Dissociable effects of retrosplenial cortex and hippocampal lesions on performance of spatial memory tasks in rats

（ラットの空間記憶課題遂行に及ぼす後部帯状皮質損傷及び海馬損傷の効果の比較）

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Introduction

Recent studies indicate that the retrosplenial cortex (RS) may contribute to spatial learning and memory. In humans, damage to RS caused anterograde and retrograde amnesia and spatial disorientation (Valenstein et al., 1987; Bowers et al., 1988; Yasuda et al., 1997; Maeshima et al., 2001; McDonald et al., 2001; Maguire, 2001). In addition, functional imaging studies demonstrated that RS was activated during performance of certain memory tasks (Maguire et al., 1997; Wiggs et al., 1999; Shannon & Buckner, 2004; Wagner et al., 2005), and spatial problems (Ino et al., 2002; Rosenbaum et al., 2004; Wolbers et al., 2004; Spiers & Maguire, 2006). In rodents, RS lesions induced impairments of performance in spatial memory tasks such as radial maze (Vann & Aggleton, 2002, 2004), water maze (Sutherland et al., 1988; Vann & Aggleton, 2002, 2004; Harker & Whishaw, 2004), and object-in-place recognition tasks (Ennaceur et al., 1997; Vann & Aggleton, 2002; Parron & Save, 2004). Immunohistochemical studies of the expression of immediate early genes have shown that RS was activated during performance of radial maze task (Vann et al., 2000a) and remote spatial memory test (Maviel et al., 2004).

RS might cooperate with the hippocampus (HPC) to engage in spatial learning and memory. Neuroanatomical studies have revealed that RS has dense reciprocal connections with the hippocampal formation (Vogt, 1986; Wyss & van Goren, 1992; Burwell & Amaral, 1998), which have a central role in learning and memory. Electrophysiological studies showed that both RS and HPC contain neurons with spatial firing properties: RS contains 'head direction cells' that fire when a rat is facing a particular direction (Chen et al., 1994; Cho & Sharp, 2001), while HPC contains 'place cells' that discharge when a rat is in specific place (O'Keefe & Nadel, 1978). In addition, the neural activity of RS affects the hippocampal spatial coding (Cooper & Mizumori, 2001), and the hippocampal theta wave activity (Destrade & Ott, 1982;

Vanderwolf et al., 1985), which is thought to be related to learning and memory (Pan & McNaughton, 1997; Vertes, 2005). Furthermore, fornix transection or HPC lesions caused a reduction of c-Fos expression in RS during radial maze performance (Vann et al., 2000b; Albasser et al., 2007).

It has been considered that memory function depends on an interaction between HPC and the neocortical areas (e.g. Squire, 1992; Eichenbaum et al., 1994; Nadel and Moscovitch, 1997). Most of these perspectives assumed that long-term memory is stored in the neocortex. Thus, RS might be one of the candidate brain regions that form a network with hippocampal formation in memory function, especially in long-term memory processing. However, most of previous studies examined RS-lesioned rats for the learning process of the spatial memory tasks rather than the retention of the spatial memory. In addition, there were few lesion studies that demonstrated the role of RS in spatial memory processes, and directly compared the effects of lesions of RS and HPC. Thus, it remains unclear whether RS is important for spatial working memory and/or spatial long-term memory, and whether the roles of RS and HPC in spatial memory could be clearly differentiated. Therefore, it is important to investigate the retention of the spatial memory to elucidate the involvement of RS in spatial memory processes and functional relevance between RS and HPC.

Here I compared the effects of RS and HPC lesions on performance of two spatial memory tasks. The present study made dorsal HPC lesions since dorsal HPC has been reported more important for spatial memory than ventral HPC (e.g. Pothuizen et al., 2004). In experiment 1, to examine the roles of RS and HPC in spatial working memory, I employed the delayed nonmatching-to-place (DNMTP) task (Sasaki and Iwasaki, 2003), in which animals were required to maintain the information during delay period, but not after the trial. In experiment 2 and 3, I tested retrograde and anterograde amnesic effects of RS and HPC lesions on 2-arm place discrimination to

investigate the role in long-term memory (Cho and Kesner, 1996). Retrograde amnesia is defined as an inability to remember events that occurred prior to the brain lesion. On the other hand, anterograde amnesia refers to the loss of memory for events and experiences that occurred subsequent to the brain lesion. To test retrograde amnesia (experiment 2), animals were trained at different time periods prior to surgery, and the retention test was conducted after surgery. Anterograde amnesia (experiment 3) was assessed both by post-surgical acquisition of the 2-arm place discrimination and by retention test of the task after various retention intervals.

General Method

Subjects

Experimentally naive male Wistar-Imamichi rats (n=26, 22 and 23 in experiment 1, 2 and 3, respectively), 3-4 months old (weighing 273-351g), were used in this study. They were maintained at 80-85% of their free feeding weight, and housed individually and kept on a 12 hr light-dark cycle. All behavioral testing was conducted during the light cycle (08:00-20:00). Animal experiments were approved by the University of Tsukuba Committee on Animal Research. All efforts were made to minimize the number of animals used and their suffering.

Apparatus

An elevated eight-arm radial maze made of gray polyvinyl chloride was used. It consisted of an octagonal central platform (35 cm in diameter) and eight arms (60×12 cm) radiating from the central platform. The side wall of each arm was 4 cm high. A food well (3 cm in diameter and 1 cm deep) for three food pellets (45 mg each, Bio-Serv Inc., USA) as a reward was placed 1 cm from the end of each arm. A transparent guillotine door that controlled rats' access to the central platform was placed between each arm and the central platform. There were many kinds of extra-maze cues (e.g. blackboard, shelf, desk, etc.) around the maze in the experiment room (2.4×2.4 m).

Surgery

Surgery was conducted under sodium pentobarbital anesthesia (40 mg/kg, i.p.) using a stereotaxic instrument (David Kopf, USA). With the skull flat (the incisor bar set at -2.8 mm), the bone overlying injection sites was removed. The excitotoxic lesions were produced by *N*-methyl-D-aspartic acid (NMDA; Sigma, USA), which was

dissolved in 0.1 M phosphate buffered saline (PBS; pH 7.4) at a concentration of 10 mg/ml. NMDA was injected bilaterally into each site at a rate of 0.3 μl/min for 1 min (i.e. 3 μg of NMDA) through a stainless steel injection cannula (30 gauge) connected to a 10 μl Hamilton syringe. The cannula was left in place for 3 min at each site. The lesion coordinates were derived from Paxinos and Watson (1998). The stereotaxic coordinates for RS lesions were as follows: AP -2.5, LM ± 0.5 (from bregma), DV -0.7 (from dura); AP -3.6, LM ± 0.5, DV -0.7; AP -4.7, LM ± 0.6, DV -1.0; AP -5.8, LM ± 0.8, DV -1.2; AP -6.9, LM ± 0.9, DV -1.5; AP -8.0, LM ± 1.2 , DV -2.2. Those for the dorsal HPC lesions were as follows: AP -2.8, LM ± 1.5, DV -3.0; AP -3.3, LM ± 2.5, DV -3.3; AP -3.9, LM ± 2.0, DV -3.3; AP -3.9, LM ± 3.0, DV -3.0; AP -4.5, LM ± 2.5, DV -3.5; AP -4.5, LM ± 3.5, DV -3.5. Sham operated control (Cont) rats received the same procedure as RS or dorsal HPC lesion except that they were injected with the same volume of PBS.

Histology

After the completion of behavioral tests, the rats were deeply anesthetized with sodium pentobarbital (i.p.), and perfused intracardinally with 0.9% saline solution followed by 10% formalin solution. Brains were removed and postfixed in formalin solution for 24 h and then submerged in a 20% sucrose solution. Coronal sections (40 μm) were cut in a cryostat (CM3000; Leica, Germany), and stained with cresyl violet. When completed, sections from lesioned and Cont subjects were examined under a light microscope to determine the extent of damage (defined as tissue that was either missing or necrotic).

Histological Analysis

Sham operated controls

Examination of the histological materials from Cont animals in all three

experiments confirmed that there was no discernible damage to RS or HPC due to PBS injection. Small superficial damage to the cortical mantle could be detected in some Cont subjects that were injected with PBS in HPC.

Retrosplenial cortex lesions

No substantial differences were observed among RS lesions in all three experiments. Fig. 1 shows the reconstruction of the largest and the smallest extent of RS lesions in each experiment. Fig. 2A displays photomicrograph of a representative RS lesion in this study. Most RS-lesioned rats sustained bilateral damage of large extent of RS. There was no damage extended to the posterior parietal cortex. Small unilateral damage to the CA1 subfield of the dorsal HPC was observed in 2 cases in experiment 1, and in one case in experiment 2. Partial damage to the most caudal area of the anterior cingulate cortex occurred in 2 cases in experiment 2, and in 3 cases in experiment 3.

Hippocampal lesions

Excitotoxic dorsal HPC lesions were comparable in all three experiments. Fig. 3 illustrates the largest and smallest extent of HPC lesions in each experiment. Fig. 2B shows photomicrograph of a representative HPC lesion in this study. Most HPC-lesioned rats had bilateral damage to the CA1-CA3 subfields of the dorsal HPC and dorsal dentate gyrus. The smallest lesioned animals in each experiment spared some bilateral CA1 subfield. None of HPC-lesioned rats had any damage to RS. Partial damage to the posterior parietal cortex located dorsal to HPC occurred in 4 cases in experiment 1, in 3 cases in experiment 2, and in one case in experiment 3. Slight bilateral damage to the dorsal thalamus was observed in 3 cases in experiment 1 and 2, and in 2 cases in experiment 3.

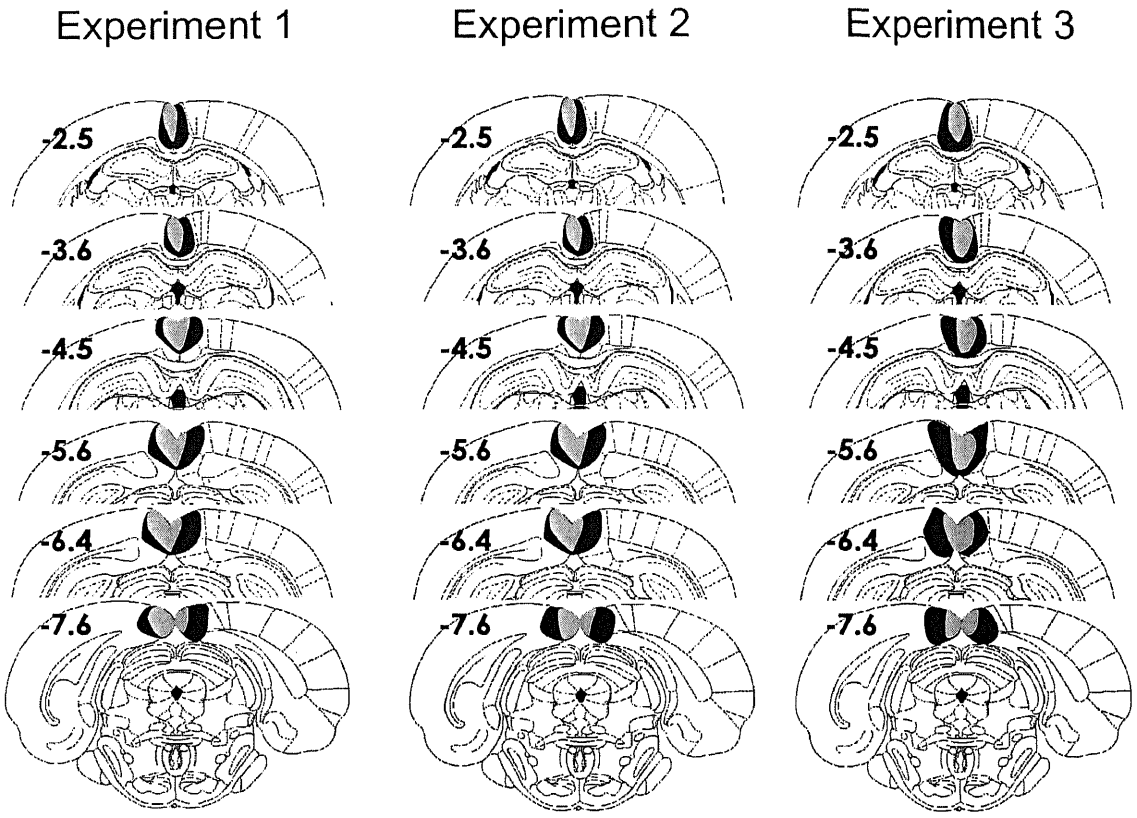

Figure 1. Coronal brain sections illustrating the extent of the largest (black) and smallest (gray) lesions of RS in each experiment. Numbers represent the distance (mm) posterior to bregma (Paxinos & Watson, 1998).

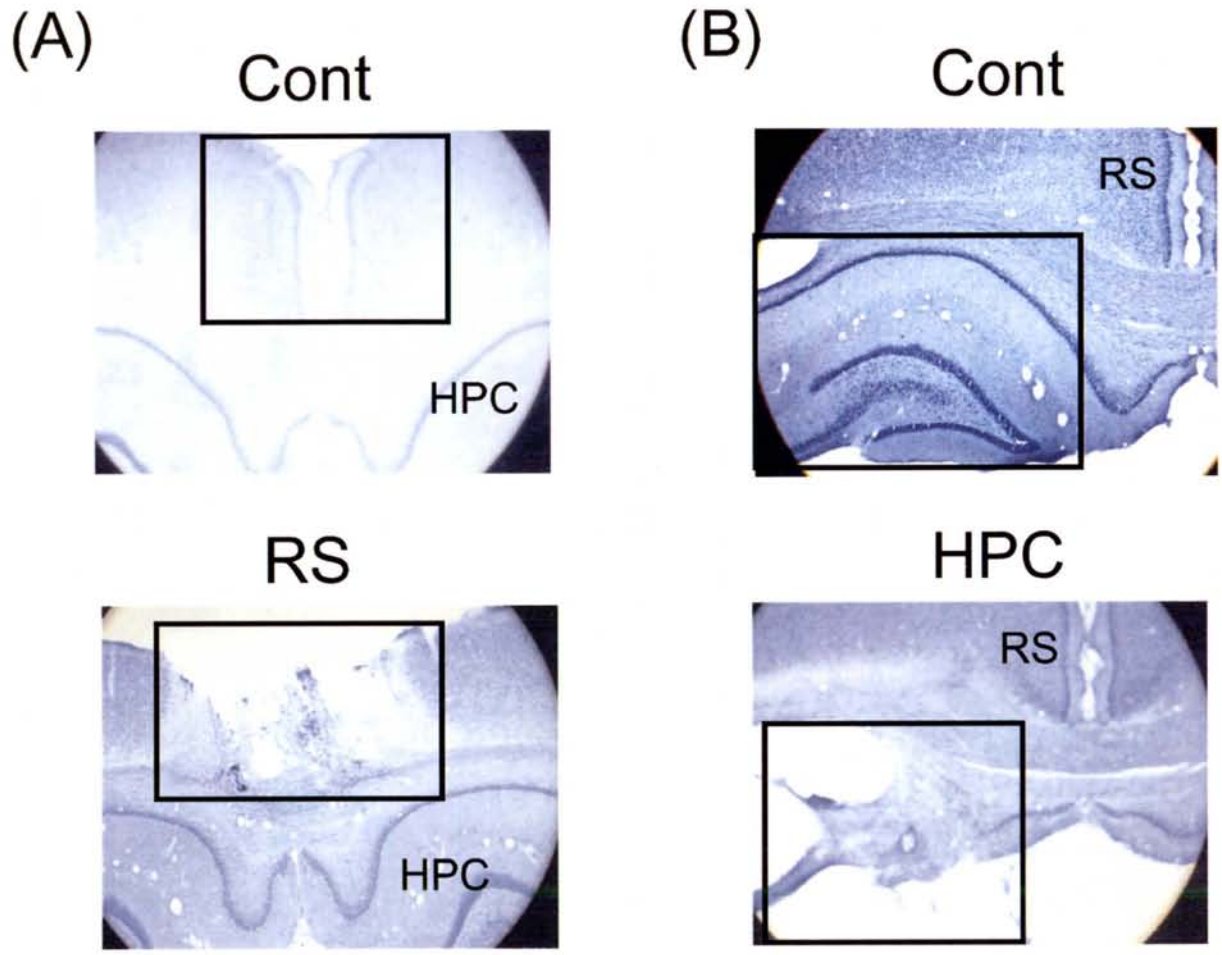

Figure 2. Photomicrographs of a representative RS lesion (A) and HPC lesion (B). Squares indicate zone of RS region (A) or HPC region (B).

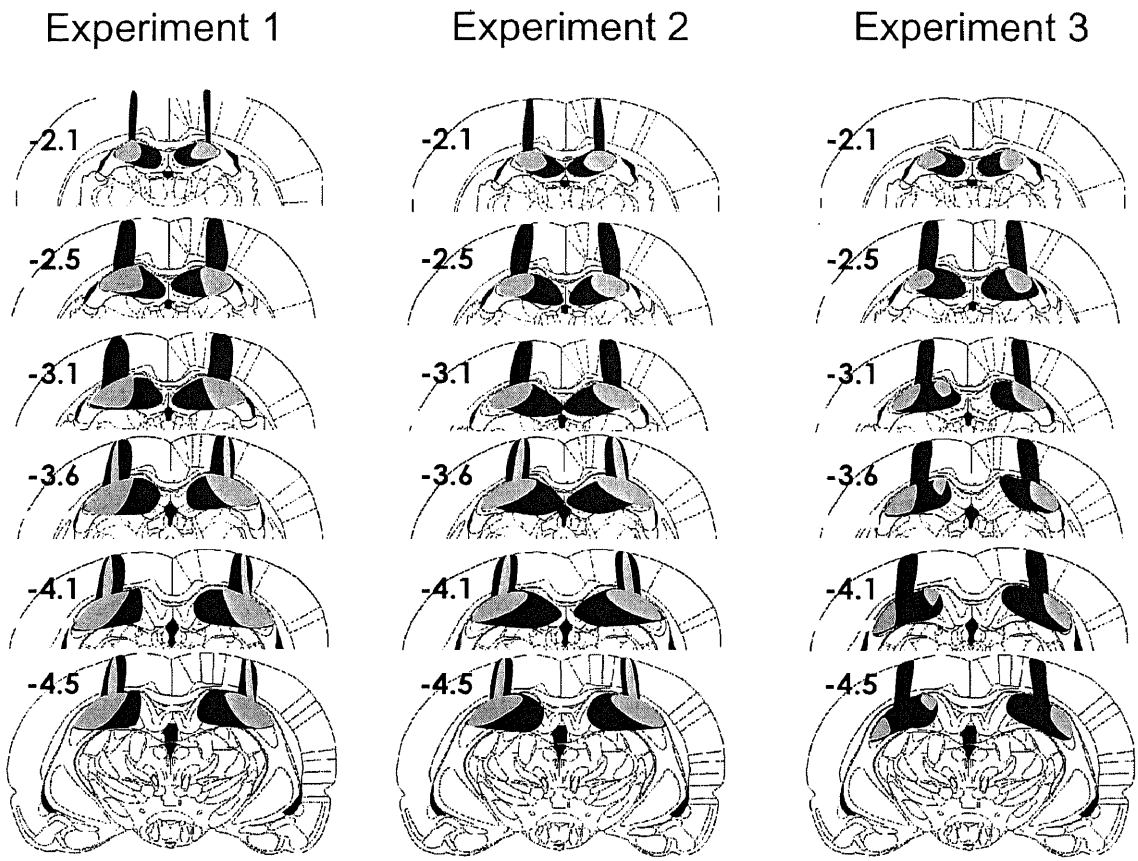

Figure 3. Coronal brain sections illustrating the extent of the largest (black) and smallest (gray) lesions of HPC in each experiment. Numbers represent the distance (mm) posterior to bregma (Paxinos & Watson, 1998).

Experiment 1 : Test of delayed nonmatching-to-place

In this experiment, I investigated the involvement of RS and HPC in spatial working memory employing delayed nonmatching-to-place task in a radial maze. This task is considered to be suitable for examining the spatial working memory, since it requires the animals to maintain the information during delay period, but not after the trial. In addition, this task has some advantages in the following aspects compared to other working memory tasks employed by the previous studies (Cho et al., 1992; Cho and Jaffard, 1994; Lee and Kesner, 2002). First, this task could minimize the proactive interference since each arm was used only once a day, while previous studies employing T-maze or Y-maze used repeatedly the same arms within a day. Second, presentation of arm pairs with randomly selected angles (45, 90, 135, and 180 degrees) could prevent the animals from using a response strategy based on proprioceptive cues other than spatial cues. Third, this task could differentiate each trial by using the procedure that animals were removed from the apparatus and put in the waiting cage after the sample run (i.e. during delay period), and, on the other hand, put in their homecage after the choice run.

Behavioral procedures

Nonmatching-to-place (NMTP) task training

After 3 days of handling and 5 days of habituation to the apparatus, the rats were trained in NMTP task. Each trial of this task was composed of the sample run and the choice run. At the sample run, the rat was confined to the central platform for 5 sec and then one door of eight arms that was randomly selected was opened, and the rat was allowed to run down to the baited arm. After consuming the reward, the animal was picked up to be transferred to a waiting cage which was made of stainless steel (22×40×20 cm), and immediately returned to the central platform with all the

doors closed. Then the choice run began. At the choice run, the doors of two arms were opened at once. One was the baited arm which was randomly selected from arms not presented at the sample run, and the other was the non-baited arm which was presented at the sample run. Correct response was defined as choosing the former. After each choice run, the subject was returned to the homecage. Four trials with 10-15 min inter-trial interval (ITI) were conducted each day, and each arm was used once a day. The angles made by paired arms at the choice run were 45, 90, 135, and 180 degrees, and each angle was used once a day in a random order across training period. The training was continued until the rat reached the learning criterion of 22 correct responses out of 24 successive trials.

Preoperative delayed nonmatching-to-place (DNMTP) test

After reaching the criterion, the rat was given preoperative DNMTP task for 12 days. The procedure was the same as NMTP task except that the delays of 0, 3, 9, and 27 min were randomly interposed in the waiting cage between the sample run and the choice run. On the day after completion of preoperative DNMTP test, the animals were assigned to RS (n=8), HPC (n=9), or Cont (n=9) group on the basis of preoperative scores on DNMTP test.

Postoperative DNMTP test

After 1 week recovery period from surgery, each rat was given postoperative DNMTP test for 12 days which was identical to preoperative DNMTP test.

Results

In the acquisition of NMTP task, mean trials to criterion (± SEM) including 24 criterion trials were 48.4 ± 1.82 for Cont group, 41.0 ± 4.05 for RS group, 45.3 ± 2.67 for HPC group. A one-way analysis of variance (ANOVA) showed no significant

difference among the groups. Fig. 4 shows mean percent of correct responses for each group in the preoperative DNMTP test. All three groups performed almost 100% correct at 0 min delay, and showed a delay-dependent decline of correct responses. A two-way ANOVA revealed that there was a significant main effect of delay ($F_{(3,69)}$=14.23, p<.001), but there was not a significant effect of group and a group × delay interaction. In the postoperative DNMTP test (Fig. 5), Cont and RS rats performed well as in the preoperative DNMTP test, whereas HPC rats showed a remarkable delay-dependent decline of correct responses. A two way ANOVA showed significant main effects of group ($F_{(2,23)}$=15.24, p<.001), and delay ($F_{(3,69)}$=36.24, p<.001). A significant interaction of group × delay ($F_{(6,69)}$=10.63, p<.001) was also detected. Further analysis of simple main effects at each delay condition revealed that there were significant main effects of group at delay 9 min ($F_{(2,92)}$=4.19, p<.05), and 27 min ($F_{(2,92)}$=20.48, p<.01). Post-hoc comparison by a Tukey's HSD test for each delay indicated that HPC group showed significantly lower level of correct responses compared with Cont group (p<.05 for each pair). There was not a significant difference of percent correct responses among various angle conditions of arm pairs in preoperative and postoperative DNMTP in all groups.

Discussion

In DNMTP task in the radial maze as a test of spatial working memory, performance of RS group did not differ from Cont group, while HPC group showed a marked delay-dependent deficit. The results of the present study are consistent with a previous study in which RS-lesioned rats could perform the delayed nonmatching-to-position task accurately in an operant chamber, while fornix-lesioned animals were impaired in the task (Neave et al., 1994). HPC-lesioned rats performed comparable to Cont rats at shorter delays (0-3 min), but were increasingly impaired as the delay interval extended. This delay-dependent impairment of HPC group is

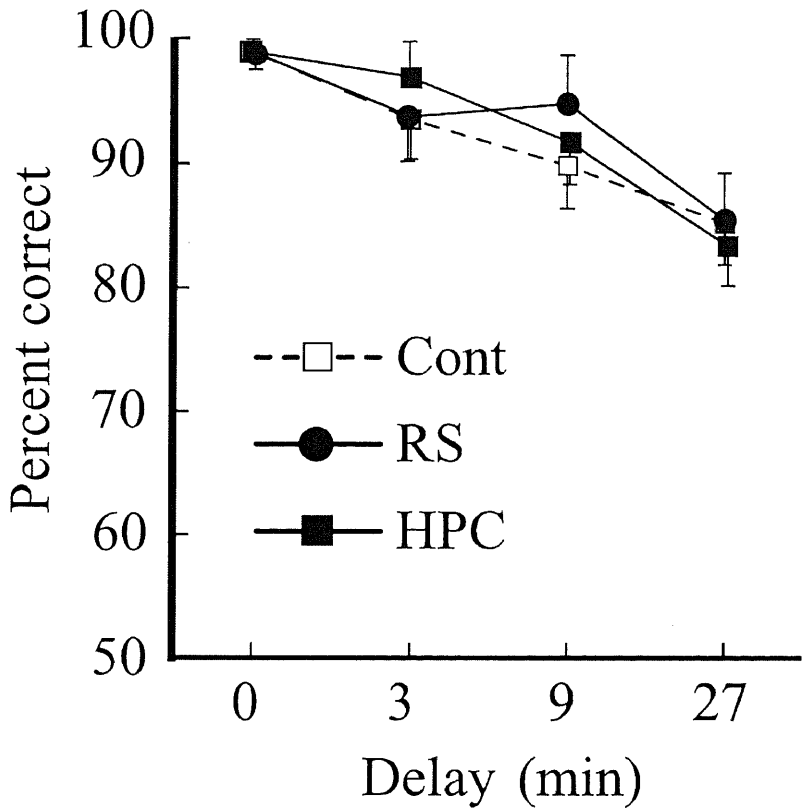

Figure 4. Mean percent correct responses in preoperative DNMTP test in experiment 1 (mean ± SEM). Cont = rats with vehicle injection; RS = rats with NMDA lesion of the retrosplenial cortex; HPC = rats with NMDA lesion of the dorsal hippocampus.

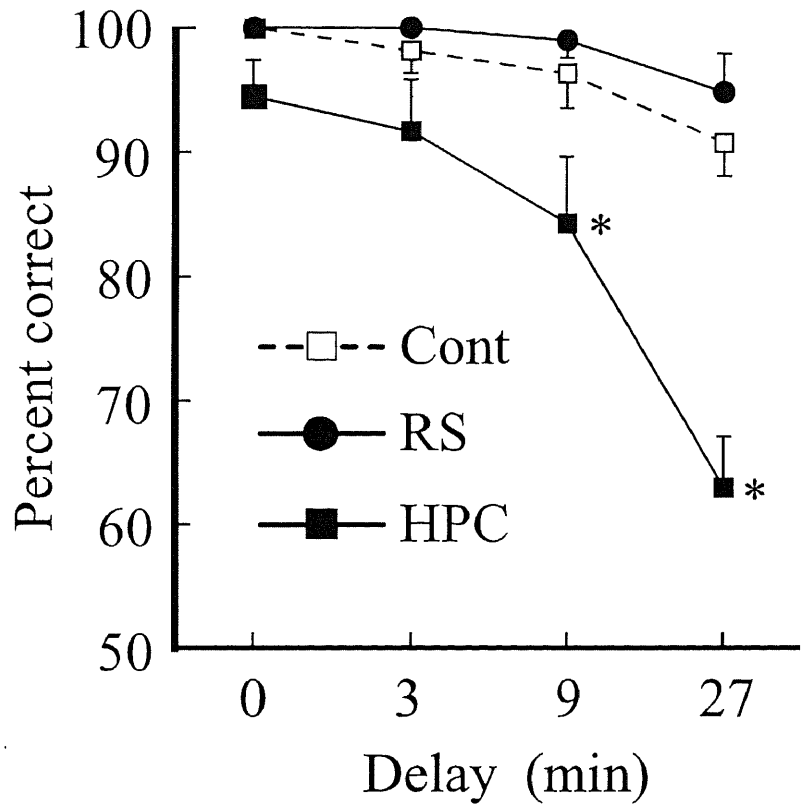

Figure 5. Mean percent correct responses in postoperative DNMTP test in experiment 1 (mean ± SEM). Cont = rats with vehicle injection; RS = rats with NMDA lesion of the retrosplenial cortex; HPC = rats with NMDA lesion of the dorsal hippocampus. Asterisks show significant differences from Cont group (*p<.05).

considered to reflect a deficit of short-term retention rather than non-mnemonic factors, since HPC-lesioned rats did not show an impairment at 0 min delay. In addition, previous studies reported that hippocampal lesions spared immediate memory (Porter et al., 2000), and hippocampal-lesioned rats could perform as well as intact rats when the delay was 0 min in delayed nonmatching-to-position or delayed nonmatching-to-sample task (Neave et al., 1994; Hampson and Deadwyler, 1998; Porter et al., 2000).

The present result of RS group and a previous study suggest that RS does not appear to be involved in spatial working memory. In contrast, however, it has also been reported that RS-lesioned rats showed an impairment in the matching-to-place task in the water maze (Harker and Whishaw, 2004). The difference of this effect of RS lesions from the effects on DNMTP performance in the present study and delayed nonmatching-to-position performance (Neave et al., 1994) may come from several possibilities.

First, in Harker and Whishaw's experiment, the rats were trained post-surgically. There are reports that RS-lesioned animals trained post-surgically displayed place navigation learning deficit in the water maze task, while pre-surgically trained rats showed less impairment in the same task post-surgically (Lukoyanov et al., 2005; Cain et al., 2006). This suggests that RS-lesioned animals were deficient in ability to learn place navigation strategy itself rather than in ability of spatial working memory, since spatial working memory was thought to be required evenly both in pre- and post-surgical training. In the present study, subjects were trained DNMTP task before RS lesions. Place discrimination strategy was already learned at the time of surgery, and thus these animals are thought to have avoided disruptive effect of RS lesions.

Second, RS is considered to be involved in spatial memory based on neuroanatomical and electrophysiological findings. In addition, several behavioral

studies suggested that RS plays a role in spatial navigation and/or path integration (Cooper and Mizumori, 1999, 2001; Whishaw et al., 2001). Although the matching-to-place task might have highly demanded spatial navigation ability of rats, DNMTP task in the present study is thought to require the ability of discriminating arm positions based on spatial cues around the maze, and thus the animals were demanded relatively less spatial navigation and/or path integration in this task. Delayed nonmatching-to-position in an operant chamber (Neave et al., 1994) is not thought to have required spatial navigation, either. Therefore, it is plausible that RS-lesioned rats were impaired only in the matching-to-place in the water maze, and not impaired in the present DNMTP task and delayed nonmatching-to-position in an operant chamber.

Third, difference of the extent of lesions should be considered as factors that might have caused the different results. Harker and Whishaw (2004) conducted more extensive RS lesions than Neave et al. (1994). It is suggested that caudal area of RS is more important for the performance of spatial memory tasks (Vann & Aggleton, 2002, 2004; Vann et al., 2003). The RS lesions in the present study, however, was more extensive than those in Neave et al.'s experiment, and larger part of RS was damaged. Nevertheless, the DNMTP performance was not impaired at all, suggesting that caudal area of RS is not necessarily important for spatial working memory, and thus the above-mentioned two possibilities are more plausible than the third one to interpret the inconsistency of previous studies. Taken together, the present results suggest that RS is not important for spatial working memory, while HPC plays a critical role in it.

Experiment 2: Test of retrograde amnesia of place discrimination

In this experiment, I examined retrograde amnesic effects of RS and HPC lesions on 2-arm place discrimination to investigate the role in long-term memory. In this task, animals can acquire a place discrimination within a day, while previous studies employing other spatial memory tasks such as radial maze task or water maze task took several days to train the animals. Thus, this task has the advantage of being able to train animals at the specific day and at several time points prior to surgery. Since the aim of the present study was to investigate the retention rather than acquisition, 2-arm place discrimination task was considered to be suitable for the aim of this study. In addition, since this task utilizes four arm pairs, it could train and test animals with different episodes (or information) in the same experimental environment. Thus, the present experiment could employ a within-subject design that could minimize the individual difference, although most previous studies used a between-subject design.

Behavioral procedures (Fig. 6)

For the place discrimination task, pairs of four adjacent arms out of eight arms were used (named P1-P4 in a trained order). These arm pairs differed among subjects. One arm of each pair was always baited as a positive arm (S+), whereas the other was not baited throughout experiment as a negative arm (S-). The position of S+ arm (i.e. left or right) was counterbalanced across arm pairs and lesion groups.

Each trial began by opening both doors of the arm pair after a confinement of the subject into the central platform for 20 sec. If the rat ran down to the S+ arm, it could get the reward (correct response). If the rat ran down to an arm end of the arm pair, the rat was picked up and immediately returned to the central platform. After a 20-sec confinement (i.e. ITI), the same pair of doors were reopened for the next trial.

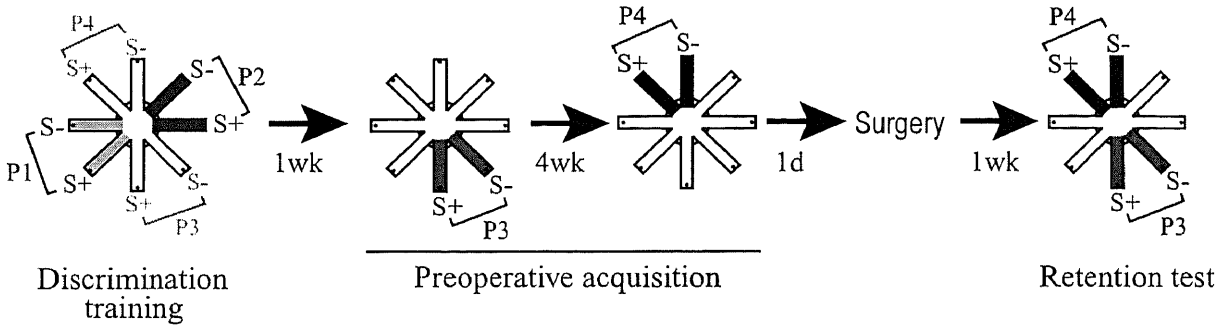

Figure 6. Test procedure of retrograde amnesia (Experiment 2) using two-arm place discrimination task in a radial maze. S+ and S- represent always-baited and always-unbaited arms, respectively. P1 to P4 denote the order of acquisition in place discrimination training. Subjects were preliminary trained P1 and P2 in discrimination training in 2 days. One week after P2 acquisition, rats were trained P3 and P4 4 weeks or 1 day before surgery, respectively. One week after surgery, they were given retention test for P3 and P4 within a day. The arm pairs and S+ arms were different among subjects.

By using this length of confinement time, I could prevent animals from developing a bias such that when the door was opened, their orientation was not random, since animals did not remain stationary for 20 sec and moved around in the central platform. Thus, animals could not use a response/cue strategy and I could test spatial memory in this task. Each session consisted of 12 consecutive trials and the inter-session interval was 10-20 min. The animals received continuous training in a day until they reached the learning criterion of 11 or more correct responses in one session. In retention test, only one session under the same procedure was conducted.

Preliminary place discrimination training

After 3 days of handling and 5 days of habituation to the apparatus, each rat was trained P1 and P2 within 2 days. These 2 pairs were used for training of the task itself and they were not used for the retention test.

Preoperative acquisition

One week after P2 acquisition, the animals were trained P3 and P4 for acquisition. P3 was trained 4 weeks before surgery, and P4 was 1 day before surgery. On the next day of P4 acquisition, the animals were assigned to RS (n=8), HPC (n=7), or Cont (n=7) group on the basis of sessions to criterion in P3 and P4.

Retention test

After 1 week recovery period from surgery, they were given retention test (12trials) of P3 and P4 within a day. The order of P3 and P4 test was counterbalanced.

Results

In preoperative acquisition in experiment 2, all subjects acquired place

discrimination of P3 and P4 at 4 weeks or 1 day before surgery. Mean number of sessions to learning criterion (± SEM) including criterion session for P3 and P4 were 1.91 ± 0.12, and 1.60 ± 0.15, respectively. A two-way ANOVA showed that there were no significant differences among three groups and between two pairs of training-lesion intervals (i.e. P3 and P4). Fig. 7 shows mean percentage of correct responses in retention test at each training-lesion interval. Cont rats performed well in retention test, and the correct responses declined in accordance with the interval length. Conversely, both lesion groups showed a marked impairment at both 1 day and 4 week intervals. A two-way ANOVA showed a significant main effect of group ($F_{(2,19)}$=13.84, $p<.001$). Post hoc analysis by a Tukey's HSD test indicated that scores of both RS and HPC groups were significantly lower than that of Cont group ($p<.05$). Although RS group was somewhat better than HPC group, there was not a significant difference between two groups in both interval conditions. There was neither significant main effect of interval nor interaction between lesion × interval effects. Since the retention performance in Fig. 7 was based on all 12 trials with reinforcement, I also analyzed the animals' correct response in the first trial of the test as an accurate measure of retrograde amnesia. As shown in Table 1, percent animals which showed a correct choice in the first trial of retention test almost paralleled the scores of whole the session in Fig. 7.

Discussion

In experiment 2, both RS and HPC groups showed a severe impairment of performance in postoperative retention test. The correct responses of both lesion groups were very poor (51-61%) irrespective of the length of training-lesion interval. Analysis of the first trial responses in retention test (Table 1) also showed almost the same levels of correct responses in each group, although it could not provide statistical support because of small group sizes. Thus, both lesion groups showed a severe and

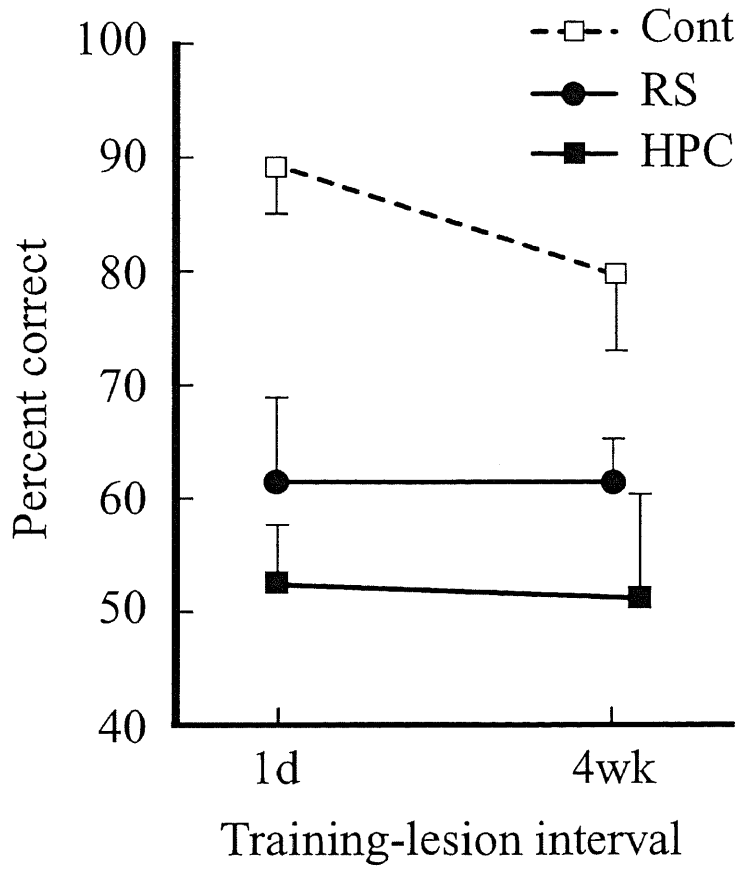

Figure 7. Mean percent correct responses (± SEM) in retention test (12 consecutive trials) in experiment 2. Cont = rats with vehicle injection; RS = rats with NMDA lesion of the retrosplenial cortex; HPC = rats with NMDA lesion of the dorsal hippocampus.

Table 1

The number of rats in each group that chose the correct arm on the first trial of retention test

Group	Training-lesion interval	
	1d	4wk
Cont	7/7(100%)	5/7 (71%)
RS	4/8(50%)	5/8 (63%)
HPC	3/7(43%)	4/7 (57%)

Cont = rats with vehicle injection; RS = rats with NMDA lesion of the retrosplenial cortex; HPC = rats with NMDA lesion of the dorsal hippocampus.

temporally ungraded retrograde amnesia of place discrimination.

Retrograde amnesic effect of HPC lesions have been repeatedly investigated in various tasks, however the different patterns of results have been reported. Some studies showed that HPC lesions caused temporally graded retrograde amnesia (e.g. Kim et al., 1995; Clark et al., 2002), which is the phenomenon of premorbid memory loss whereby information acquired recently is more impaired than that acquired more remotely. The hypothesis based on this pattern of findings proposed that HPC might have a time-limited role in retention and retrieval of memory until the consolidation is complete, after which memories become gradually independent of HPC, and stored in the neocortex (Squire and Alvalez, 1995; Frankland and Bontempi, 2005). The other studies showed that HPC lesions induced temporally ungraded retrograde amnesia (e.g. Mumby et al., 1999; Lehmann et al., 2007), in which both recent and remote memories acquired prior to lesion were impaired equally. This phenomenon has led to the proposition that HPC can contribute indefinitely to memory storage and retrieval (Nadel and Moscovitch, 1997; Rosenbaum et al., 2001).

The present results are consistent with the latter model pattern, and suggest that HPC is important for both recent and remote spatial memory. Thus, HPC might be involved in all stages of spatial long-term memory processes which include consolidation, storage and retrieval. Nevertheless, this does not mean that I can totally rule out the possibility that HPC is involved in memory only for a limited period of time after learning. First, brain imaging studies using [^{14}C]2-deoxyglucose uptake and Zif268 expression technique have shown that remote spatial memory could become independent of HPC with the passage of time (Bontempi et al., 1999; Maviel et al., 2004; Frankland and Bontempi, 2005).

Second, most of previous studies using spatial memory tasks reported temporally ungraded retrograde amnesia after HPC lesions (e.g. Bolhuis et al., 1994; Martin et al., 2005), while in non-spatial memory tasks such as social transmission of

food preference (Clark et al., 2002) HPC-lesioned rats showed temporally graded retrograde amnesia. I hypothesize that HPC plays an important role not only in spatial memory but also in on-line spatial information processing (O'Keefe and Nadel, 1978; Poucet et al., 2003). Since spatial memory tasks require on-line spatial information processing as well as spatial memory, HPC lesions could disturb the task performance even after a limited period of time, and thus produced temporally ungraded retrograde amnesia. Based on this view, one may or may not observe a temporally graded retrograde amnesia depending on the details of the test situation. However, the second possibility is not fully applicable in this study, since in Experiment 1 HPC-lesioned rats could perform well at the delay of 0 and 3 min in postoperative DNMTP task. Taken together, temporally ungraded retrograde amnesia obtained in the present study suggests the long-lasting HPC contribution to memory formation, although another possibility that HPC has a time-limited role in spatial long-term memory could not be fully excluded.

Concerning the function of RS, I have found evidence for temporally ungraded retrograde amnesia induced by the lesion of this area for the first time. The result suggests the possibility that RS is needed indefinitely for spatial long-term memory. However, increased Zif268 expression in RS has been reported if animals were tested 30 days, but not 1 day, after training in a five-arm radial maze as a reference memory task (Maviel et al., 2004), and this pattern of expression was in contrast to the expression in HPC. Thus, it seems that RS has a time-limited role in spatial memory, and is important for remote memory, but not for recent memory. On the other hand, RS contains cells with strong spatial correlation (Chen et al., 1994; Cho and Sharp, 2001), and this suggests that RS is necessary for on-line spatial information processing to perform spatial tasks as well as HPC; the present result could be due to the deficit of spatial processing itself during retention test as well as the deficit of spatial memory. However, since RS-lesioned rats could perform well in postoperative DNMTP in

Experiment 1, this explanation might be inapplicable in this study. Taken together, the present results suggest that RS might contribute to both recent and remote memories acquired prior to lesions. However, further investigation is needed whether the same kind of results would be obtained even if non-spatial tasks were tested.

Experiment 3: Test of anterograde amnesia of place discrimination

In this experiment, I examined anterograde amnesic effects of RS and HPC lesions on 2-arm place discrimination to investigate the role of these brain regions in long-term memory. As described above, I employed 2-arm place discrimination task since this task could train animals at the specific day and examine the retention at several time points after surgery, employing a within-subject design.

Behavioral procedures (Fig. 8)

Preliminary place discrimination training

The procedure of place discrimination was same as in experiment 2. After 3 days of handling and 5 days of habituation to the apparatus, the rats were trained P1 within a day. P1 was used for training of the task itself and it was not used for the retention test. On the next day of P1 acquisition, the animals were assigned to RS (n=8), HPC (n=7), or Cont (n=8) group on the basis of sessions to criterion in P1.

Postoperative acquisition and retention test

After 1 week recovery period from surgery, they were given acquisition training of P2-P4 within a day. After acquisition of these pairs, 1, 2, or 4 week interval was interposed, and one of these pairs was tested for retention (12 trials), respectively. The assignment of which interval was interposed for P2-P4 was counterbalanced.

Results

In preoperative place discrimination training in experiment 3, animals acquired P1 place discrimination task in 6.4 ± 0.3 sessions (mean ± SEM) including

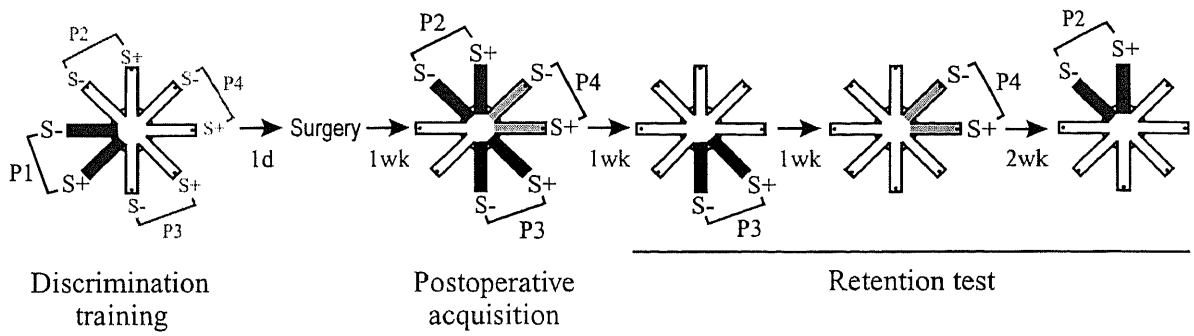

Figure 8. Test procedure of anterograde amnesia (Experiment 3) using two-arm place discrimination task in a radial maze. S+ and S- represent always-baited and always-unbaited arms, respectively. P1 to P4 denote the order of acquisition in place discrimination training. Rats were trained P1 on the previous day of surgery. After 1 week recovery period, the animals were given acquisition training for P2 to P4 within a day. After acquisition of these pairs, 1, 2, or 4 week interval was interposed, and one of these pairs was tested for retention, respectively. The arm pairs and S+ arms were different among subjects.

the criterion session. There was not a significant difference among three groups. Fig. 9 shows mean number of sessions to learning criterion of P2-P4 in postoperative acquisition. All groups of animals could acquire P2-P4 place discrimination, and the number of sessions to criterion was smaller in all three groups than that of preoperative acquisition of P1. RS rats acquired these pairs as fast as Cont rats, while HPC animals required more sessions to criterion for each pair as compared to Cont rats. A one-way ANOVA for each arm pair showed significant main effects of group ($F_{(2,20)}$=4.74, $p<.05$ for P2; $F_{(2,20)}$=7.84, $p<.01$ for P3; $F_{(2,20)}$=3.92, $p<.05$ for P4). Post hoc analysis by a Tukey's HSD test in each pair revealed that HPC group, but not RS group, needed more sessions to criterion compared to Cont group in all three pairs ($p<.05$ for P2 and P4; $p<.01$ for P3).

Fig. 10 shows mean percentage of correct responses at each retention interval in retention test. The correct responses in Cont and RS group declined as the retention interval extended, and RS rats showed a more accelerated decline. Conversely, HPC rats' performance was at about 70% level irrespective of the retention interval. A two-way ANOVA showed significant main effects of group ($F_{(2,20)}$=3.96, $p<.05$) and retention interval ($F_{(2,40)}$=10.43, $p<.01$), and a significant interaction of group × retention interval ($F_{(4,40)}$=3.13, $p<.05$). Further analyses of simple main effects at each retention interval revealed that there were significant main effects of group at 1 week ($F_{(2,60)}$=3.83, $p<.05$) and 4 weeks ($F_{(2,60)}$=4.84, $p<.05$). Post hoc comparison by a Tukey's HSD test for each retention interval indicated that HPC animals in 1 week interval was lower than Cont animals ($p<.05$), and RS group in 4 week interval was lower than Cont group ($p<.05$). I also analyzed the first choice response in retention test as in experiment 2. Similar to the scores based on the whole retention test trials, correct responses in RS group tended to decrease as retention interval prolonged, while those in HPC group remained stable irrespective of retention intervals (Table 2). Furthermore, I analyzed if there was a significant

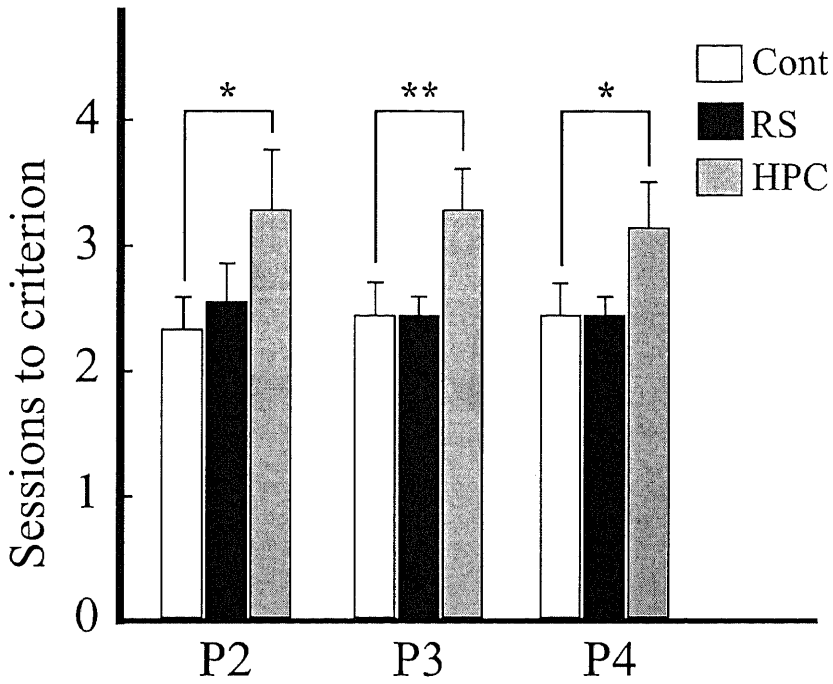

Figure 9. Mean number of sessions to criterion (± SEM) in P2 to P4 acquisition in experiment 3. Asterisks show significant differences from Cont group (*p<.05, **p<.01). Cont = rats with vehicle injection; RS = rats with NMDA lesion of the retrosplenial cortex; HPC = rats with NMDA lesion of the dorsal hippocampus.

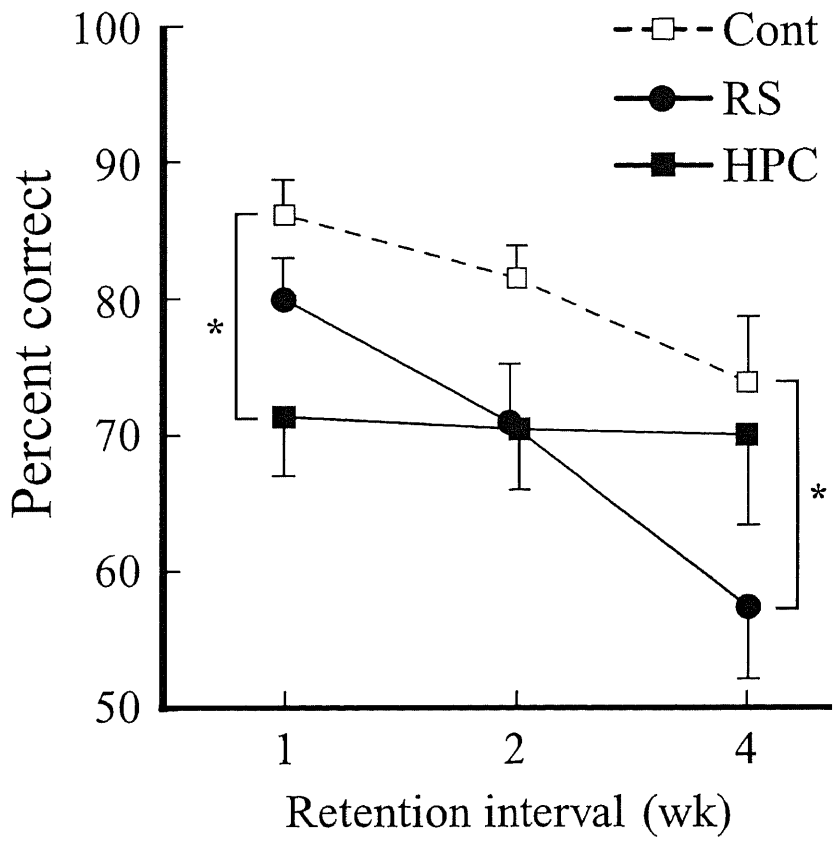

Figure 10. Mean percent correct responses (± SEM) in retention test (12 consecutive trials) in experiment 3. Asterisks show significant differences from Cont group (*p<.05). Cont = rats with vehicle injection; RS = rats with NMDA lesion of the retrosplenial cortex; HPC = rats with NMDA lesion of the dorsal hippocampus.

Table 2

The number of rats in each group that chose the correct arm on the first trial of retention test

Group	Retention interval		
	1wk	2wk	4wk
Cont	6/8 (75%)	6/8 (75%)	6/8 (75%)
RS	6/8 (75%)	5/8 (63%)	3/8 (38%)
HPC	3/7 (43%)	5/7 (71%)	4/7 (57%)

Cont = rats with vehicle injection; RS = rats with NMDA lesion of the retrosplenial cortex; HPC = rats with NMDA lesion of the dorsal hippocampus.

increase of correct choice within the session (Fig. 11), since within-session learning could produce high level of performance irrespective of whether animals retained information which arm was baited. The performance of both lesion groups, as well as Cont group, was almost the same extent in the first and second 6 trials at all interval conditions. Student's t-test between the first and second halves showed there were no significant differences of percent correct responses for all lesion groups at all retention intervals. The correct responses of Cont rats declined as the retention interval extended, while the number of animals which showed a correct choice in the first trial of retention test was constant at different retention intervals. With regard to this discrepancy, it seems that the correct performance of Cont rats improved during retention test at 1 week interval, while it did not ameliorate at 4 weeks interval. A previous study reported that a reminder treatment such as re-exposing the animals to the contextual or apparatus cues increased test performance, when this treatment was employed 10 days, but not 6 weeks after learning (Martin et al., 2005). Thus, the result of Cont group in this study might be due to this kind of amelioration effect, since in this study the retention was tested by a session containing 12 consecutive trials. However, within-session learning of Cont group was not detected when the correct responses were analyzed in the first and second 6 trials (Fig. 11).

Discussion

In experiment 3, clearly different patterns of anterograde amnesia between RS and HPC groups were observed. In postoperative place discrimination training, all three groups could acquire P2-P4, and needed less sessions to acquire them than to acquire P1 in preoperative training. . This suggests that all groups of animals could retain the pre-surgically acquired memory of how to solve the problem in the apparatus. However, only HPC-lesioned rats took significantly more sessions to criterion than Cont group. This is consistent with a previous study in which HPC-lesioned rats

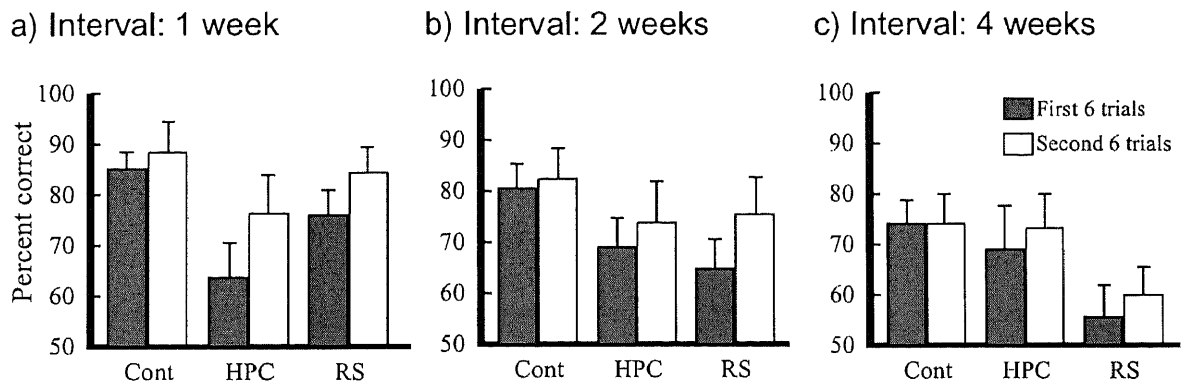

Figure 11. Mean percent correct responses (± SEM) in the first and second halves (6 trials) of retention test at each retention interval in experiment 3. Cont = rats with vehicle injection; RS = rats with NMDA lesion of the retrosplenial cortex; HPC = rats with NMDA lesion of the dorsal hippocampus.

were impaired to learn place discrimination of a new arm pair (Cho et al., 1995). In order to acquire place discrimination in the present study, subjects might have been required to maintain information which arm was baited during training. Therefore, HPC rats might have a difficulty in maintaining this information even for a limited, short period of training session (up to 10-20 min). Thus, these animals showed anterograde amnesia, although the deficit was not so severe. Conversely, RS group could acquire P2-P4 as fast as Cont group. Previous studies reported that RS-lesioned rats which had preoperative training showed mild impairment compared to those without preoperative training (Lukoyanov et al., 2005; Cain et al., 2006). Thus, since in the present experiment I conducted preoperative training using different alternatives, RS-lesioned rats might have been able to perform normally in postoperative place discrimination training.

In retention test of postoperatively acquired discrimination (Fig. 10, Table 2), correct performance of Cont animals showed a gradual decrease as the retention interval increased. RS group showed a remarkable retention interval-dependent impairment. It must be noticed that RS-lesioned rats completely failed to discriminate adjacent two arms that they learned 4 weeks before, although they could discriminate other adjacent two arms almost to the same extent as Cont animals if they learned it 1 week before. This pattern of impairment strongly suggests that the role of RS in spatial memory required for this task changes depending on the time after acquisition. The present results might reflect a deficit of long-term storage and/or retrieval after long-term storage, since performance of RS animals was impaired only with a long retention interval. This may be consistent with the finding that the expression of Zif268 was elevated in RS after the test of remote spatial memory, but not of recent memory (Maviel et al., 2004). RS is proposed as a candidate brain region that forms a network with hippocampal formation in memory function, since RS has dense neural connections with hippocampal formation. Consistent with this

view, the present result suggests that RS may contribute to a more permanent store of the information in the cortical area.

Conversely, HPC-lesioned rats performed the task at the same level regardless of the retention interval. Accordingly, correct performance level of HPC group was inferior to that of Cont group when tested 1 week after training, while it was almost the same as Cont group when tested 4 weeks after training. This result suggests that HPC might have more important role for recent memory rather than for remote memory. In addition, the performance of HPC group in retention test was clearly different from that of RS group. This might reflect the functional difference between RS and HPC in spatial long-term memory, and suggests the possibility that the relative importance of RS and HPC function had changed as time passed. However, it should be considered that the performance of HPC group did not show any gradient as retention interval increased, and it was about 70% correct level, but not 50% level, both in the shortest and the longest retention intervals. It should be noticed that HPC-lesioned rats could acquire the discrimination of all three arm pairs without intact dorsal HPC function. Although the learning was slower than Cont animals, HPC-lesioned animals could acquire the task, suggesting that other brain regions, including residual HPC areas, might have compensated for the lesioned dorsal HPC. One may suppose that within-session learning in retention test could produce performance above chance level and a flat gradient amnesia. However, the significant increase of correct performance within the session was not observed in all retention intervals (Fig. 11). Thus, this possibility could not explain results of HPC group in the present study. The reason why HPC group could maintain 70% level performance at all intervals despite the loss of sufficient HPC function is not clear at present. However, my view that residual HPC or other brain regions might have compensated for the HPC function may partly explain these results. In order to solve this problem, further study employing additional lesion groups will be needed.

General Discussion

The present study aimed to reveal the role of RS in spatial memory. Based on the previous studies and memory models that have been proposed, it could be hypothesized that RS might be involved in spatial long-term memory processes, and the function of RS might be different from that of HPC.

The main findings of the present study were (1) RS-lesioned rats performed well in DNMTP task, while HPC-lesioned rats showed a remarkable delay-dependent impairment. (2) In retention test of preoperatively acquired place discrimination, both RS and HPC lesions produced temporally ungraded retrograde amnesia. (3) By contrast, animals with these lesions revealed a different pattern of anterograde amnesia: RS-lesioned rats showed retention interval-dependent anterograde amnesia producing a significant impairment compared with Cont group at the longest interval, while performance of HPC-lesioned rats did not change depending on the retention interval yielding a significant difference with Cont group at the shortest interval.

In accordance with the above-mentioned hypothesis, these results of the present study suggest that RS and HPC have different roles in spatial memory processes. RS might have more important role in long-term memory processes, especially in remote memory, rather than spatial working memory, whereas HPC might be involved in spatial working memory, and also in the process of spatial long-term memory.

It has been unclear which brain region is important for long-term memory processing. Since RS-lesioned rats showed both retrograde and anterograde amnesia of spatial memory in the present study, it is suggested that RS is one of the candidate brain region which has an important role in spatial long-term memory process. In addition, the present results confirmed the involvement of HPC in spatial long-term memory in accordance with the previous studies. Therefore, RS and HPC might

interact with each other. Based on the results of Experiment 3, the involvement of RS in spatial long-term memory might be different from that of HPC. One possible mechanism is that the information which is temporarily stored in HPC might be transferred to RS as a long-term memory. However, whether the involvement of HPC in spatial long-term memory is time-limited or not was not clearly revealed in this study. Furthermore, RS-lesioned rats showed an impairment irrespective of remoteness of memory in Experiment 2, while they showed a deficit when tested remotely, but not recently, after learning in Experiment 3. This discrepancy should be solved by additional experiments.

To elucidate the memory function of RS and RS-HPC interaction in spatial long-term memory process, further study will be needed. Although I merely focused in this study on the comparison of the memory function of RS and HPC, I should take into account the memory function of other brain regions such as the entorhinal cortex and the perirhinal cortex in the future, since these areas have dense reciprocal neural connections both with RS and HPC.

References

Albasser, M.M., Poirier, G.L., Warburton, E.C., and Aggleton, J.P. 2007 Hippocampal lesions halve immediate-early gene protein counts in retrosplenial cortex: distal dysfunctions in a spatial memory system. *European Journal of Neuroscience,* **26**, 1254-1266.

Bolhuis, J.J., Stewart, C.A., and Forrest, E.M. 1994 Retrograde amnesia and memory reactivation in rats with ibotenate lesions to the hippocampus and subiculum. *Quarterly Journal of Experimental Psychology B,* **47B**, 129-150.

Bontempi, B., Laurent-Demir, C., Destrade, C., and Jaffard, R. 1999 Time-dependent reorganization of brain circuitry underlying long-term memory storage. *Nature*, **400**, 671-675.

Bowers, D., Verfaellie, M., Valenstein, E., and Heilman, K.M. 1988 Impaired acquisition of temporal information in retrosplenial amnesia. *Brain and Cognition*, **8**, 47-66.

Burwell, R.D. and Amaral, D.G. 1998 Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *Journal of Comparative Neurology*, **398**, 179-205.

Cain, D.P., Humpartzoomian, R., and Boon, F. 2006 Retrosplenial cortex lesions impair water maze strategies learning or spatial place learning depending on prior experience of the rat. *Behavioural Brain Research*, **170**, 316-325.

Chen, L.L., Lin, L.H., Green, E.J., Barnes, C.A., and McNaughton, B.L. 1994 Head-direction cells in the rat posterior cortex. I. Anatomical distribution and behavioral modulation. *Experimental Brain Research*, **101**, 8-23.

Cho, J. and Sharp, P.E. 2001 Head direction, place and movement correlates for cells in the rat retrosplenial cortex. *Behavioral Neuroscience*, **115**, 3-25.

Cho, Y.H. and Jaffard, R. 1994 The entorhinal cortex and a delayed non-matching-to-place task in mice: emphasis on preoperative training and

presentation procedure. *European Journal of Neuroscience*, **6**, 1265-1274.

Cho, Y.H. and Kesner, R.P. 1996 Involvement of entorhinal or parietal cortex in long-term spatial discrimination memory in rats: retrograde amnesia. *Behavioral Neuroscience*, **110**, 436-442.

Cho, Y.H., Beracochea, D., and Jaffard, R. 1992 Differential effects of ibotenate lesions of the CA1 subfield of the hippocampus on a delayed-non-matching-to-place task as a function of preoperative training in mice. *Psychobiology*, **20**, 261-269.

Cho, Y.H., Kesner, R.P., and Brodale, S. 1995 Retrograde and anterograde amnesia for spatial discrimination in rats: role of hippocampus, entorhinal cortex, and parietal cortex. *Psychobiology*, **23**, 185-194.

Clark, R.E., Broadbent, N.J., Zola-Morgan, S., and Squire, L.R. 2002 Anterograde amnesia and temporally-graded retrograde amnesia for a nonspatial memory task following lesions of hippocampus and subiculum. *Journal of Neuroscience*, **22**, 4663-4669.

Cooper, B.G. and Mizumori, S.J.Y. 1999 Retrosplenial cortex inactivation selectively impairs navigation in darkness. *NeuroReport*, **10**, 625-630.

Cooper, B.G. and Mizumori, S.J.Y. 2001 Temporary inactivation of the retrosplenial cortex causes a transient reorganization of spatial coding in the hippocampus. *Journal of Neuroscience*, **21**, 3986-4001.

Destrade, C. and Ott, T. 1982 Is a retrosplenial (cingulate) pathway involved in the mediation of high frequency hippocampal rhythmical slow activity (theta) ? *Brain Research*, **252**, 29-37.

Eichenbaum, H., Otto, T., and Cohen, N.J. 1994 Two functional components of the hippocampal memory system. *Behavioral and Brain Sciences*, **17**, 449-518.

Ennaceur, A., Neave, N., and Aggleton, J.P. 1997 Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental Brain*

Research, **113**, 509-519.

Frankland, P.W. and Bontempi, B. 2005 The organization of recent and remote memories. *Nature Reviews Neuroscience*, **6**, 119-130.

Hampson, R.E. and Deadwyler, S.A. 1998 Role of Cannabinoid Receptors in Memory Storage. *Neurobiology of Disease*, **5**, 474–482.

Harker, K.T. and Whishaw, I.Q. 2004 Impaired place navigation in place and matching-to-place swimming pool tasks follows both retrosplenial cortex lesions and cingulum bundle lesions in rats. *Hippocampus*, **14**, 224-231.

Ino, T., Inoue, Y., Kage, M., Hirose, S., Kimura, T., and Fukuyama H. 2002 Mental navigation in humans is processed in the anterior bank of the parieto-occipital sulcus. *Neuroscience Letters*, **322**, 182-186.

Kim, J.J., Clark, R.E., and Thompson, R.F. 1995 Hippocampectomy impairs the memory of recently, but not remotely, acquired trace eyeblink conditioned responses. *Behavioral Neuroscience*, **109**, 195-203.

Lee, I. and Kesner, R.P. 2002 Differential contribution of NMDA receptors in hippocampal subregion to spatial working memory. *Nature Neuroscience*, **5**, 162-168.

Lehmann, H., Lacanilao, S., and Sutherland, R.J. 2007 Complete or partial hippocampal damage produces equivalent retrograde amnesia for remote contextual fear memories. *European Journal of Neuroscience*, **25**, 1278-1286.

Lukoyanov, N.V., Lukoyanova, E.A., Andrade, J.P., and Paula-Barbosa, M.M. 2005 Impaired water maze navigation of Wistar rats with retrosplenial cortex lesions: effect of nonspatial pretraining. *Behavioural Brain Research*, **158**, 175-182.

Maeshima, S., Ozaki, F., Masuo, O., Yamaga, H., Okita, R., and Moriwaki, H. 2001 Memory impairment and spatial disorientation following a left retrosplenial lesion. *Journal of Clinical Neuroscience*, **8**, 450-451.

Maguire, E.A. 2001 The retrosplenial contribution to human navigation: a review of

lesion and neuroimaging findings. *Scandinavian Journal of Psychology*, **42**, 225-238.

Maguire, E.A., Frackowiak, R.S.J. and Frith, C.D. 1997 Recalling routes around London: Activation of the right hippocampus in taxi drivers. *Journal of Neuroscience*, **17**, 7103-7110.

Martin, S.J., de Hoz, L., and Morris, R.G.M. 2005 Retrograde amnesia: neither partial nor complete hippocampal lesions in rats result in preferential sparing of remote spatial memory, even after reminding. *Neuropsychologia*, **43**, 609-624.

Maviel, T., Durkin, T.P., Menzaghi, F., and Bontempi, B. 2004 Sites of neocortical reorganization critical for remote spatial memory. *Science*, **305**, 96-99.

McDonald, C.R., Crosson, B., Valenstein, E., and Bowers, D. 2001 Verbal encoding deficits in a patient with a left retrosplenial lesion. *Neurocase*, **7**, 407-17.

Mumby, D.G., Astur, R.S., Wiesend, M.P., and Sutherland, R.J. 1999 Retrograde amnesia and selective damage to the hippocampal formation: Memory for places and object discriminations. *Behavioural Brain Research*, **106**, 97-107.

Nadel, L. and Moscovitch, M. 1997 Memory consolidation, retrograde amnesia and the hippocampal complex. *Current Opinion in Neurobiology*, **7**, 217-227.

O'Keefe, J. and Nadel, L. 1978 *The Hippocampus as a Cognitive Map.* Clarendon, Oxford.

Pan, W-X. and McNaughton, N. 1997 The medial supramammillary nucleus, spatial learning and the frequency of hippocampal theta activity. *Brain Research*, **764**, 101-108.

Parron, C. and Save, E. 2004 Comparison of the effects of entorhinal and retrosplenial cortical lesions on habituation, reaction to spatial and non-spatial changes during object exploration in the rat. *Neurobiology of Learning and Memory*, **82**, 1-11.

Paxinos, G. and Watson, C. 1998 *The Rat Brain in Stereotaxic Coordinates.* 4th ed. Academic Press, San Diego, CA.

Porter, M.C., Burk, J.A., and Mair, R.G. 2000 A comparison of the effects of hippocampal or prefrontal cortical lesions on three versions of delayed non-matching-to-sample based on positional or spatial cues. *Behavioural Brain Research*, **109**, 69-81.

Pothuizen, H.H., Zhang, W.N., Jongen-Rêlo, A.L., Feldon, J., and Yee, B.K. 2004 Dissociation of function between the dorsal and the ventral hippocampus in spatial learning abilities of the rat: a within-subject, within-task comparison of reference and working spatial memory. *European Journal of Neuroscience.* **19**, 705-712.

Poucet, B., Lenck-Santini, P.P., Paz-Villagran, V., and Save, E. 2003 Place cells, neocortex and spatial navigation : a short review. *Journal of Physiology-Paris*, **97**, 537-546.

Rosenbaum, R.S., Winocur, G., and Moscovitch, M. 2001 New views on old memories: re-evaluating the role of the hippocampal complex. *Behavioural Brain Research*, **127**, 183-197.

Rosenbaum, R.S., Ziegler, M., Winocur, G., Grady, C.L., and Moscovitch, M. 2004 "I have often walked down this street before": fMRI studies on the hippocampus and other structures during mental navigation of an old environment. *Hippocampus*, **14**, 826-835.

Sasaki, Y. and Iwasaki, T. 2003 Differential effects of entorhinal and hippocampal lesions on DNMTP performance in rats. *Japanese Journal of Physiological Psychology and Psychophysiology*, **21**, 231-238.

Shannon, B.J. and Buckner, R.L. 2004 Functional-anatomic correlates of memory retrieval that suggest nontraditional processing roles for multiple distinct regions within posterior parietal cortex. *Journal of Neuroscience*, 24, 10084-10092.

Spiers, H.J. and Maguire, E.A. 2006 Thoughts, behaviour, and brain dynamics during navigation in the real world. *Neuroimage*, **31**, 1826-1840.

Squire, L.R. 1992 Memory and the hippocampus: a synthesis from findings with rats,

monkeys, and humans. *Psychological Review*, **99**, 195-231.

Squire, L.R. and Alvarez, P. 1995 Retrograde amnesia and memory consolidation: a neurobiological perspective. *Current Opinion in Neurobiology*, **5**, 169-177.

Sutherland, R.J., Whishaw, I.Q., and Kolb, B. 1988 Contributions of cingulate cortex to two forms of spatial learning and memory. *Journal of Neuroscience*, **8**, 1863-1872.

Valenstein, E., Bowers, D., Verfaellie, M., Heilman, K.M., Day, A., and Watson, R.T. 1987 Retrosplenial amnesia. *Brain*, **110**, 1631-1646.

Vanderwolf, C.H., Leung, L-WS., and Cooley, R.K. 1985 Pathways through cingulate, neo- and entorhinal cortices mediate atropine-resistant hippocampal rhythmical slow activity. *Brain Research*, **347**, 58-73.

Vann, S.D. and Aggleton, J.P. 2002 Extensive cytotoxic lesions of the rat retrosplenial cortex reveal consistent deficits on tasks that tax allocentric spatial memory. *Behavioral Neuroscience*, **116**, 85-94.

Vann, S.D. and Aggleton, J.P. 2004 Testing the importance of the retrosplenial guidance system: effects of different sized retrosplenial cortex lesions on heading direction and spatial working memory. *Behavioural Brain Research*, **155**, 97-108.

Vann, S.D., Brown, M.W., and Aggleton, J.P. 2000a Fos expression in the rostral thalamic nuclei and associated cortical regions in response to different spatial memory tests. *Neuroscience*, **101**, 983-991.

Vann, S.D., Brown, M.W., Erichsen, J.T., and Aggleton, J.P. 2000 Using fos imaging in the rat to reveal the anatomical extent of the disruptive effects of fornix lesions. *Journal of Neuroscience*, **20**, 8144-8152.

Vann, S.D., Brown, M.W., Erichsen, J.T., and Aggleton, J.P. 2000b Using fos imaging in the rat to reveal the anatomical extent of the disruptive effects of fornix lesions. *Journal of Neuroscience*, **20**, 8144-8152.

Vann, S.D., Wilton, L.A.K., Muir, J.L., and Aggleton, J.P. 2003 Testing the importance

of the caudal retrosplenial cortex for spatial memory in rats. *Behavioural Brain Research*, **140**, 107-118.

Vertes, R.P. 2005 Hippocampal theta rhythm: a tag for short-term memory. *Hippocampus*, **15**, 923-935.

Vogt, B.A. 1986 Cingulate cortex. In: *Cerebral Cortex* (A. Peters and E. G. Jones, Eds), pp. 89-149. Plenum Press, New York.

Wagner, A.D., Shannon, B.J., Kahn, I., and Buckner, R. 2005 Parietal contributions to episodic memory retrieval. *Trends in Cognitive Sciences*, **9**, 445-453.

Whishaw, I.Q., Maaswinkel, H., Gonzalez, C.L., and Kolb, B. 2001 Deficits in allothetic and idiothetic spatial behavior in rats with posterior cingulate cortex lesions. *Behavioural Brain Research*, **118**, 67-76.

Wiggs, C.L., Weisberg, J., and Martin, A. 1999 Neural correlates of semantic and episodic memory retrieval. *Neuropsychologia*, **37**, 103-118.

Wolbers, T., Weiller, C., and Büchel, C. 2004 Neural foundations of emerging route knowledge in complex spatial environments. *Cognitive Brain Research*, **21**, 401-411.

Wyss, J.M. and van Goren, T. 1992 Connections between the retrosplenial cortex and the hippocampal formation in the rat: a review. *Hippocampus*, **2**, 1-12.

Yasuda, Y., Watanabe, T., Tanaka, H., Tadashi, I., and Akiguchi, I. 1997 Amnesia following infarction in the right retrosplenial region. *Clinical Neurology and Neurosurgery*, **99**, 102-105.

Summary

I investigated the effects of bilateral excitotoxic lesions of the rat retrosplenial cortex (RS) and hippocampus (HPC) on performance in spatial memory tasks using a radial maze. In experiment 1, animals were tested in delayed nonmatching-to-place task as a test of working memory. RS-lesioned rats could perform this task as well as sham-operated controls (Cont), while HPC-lesioned rats showed a remarkable delay-dependent decline of correct performance. In experiment 2, to investigate the involvement in long-term memory, retrograde and anterograde amnesic effects of RS and HPC lesions on 2-arm place discrimination were examined. To test retrograde amnesia, rats were trained on place discrimination 4 weeks or 1 day before surgery. In retention test conducted 1 week after surgery, both RS and HPC groups showed temporally ungraded retrograde amnesia. To test anterograde amnesia, animals were trained 1 week after surgery to discriminate three arm pairs successively within a day, and then after interposition of 1, 2, or 4 week interval, one of these pairs respectively was tested for retention. RS-lesioned rats could acquire three arm pairs of place discriminations as fast as Cont animals, but showed a retention interval-dependent impairment in retention test. Conversely, HPC-lesioned rats took significantly more sessions to acquire the place discriminations than Cont group, and their retention test performance was about 70% correct performance regardless of retention interval. Results suggest that RS and HPC might have different roles in spatial memory, and RS might be important for long-term memory processes, and not for spatial working memory.