocampus, the heat was applied at 57°C for 55 sec with a bilaterally inserted electrode 4.0 and 3.8 mm from the skull surface and through the bore holes 3.8 mm posterior to the bregma and 1.4 and 3.4 mm lateral of the midline. The incisor-bar was set at -3.3 relative to the horizontal plane. The rats in group C had only an incision of the skin. After surgical procedures, the incised skin was sutured and sterilized in all rats.

**Behavioral procedure:** We referred to the method in a preceding study (Ennaceur & Delacour, 1988). Two days before testing, all rats were allowed to explore a test box for 15 min once a day. Rats received one test session in a day. A test session consisted of a sample phase, a delay period, and a choice phase (Fig. 1). In the sample phase, rats were exposed to two identical objects (A1 and A2) for 3 min. These objects were placed in the back corners of the box 10 cm from the wall. The distance between the two identical objects was 70 cm. After the sample phase ended, the delay period started and lasted for 15 or 25 min. During the delay period, we picked up the rats from the box. Then we removed the objects. Next, for the spontaneous object recognition task, a third identical copy of the familiar object (A3) and a novel object (B) were placed in the box. A3 and B were placed in the same locations as A1 and A2. In the spontaneous place recognition task, third and fourth identical copies of the familiar object (A3 and A4) were placed in the box. A3 was placed in the same location as A1 or A2.
and A4 was placed in a novel location. In the choice phase, rats were allowed to explore A3 and B (A4) for 3 min. The locations where the objects were placed were counterbalanced for each session. The time spent exploring each of the objects was assessed from videotape recordings of each session. The sawdust on the floor was stirred after every phase to prevent specific odor from remaining in a specific location. Exploration of an object was defined as directing the nose to the object at a distance of less than 2 cm and/or touching it with the nose. Each rat received four sessions (two for 15-min delay and two for 25-min delay) with four sets of different objects in object or place recognition.

Statistical analysis: We analyzed three measures. The total time spent exploring the two sample objects in the sample phase (A1+A2). The total time spent exploring the two objects in the choice phase (A3+B or A3+A4). Discrimination ratio, which is the difference in exploration time divided by the total time spent exploring the two objects in the choice phase (\((B-A3)/(B+A3)\) or \((A4-A3)/(A4+A3)\)).

Histological analysis: After behavioral testing, the rats were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and perfused intracardially with saline followed by 10% formal saline. Brains were removed and sectioned in the coronal plane (50 μm) on a frozen microtome and stained with cresyl violet. These sections were assessed through a microscope for the location and extent of the lesion.

Results

Hippocampal lesions: Figure 2 shows the extent of the hippocampal lesion in each of the animals in group H. In all cases there was removal of the dorsal hippocampus, but in all cases there was also some sparing of the dorsal hippocampus and some damage to the parietal cortex spreading over the dorsal hippocampus.
Perirhinal cortex lesions: Figure 3 shows the extent of the perirhinal cortex lesion in each of the animals in group P. The sections from the brains were matched to a separately prepared set of sections of brains with normal perirhinal cortex, in order to identify the limits of the lesions by cytoarchitecture. In all cases there was substantial removal of the perirhinal cortex through layers I to VI, but in all cases there was also some sparing of the perirhinal cortex and some damage to the lateral entorhinal cortex and the ventral part of area TE. In only one case, in which the object discrimination task was performed, damage had spread to some parts of the ventral hippocampus. There was not any damage in the postrhinal cortex in any of the cases.

Spontaneous object recognition task: The total exploring time in the sample phase (grand average was 8.8 ± 1.1 sec (mean ± s.e.m.)) of each group in the 15- and 25-min delay sessions was evaluated by a two-factor analysis of variance with repeated measures (group x delay). There were not any significant effects of group, delay, or group x delay interaction.

The total exploring time in the choice phase in 15- and 25-min delay sessions (Figure 4A) in three groups was evaluated by a two-factor analysis of variance with repeated measures (group x delay). There was not any significant effect (group: F(2,29)=0.54, p>0.1, delay: F(1,29)=1.26, p>0.1, interaction: F(2,29)=0.84, p>0.1).

The discrimination ratio in the 15- and 25-min delay sessions (Figure 5A) in three groups was evaluated by a two-factor analysis of variance with repeated measures (group x delay). There were significant main effects of group (F(2,29)=4.0, p<0.05) and delay (F(1,29)=7.5, p<0.05), and a significant interaction (F(2,29)=3.5, p<0.05). The simple main effect of groups was significant for the 25-min delay sessions (F(2,58)=5.5, p<0.01). Tukey’s post-hoc HSD tests showed that there was a significant difference (p<0.01) between groups C and P.

Spontaneous place recognition task: The total exploring time in the sample phase (grand average was 27.2 ± 1.2 sec) of each group in 15- and 25-min delay sessions was evaluated by a two-factor analysis of variance with repeated measures (group x delay). There were significant main effects of group (F(2,29)=4.0, p<0.05) and delay (F(1,29)=7.5, p<0.05), and a significant interaction (F(2,29)=3.5, p<0.05). The simple main effect of groups was significant for the 25-min delay sessions (F(2,58)=5.5, p<0.01). Tukey’s post-hoc HSD tests showed that there was a significant difference (p<0.01) between groups C and P.
A delay sessions was evaluated by two-factor analysis of variance with repeated measures (group x delay). There were not any significant effects of group, delay or group x delay interaction.

The total exploring time in the choice phase in 15- and 25-min delay sessions (Figure 4B) in three groups was evaluated by a two-factor analysis of variance with repeated measures (group x delay). A significant main effect of group was revealed, but the main effect of delay and group x delay interaction were not significant (group: F(2,32)=12.1, p<0.01, delay: F(1,32)=1.3, p>0.1, interaction: F(2,32)=0.3, p>0.1). Tukey’s post-hoc HSD tests showed that there was a significant difference between groups H and C (p<0.01 for each delay).

The discrimination ratio in 15- and 25-min delay sessions (Figure 5B) in three groups was evaluated by a two-factor analysis of variance with repeated measures (group x delay). There was a significant main effect of group (F(2,32)=12.7, p<0.01). Tukey’s post-hoc HSD tests showed that there was a significant difference (p<0.01) between groups C and H. There was not any significant difference between groups C and P (p>0.1).

Discussion

The lesion of the hippocampus did not deteriorate the performance of the spontaneous object recognition task. On the other hand, the importance of the hippocampus in place recognition memory was confirmed. Compared with group C, group H spent significantly more time exploring objects in the choice phase of the spontaneous place recognition task (Figure 4B). Although it is known that hippocampectomy changes emotional behaviors including exploration (Douglas, 1967; Kimble, 1968), the hippocampectomy did not increase the time spent exploring in the choice phase of the spontaneous object recognition task or in the sample phase of both tasks. Therefore, it is possible that rats with the lesion of the hippocampus might recognize the changes in the spatial arrangement, and they might spend more time exploring to examine the changes in compensation because of their deficits. However, it was also reported that object exploration to spatial changes was not affected by the lesion of the hippocampus (Save, Poucet, Foreman & Buhot, 1992). Further studies should be needed to make it clear whether the increase of the exploration in the present study was related to the changes in the spatial arrangement.

The lesion of the temporal cortex, including the perirhinal cortex, deteriorated the performance in the spontaneous object recognition memory task (Aggleton et al., 1997; Ennaceur & Aggleton, 1997; Ennaceur et al., 1996). The present results showed that the lesion of the perirhinal cortex by itself caused the deficits in the task.

It was reported that the combined lesion of the perirhinal cortex and the dorsal part of the entorhinal cortex (Mumby & Pinel, 1994) and lesion of the perirhinal cortex by itself (Wiig & Bilkey, 1995) both deteriorated the performance of the object DNMS (delayed non-matching to sample) task in a delay-dependent manner. In both findings, the lesion...
groups, compared with the control group, showed a deficit on the delay test for delays longer than 30 sec. On the other hand, the present results showed that the lesion of the perirhinal cortex did not cause deficits in the 15-min delay sessions, but did cause deficits in the 25-min delay sessions in the spontaneous object recognition memory task. This difference may depend on the difference in difficulty level between tasks. That is, with a 15-sec inter-trial-interval, fifteen trials with thirty objects (Wiig & Bilkey, 1995) or twenty-five trials with fifty objects (Mumby & Pinel, 1994) were performed per day in the object DNMS task. On the other hand, one trial with two objects was performed in the present spontaneous object recognition memory task. Compared with the object DNMS task, the interference between trials might be mild in the spontaneous object recognition memory task. This may cause the difference in the lifetime of object recognition memory. Furthermore, the spontaneous object recognition memory task might be easier than the object DNMS task because the former did not need any rule-learning (reference memory) while the latter did. Although the object DNMS task is designed to examine recognition memory, it also needs some reference memories: 1) when rats move the sample object and are rewarded, the sample phase ends and the delay phase starts, 2) rats are rewarded if they choose the novel object in the choice phase, 3) rats must move to a start box, a sample presentation box, and a choice box appropriately. On the other hand, such rule-learning is not needed in the present spontaneous object recognition memory task. They could perform correctly with only “familiarity judgment” (Ennaceur & Delacour, 1988). Rats normally spend more time exploring a novel object than a familiar one. If they can only judge which object is novel or familiar (familiarity judgment), then good performance is expected in the spontaneous object recognition memory task.

In the spontaneous place recognition memory task, the lesion of the perirhinal cortex did not deteriorate the performance. This finding coincides with some of other reported findings, in which the lesion of the perirhinal cortex did not cause DNMP performance deficits in the Morris water maze (Glenn & Mumby, 1998) and excitotoxic lesion of the perirhinal cortex with NMDA did not cause DNMP deficits in a T-maze (Ennaceur et al., 1996). However, contradictory findings have also been reported. For example, following electrical lesion or excitotoxic lesion with ibotenic acid of the perirhinal cortex, delay-dependent deficits were reported in the DNMP task in a T-maze (Liu & Bilkey, 1998a; Wiig & Bilkey, 1994). Furthermore, the lesion of the perirhinal cortex and the combined lesion of the perirhinal and entorhinal cortices deteriorated the place recognition memory. That is, they increased the number of errors in which rats reentered the arm from which they had already obtained a food pellet (Liu & Bilkey, 1998b; Otto, Wolf & Walsh, 1997). We could not conclude that the main factor causing performance deficits in the place recognition memory task was simply the extent of the lesion or the method of making the lesion. For example, the broader lesion including the perirhinal, dorsal entorhinal, and rostral postrhinal cortices and ventral area TE did not deteriorate the place recognition memory performance (Ennaceur et al., 1996). On the other hand, only a limited lesion inside the perirhinal cortex caused deficits (Wiig & Bilkey, 1994). Because lesions (produced electrically or by heat or aspiration) disrupted fiber connections input to and output from the target area, such lesions tended to cause more severe deficits than lesions produced by excitotoxic acid, ibotenic acid, or NMDA. However, lesions produced by heat did not deteriorate performance, while lesions produced by excitotoxic acid did cause performance deficits (Liu & Bilkey, 1998a).

There are two possible reasons why the lesion of the perirhinal cortex did not deteriorate the performance in the present place recognition memory task. The conventional recognition memory task for places includes some rule-learning (reference memory) for correct performance like the object DNMS task.
On the other hand, in the present spontaneous place recognition memory task, rats could perform correctly simple by “familiarity judgment” and did not need any rule-learning. The second reason concerns the number of stimuli used in the task. It is known that the lesion of the perirhinal cortex causes more severe deficits in the object DNMS task as the number of stimuli increases in the monkey (Eacott et al., 1994). In the present study, the discriminanda were two locations. Perhaps, no deficits might be found in the place memory task with a relatively small number of discriminanda. However, performance deficits were also reported on the DNMP task in the T-maze where the two arms were discriminanda (Liu & Bilkey, 1998a; Wiig & Bilkey, 1994). Whether the number of stimuli determines the severity of the deficits in place recognition memory should also be examined. However, in the present spontaneous object recognition memory task, the lesion of the perirhinal cortex caused the performance deficits, even though the number of discriminanda was the same as in the spontaneous place recognition memory task. Therefore, it is clear that the perirhinal cortex participates in object recognition memory more closely than in place recognition memory.

Although, it was quite likely that the lesion of the perirhinal cortex could bring about similar deficits to hippocampal lesions by blocking the cortical information to the hippocampus, it was not the case. The existence of the other route of the cortical information to the hippocampus would be assumed. The postrhinal cortex, which is located posterior to the perirhinal cortex, might be able to be raised as a candidate. Although the mnemonic function of the postrhinal cortex has not been clarified, they may be relaying the spatial information to the hippocampus (Burwell, 2001).

In conclusion, these results suggested that the hippocampus and the perirhinal cortex have different functions in recognition memory based on “familiarity judgment”, and the hippocampus participates in place recognition memory, while the perirhinal cortex participates in object recognition memory.

References


