



SHORT COMMUNICATION

The Serotonin_{1A} Receptor Agonist 8-OHDPAT Reverses Δ^9 -Tetrahydrocannabinol-induced Impairment of Spatial Memory and Reduction of Acetylcholine Release in the Dorsal Hippocampus in Rats

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We studied the effects of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT), a 5-HT_{1A} receptor agonist, on both Δ^9 -tetrahydrocannabinol-induced spatial memory impairment in an 8-arm radial maze, and the reduction of acetylcholine release in the dorsal hippocampus as assessed by *in vivo* microdialysis in rats. A 6 mg/kg i.p. dose of Δ^9 -tetrahydrocannabinol impaired spatial memory in the 8-arm radial maze and decreased the acetylcholine release in the dorsal hippocampus. 8-OHDPAT, at very low doses of 0.1-0.3 μ g/kg, reversed both the impairment of spatial memory and the decrease in acetylcholine release induced by Δ^9 -tetrahydrocannabinol. These findings suggest that low doses of 8-OHDPAT may improve Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory by enhancing acetylcholine release in the dorsal hippocampus.

Keywords: Δ^9 -Tetrahydrocannabinol; Spatial memory; Acetylcholine; Serotonin; 5-HT_{1A} receptor; 8-OHDPAT; Hippocampus

It is reported that the serotonin_{1A} (5-HT_{1A}) receptor plays an important role in the mechanisms of learning and memory, and attention. Low doses of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT), a 5-HT_{1A}

receptor agonist, reversed the scopolamine-induced impairment of spatial learning in the water maze and of short term memory in delayed matching-to-position tasks in rats, whereas high doses of 8-OHDPAT impaired spatial learning in the water maze (Carli *et al.*, 1992; 1998; 2000). These findings suggest that 5-HT_{1A} receptors may have a biphasic action on learning and memory in rats. In addition, 5-HT_{1A} receptor agonists have been reported to significantly enhance acetylcholine release in cortex and hippocampus as assessed by *in vivo* microdialysis (Koyama *et al.*, 1999; Katsu, 2001).

Δ^9 -tetrahydrocannabinol has been recognized as the major psychoactive component of marijuana. Δ^9 -tetrahydrocannabinol and synthetic cannabinoid CB₁ receptor agonists have also been shown to impair short-term memory and attention in humans, other primates and rodents (Sullivan, 2000). We have also demonstrated that Δ^9 -tetrahydrocannabinol might produce selective impairment of working memory through cannabinoid CB₁ receptors of dorsal and ventral hippocampus in an 8-arm radial maze (Mishima *et al.*, 2001; Egashira *et al.*, 2002a). Δ^9 -tetrahydrocannabinol and other cannabinoids have been recently reported to reduce acetylcholine release *in vivo* from rat hippocampus (Nava *et al.*, 2000; Schlicker and Kathmann, 2001; Mishima *et al.*, 2002). These findings suggest that

cannabinoid-induced impairment of learning and memory might be related to a reduction in acetylcholine neurotransmission (Gessa *et al.*, 1998). In addition, Δ^9 -tetrahydrocannabinol decreases 5-HT release in the ventral hippocampus, and low doses of four different 5-HT related agonists attenuate the Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory, suggesting that the impairments play an important role in not only the cholinergic neuronal system but also the 5-HT neuronal system (Egashira *et al.*, 2002b). Moreover, it was recently reported that 5-HT_{1A} receptors were implicated in anxiety-related behavioral responses to a cannabinoid agonist (Marco *et al.*, 2004).

The purpose of the present study was to clarify whether 8-OHDPAT reverses the Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory in an 8-arm radial maze. Moreover, we examined the effect of 8-OHDPAT on the Δ^9 -tetrahydrocannabinol-induced decrease in acetylcholine release in the dorsal hippocampus using microdialysis.

Male Wistar rats (200–250 g, Kyudo Co. Ltd., Saga, Japan) were housed in groups of 4 to 5 per cage in a room at $23 \pm 2^\circ\text{C}$ with a 12 h light-dark cycle (light on at 7:00 a.m.). The animals were restricted in their food intake (10–12 g every day, CE-2; Clea Japan, Tokyo, Japan) and maintained at approximately 80% of the body weight they had under free-feeding conditions during the experimental period, but had free access to water. All procedures regarding animal care and use were carried out based on the regulations established by the Experimental Animal Care and Use Committee of Fukuoka University. Experiments in an 8-arm radial maze and microdialysis for acetylcholine release were performed as described previously (Mishima *et al.*, 2001; 2002). The maze (Neuroscience, Tokyo, Japan) consisted of a central platform 24 cm in diameter, with 8 arms extending radially. Each arm was 50 cm long, 10 cm wide with 50 cm high walls of transparent plastic. Food cups as the reinforcers were placed near the end of each arm, and each rat's movement in the maze was monitored with a CCD camera equipped with a personal computer, and was analyzed by measuring three parameters: the number of correct choices in the initial 8 chosen arms, the number of errors which was defined as choosing arms that had already been visited, and the time elapsed before the animal ate all 8 pellets. If the animals made 7 or 8 correct choices and less than one error in three successive sessions, Δ^9 -tetrahydrocannabinol (6 mg/kg, i.p., isolated by Professor Y. Shoyama, Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Kyushu University) or 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT,

0.03–0.3 $\mu\text{g}/\text{kg}$, i.p., RBI, Natick, MA) was injected 60 min or 15 min prior to the test, respectively.

For experiments on brain microdialysis, only the animals that fulfilled the criteria for the 8-arm radial maze were stereotaxically implanted with a guide cannula (AG-8; Eicom, Kyoto, Japan) under pentobarbital anesthesia (50 mg/kg, i.p.). The guide cannula was placed in the dorsal hippocampus (A: - 3.8, L: 2.0, V: 2.0 from bregma) according to the atlas of Paxinos and Watson. A microdialysis probe (Eicom) was perfused with Ringer's solution containing 0.1 mM eserine sulfate (Sigma, St Louis, MO, USA) at a flow rate of 2 $\mu\text{l}/\text{min}$ by means of a syringe pump (CMA/100; Carnegie Medicine, Stockholm, Sweden). The sample (20 μl) was collected by a refrigerated collector (CMA/140; Carnegie Medicine) at 20-minute intervals over 120-min period after Δ^9 -tetrahydrocannabinol administration (6 mg/kg, i.p.). The sampled acetylcholine concentrations were then measured by a high-performance liquid chromatography-electrochemical detector (HPLC-ECD) system (Waters Assoc., Milford, MA.), using an EicomPak AC column and enzyme column (EICOMPAK AC-GEL, Eicom).

Data from the 8-arm radial maze were evaluated for significant differences using the Kruskal-Wallis test followed by the Bonferroni's (Dunn's) test for non-parametric multiple comparisons. Acetylcholine release was analyzed by a two-way (with repeated measures) analysis of variance (ANOVA) and Tukey test. Values were considered significant at $P < 0.05$.

As shown in Fig. 1A, 8-OHDPAT at high doses of 1 and 3 mg/kg significantly impaired spatial memory (correct choices: $H(4)=15.058$, $P < 0.01$ and errors: $H(4)=21.902$, $P < 0.001$ by the Kruskal-Wallis test, 1 mg/kg: correct choices, $P < 0.05$ and errors, $P < 0.01$; 3 mg/kg: correct choices and errors, $P < 0.05$ by the Bonferroni's test). 8-OHDPAT significantly increased the running time in the 8-arm radial maze (running time, $H(4)=16.856$, $P < 0.01$ by the Kruskal-Wallis test, vehicle: 73.0 ± 13.1 sec; 8-OHDPAT, 1 mg/kg: 409.5 ± 72.1 sec, $P < 0.01$ by the Bonferroni's test). 8-OHDPAT at higher doses of 1 and 3 mg/kg produced abnormal behavior such as flat body posture, being a component of the 5-HT syndrome.

Fig. 1B shows the effects of 8-OHDPAT on Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory in the 8-arm radial maze. Δ^9 -tetrahydrocannabinol (6 mg/kg, i.p.) impaired spatial memory in the 8-arm radial maze. 8-OHDPAT at very low doses significantly reversed the decrease in the number of correct choices and the increase in errors induced by Δ^9 -tetrahydrocannabinol (correct choices: $H(3)=9.793$,

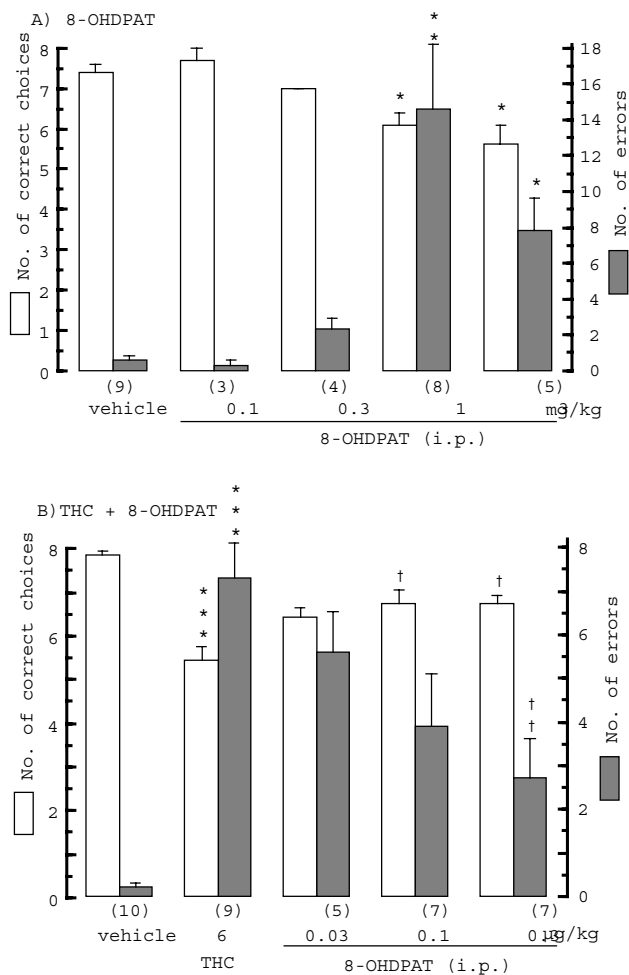


FIGURE 1 Effects of 8-OHDPAT on spatial memory, (A) on Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory (B) in the 8-arm radial maze. Δ^9 -tetrahydrocannabinol and 8-OHDPAT or their vehicle were injected i.p. 60 min or 15 min prior to the test, respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs vehicle, $^{\dagger}P < 0.05$, $^{\ddagger}P < 0.01$ vs Δ^9 -tetrahydrocannabinol (Kruskal-Wallis test followed by the Bonferroni's test). Number of rats is given at the bottom of each column.

$P < 0.05$ and errors: $H(3)=11.414$, $P < 0.01$ by the Kruskal-Wallis test). 8-OHDPAT at doses of 0.1 and 0.3 $\mu\text{g}/\text{kg}$ significantly improved the Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory (0.1 $\mu\text{g}/\text{kg}$: error, $P < 0.05$; 0.3 $\mu\text{g}/\text{kg}$: correct choices, $P < 0.01$ and errors, $P < 0.05$ by the Bonferroni's test). Δ^9 -Tetrahydrocannabinol significantly increased the running time in the 8-arm radial maze (running time, vehicle: 65.6 ± 6.6 sec; Δ^9 -tetrahydrocannabinol: 333.6 ± 47.0 sec, $P < 0.01$). 8-OHDPAT at a dose of 0.1 and 0.3 $\mu\text{g}/\text{kg}$ (running time, $H(4)=14.769$, $P < 0.01$ by the Kruskal-Wallis test, 0.1 $\mu\text{g}/\text{kg}$: 114.7 ± 22.3 sec, $P < 0.01$; 0.3 $\mu\text{g}/\text{kg}$: 121.0 ± 16.9 sec, $P < 0.05$ by the Bonferroni's test) significantly decreased the increase

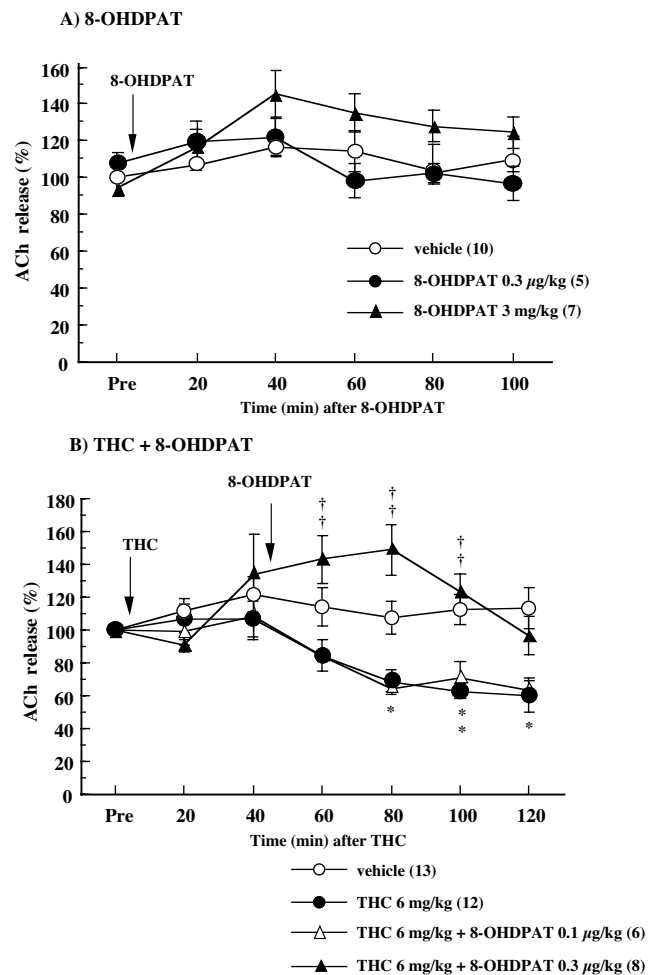


FIGURE 2 Effect of 8-OHDPAT on acetylcholine release in the dorsal hippocampus as assessed by *in vivo* microdialysis in (A) intact and (B) Δ^9 -tetrahydrocannabinol-treated rats. 8-OHDPAT at a dose of 3 mg/kg tended to increase acetylcholine release in the dorsal hippocampus during 40 to 80 min after the injection, but there were no significant differences between the groups. Δ^9 -tetrahydrocannabinol (6 mg/kg , i.p.) was injected immediately after sampling the pre-fraction and 8-OHDPAT (0.1-0.3 $\mu\text{g}/\text{kg}$) was then injected i.p. after sampling the 40-min fraction. There were no significant differences between vehicle and tetrahydrocannabinol plus 8-OHDPAT (0.3 $\mu\text{g}/\text{kg}$). The acetylcholine concentrations are expressed as a percent of the average pre-fraction. * $P < 0.05$, ** $P < 0.01$, vs vehicle, $^{\dagger}P < 0.01$ vs Δ^9 -tetrahydrocannabinol (two-way ANOVA test followed by Tukey test).

of running time induced by Δ^9 -tetrahydrocannabinol.

As shown in Fig. 2A, 8-OHDPAT alone at 0.3 $\mu\text{g}/\text{kg}$ did not change acetylcholine release and at 3 mg/kg tended to increase acetylcholine release in the dorsal hippocampus during 40 to 80 min after the injection.

8-OHDPAT at a dose of 0.3 $\mu\text{g}/\text{kg}$, which improved the impairment of spatial memory, reversed the decrease in acetylcholine release induced by Δ^9 -tetrahydrocannabinol in the dorsal hippocampus (a

group difference [$F(2,13) = 2.257, P=0.1441$], effect of time [$F(5,65) = 8.320, P<0.001$], a group X time interaction [$F(10,65) = 2.400, P<0.05$]. 8-OHDPAT significantly increased acetylcholine release in the dorsal hippocampus during 60 to 100 min after the Δ^9 -tetrahydrocannabinol injection in comparison with Δ^9 -tetrahydrocannabinol alone (60-100 min, $P < 0.01$, Tukey test, FIG. 2B).

The findings of this study show that Δ^9 -tetrahydrocannabinol impaired spatial memory in rats in the 8-arm radial maze, and reduced acetylcholine release in the dorsal hippocampus as assessed by microdialysis. These findings support our previous report that tetrahydroaminoacridine, an acetylcholinesterase inhibitor, reversed both Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory and the reduction of acetylcholine release in the dorsal hippocampus (Mishima *et al.*, 2002). It is reported that different cannabinoid agonists inhibit acetylcholine release in different brain regions *in vivo*, whereas the cannabinoid CB₁ receptor antagonist, SR141716A, enhances acetylcholine release in the hippocampus and medial-prefrontal cortex (Gessa *et al.*, 1998). Moreover, SR141716A reversed the decrease in acetylcholine release induced by Δ^9 -tetrahydrocannabinol (Gessa *et al.*, 1998). We also have demonstrated that SR141716A greatly improved Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory in the 8-arm radial maze (Mishima *et al.*, 2001). Thus, these findings suggest that Δ^9 -tetrahydrocannabinol may impair spatial memory via an inhibition of cholinergic transmission through cannabinoid receptors in the dorsal hippocampus.

Koyama *et al.* (1999), using a sensitive and specific radioimmunoassay without cholinesterase inhibitor in the perfusion fluid, reported that BAYx3702, a higher-affinity 5-HT_{1A} receptor agonist, significantly increased acetylcholine release; and the effects were inhibited by NAN-190 or WAY-100135, 5-HT_{1A} receptor antagonists. This suggests that acetylcholine release in the hippocampus is at least partly regulated via 5-HT_{1A} receptors on presynaptic cholinergic neurons. In the present study, using standard microdialysis technique with cholinesterase inhibitor in the perfusion fluid, 8-OHDPAT increased acetylcholine release but not significantly. Although the differences in the potentiation of acetylcholine release remain unclear, it may be related to the affinities of 5-HT_{1A} receptor agonists and the microdialysis method. In this study, 8-OHDPAT reversed the Δ^9 -tetrahydrocannabinol-induced reduction of acetylcholine release in the dorsal hippocampus. The cannabinoid CB₁ receptors are known to control acetylcholine release by being located on

cholinergic nerve terminals in the hippocampus and cortex, as shown in autoradiographic and immunohistochemical studies (Tsou *et al.*, 1997). These findings suggest that the ameliorative effects of 8-OHDPAT on the Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory may be mediated by the concomitant activation of the cannabinoid and 5-HT_{1A} receptors on cholinergic neuron terminals.

Δ^9 -tetrahydrocannabinol (6 mg/kg, i.p.) significantly increased the running time in the 8-arm radial maze, suggesting that this suppressive effect on the locomotor activity may affect the performance itself in the 8-arm radial maze. However, we already reported that none of the Δ^9 -tetrahydrocannabinol (6 mg/kg)-treated rats showed any other abnormal behaviour including pivoting and walking backward, 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. Δ^9 -tetrahydrocannabinol (6 mg/kg) selectively impaired working memory in a reference and working memory task of the 8-arm radial maze. Moreover, we found that serotonergic agonists at effective doses did not always decrease the increase of running time induced by Δ^9 -tetrahydrocannabinol (Egashira *et al.*, 2002). In addition, Δ^9 -tetrahydrocannabinol has been reported to impair memory at doses lower than those producing locomotor suppression (Nakamura *et al.*, 1991; Lichtman and Martin, 1996; Varvel *et al.*, 2001). It is not likely that Δ^9 -tetrahydrocannabinol impairs the spatial memory by this suppressive effect on locomotor activity.

Of particular interest is the finding that high doses of 8-OHDPAT impaired performance in the water maze (Carli and Samanin, 1992), whereas lower doses improved the impairment of spatial learning induced by scopolamine, a muscarinic receptor antagonist (Carli *et al.*, 1998; 2000). In support of this, we have also demonstrated that high doses (1, 3 mg/kg) of 8-OHDPAT impaired spatial memory while lower doses (0.1, 0.3 μ g/kg) improved Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory. However, the spatial memory impairment observed at high doses may not be specific, because higher doses caused profound changes in the animals' motor behavior, such as flat body posture which is a component of the 5-HT syndrome.

In summary, the findings here indicate that 8-OHDPAT improved Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory by enhancing acetylcholine transmission in the dorsal hippocampus, and that the 5-HT_{1A} receptor may be involved in Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory.

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References

- Carli M and R Samanin (1992) 8-Hydroxy-2-(di-n-propylamino)tetralin impairs spatial learning in a water maze: role of postsynaptic 5-HT_{1A} receptors. *Br. J. Pharmacology* **105**, 720-726.
- Carli M, P Bonalumi and R Samanin (1998) Stimulation of 5-HT_{1A} receptors in the dorsal raphe reverses the impairment of spatial learning caused by intrahippocampal scopolamine in rats. *Eur. J. Neuroscience* **10**, 221-230.
- Carli M, C Balducci and R Samanin (2000) Low doses of 8-OH-DPAT prevent the impairment of spatial learning caused by intrahippocampal scopolamine through 5-HT_{1A} receptors in the dorsal raphe. *Br. J. Pharmacology* **131**, 375-381.
- Egashira N, K Mishima, K Iwasaki and M Fujiwara (2002a) Intracerebral microinjections of Δ^9 -tetrahydrocannabinol: search for the impairment of spatial memory in the 8-arm radial maze in the rat. *Brain Res.* **952**, 239-245
- Egashira N, K Mishima, S Katsurabayashi, Y Matsumoto, T Yoshitake, J Ishida, M Yamaguchi, K Iwasaki and M Fujiwara (2002b) Involvement of 5-hydroxytryptamine in Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory in the 8-arm radial maze in the rat. *Eur. J. Pharmacology* **445**, 221-229
- Katsu H (2001) Selective 5-HT_{1A} receptor agonist, 8-OH-DPAT, locally administered into the dorsal raphe nucleus increased extracellular acetylcholine concentrations in the medial prefrontal cortex of conscious rats. *Jpn. J. Neuropsychopharmacology* **21**, 121-123
- Koyama T, Y Nakajima, T Fujii and K Kawashima (1999) Enhancement of cortical and hippocampal cholinergic neurotransmission through 5-HT₁ receptor-mediated pathways by BAY x 3702 in freely moving rats. *Neurosci. Lett.* **265**, 33-36.
- Lichtman AH and BR Martin (1996) Δ^9 -Tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. *Psychopharmacology* **126**, 125-131.
- Marco EM, L Perez-Alvarez, E Borcel, M Rubio, C Guaza, E Ambrosio, SE File and MP Viveros (2004) Involvement of 5-HT_{1A} receptors in behavioural effects of the cannabinoid receptor agonist CP 55,940 in male rats. *Behav. Pharmacol.* **15**, 21-27.
- Mishima K, N Egashira, N Hirosawa, M Fujii, T Egawa, Y Matsumoto, K Iwasaki and M Fujiwara (2001) Selective impairment of working memory Induced by Δ^9 -tetrahydrocannabinol in rats. *Jpn. J. Pharmacology* **87**, 297-308.
- Mishima K, N Egashira, Y Matsumoto, K Iwasaki and M Fujiwara (2002) Involvement of reduced acetylcholine release in Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory in the 8-arm radial maze. *Life Sci.* **72**, 397-407
- Nakamura EM, EA Da Silva, GV Concilio, DA Wilkinson and J Masur (1991) Reversible effects of acute and long-term administration of Δ^9 -tetrahydrocannabinol (THC) on memory in the rat. *Drug Alcohol Depend.* **28**, 167-175.
- Nava F, G Carta, AM Battasi and GL Gessa (2000) D₂ dopamine receptors enable Δ^9 -tetrahydrocannabinol induced memory impairment and reduction of hippocampal extracellular acetylcholine concentration. *Br. J. Pharmacology* **130**, 1201-1210.
- Schlicker E and M Kathmann (2001) Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol. Sci.* **22**, 565-572.
- Sullivan JM (2000) Cellular and molecular mechanisms underlying learning and memory impairments produced by cannabinoids. *Learn. Mem.* **7**, 132-139.
- Tsou K, S Brown, MC Sanudo-Pena, K Mackie and JM Walker (1997) Immunohistochemical distribution of cannabinoid CB₁ receptors in the rat central nervous system. *Neuroscience* **83**, 393-411.
- Varvel SA, RJ Hamm, BR Martin and AH Lichtman (2001) Differential effects of Δ^9 -THC on spatial reference and working memory in mice. *Psychopharmacology* **157**, 142-150.

