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# Involvement of 5-hydroxytryptamine neuronal system in $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory

Nobuaki Egashira [a], Kenichi Mishima [a], Shutaro Katsurabayashi [a], Tomohiro Yoshitake [b,c], Yoshiaki Matsumoto [a,d], Junichi Ishida [b], Masatoshi Yamaguchi [b], Katsunori Iwasaki [a], Michihiro Fujiwara [a,*]

[a] *Department of Physiology and Pharmacology, Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka 814-0180, Japan*
[b] *Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka 814-0180, Japan*
[c] *Chemical Biotesting Center, Chemical Inspection and Testing Institute, Oita, Japan*
[d] *School of Nursing, Kyushu Women's Jr. College, Kitakyushu 807-8586, Japan*

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**Abstract**

The present study investigated the involvement of the serotonin (5-hydroxytryptamine, 5-HT) neuronal system in the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory in the eight-arm radial maze in rats. $\Delta^9$-Tetrahydrocannabinol (6 mg/kg, i.p.), which impairs spatial memory, significantly increased the 5-HT content in the ventral hippocampus. A microdialysis study showed that $\Delta^9$-tetrahydrocannabinol (6 mg/kg, i.p.) decreased 5-HT release in the ventral hippocampus. The 5-HT precursor, 5-hydroxy-L-tryptophan (5-HTP; 50 mg/kg, i.p.), the 5-HT re-uptake inhibitor, clomipramine (0.01 and 0.1 mg/kg, i.p.), the 5-HT receptor agonist, 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT; 0.01 and 0.03 mg/kg, i.p.), and the 5-$HT_2$ receptor agonist, 1-(2,5-dimethoxy 4-iodophenyl)-2-amino propane (DOI; 10 μg/kg, i.p.), significantly attenuated the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory. These results suggest that the 5-HT neuronal system may be involved in the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory. 

*Keywords:* $\Delta^9$-Tetrahydrocannabinol; 5-HT (5-hydroxytryptamine, serotonin); Hippocampus; Spatial memory; DOI (1-(2,5-dimethoxy 4-iodophenyl)-2-amino propane)

## 1. Introduction

$\Delta^9$-Tetrahydrocannabinol, the principal psychoactive component of marihuana, is known to impair immediate memory, short-term memory and spatial cognition in humans (Tinklenberg et al., 1970; Dornbuch et al., 1971; Miller et al., 1977; Miller and Branconnier, 1983). $\Delta^9$-Tetrahydrocannabinol has also been reported to impair performance in the radial maze in rats (Nakamura et al., 1991; Lichtman et al., 1995). In addition, the impairment of spatial memory was attenuated by the cannabinoid $CB_1$ receptor antagonist SR141716A [*N*-(piperidine-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride] (Lichtman and Martin, 1996). Similarly, synthetic cannabinoid receptor agonists impaired performance in the radial maze task (Lichtman et al., 1995). Moreover, SR141716A alone enhanced spatial memory in the delayed task in the radial maze (Lichtman, 2000). In a recent study, it was demonstrated that $\Delta^9$-tetrahydrocannabinol selectively impairs working memory, and these memory deficits are more sensitive than other pharmacological effects of $\Delta^9$-tetrahydrocannabinol (Varvel et al., 2001). This study reported that $\Delta^9$-tetrahydrocannabinol impaired working memory (3 mg/kg) at lower doses than those required to elicit hypomotility (30 mg/kg), antinociception (10 mg/kg), catalepsy (10 mg/kg) and hypothermia (30 mg/kg). We previously reported that $\Delta^9$-tetrahydrocannabinol (6 mg/kg, i.p.) impaired spatial memory in a standard task of the eight-arm radial maze, and that this impairment was reversed by SR141716A (Mishima et al., 2001). Moreover, $\Delta^9$-tetrahydrocannabinol (4 mg/kg) selectively impaired working memory in a reference and working memory task of the eight-arm radial maze in which the food was baited at four of eight arms (Mishima et al., 2001). We

* Corresponding author. Tel.: +81-92-871-6631x6634; fax: +81-92-863-0389.
*E-mail address:* mfuji@fukuoka-u.ac.jp (M. Fujiwara).

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also reported that $\Delta^9$-tetrahydrocannabinol can be used as a pharmacological tool to evaluate the effects of cognitive enhancers on deficits of learning and memory in rats (Iwasaki et al., 1992). Thus, cannabinoid $CB_1$ receptors appear to play an important role in learning and memory, especially working memory.

Serotonin (5-hydroxytryptamine, 5-HT) has been linked to learning and memory in rats (Lalonde and Vikis-Freibergs, 1985). It has been reported that acute treatment with $\Delta^9$-tetrahydrocannabinol reduces 5-HT turnover in areas related to memory such as the hippocampus (Molina-Holgado et al., 1993). The selective 5-HT re-uptake inhibitor fluoxetine has been shown to modify the hypothermia induced by $\Delta^9$-tetrahydrocannabinol (Malone and Taylor, 1998). Nakazi et al. (2000) also reported that synthetic cannabinoid receptor agonists inhibited both the electrically and $Ca^{2+}$-induced release of 5-HT in mouse brain cortex slices via presynaptic cannabinoid $CB_1$ receptors. In a recent study, it was reported that SR141716A produced head twitching in mice, and the severity of this behaviour was attenuated by $\Delta^9$-tetrahydrocannabinol or a 5-$HT_{2A}$ receptor antagonist (Darmani and Pandya, 2000). Moreover, $\Delta^9$-tetrahydrocannabinol and synthetic cannabinoid receptor agonists have been reported to reduce the extent of head twitching in mice induced by the 5-$HT_2$ receptor agonist DOI [1-(2,5-dimethoxy 4-iodophenyl)-2-amino propane] (Darmani, 2001). These findings suggest that there is an interaction between cannabinoid $CB_1$ receptors and the 5-HT neuronal system. However, whether the 5-HT neuronal system is involved in the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory remains unknown. Therefore, the purpose of the present study was: (1) to investigate whether $\Delta^9$-tetrahydrocannabinol (6 mg/kg, i.p.), which impairs spatial memory, produces changes in 5-HT content in the frontal and frontoparietal cortex, the dorsal and ventral hippocampus and the dorsal and median raphe nucleus, (2) to examine the change in 5-HT release on treatment with $\Delta^9$-tetrahydrocannabinol (6 mg/kg, i.p.) using in vivo microdialysis and (3) to determine whether 5-HT related drugs can modify the impairment of spatial memory induced by $\Delta^9$-tetrahydrocannabinol (6 mg/kg, i.p.) in the standard task of the eight-arm radial maze. The following drugs were used: the 5-HT precursor 5-hydroxy-L-tryptophan (5-HTP), the 5-HT re-uptake inhibitor clomipramine, the 5-HT receptor agonist 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT) and the 5-$HT_2$ receptor agonist DOI.

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats, aged 7 weeks and weighing 200–250 g, were obtained from Kyudo (Saga, Japan), and were housed in groups of four to five per cage in a room with the temperature controlled at $23 \pm 2$ °C, a relative humidity of $60 \pm 10\%$ and the lights on from 7:00 to 19:00. The animals were restricted in their food intake (10–12 g every day, CE-2; Clea Japan, Tokyo, Japan) and were kept at approximately 80% of the body weight they had under free-feeding conditions during the experimental period. All animals had free access to water in their home cages. All procedures regarding animal care and use were carried out based on the regulations established by the Experimental Animal Care and Use Committee at Fukuoka University, at the Facilities for Experimental Animals.

### 2.2. Apparatus

Behavioural testing was conducted as previously reported (Iwasaki et al., 1996), using an eight-arm radial maze (Neuroscience, Tokyo, Japan), which was a modification of the maze originally developed by Olton and Samuelson (1976). The maze was elevated 50 cm from the floor. It consisted of a central platform 24 cm in diameter, with eight arms extending radially. Each arm was 50 cm long, 10 cm wide and 50 cm high with transparent plastic sidewalls. Food cups for the reinforcers were placed near the end of each arm. The maze was located in a room containing many extra-maze visual cues. For the behavioural analysis, an image motion analyser, AXIS-30 (Neuroscience), was used to quantify the task performance of rats in the eight-arm radial maze. This high-speed analyser has an automatic tracking system that allows the movement of each rat to be tracked in the maze with a CCD camera equipped with a personal computer, which then analyses the movement in real time and assesses the length of the route, frequency of arm visits, velocity of walking and time required to accomplish the task.

### 2.3. Preparation of animals for the eight-arm radial maze

A group of animals was trained so that they would become habituated to the apparatus and food pellets for 3 days before each test. A 10-min period of habituation was repeated three times a day, at intervals of more than 1 h. In each training session, the animal was placed within a circular plastic wall on the platform in the middle of the eight-arm radial maze. After 1 min, the wall was lifted and the animal was allowed to move freely in the maze. The trial continued until the animal had either entered all eight arms or 10 min had elapsed. Animals that proceeded through the maze using nonspatial strategies, that is, repeatedly choosing the arm adjacent to (45°) or three arms away from (135°) the one currently visited, were excluded from the present experiment because they were thought not to have acquired spatial memory. The performance of the animal in each trial was assessed using three parameters: the number of correct choices in the initial eight chosen arms, the number of errors, which was defined as choosing arms which had already been visited, and the time elapsed before the animal ate all eight pellets. If the animals made seven or

eight correct choices and less than one error in three successive sessions, they were then used for drug evaluation the next day.

### 2.4. Drug testing

If a test animal met the above criterion, it was administered $\Delta^9$-tetrahydrocannabinol i.p. 60 min prior to the test. In line with our previous report (Iwasaki et al., 1992), $\Delta^9$-tetrahydrocannabinol (6 mg/kg, i.p.)-treated rats made less than six correct choices and more than three errors. That is, they showed impairment of spatial memory. After 3 days, all rats were retrained until they met the above criterion. If the rats met the criterion, they were injected with $\Delta^9$-tetrahydrocannabinol and 5-HT-related drugs the next day. The following drugs were administered according to each injection time: $\Delta^9$-tetrahydrocannabinol and 5-MeODMT were administered i.p. 60 min before the test, and 5-HTP, clomipramine and DOI were administered i.p. 30 min before the test, respectively.

$\Delta^9$-Tetrahydrocannabinol was isolated from cannabis by Professor Y. Shoyama (Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Kyushu University) and emulsified in a 1% Tween 80 solution. 5-HTP (Sigma, USA), clomipramine HCl (Sigma) and DOI (Research Biochemicals, Natick, MA) were dissolved in saline. 5-MeODMT (Sigma) was suspended in 0.5% CMC-Na.

### 2.5. Determination of brain 5-HT content

After performance in the eight-arm maze was monitored, the rats of the vehicle treated and the impaired group were anaesthetized with ether and decapitated, and the brain was immediately placed in a cryostat. The brain was cut into 1-mm coronal cryosections and punched out on an ice-cooled glass stage. Brain tissue was sampled from six coronal sections, the frontal and frontoparietal cortex, the dorsal and ventral hippocampus and the dorsal and median raphe nucleus, at +2.7, +1.2, −3.3, −4.8, −7.8 and −7.8 mm anterior from the bregma, respectively, according to the atlas of Paxinos and Watson (1998). The regional samples were homogenized in 200 μl of 0.5 M perchloric acid (all reagents for high-performance liquid chromatography–electrochemical detector (HPLC–ECD) studies were purchased from Sigma). Following centrifugation, the supernatant was injected into an HPLC–ECD system. The system (Waters Assoc., Milford, MA) used a Shodex OD Spak column (Showa Denko, Tokyo, Japan) set at a potential of +0.75 V versus the reference electrode. The HPLC mobile phase was 0.1 M citrate–phosphate buffer containing 1.5 mM sodium octyl sulphate (PIC-$B_8$) and 11% methanol with 20 μM $Na_2$EDTA (JJP-14). The flow rate was maintained at 0.9 ml/min. Brain 5-HT content was quantified by calculating the area under the curve using an integrator (Waters Model 730, Waters Assoc.) and then determined based on standard curves.

### 2.6. Procedure of brain microdialysis for 5-HT release with a HPLC precolumn fluorescence system

Brain microdialysis was performed as previously described (Iwasaki et al., 1996). Briefly, only the animals that completed the eight-arm radial maze task were stereotaxically implanted with a guide cannula (AG-8; EICOM, Kyoto, Japan) under pentobarbital anaesthesia (40 mg/kg, i.p.; Tokyo Kasei, Tokyo, Japan). The guide cannula was placed in the ventral hippocampus (*A*: −4.8 mm, *L*: 5.0 mm, *V*: 6.0 mm from the bregma) according to the atlas of Paxinos and Watson (1998) and held in place by a stainless-steel screw and dental cement. A microdialysis probe (A-I-8-03; 3.0 mm dialysis membrane, EICOM) was inserted into the guide cannula of rats housed in plastic cages (30 × 30 × 35 cm, 3–7 days following surgery), and the brain was perfused with Ringer's solution at a flow rate of 2.0 μl/min by means of a syringe pump (CMA/100; Carnegie Medicine, Stockholm, Sweden). Samples (10 μl) were collected at 5-min intervals over a 300-min period. To achieve stable baseline readings, the microdialysis was allowed to proceed for 30 min before the collection of fractions. The 5-HT concentrations of the samples were then measured using the HPLC precolumn fluorescence system as we reported previously (Ishida et al., 1998). Briefly, chromatography was performed with an EP-300 HPLC system (EICOM) and an L-7480 fluorescence spectromonitor (2 μl flow-cell, Hitachi, Tokyo, Japan). The latter was operated at an excitation wavelength of 345 nm and an emission wavelength of 481 nm. The column was of TSK gel ODS-80 TM (100 × 1.0 mm I.D.; particle size 5 μm; Tosoh, Japan). The separation of the benzylamine derivative of 5-HT was achieved by using a mixture of acetonitrile and 40 mM phosphate buffer (pH = 7.5) (53:47, v/v) containing 1 mM disodium EDTA. The flow rate was 50 μl/min. The column temperature was ambient (20–23 °C).

### 2.7. Histology

After completion of the microdialysis experiment, the animals were anaesthetized with ether and decapitated. The brain was removed, frozen and cut into 40-μm slices. The position of the guide cannula in the ventral hippocampus site was confirmed by microscopic examination. Only data from animals in which the implantation was made at the desired site were analysed.

### 2.8. Statistical analyses

Data for the eight-arm radial maze task were evaluated for statistical significance using the nonparametric analysis of variance (Kruskal–Wallis test) followed by the nonparametric Bonferroni test (Dunn's test). 5-HT content was analysed by Student's *t*-test. 5-HT release was analysed by a one-way analysis of variance (ANOVA) and Student's

*t*-test. The level of statistical significance was set at $P < 0.05$. The results are expressed as the means ± S.E.M.

## 3. Results

### 3.1. Changes in brain 5-HT content in $\Delta^9$-tetrahydrocannabinol-treated rats

The 5-HT content of the ventral hippocampus was significantly increased ($P < 0.001$ by Student's *t*-test, Table 1) in $\Delta^9$-tetrahydrocannabinol (6 mg/kg)-treated rats compared to 1% Tween 80-treated rats. On the other hand, $\Delta^9$-tetrahydrocannabinol did not affect the 5-HT content of other brain areas.

### 3.2. Effect of $\Delta^9$-tetrahydrocannabinol on 5-HT release in the ventral hippocampus

As shown in Fig. 1, the administration of $\Delta^9$-tetrahydrocannabinol at the dose of 6 mg/kg markedly decreased the extracellular 5-HT concentration in the ventral hippocampus ($F(10,33) = 6.477$, $P < 0.001$, ANOVA). The inhibition was maximal at 120 min after treatment (41.7 ± 8.4% of prefraction, $P < 0.001$, Student's *t*-test), and no longer significant at 300 min.

### 3.3. Effects of 5-HTP and clomipramine on $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory

As shown in Fig. 2A, the 5-HT precursor 5-HTP dose dependently improved the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory (correct choices: $H(3) = 9.695$, $P < 0.05$ and errors: $H(3) = 14.364$, $P < 0.01$ by the Kruskal–Wallis test). At a dose of 50 mg/kg, the number of correct choices significantly increased while that of errors decreased (correct choices, $P < 0.05$ and errors, $P < 0.01$ by Dunn's test). $\Delta^9$-Tetrahydrocannabinol significantly increased the running time in the eight-arm radial maze (running time, vehicle: 52.6 ± 7.7 s; $\Delta^9$-tetrahydrocannabinol: 355.2 ± 57.6 s, $P < 0.01$ by Dunn's test). 5-HTP at a dose of 50 mg/kg (running time: 145.4 ± 22.3 s) significantly decreased the increase in running time induced by $\Delta^9$-tetrahydrocannabinol ($P < 0.01$ by Dunn's test).

Table 1
Changes in 5-HT content in the brains of $\Delta^9$-tetrahydrocannabinol-treated rats

| Brain region | Group | 5-HT (ng/mg) |
|---|---|---|
| Frontal cortex | Tween | 0.38 ± 0.03 |
| | $\Delta^9$-tetrahydrocannabinol | 0.34 ± 0.03 |
| Frontoparietal cortex | Tween | 0.18 ± 0.02 |
| | $\Delta^9$-tetrahydrocannabinol | 0.19 ± 0.02 |
| Dorsal hippocampus | Tween | 0.14 ± 0.02 |
| | $\Delta^9$-tetrahydrocannabinol | 0.15 ± 0.01 |
| Ventral hippocampus | Tween | 0.30 ± 0.03 |
| | $\Delta^9$-tetrahydrocannabinol | 0.43 ± 0.02[a] |
| Dorsal raphe nucleus | Tween | 0.29 ± 0.08 |
| | $\Delta^9$-tetrahydrocannabinol | 0.34 ± 0.04 |
| Median raphe nucleus | Tween | 0.68 ± 0.22 |
| | $\Delta^9$-tetrahydrocannabinol | 0.89 ± 0.13 |

The data are expressed as the means ± S.E.M., $n = 17$ for each group. Effect of $\Delta^9$-tetrahydrocannabinol on 5-HT content in the frontal and frontoparietal cortex, the dorsal and ventral hippocampus and the dorsal and median raphe nucleus, respectively.

[a] $P < 0.001$ compared with the Tween-treated group (by Student's *t*-test).

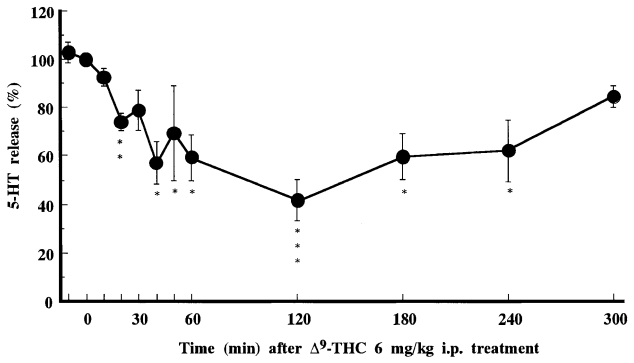


Fig. 1. Effect of $\Delta^9$-tetrahydrocannabinol on 5-HT release in the ventral hippocampus as assessed by in vivo microdialysis. $\Delta^9$-Tetrahydrocannabinol (6 mg/kg, i.p.) was administered immediately after sampling the prefraction. The data are expressed as percentages (mean ± S.E.M.: $n = 4$) of the baseline concentration. The basal value of the extracellular 5-HT concentration prior to drug administration was 0.15 ± 0.0073 fmol/μl. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the prefraction (determined by ANOVA, followed by Student's *t*-test).

The 5-HT re-uptake inhibitor clomipramine also improved the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory (correct choices: $H(4) = 17.397$, $P < 0.01$ and errors: $H(4) = 16.056$, $P < 0.01$ by the Kruskal–Wallis test). At the dose of 0.01 and 0.1 mg/kg, the number of correct choices significantly increased while that of errors decreased (0.01 mg/kg: correct choices and errors, $P < 0.05$; 0.1 mg/kg: correct choices and errors, $P < 0.01$ by Dunn's test, Fig. 2B). However, clomipramine at the same doses (running time, $\Delta^9$-tetrahydrocannabinol alone: 325.6 ± 53.8 s; 0.01 mg/kg: 241.3 ± 81.0 s; 0.1 mg/kg: 176.9 ± 62.0 s) had no effect on the increase in running time induced by $\Delta^9$-tetrahydrocannabinol. A higher dose of 1 mg/kg had no effect on the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory. In addition, the same dose of 5-HTP or clomipramine alone did not affect spatial memory, but 5-HTP at the higher dose of 100 mg/kg impaired spatial memory in the eight-arm radial maze (data not shown).

### 3.4. Effects of 5-MeODMT and DOI on $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory

As shown in Fig. 3A, the 5-HT receptor agonist 5-MeODMT improved the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory (correct choices: $H(5) = 15.427$, $P < 0.01$ and errors: $H(5) = 18.823$, $P < 0.01$ by

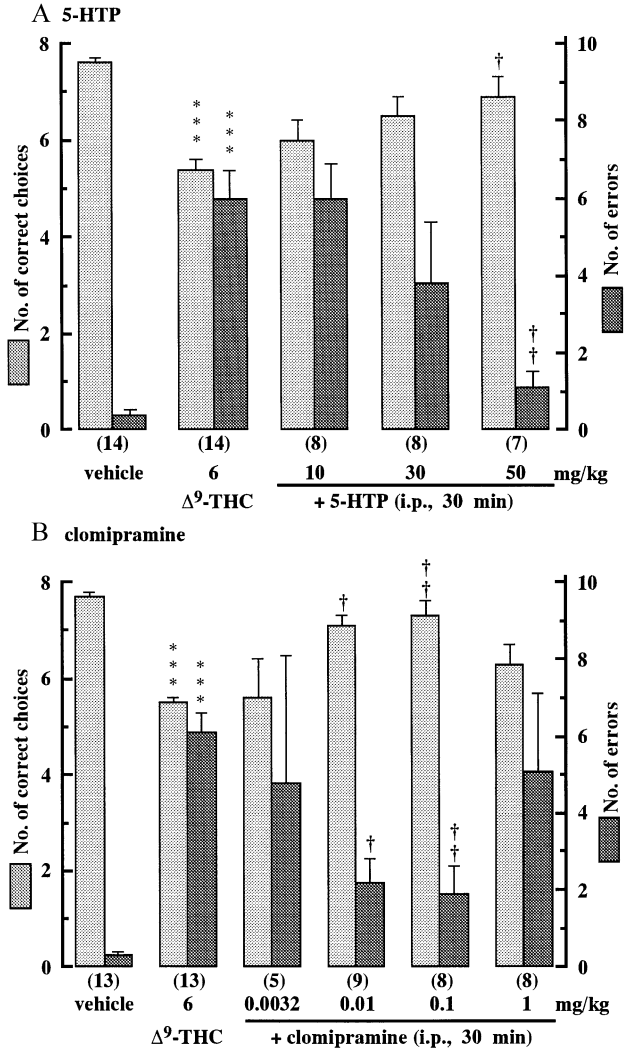


Fig. 2. Effects of 5-HTP and clomipramine on $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory in the eight-arm radial maze. $\Delta^9$-Tetrahydrocannabinol, 5-HTP and clomipramine were administered i.p. 60, 30 and 30 min prior to the test, respectively. *** $P<0.001$ compared with vehicle, † $P<0.05$, †† $P<0.01$ compared with $\Delta^9$-tetrahydrocannabinol (determined by the Kruskal–Wallis test followed by Dunn's test). The number of rats is given at the bottom of each column.

the Kruskal–Wallis test). At the dose of 0.01 and 0.03 mg/kg, the number of correct choices significantly increased while that of errors decreased (0.01 mg/kg: correct choices and errors, $P<0.01$; 0.03 mg/kg: correct choices and errors, $P<0.05$ by Dunn's test, Fig. 3A). However, 5-MeODMT at the same doses (running time, $\Delta^9$-tetrahydrocannabinol alone: $242.8 \pm 34.9$ s; 0.01 mg/kg: $172.6 \pm 66.5$ s; 0.03 mg/kg: $170.1 \pm 63.5$ s) had no effect on the increase in running time induced by $\Delta^9$-tetrahydrocannabinol. A higher dose of 0.1 mg/kg had no effect on the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory.

The 5-$HT_2$ receptor agonist DOI also improved the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory (correct choices: $H(4)=19.487$, $P<0.001$ and errors: $H(4)=15.884$, $P<0.01$ by the Kruskal–Wallis test). At very low doses of 0.3, 1 and 10 μg/kg, the number of correct choices significantly increased while that of errors decreased (correct choices: 0.3 μg/kg, $P<0.05$, 1 and 10 μg/kg, $P<0.01$; errors: 10 μg/kg, $P<0.05$ by Dunn's test, Fig. 3B). However, DOI at the same doses (running time, $\Delta^9$-tetrahydrocannabinol alone: $288.4 \pm 46.4$ s; 0.3 μg/kg: $167.3 \pm 57.9$ s; 1 μg/kg: $158.6 \pm 18.7$ s; 10 μg/kg: $158.6 \pm 44.2$ s) had no effect on the increase in running time induced by $\Delta^9$-tetrahydrocannabinol. In addition, the same dose of 5-MeODMT or DOI alone did not affect spatial memory, but DOI at the higher dose of 3 mg/kg impaired spatial memory in the eight-arm radial maze (data not shown).

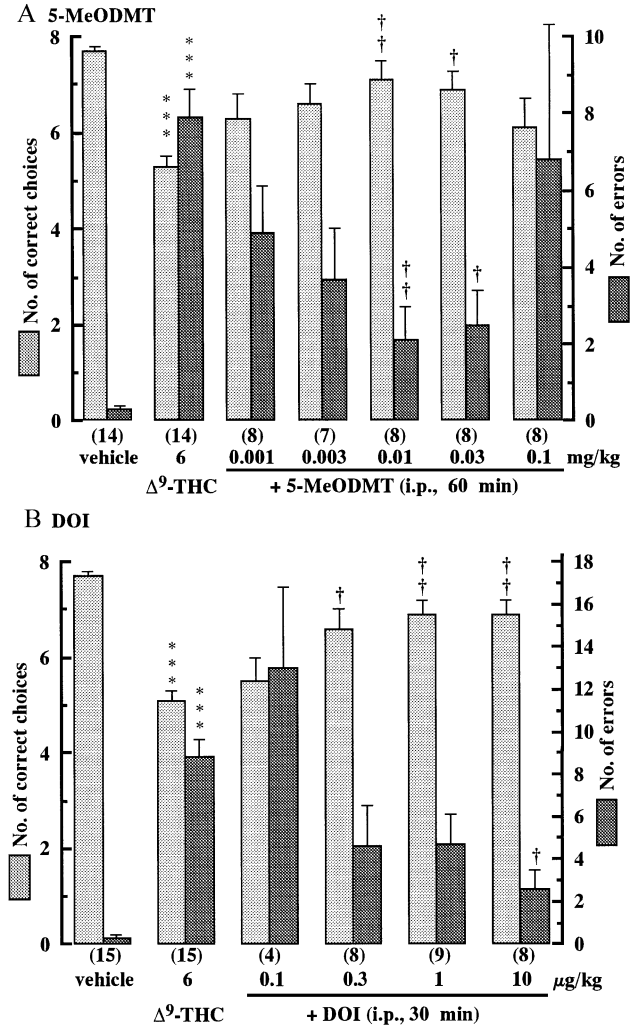


Fig. 3. Effects of 5-MeODMT and DOI on $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory in the eight-arm radial maze. $\Delta^9$-Tetrahydrocannabinol, 5-MeODMT and DOI were administered i.p. 60, 60 and 30 min prior to the test, respectively. *** $P<0.001$ compared with vehicle, † $P<0.05$, †† $P<0.01$ compared with $\Delta^9$-tetrahydrocannabinol (determined by the Kruskal–Wallis test followed by Dunn's test). The number of rats is given at the bottom of each column.

## 4. Discussion

In the present study, the 5-HT content significantly increased in the ventral hippocampus in $\Delta^9$-tetrahydrocannabinol (6 mg/kg)-treated rats. This finding suggests an involvement of the 5-HT neuronal system in the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory. No changes were seen in the other regions. Thus, the serotonergic nerve in the ventral hippocampus was more sensitive to $\Delta^9$-tetrahydrocannabinol. Several reports have provided evidence for higher levels of serotonergic markers, such as 5-HT content (Lombardi et al., 1987) and release (McQuade and Sharp, 1997) in the ventral hippocampus. Furthermore, a higher neuronal activity of the ventral hippocampus has also been reported (Jackisch et al., 1995). Importantly, evidence obtained previously shows that the release of acetylcholine in the ventral hippocampus closely parallels the induction of scopolamine (muscarinic receptor antagonist)-induced impairment of spatial memory in the eight-arm radial maze (Mishima et al., 2000). Moreover, we already confirmed that $\Delta^9$-tetrahydrocannabinol impaired spatial memory when injected into the ventral hippocampus (paper in preparation). These facts suggest that the ventral hippocampus plays an important role behaviourally and biochemically as a site of action in spatial memory in the eight-arm radial maze. Taken in the light of the existence of a high density of cannabinoid $CB_1$ receptors in the hippocampal formation (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992; Matsuda et al., 1993; Moldrich and Wenger, 2000), these findings indicate that the ventral hippocampus may be a critical site for the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory.

We next focused on 5-HT release in the ventral hippocampus, where changes in 5-HT content were observed during the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory. It is difficult to measure 5-HT, especially a decrease in 5-HT after treatment. For this reason, we developed a method for measuring the concentration of 5-HT, using microbore-liquid chromatography coupled to precolumn fluorescence derivatization with benzylamine (Ishida et al., 1998). This method allows for a highly sensitive, selective and quick determination of 5-HT. We found that $\Delta^9$-tetrahydrocannabinol (6 mg/kg, i.p.), which impairs spatial memory, decreased 5-HT release in the ventral hippocampus. It is recognized that there are two pathways for the 5-HT system in the ventral hippocampus, both receiving projections from the dorsal and median raphe nucleus (Azmitia and Segal, 1978). We also found that $\Delta^9$-tetrahydrocannabinol did not affect the 5-HT content in the dorsal and median raphe nucleus. Moreover, we already confirmed that the microinjection of $\Delta^9$-tetrahydrocannabinol into the ventral hippocampus impaired spatial memory, whereas microinjection of $\Delta^9$-tetrahydrocannabinol into the dorsal or median raphe nucleus had no effect on spatial memory (paper in preparation). Therefore, the increase in 5-HT content in the ventral hippocampus in $\Delta^9$-tetrahydrocannabinol (6 mg/kg)-treated rats is thought to result from inhibition of 5-HT release in this area. This finding suggests that the inhibition of 5-HT neurotransmission in the ventral hippocampus may be involved in the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory.

In recent studies, $\Delta^9$-tetrahydrocannabinol at low doses (75 and 150 μg/kg, i.v.), which induces behavioural stimulation, has been reported to increase the release of acetylcholine in the prefrontal cortex and hippocampus through cannabinoid $CB_1$ receptors (Acquas et al., 2001). In contrast, $\Delta^9$-tetrahydrocannabinol at higher doses (2.5–7.5 mg/kg, i.p.), which impairs memory, has been reported to decrease the release of acetylcholine in the medial–prefrontal cortex and hippocampus through cannabinoid $CB_1$ receptors (Carta et al., 1998; Gessa et al., 1998; Nava et al., 2000). Moreover, cannabinoids inhibit the release of noradrenaline (Schlicker et al., 1997) and glutamate (Shen et al., 1996) from the hippocampus. Nakazi et al. (2000) reported that synthetic cannabinoid receptor agonists inhibited both the electrically and $Ca^{2+}$-induced release of 5-HT in mouse brain cortex slices via presynaptic cannabinoid $CB_1$ receptors. We found that $\Delta^9$-tetrahydrocannabinol (6 mg/kg, i.p.), which impairs spatial memory, markedly decreased 5-HT release in the ventral hippocampus. The existence of cannabinoid receptors on presynaptic nerve endings has been suggested on noradrenergic neurons, because cannabinoid $CB_1$ receptor mRNA has been found to be present in sympathetic ganglia (Ishac et al., 1996). Hence, the possibility exists that cannabinoid $CB_1$ receptors are located presynaptically on other types of neurons such as serotonergic neurons. The release of 5-HT in the rat ventral hippocampus is reported to be mediated by N-type $Ca^{2+}$ channels (Sharp et al., 1990), and activation of cannabinoid $CB_1$ receptors inhibits N- and P/Q-type $Ca^{2+}$ channels in cultured hippocampal neurons (Twitchell et al., 1997; Shen and Thayer, 1998). These channels are known to be required for the release of transmitter from hippocampal synapses (Takahashi and Momiyama, 1993; Wheeler et al., 1994). Moreover, cannabinoids have been shown to inhibit adenylate cyclase activity (Howlett and Fleming, 1984; Barg et al., 1995) and enhance voltage-sensitive $K^+$ channels (Deadwyler et al., 1995). These cellular effects would be expected to inhibit neurotransmitter release. Therefore, it is possible that presynaptic cannabinoid $CB_1$ receptor activation inhibits the release of 5-HT. However, immunocytochemical and electrophysiological studies revealed that in the hippocampus, cannabinoid $CB_1$ receptors are expressed on axon terminals of γ-aminobutyric acid-mediated (GABAergic) inhibitory interneurons (Tsou et al., 1999; Katona et al., 1999) and that activation of these receptors decreases GABA release (Hajos et al., 2000). Hence, the possibility exists that $\Delta^9$-tetrahydrocannabinol inhibits 5-HT release, probably through GABAergic neurons but not through a direct action at serotonergic nerve endings. It has been reported that the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory is reversed by the cannabi-

noid $CB_1$ receptor antagonist SR141716A (Lichtman and Martin, 1996; Mishima et al., 2001). These facts would suggest that $\Delta^9$-tetrahydrocannabinol impairs spatial memory through a direct action at cannabinoid $CB_1$ receptors. Therefore, the present results indicate that there is a possible functional relationship between cannabinoid $CB_1$ receptors and 5-HT neurotransmission in the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory.

In the present study, we found that the 5-HT precursor 5-HTP and the 5-HT re-uptake inhibitor clomipramine significantly improved the $\Delta^9$-tetrahydrocannabinol (6 mg/kg)-induced impairment of spatial memory. Clomipramine has been shown to increase the extracellular concentration of 5-HT by inhibition of neuronal 5-HT uptake (Gross et al., 1987; Carboni and Di Chiara, 1989). The ameliorative effect of these drugs is thought to be due to activation of the 5-HT neuronal system via an increase in 5-HT levels in the 5-HT synaptic cleft, because $\Delta^9$-tetrahydrocannabinol decreased 5-HT release in the ventral hippocampus. We also found that the 5-HT receptor agonist 5-MeODMT significantly improved the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory. This ameliorative effect is thought to be due to activation of the 5-HT neuronal system via a stimulation of postsynaptic 5-HT receptors. Our findings thus indicate that suppression of the 5-HT neuronal system is involved in the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory. Moreover, we found that the $5\text{-HT}_2$ receptor agonist DOI also significantly improved the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory at a very low dose. This ameliorative effect of the drug is thought to be due to activation of the 5-HT neuronal system via a stimulation of postsynaptic $5\text{-HT}_2$ receptors. In a recent study, it was reported that $\Delta^9$-tetrahydrocannabinol and synthetic cannabinoid receptor agonists reduced the severity of DOI-induced head-twitch behaviour (Darmani, 2001), which is thought to be mediated by postsynaptic $5\text{-HT}_2$ receptors (Darmani et al., 1990). Conversely, SR141716A evoked head twitching in mice, the extent of which was attenuated by $\Delta^9$-tetrahydrocannabinol or a $5\text{-HT}_{2A}$ receptor antagonist (Darmani and Pandya, 2000). Moreover, a high concentration of arachidonylethanolamide (anandamide), an endogenous cannabinoid receptor ligand, has been reported to decrease the density of $5\text{-HT}_2$ receptors (Kimura et al., 1998). Though the mechanism of the inhibition of $5\text{-HT}_2$ receptors induced by $\Delta^9$-tetrahydrocannabinol is still unclear, it is possible that there is an interaction between cannabinoid $CB_1$ receptors and $5\text{-HT}_2$ receptors, and that suppression of postsynaptic $5\text{-HT}_2$ receptors may be involved in the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory.

$\Delta^9$-Tetrahydrocannabinol (6 mg/kg, i.p.) significantly increased the running time in the eight-arm radial maze, suggesting that this suppressive effect on the locomotor activity may affect the performance of the eight-arm radial maze. However, we already reported that none of the $\Delta^9$-tetrahydrocannabinol (6 mg/kg)-treated rats showed any other abnormal behaviour, including pivoting and walking backwards, observed at a dose of 10 mg/kg (Mishima et al., 2001). These rats moved to the same arm slowly and repeatedly after obtaining a food pellet and $\Delta^9$-tetrahydrocannabinol (6 mg/kg) selectively impaired working memory in a reference and working memory task of the eight-arm radial maze. Moreover, we found that serotonergic agonists at effective doses did not always decrease the increase in running time induced by $\Delta^9$-tetrahydrocannabinol in this study. In addition, $\Delta^9$-tetrahydrocannabinol has been reported to impair memory at lower doses than those that produce locomotor suppression (Nakamura et al., 1991; Lichtman and Martin, 1996; Varvel et al., 2001). It is not likely that $\Delta^9$-tetrahydrocannabinol impairs spatial memory by this suppressive effect on locomotor activity.

In the present study, it was observed that the higher dose of clomipramine or 5-MeODMT did not improve the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory. The anticholinergic effect of clomipramine on the central nervous system is well known (Lindbom et al., 1982). Therefore, the reduction of the ameliorative effect at a higher dose may partly result from direct antagonism of the muscarinic receptor. Clomipramine (1, 3 and 10 mg/kg, s.c.) has also been reported to produce a moderate inhibition of 5-HT release in the ventral hippocampus (Auerbach et al., 1995). Therefore, the reduction of the ameliorative effect at the higher dose may be due to inhibition of 5-HT release via an inhibitory effect on 5-HT neuronal firing. A high dose of 5-HT re-uptake inhibitors or 5-HT receptor agonists impairs memory in rats (Lalonde and Vikis-Freibergs, 1985; Winter and Petti, 1987; Cole et al., 1994), whereas a low dose improves memory task performance in normal and scopolamine-treated rats (Cole et al., 1994; Carli et al., 2000; Miura et al., 1993). We also confirmed that the higher dose of the 5-HT receptor agonist impaired spatial memory in the eight-arm radial maze in the present study. Therefore, the balance of the 5-HT neuronal system might play an important role in learning and memory in rats. A high dose of clomipramine or 5-MeODMT might cause an imbalance in the system. In addition, a high dose of 5-MeODMT induces various behavioural changes, such as the 5-HT behavioural syndrome (Tricklebank et al., 1985), and possibly contributes to the disappearance of the ameliorative effects.

In conclusion, the study presented here indicated the involvement of the 5-HT neuronal system in the $\Delta^9$-tetrahydrocannabinol-induced impairment of spatial memory in the eight-arm radial maze. Moreover, the results suggest that 5-HT-related drugs could be effective in the treatment of $\Delta^9$-tetrahydrocannabinol-induced memory deficits.

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