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Hippocampal AP5 treatment impairs both spatial working and reference memory in radial maze performance in rats

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ABSTRACT

The possible involvement of hippocampal N-methyl-D-aspartate (NMDA) receptors in spatial reference and working memory was investigated. Rats were first trained in a four-baited/four-unbaited version of the eight-arm radial maze task in which only predetermined four arms for each rat were baited with a food pellet. After rats reached the learning criterion, their performance was tested under the treatment of a NMDA antagonist, AP5 (D,L-2-amino-5-phosphonopentanoic acid, 20–40 nmol), or vehicle into the dorsal hippocampus through the bilaterally implanted guide cannulae. AP5 produced dose-dependent increments on both reference and working memory errors, but did not have any effect on the running speed. Additionally, there were significant correlations between the number of trials to criterion in acquisition and the number of reference and working memory errors induced by AP5 treatment. The results suggest that hippocampal NMDA receptors are involved in both spatial reference and working memory.

1. Introduction

It has been well known that N-methyl-D-aspartate (NMDA) receptors, subclass of glutamate receptors, play a critical role in induction of hippocampal long-term potentiation (LTP), which is regarded as a neural base of learning and memory. Since the first report of spatial learning deficit caused by intracerebroventricular treatment of a NMDA receptor antagonist, 2-amino-5-phosphonopentanoic acid (AP5) (Morris, 1989; Morris et al., 1986), many studies have demonstrated an involvement of hippocampal NMDA receptors in acquisition or performance of spatial "working" memory tasks in radial maze and water maze (e.g. Kawabe et al., 1998a, 1998b; Lee and Kesner, 2002; Steele and Morris, 1999; Yoshihara and Ichitani, 2004).

On the other hand, whether the hippocampal NMDA receptors are also involved in spatial "reference" memory, which is another spatial memory function, is still debatable. Although an early report by Olton and Papas (1979) showed that hippocampal lesions impaired only spatial working memory but not spatial reference memory, subsequent studies have shown that a systemic treatment of NMDA receptor antagonist, as well as hippocampal lesions, impaired acquisition of spatial reference memory (Galani et al., 2002; Pothuizen et al., 2004; Ramos, 2013; Wilson et al., 1999). Furthermore, retrieval of once acquired spatial reference memory was also impaired by the systemic treatment of a NMDA receptor antagonist (Enomoto et al., 2008). Since NMDA receptor antagonists have been given systemically in these previous studies, however, it has not yet been made clear whether hippocampal NMDA receptors play a role in spatial reference memory. Although the role of hippocampal NMDA receptors in memory acquisition or recall had been investigated using NMDA receptor subunit 1 (NR1) knockout mice (Nakazawa et al., 2002; Nakazawa et al., 2003; Niewoehner et al., 2007; Tsien et al., 1996), a transient inactivation of hippocampal NMDA receptors induced by drug treatment is also useful in determining the role in spatial reference memory.

Thus, in the present study, in order to determine the role of hippocampal NMDA receptors in spatial working and reference memory, we assessed the effects of intrahippocampal AP5 treatment on performance of a four-baited/four-unbaited version of the eight-arm radial maze task. Using this procedure, we could evaluate concurrently two types of spatial memory error, working memory error and reference memory error, with the same set of spatial cues, and thereby we could make a within-subject and within-task comparison between working and reference memory. In this task, animals were required to memorize which four arms were baited or unbaited across trials (spatial reference memory). Within a trial, the animals needed to recognize the arms that had already been visited from those that had not (spatial working memory). The animals were required to integrate both type of memory for the optimal performance in this task. This is the first

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attempt to compare the effects of hippocampal NMDA antagonist treatment on the spatial working and reference memory using this particular test.

2. Material and methods

2.1. Animals

Male Wistar-Imamichi rats (3 months old, $n=9$) were used. Their mean body weight was approximately 220 g at the beginning of behavioral test. They were housed in individual cages with water available ad libitum, and maintained on a 12:12 h light/dark cycle. Food was restricted so that body weight of rats was kept at 80–85% of their free-feeding weight. All experiments were carried out according to the guidelines for the Care and Use of Animals approved by the University of Tsukuba Committee on Animal Research.

2.2. Apparatus

The apparatus was an elevated 8-arm radial maze made of black polyvinyl chloride. It consisted of an octagonal center platform (34 cm in diameter) and eight arms (60 cm long, 12 cm wide) radiating from the center platform. A food well was bored at the end of each arm. The entire maze was elevated 70 cm above the floor. Transparent Plexiglas guillotine doors, which could be operated by the experimenter, were placed between the center platform and each of arms. The maze was placed in an experimental room in which many extra-maze cues were available. The illumination of the center of the platform was 140 lx.

2.3. Drugs and intrahippocampal microinjection

D,L-AP5 (Research Biochemicals, MA) was dissolved in 0.02 M phosphate buffer (PB) in concentrations of 20 and 40 mM. These solutions and PB (1 μl/side) were bilaterally injected into the dorsal hippocampus via an injection needle, which was inserted into the guide cannulae extending to the depth of 3.7 mm below the skull surface. The injection rate was kept at 0.5 μl/min using a microsyringe pump (EP-60, EICOM, Kyoto) for 2 min. At the end of each injection, the needle was left in place for additional 1 min to allow the diffusion of the drug from the needle tip.

2.4. Pre-operative training

After handling and habituation to the apparatus for 3 days, rats were trained the four-baited/ four-unbaited eight arm radial maze task 3 trials per day. At the beginning of each trial, a 45 mg food pellet (Neuroscience, Tokyo) was placed in four food wells, which were randomly assigned to be the baited arms for each animal. The baited and unbaited arms for each animal were kept constant throughout the experiment. Then the rat was placed in the center of the platform with all the guillotine doors closed. All the doors were raised, and the rat was allowed to choose one of the arms. A choice was counted if the rat completely stepped into the arm, then all the doors were lowered. An error was counted if the rat entered the unbaited arm (reference memory error) or reentered the arm that had been visited previously in the trial (working memory error). While the rat arrived at the end of the arm and consumed the food pellet, the door of the arm was raised. When the rat returned to the center platform, all the doors were lowered, confining it for 5 s. Following the confinement, all the doors were raised. A trial was terminated when all food pellets were consumed or when 5 min had elapsed. The training was continued until the rat had reached the learning criterion of 3 or 4 correct choices in the first 4 choices for 3 trials in a day, in which at least one no-error trial was included. In each trial, all the choice responses and running time to complete the trial were recorded.

2.5. Surgery

After the attainment of the criterion, the rats were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and placed into a stereotaxic instrument. Guide cannulae (22 gauge) were bilaterally implanted into the dorsal hippocampus (AP: −3.8 mm, LM: ±2.7 mm from bregma, DV: −2.7 mm from skull surface, skull flat), and fixed by dental cement and three small screws.

2.6. Post-operative training

One week after the surgery, the rats were retrained the four-baited/four-unbaited radial maze task. The position of baited arms for each rat, the procedures and the learning criterion were the same as those in the pre-operative training.

2.7. Drug test

Following the retraining, each rat was tested once under each of three drug conditions (20, 40 mM AP5 and PB) in a random order. All drugs were injected 15 min prior to the test. In each trial, the number of reference and working memory errors in total choices were recorded. Reference memory error was defined as entering one of four unbaited arms. Therefore, maximum reference memory error was four in a test. Working memory error was defined as reentering an arm that had been visited previously in the trial. Drug-free trials were interposed between each drug test. They were carried out three times per day and continued until reaching the criterion, which was the same as in the pre-operative training.

2.8. Histology

After the behavioral tests, rats were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and perfused intracardially with 0.02 M phosphate buffered saline followed by 10% formalin solution. The brains were further fixed in 10% formalin solution, and then immersed in 20% sucrose solution. They were frozen by carbon dioxide, and sectioned in the coronal plane (40 μm) using a cryostat (CM3000, Leica, Heidelberg). Sections were Nissl-stained with cresyl violet to assess the location of tips of injection cannulae.

2.9. Statistical analysis

Data were expressed as mean ± S.E.M. Statistical analyses were performed by using the Statistical Program for the Social Sciences (SPSS; IBM Corp., NY, USA). Data of preoperative learning curves and those of drug test were analyzed using a one-way analysis of variance (ANOVA) with repeated measures. Individual comparisons were evaluated by using post-hoc Bonferroni's test. Correlation analyses were performed with the number of trials to criterion in acquisition training and the number of errors in drug test to determine whether relationships existed between individual differences in radial maze learning speed and effect size of NMDA receptor blockade.

3. Results

Fig. 1 shows the mean number of correct choices in the first 4 choices (A) and the mean number of reference/working memory errors (B) until subjects reached the learning criterion prior to surgery (3 trials as the criterion were included). A maximum