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Effects of intrahippocampal cannabinoid receptor agonist and antagonist on radial maze and T-maze delayed alternation performance in rats

Toshiko Suenaga, Masami Kaku, Yukio Ichitani *

Department of Behavioral Neuroscience, Institute of Psychology, University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan

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ABSTRACT

Brain cannabinoid receptors are abundantly distributed in the hippocampus, however their detailed role in learning and memory remains unclear. This study investigated the role of hippocampal cannabinoid receptors for performing two kinds of working memory tasks. In experiment 1, intrahippocampal infusion of cannabinoid receptor agonist WIN 55,212-2 (1-2 μg/side) dose-dependently disturbed radial maze performance in rats. In experiment 2, WIN 55,212-2 (2 μg/side) disturbed the performance of delayed alternation in a T-maze by increasing the errors and successive errors, and on the other hand, a cannabinoid receptor antagonist AM 281 (1 μg/side) did not have any significant effects. Disruptive effect of WIN 55,212-2 on the number of errors in delayed alternation was blocked by the pretreatment with intraperitoneal AM 281 (2 mg/kg). Results suggest that hippocampal cannabinoid receptors are involved in the performance of working memory tasks. A possible role of endogenous cannabinoid system in the hippocampus was discussed in terms of an inhibitory adjustment of behavior based on the outcome of animals' previous response.

1. Introduction

Cannabinoids are known to affect sensory, motor and cognitive functions including learning and memory both in humans and rodents (Iversen, 2003). There are at least two types of G protein-coupled cannabinoid receptors identified presently. CB1 receptors are mainly expressed in the central nervous system (Matsuda et al., 1990), and their expression is abundant in the basal ganglia, cerebellum and hippocampus (Herkenham et al., 1990; Moldrich and Wegner, 2000; Tsou et al., 1998). The second cannabinoid receptors, CB2, are expressed in tissues of the immune system (Munro et al., 1993). In addition it is suggested recently that the third, putative cannabinoid receptors (CB3) exist in the central nervous system (Wilson and Nicoll, 2002). According to their localization, the effects of cannabinoids on cognitive functions are thought to arise through CB1 or CB3 receptor mechanisms. It has been suggested that activation of cannabinoid receptors inhibits long-term potentiation (LTP) in rat hippocampal slices (Collins et al., 1995; Misner and Sullican, 1999; Stella et al., 1997; Terranova et al., 1995), which is recognized as a neural base of learning and memory. On the other hand, hippocampal slices from mice lacking CB1 receptors exhibit larger LTP than those from wild-type animals (Bohme et al., 2000). Therefore, hippocampal CB1 receptors presumably are involved in learning and memory process.

A number of behavioral studies have shown that cannabinoid receptors play a role in the performance of various memory tasks which are closely related to the hippocampal functions. For example, systemic or intrahippocampal administration of several cannabinoid receptor agonists impaired the performance of radial maze task (Egashira et al., 2002; Iwasaki et al., 1992; Lichtman et al., 1995; Molina-Holgado et al., 1995) and of delayed alternation task in rats (Nava et al., 2000, 2001). On the other hand, systemic administration of a CB1 antagonist improved the performance in delay-interposed radial maze task (Lichtman, 2000; Wolff and Leander, 2003). These data suggest the possibility that the cannabinoid receptor blockade enhances the maintenance of memory information, while the activation deteriorates it. Furthermore, when CB1 receptor knockout mice were tested in several memory tasks, they showed better object recognition memory (Reibaud et al., 1999), and they continued to swim to the original platform position in the reversal task of Morris water maze compared to wild-type mice (Varvel and Lichtman, 2002). These observations also give support to the hypothesis that cannabinoid receptors are involved in learning and memory in an inhibitory manner.

However there is only a little evidence of hippocampal cannabinoid receptor involvement in learning and memory based on behavioral studies using direct administration of the drugs into the hippocampus. Furthermore, in previous studies, effects of cannabinoid receptor agonists and antagonists have not been examined in an identical learning task, so the effects of cannabinoid receptor activation and blockade on the memory task performance could not be

* Corresponding author. Tel./fax: +81 29 853 6094.
E-mail address: y-ichit@human.tsukuba.ac.jp (Y. Ichitani).

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compared directly. Therefore, we investigated the effects of intrahippocampal administration of cannabinoid receptor agonist and antagonist on two kinds of working memory tasks in rats. If the hippocampal cannabinoid receptors are involved in inhibition of working memory, a cannabinoid receptor agonist would disturb working memory performance, while an antagonist would improve it. First, we examined the effects of cannabinoid receptor agonist WIN 55,212-2 (R-(+)-(2,3-Dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo (1,2,3-de)-1,4-benzoxazinyl)-(1-naphthalenyl) methanone; WIN) on the radial maze performance (experiment 1). Next, we tested the effects of WIN and a cannabinoid receptor antagonist AM 281 (1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morpholinyl-1*H*-pyrazole-3- carboxamide; AM) on the delayed alternation performance in a T-maze (experiment 2). The effect of pretreatment with intraperitoneal AM prior to hippocampal WIN treatment was also tested in this experiment. We used the task since we could easily operate the difficulty of the task by changing the length of intertrial interval (ITI), and thus we could examine the effects of both activation and blockade of these receptors in the same task.

2. Materials and methods

2.1. Subjects

Forty male Wistar–Imamichi rats (8–12 weeks old) were used as subjects, and their mean body weight at the beginning of behavioral tests was 310 g. They were housed in individual cages on a 12:12 h light–dark cycle, and maintained at 80–90% of their expected free feeding weight. Water was freely available. Seven rats were used in the radial maze task (experiment 1). Thirty-three rats were used in the delayed alternation task (experiment 2), and they were assigned to one of the three groups of drug treatment, WIN ($n=13$), AM ($n=11$) or AM+WIN ($n=9$). Animal experiments were approved by the University of Tsukuba Committee on Animal Research.

2.2. Surgery

Rats pretreated with atropine sulfate (0.05 mg, i.p.) were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and ketamine (10 mg, i.m.), and placed on a stereotaxic instrument. Guide cannulae were implanted bilaterally into the dorsal hippocampus with the stereotaxic coordinates (mm) AP: −3.8 from bregma, ML: ±2.7, DV: −3.0 from skull surface (Paxinos and Watson, 1998), and they were fixed on the skull with dental cement and small screws.

2.3. Drugs

Cannabinoid receptor agonist WIN (Sigma, MO) was dissolved in 45% 2-hydroxypropyl-β-cyclodextrin (HBC, Sigma) solution. Cannabinoid antagonist AM (Tocris, MO) was dissolved in dimethyl sulfoxide (DMSO; Wako, Osaka).

In intracerebral administration, drugs were bilaterally injected into the dorsal hippocampus 10 min prior to each trial (radial maze task) or each session (delayed alternation) via injection canulae, which were inserted into the guide cannulae and advanced 1.0 mm below the tips of them. The flow rate was kept 0.5 μl/min with a microsyringe pump (ESP-32, Eicom, Kyoto). After the drug injection, the injection cannulae were held to the site for additional 1 min to diffuse the drug from the tips. In AM+WIN group of experiment 2, AM or DMSO was administered intraperitoneally 15 min prior to hippocampal WIN injection.

2.4. 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 (Wako). The brains were further fixed in 10% formalin solution, 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.

2.5. Behavioral procedures

2.5.1. Radial maze task (experiment 1)

2.5.1.1. Apparatus. An elevated eight-arm radial maze made of black polyvinyl chloride was used. The maze consisted of an octagonal center platform (32 cm in diameter) and 8 arms (60 cm×12 cm) radiated from the platform. A food well (1 cm in diameter, 0.5 cm deep) was carved out at each end of the arms. Plexiglas guillotine doors (15 cm high) divided the arms from the center platform, and each of them was operated automatically. The sidewalls of the arms were 4 cm high, except 12 cm from guillotine doors (12 cm high). The maze was elevated 70 cm above the floor. There were extra-maze visual cues (e.g. curtain, desk, colored drawing paper and door) around the maze in the experiment room. Control and analysis of the behavioral experiment were carried out using Image RM (O'Hara Co. Ltd, Tokyo), modified software based on the public domain NIH Image program (developed at the U.S. National Institutes of Health).

2.5.1.2. Training. Rats were given 5 min handling for three days and then three daily sessions of habituation to the apparatus. In the habituation session, all the guillotine doors were opened, and 20 mg food pellets (Research Diets, Inc., NJ) were placed on the platform and arms. In the first two sessions, 5 rats were placed in the maze together for 30 min, and in the last session, each rat was put in the maze individually for 15 min.

Rats were trained the radial maze task one trial a day. At the beginning of each trial, a 20 mg food pellet was placed in each food well. The rat was placed on the center platform and all the doors were opened. A choice was counted when the rat completely entered an arm, then all the doors except the chosen arm were closed. When the rat returned to the center platform, the door was closed and the rat was confined there for 5 s. After that, all the doors were reopened, and the rat was allowed the next choice. This procedure was repeated until the animal had consumed all the pellets, it had made 16 choices, or 10 min had elapsed since the start of the trial. A correct choice was defined as the rat entered an arm which had not been chosen in the trial and consumed the pellet, and the other choices were counted as errors. The learning criterion was defined as the 5 consecutive trials in which 7 or more correct choices in the first 8 choices were attained. Rats' choice responses and time spent to complete a trial were recorded. After the rats attained the criterion, they received the surgery of guide cannulae implantation.

2.5.1.3. Drug tests. After a week of recovery period from surgery, rats were retrained in the radial maze task. The procedures and the criterion were the same as in the acquisition training. Rats which reattained the criterion were given the drug tests. In the drug test, HBC (1 μl/side) and WIN (1.0–2.0 μg/1 μl/side) were tested in a random order. After drug injection trials, rats were given drug-free trials that continued until the criterion of 7 or more correct choices in the first 8 choices for 2 consecutive trials was attained.

2.5.2. Delayed alternation task (experiment 2)

2.5.2.1. Apparatus. A T-maze made of gray polyvinyl chloride was used. The maze consisted of a start box (20×12 cm, 30 cm high), an adjoining stem (40×12 cm, 30 cm high), and two arms (60×12 cm, 30 cm high). A food well (3 cm in diameter, 1 cm deep) was carved out at each end of the arms. Gray guillotine doors made of polyvinyl

chloride (20 cm high) divided the start box and two arms from the stem, and each of them could be operated by the experimenter by means of overhead lines.

2.5.2.2. Training. Rats were given 5 min handling for three days and then three daily sessions of habituation to the apparatus. Habituation was carried out with the guillotine doors opened, and 45 mg food pellets (Bio-Serv, NJ) were placed in the stem and arms. In the first session, 2 or 3 rats together were allowed to explore the maze and consume the pellets freely for 15 min. In the following two sessions, each rat was placed in the maze individually for 10 min.

From the next day, rats were trained the alternation task one session a day. A session consisted of 11 consecutive trials. At first, the rats were trained the task without delay, and then they were trained with delay. At the start of each trial, the rat was placed in the start box with the guillotine door closed. When the door was opened, the rat could enter the stem and reach the choice point. In the first trial of a session (forced trial), one of the doors of two arms was closed, thus the rat was forced to enter the other arm, which was randomly altered day by day. When four paws of the rat completely entered the arm, the door was closed. Two food pellets had been placed in the food well at the end of arm. After the rat consumed the pellets, it was removed from the arm and returned to the start box, and the next trial started. In the following 10 trials, the guillotine doors of the 2 arms were opened, and the rat could choose an arm freely (free-choice trials). If four paws of the rat completely entered either arm, the door was closed and the rat was confined in it. The rat was rewarded with two pellets only when it entered the opposite arm to that which had been chosen in the previous trial. A correct choice was defined as the rat alternated the arm and consumed the pellets within 2 min. The other responses were defined as errors; entering the same arm that had been chosen in the previous trial, no consuming the pellets within 2 min in the correct arm, or no entering into arms. Then the rat was immediately returned to the start box, and the next trial started. The learning criterion was defined as 9 or more correct choices in the 10 free-choice trials for 3 consecutive sessions were attained. After that the training of delayed alternation started. In this training, rats were given ITIs of 10 s during which the rats were removed from the maze and placed in their home cage. The learning criterion in this training was 8 or more correct choices for 3 consecutive sessions, and among these, one or more sessions of 9–10 correct choices were contained. The experimenter recorded all the rats' choices and running time on each trial. The rats received the surgery of guide cannulae implantation within 1–2 days after they attained the criterion.

2.5.2.3. Drug tests. After a week of recovery period from surgery, the rats were retrained the delayed alternation task with the same procedure and criterion as in the acquisition training. After the rats attained the criterion, they were given the drug tests. For WIN group and AM group, ITIs used in the drug tests were 10, 60 and 120 s, and they were unchanged within a session. The drug conditions tested for WIN group were HBC (1 μl/side) and WIN (2.0 μg/1 μl/side), and those for AM group were DMSO (0.5 μl/side) and AM (1.0 μg/0.5 μl/side). Six conditions based on the combination of ITI length (3) and drugs (2) were tested in a random order. In AM+WIN group, ITIs of 10 and 60 s were tested. AM (2 mg/ 0.5 ml/kg) or DMSO (0.5 ml/kg) was administered intraperitoneally 15 min prior to hippocampal WIN (2.0 μg/1 μl/side) injection. Four conditions based on the combination of ITI length (2) and drugs (2) were tested in a random order. After each drug test, rats were given drug-free sessions that continued until a session of 8 or more correct choices in the 10 free-choice trials with 10 s-ITI was attained.

2.6. Statistical analysis

In the radial maze task, the number of correct choices in the first eight choices, the number of errors, and the running time per choice were analyzed by a one-way analysis of variance (ANOVA) for repeated measure. Running time per choice was calculated from the running time in each trial divided by the total number of choices in the trial.

In the delayed alternation task, the number of errors in the session, the successive error scores, and the running time per trial were analyzed. The successive error score was calculated from the choice record in each session; i.e. one point was added when the rat made successive error responses by entering the same arm repeatedly that had been chosen in the previous trial (3 successive responses to one arm). Running time per trial was the mean of 10 free-choice trials. A two-way ANOVA (delay×drug) for repeated measures was used to analyze the number of errors and running time followed by post-hoc comparisons using a Bonferroni test ($p<.05$). The successive error score was analyzed in each delay condition by a paired *t*-test or Wilcoxon matched-pairs signed-ranks test, since the scores in the shortest delay (10 s) included zero.

3. Results

3.1. Histology

The locations of the tips of injection cannulae are shown in Figs. 1D and 2. All the tips were identified in the dorsal hippocampus both in radial maze (Fig. 1D) and delayed alternation (Fig. 2) experiments.

3.2. Behavioral results

3.2.1. Radial maze task

Effects of WIN on the radial maze performance are shown in Fig. 1. Mean number of correct choices in the first eight choices (Fig. 1A) decreased dose-dependently, and a one-way ANOVA showed a significant effect of drug [$F(2,12)=4.06$, $p<.05$]. Post-hoc test revealed that the number of correct choices of rats under WIN 2.0μg was lower

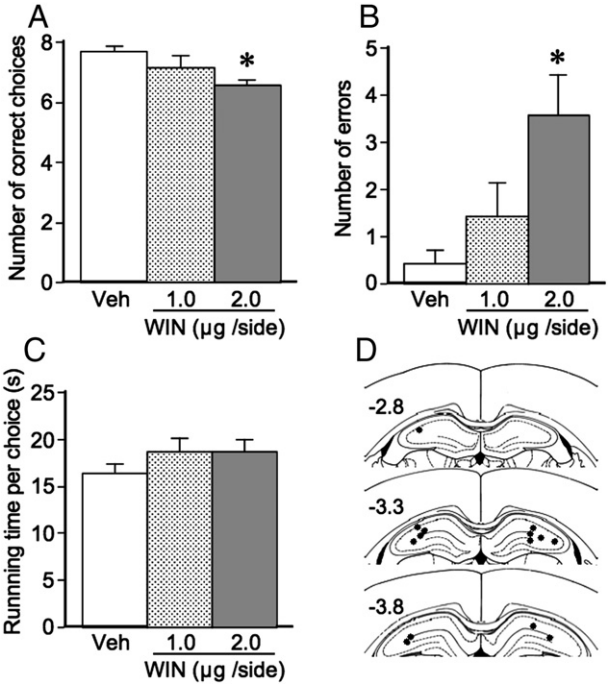

Fig. 1. Effects of hippocampal WIN treatment on the performance of radial maze task. (A) Number of correct choices in the first eight choices. (B) Number of errors in the trial. (C) Running time per choice. Each column represents mean ± S.E.M. *$p<.05$ as compared with vehicle (Veh) condition. (D) Histological coronal sections showing the location of the tips of injection cannulae (closed circles). The numbers in each section show the anteroposterior distance (mm) from bregma (Paxinos and Watson, 1998).

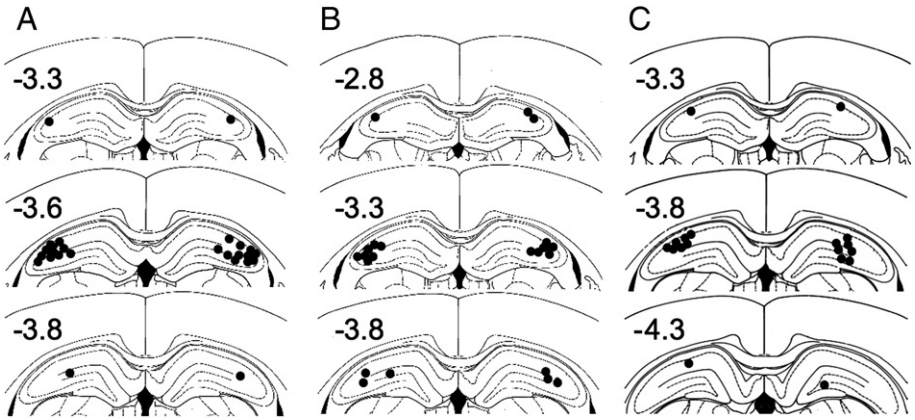

Fig. 2. Histological coronal sections showing the location of the tips of injection cannulae in delayed alternation experiment. The numbers in each section show the anteroposterior distance (mm) from bregma (Paxinos and Watson, 1998). (A) WIN-treated, (B) AM-treated, and (C) AM+WIN-treated rats.

than that under vehicle ($p<.05$). Mean number of errors (Fig. 1B) increased under the treatment of WIN. A one-way ANOVA showed a significant effect of drug [$F(2,12)=4.12$, $p<.05$], and post-hoc test revealed that rats with WIN 2.0 μg made more errors compared to those with vehicle ($p<.05$). There was not a significant change of the running time per choice by WIN treatment (Fig. 1C).

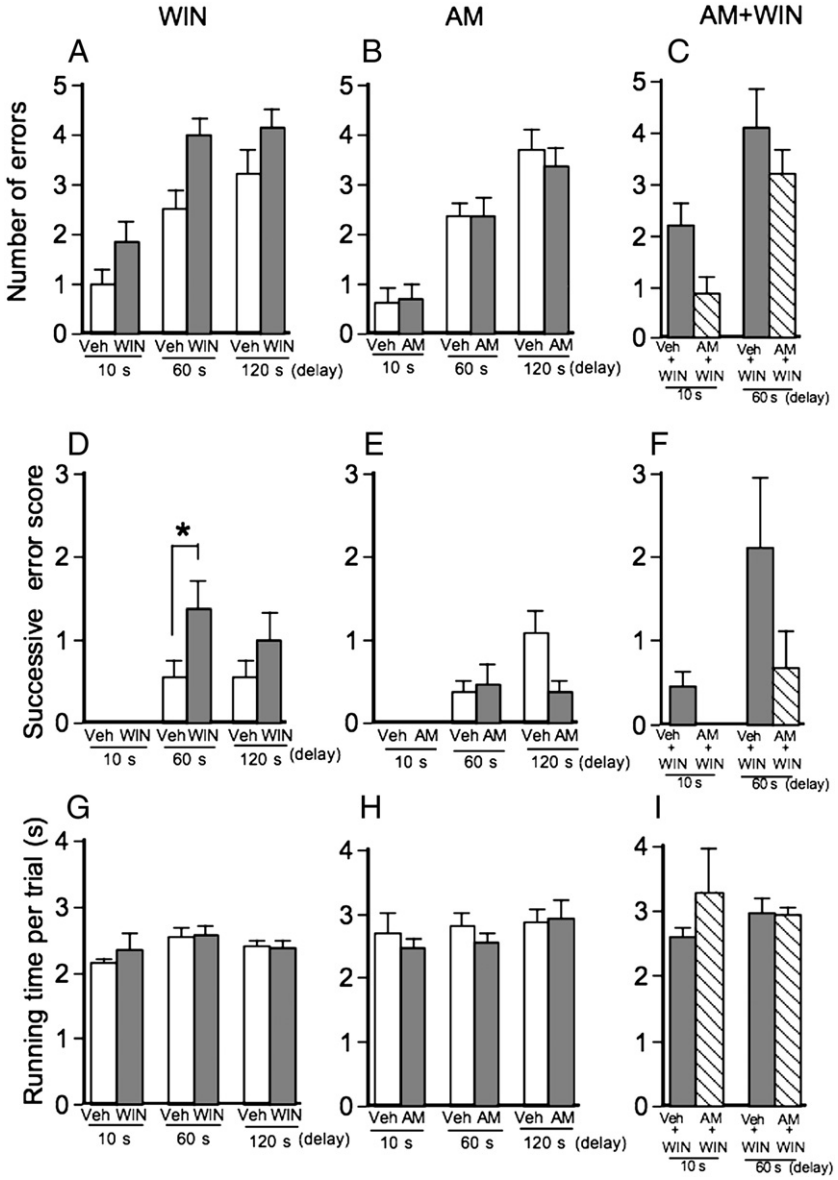

Fig. 3. Effects of hippocampal WIN (A, D, G), AM (B, E, H), and AM+WIN (C, F, I) treatments on the performance of delayed alternation task. (A, B, C) Number of errors in the session. (D, E, F) Successive error score in the session. (G, H, I) Running time per trial. Each column represents mean±S.E.M. *$p<.05$.

3.2.2. Delayed alternation task

Fig. 3 shows the effects of WIN (Fig. 3A, D, G), AM (Fig. 3B, E, H), and combined AM+WIN treatments (Fig. 3C, F, I) respectively on the performance of delayed alternation in a T-maze. In all groups of rats, the number of errors increased delay-dependently. In the WIN group (Fig. 3A), WIN treatment increased the errors in all delay conditions. A two-way ANOVA showed a significant main effect of delay [$F(2,24)=30.34$, $p<.01$], and a post-hoc test revealed that rats made significantly more errors at 60 and 120 s delays than at 10 s delay ($p<.05$). In addition, the main effect of drug was also significant [$F(1,12)=13.31$, $p<.01$]: thus the WIN treatment made more errors than vehicle. The interaction between the delay×drug effects was not significant. In the AM group (Fig. 3B), the number of errors under AM condition was almost equal to that in vehicle condition. A two-way ANOVA showed a significant main effect of delay [$F(2,20)=37.12$, $p<.01$], and a post-hoc test revealed that the errors at 120 s delay were higher than those at other two delay conditions, and the errors at 60 s delay were higher than those at 10 s delay ($p<.05$). However, the main effect of drug and its interaction with delay were not significant. In the AM+WIN group (Fig. 3C), rats under AM+WIN made less errors compared with those under vehicle+WIN in both delay conditions. A two-way ANOVA showed significant main effects of delay [$F(1,8)=15.36$, $p<.01$] and drug [$F(1,8)=6.90$, $p<.05$]. The interaction between the delay×drug effects was not significant.

Fig. 3D–F show the successive error scores. WIN made this score higher compared with vehicle condition especially in 60 s delay (Fig. 3D). According to a *t*-test adopted to the data of 60 and 120 s delay respectively, rats under WIN treatment showed higher score at 60 s delay [$t(12)=2.17$, $p=.05$]. The difference at 120 s delay was not significant. On the other hand, AM decreased successive error score at 120 s delay (Fig. 3E), and there was a tendency of statistical difference between vehicle and AM conditions at 120 s delay [$t(10)=2.03$, $p<.10$]. In the AM+WIN group (Fig. 3F), rats pretreated with AM (AM+WIN) showed lower score compared with vehicle-pretreated condition (Veh+WIN) in both delay condition. However, the difference between Veh+WIN and AM+WIN conditions did not reach a significant level according to Wilcoxon matched-pairs signed-ranks test adopted to the data of 10 s delay ($z=1.83$, $p<.10$), and a *t*-test adopted to the data of 60 s delay [$t(8)=2.04$, $p<.10$].

Mean running time per trial in 10 free-choice trials is shown in Fig. 3G–I. In all groups of rats, there was not a marked difference of running time due to the delay or the drug, and statistical analysis did not show any significant differences.

4. Discussion

The present study investigated the role of hippocampal cannabinoid receptors for the performance of two kinds of working memory tasks. In the radial maze task, intrahippocampal cannabinoid agonist WIN dose-dependently decreased correct choices and increased the number of errors without changing the running speed. In the delayed alternation task, intrahippocampal WIN increased the number of errors and it also increased the successive errors. On the other hand, intrahippocampal cannabinoid receptor antagonist AM did not have any significant effects on the performance. Disruptive effect of WIN on the number of errors in this task was significantly antagonized by the pretreatment with systemic AM.

In the radial maze task, WIN significantly decreased correct choices and increased the number of errors. This is consistent with previous reports in which CP 55,940 (Lichtman et al., 1995) and Δ^9-tetrahydrocannabinol (Δ^9-THC) (Egashira et al., 2002), cannabinoid receptor agonists, increased errors in the radial maze when injected into the dorsal hippocampus. Intrahippocampal WIN administration in the present study did not affect the running speed in the radial maze. This suggests that these animals could move in the maze as quickly as in the normal state and they were fully motivated to forage food pellets. Taken together it is plausible that cannabinoid receptor activation in the dorsal hippocampus substantially disturbed working memory process required for the radial maze performance without alternating the motor response ability and the motivational level. It has been reported that systemic treatment with WIN produces motor deficits and retards maze performance (Lichtman et al., 1995). However, as we have shown in the present study, intrahippocampal WIN did not affect motor response at all at the dose that effectively induced more errors. Thus, the local infusion of WIN has an advantage that makes roles of hippocampal cannabinoid receptors clearer independent of other systemic cannabinoid effects.

In the delayed alternation task, we have examined the effects of both agonist and antagonist in the same procedures. In this task, rats showed more errors as the delay time increased from 10 s to 120 s (Fig. 3A, B), suggesting the delay-dependent increase of difficulty. Intrahippocampal WIN increased the number of errors regardless of the delay length, and this effect was clearly antagonized by the pretreatment with AM (Fig. 3C). Previous studies showed that systemic administration of Δ^9-THC impaired the performance of delayed alternation task (Nava et al., 2000, 2001). Our present study is consistent with the reports, and further suggests that the disruption of delayed alternation performance was produced, at least in part, by the activation of hippocampal cannabinoid receptors. On the other hand, intrahippocampal AM did not affect the number of errors in the present study. A possibility is left that higher doses of AM might have improved the performance. However, it is reported that systemic cannabinoid receptor antagonist SR141716A improved performance in delay (6–7 h)-interposed radial maze task (Lichtman, 2000; Wolff and Leander, 2003). Besides, in the object recognition task, CB1 receptor knock-out mice showed an enhancement of memory over a period of 48 h (Reibaud et al., 1999). Therefore, there is another possibility that the facilitatory effects of cannabinoid receptor blockade on memory could be more easily detected when the tasks in which information should be maintained over several hours are employed.

When we focus on the successive errors (3 successive responses to one arm) under the treatment of WIN, animals showed significantly more errors in 60 s-delay condition (Fig. 3D), although this effect was not fully antagonized by the preteatment of AM (Fig. 3F). This suggests that cannabinoid receptors in the hippocampus are involved in the adjustment of behavior based on the outcome of their previous response, since successive error was scored only when rats showed perseverative response to the same direction of arm after they experienced the non-reward in the arm in the previous trail. One possible mechanism is that endogenous cannabinoid system has an inhibitory action to the adjustment system since a cannabinoid receptor agonist (WIN), not antagonist, had a disruptive effect on successive errors in the present study. This hypothesis would be better supported if an opposite tendency could be obtained by a cannabinoid receptor antagonist (AM) in the same task. However the tendency was not clearly observed in the present study (Fig. 3E).

Based on the finding that cannabinoid receptor agonists (WIN and Δ^9-THC) produced dose-dependent reduction in the firing rate during the sample phase of delayed match- and nonmatch-to-sample tasks as well as significant decrease of performance level of the tasks, Deadwyler's group (Hampson and Deadwyler, 1999, 2000; Heyser et al., 1993) hypothesized cannabinoid exposure disrupts the ability of encoding of sample information. They also hypothesized that there is a system in the hippocampus that correct performance (on short-delay trials) successively weakens the sample response code and this leads to an error, and cannabinoids prevent the adjustment of encoding strength as a function of performance outcome (error) on the previous trial. We did not use a delayed nonmatch-to-sample task in this study but used a delayed alternation task, in which the alternation response is at the same time the sample response for the next trial. In this sense, it was different from the delayed (non)match-to-sample task. However, if we adopt the second hypothesis of these previous studies,

we are able to explain the increase of successive errors under WIN treatment in the present study. Direct stimulation of hippocampal cannabinoid receptors by WIN may have prevented the adjustment of encoding strength even if the animal made an error on the previous trial.

Taken together, our results in the two experiments suggest that hippocampal cannabinoid receptors are involved in the adjustment of animal behavior based on the outcome of their own previous response, and this leads to the effects of the drugs used here on their performance in working memory tasks such as radial maze and delayed alternation tasks. This interpretation can be supported by the fact that a cannabinoid receptor agonist Δ^9-THC had differential effects on working and reference memory in Morris water maze, and working memory was selectively impaired in mice (Varvel et al., 2001).

In conclusion, the present study showed that a cannabinoid receptor agonist administered directly into the hippocampus produced more errors in the radial maze and delayed alternation behavior. These data are consistent with the hypothesis that hippocampal cannabinoid receptors are involved in working memory probably through an inhibitory action to the behavior adjustment system based on the outcome of animals' previous response, although the detailed mechanism of this adjustment system and the interaction of endogenous cannabinoid system with other transmitter-receptor systems remain unclear.

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References

Bohme GA, Laville M, Ledent C, Parmentier M, Imperato A. Enhanced long-term potentiation in mice lacking cannabinoid CB_1 receptors. Neuroscience 2000;95:5–7.
Collins DC, Pertwee RG, Davies SN. Prevention by the cannabinoid antagonist, SR141716A, of cannabinoid-mediated blockade of long-term potentiation in the rat hippocampal slice. Br J Pharmacol 1995;115:869–70.
Egashira N, Mishima K, Iwasaki K, Fujiwara M. Intracerebral microinjections of Δ^9-tetrahydrocannabinol: search for the impairment of spatial memory in the eight-arm radial maze in rats. Brain Res 2002;952:239–45.
Hampson RE, Deadwyler SA. Cannabinoids, hippocampal function and memory. Life Sci 1999;65:715–23.
Hampson RE, Deadwyler SA. Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. J Neurosci 2000;20:8932–42.
Herkenham M, Lynn AB, Little MD, Ross Johnson M, Melvin LS, De Costa BR, et al. Cannabinoid receptor localization in brain. Proc Natl Acad Sci USA 1990;87:1932–6.
Heyser CJ, Hampson RE, Deadwyler SA. Effects of delta-9-tetrahydrocannabinol on delayed match to sample performance in rats: alternations in short-term memory associated with changes in task specific firing of hippocampal cells. J Pharmacol Exp Ther 1993;264:294–307.
Iversen L. Cannabis and the brain. Brain 2003;126:1252–70.
Iwasaki K, Matsumoto Y, Fujiwara M. Effect of nebracetam on the disruption of spatial cognition in rats. Jpn J Pharmacol 1992;58:117–26.
Lichtman AH, Dimen KR, Martin BR. Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. Psychopharmacology 1995;119:282–90.
Lichtman AH. SR 141716A enhances spatial memory as assessed in a radial-arm maze task in rats. Eur J Pharmacol 2000;404:175–9.
Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 1990;346:561–4.
Misner DL, Sullivan JM. Mechanism of cannabinoid effects on long-term potentiation and depression in hippocampal CA1 neurons. J Neurosci 1999;19:6795–805.
Moldrich G, Wegner T. Localization of the CB_1 cannabinoid receptor in the rat brain: an immunohistochemical study. Peptides 2000;21:1735–42.
Molina-Holgado F, González MI, Leret ML. Effect of Δ^9-tetrahydrocannabinol on short-term memory in the rat. Physiol Behav 1995;57:177–9.
Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61–5.
Nava F, Carta G, Battasi AM, Gessa GL. D_2 dopamine receptors enable Δ^9-tetrahydrocannabinol induced memory impairment and reduction of hippocampal extracellular acetylcholine concentration. Br J Pharmacol 2000;130:1201–10.
Nava F, Carta G, Colombo G, Gessa GL. Effects of chronic Δ^9-tetrahydrocannabinol treatment on hippocampal extracellular acetylcholine concentration and alternation performance in the T-maze. Neuropharmacology 2001;41:392–9.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4th edn. San Diego: Academic Press; 1998.
Reibaud M, Obinu MC, Ledent C, Parmentier M, Böhme GA, Imperato A. Enhancement of memory in cannabinoid CB_1 receptor knock-out mice. Eur J Pharmacol 1999;379:R1–2.
Stella N, Schweitzer P, Piomelli D. A second endogenous cannabinoid that modulates long-term potentiation. Nature 1997;388:773–7.
Terranova JP, Michaud JC, Le Fur G, Soubrié P. Inhibition of long-term potentiation in rat hippocampal slices by anandamide and WIN 55212-2: reversal by SR141716A, a selective antagonist of CB1 cannabinoid receptors. Naunyn-Schmiedebergs Arc Pharmacol 1995;352:576–9.
Tsou K, Brown S, Sañudo-Peña MC, Mackie K, Walker JM. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 1998;83:393–411.
Varvel SA, Hamm RJ, Martin BR, Lichtman AH. Differential effects of Δ^9-THC on spatial reference and working memory in mice. Psychopharmacology 2001;157:142–50.
Varvel SA, Lichtman AH. Evaluation of CB_1 receptor knockout mice in the Morris water maze. J Pharmacol Exp Ther 2002;301:915–24.
Wilson RI, Nicoll RA. Endocannabinoid signaling in the brain. Science 2002;296:678–82.
Wolff MC, Leander JD. SR141716A, a cannabinoid CB_1 receptor antagonist, improves memory in a delayed radial maze task. Eur J Pharmacol 2003;477:213–7.