

## Differential effects of THC- or CBD-rich cannabis extracts on working memory in rats

Paola Fadda<sup>a,b</sup>, Lianne Robinson<sup>a</sup>, Walter Fratta<sup>b</sup>, Roger G. Pertwee<sup>a</sup>, Gernot Riedel<sup>a,\*</sup>

<sup>a</sup> Department of Biomedical Science, School of Medical Sciences, College of Life Sciences and Medicine, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, UK

<sup>b</sup> B.B. Brodie Department of Neuroscience and Center of Excellence “Neurobiology of Dependence”, University of Cagliari, Cittadella Universitaria Monserrato, Cagliari, Italy

Received 22 April 2004; received in revised form 29 June 2004; accepted 17 August 2004

### Abstract

Cannabinoid receptors in the brain (CB<sub>1</sub>) take part in modulation of learning, and are particularly important for working and short-term memory. Here, we employed a delayed-matching-to-place (DMTP) task in the open-field water maze and examined the effects of cannabis plant extracts rich in either  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), or rich in cannabidiol (CBD), on spatial working and short-term memory formation in rats.  $\Delta^9$ -THC-rich extracts impaired performance in the memory trial (trial 2) of the DMTP task in a dose-dependent but delay-independent manner. Deficits appeared at doses of 2 or 5 mg/kg (i.p.) at both 30 s and 4 h delays and were similar in severity compared with synthetic  $\Delta^9$ -THC. Despite considerable amounts of  $\Delta^9$ -THC present, CBD-rich extracts had no effect on spatial working/short-term memory, even at doses of up to 50 mg/kg. When given concomitantly, CBD-rich extracts did not reverse memory deficits of the additional  $\Delta^9$ -THC-rich extract. CBD-rich extracts also did not alter  $\Delta^9$ -THC-rich extract-induced catalepsy as revealed by the bar test. It appears that spatial working/short-term memory is not sensitive to CBD-rich extracts and that potentiation and antagonism of  $\Delta^9$ -THC-induced spatial memory deficits is dependent on the ratio between CBD and  $\Delta^9$ -THC.

© 2004 Elsevier Ltd. All rights reserved.

**Keywords:** Cannabis extract; THC; Cannabidiol; Working memory; Water maze; Rat

### 1. Introduction

Several studies provide compelling evidence that cannabis and its major psychoactive component  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) can cause impairments in immediate recall (Darley et al., 1974), working and short-term memory (Miller and Branconnier, 1983; Fletcher et al., 1996) and memory retrieval (Block and Ghoneim, 1993) in man. Similar deficits, especially in short-term memory, have been confirmed in animal studies following systemic administration of  $\Delta^9$ -THC or other synthetic cannabinoids as well as endogenous ligands such as anandamide. Working memory reflects the transient storage and processing of information and

is dependent on the online processing within neuronal circuits. Typically, working memory in animals is tested by means of delayed-non-matching to sample (DNMTS) tasks or in maze learning like the 8-arm radial maze or T-maze.  $\Delta^9$ -THC or WIN55,212-2 treatment led to delay-dependent performance deficits in DNMTS paradigms (Hampson and Deadwyler, 1998, 1999, 2000) and selective working memory impairments in radial maze and T-maze in rat and mice (Nakamura et al., 1991; Molina-Holgado et al., 1995; Lichtman and Martin, 1996; Jentsch et al., 1997; Nava et al., 2001). These tasks have in common that animals must adopt a win-shift strategy and performance falls to chance within several seconds or minutes. In contrast, working memory in the open-field water maze requires a win-stay strategy. It is also sensitive to  $\Delta^9$ -THC treatment in mice (Varvel et al., 2001; Da Silva and Takahashi, 2002).

\* Corresponding author. Tel.: +44-1224-555758; fax: +44-1224-555719.

E-mail address: [g.riedel@abdn.ac.uk](mailto:g.riedel@abdn.ac.uk) (G. Riedel).

Despite this similarity in memory disruption between humans and animals, direct comparisons are hampered due to the fact that humans generally smoke cannabis and this contains a considerable number of other, yet uncharacterized cannabinoid and terpenoid constituents. Administration of synthetic compounds in man is limited to  $\Delta^9$ -THC as in certain medications (e.g. Marinol). Overall, effects of smoked cannabis or synthetic  $\Delta^9$ -THC are rated similarly (Hart et al., 2002) and cause deficits in explicit, but not implicit memory (Curran et al., 2002). For animals, monkeys or rodents, such comparisons are widely lacking. Therefore, one aim of this study was to evaluate whether  $\Delta^9$ -THC extracted from cannabis plants would impair spatial working memory in rats tested in the water maze in a manner similar to its synthetic counterpart. Delayed-matching-to-place (DMTP) testing in the water maze is an episodic-like memory task (Morris et al., 2003) expected to be sensitive to cannabinoid treatment.

It is difficult to predict the outcome of drug treatment with cannabis extracts due to the relative paucity of comparable studies on spatial learning in the water maze. Null mutant mice for the CB<sub>1</sub> receptor showed no phenotype when trained in a reference memory paradigm in the water maze suggesting that there is no endogenous activation of CB<sub>1</sub> receptors during this behavior (Varvel and Lichtman, 2002). By contrast, systemic administration of  $\Delta^9$ -THC in mice impaired both spatial reference and working memory (Varvel et al., 2001; Da Silva and Takahashi, 2002). However, there was no effect on memory consolidation and retrieval in mice (Da Silva and Takahashi, 2002; Varvel et al., 2001) or rats (Mishima et al., 2001). Effective doses of  $\Delta^9$ -THC ranged from 0.3 to 10 mg/kg injected intraperitoneally. Effects of  $\Delta^9$ -THC or any other synthetic cannabinoid on spatial working memory in the water maze in rats has not been assessed so far. Rats have the advantage of readily acquiring the DMTP protocol and would also allow implementation of longer delays between trials 1 and 2 up to several hours (Steele and Morris, 1999; Von Linstow Roloff et al., 2002a,b). Mice, by contrast, require a long-lasting acquisition period of >30 days in this task and delays need to be rather short (several minutes at maximum: Riedel et al. unpublished data). It is therefore of considerable interest to examine spatial working memory in rats in the water maze and modulate task difficulty by extending delays to several hours. Especially long delays are proposed as being susceptible to cannabinoid treatment (Lichtman, 2000) such as  $\Delta^9$ -THC-rich extracts and synthetic  $\Delta^9$ -THC.

Some cannabis extracts are also rich in cannabidiol (CBD), which is chemically similar to  $\Delta^9$ -THC but devoid of any psychoactivity (Pertwee, 1988,2004). CBD is anxiolytic (Musty, 1984; Guimarães et al., 1990; Zuardi et al., 1982), anticonvulsant (Karler and

Turkanis, 1981; Wallace et al., 2001), neuroprotective (Hampson et al., 1998), anti-inflammatory (Sofia et al., 1973; Evans et al., 1987), affects growth and proliferation of cancer cells (Jacobsson et al., 2000) and improves motor function in dystonic patients (Sandyk et al., 1986). These data suggest that CBD has some effects in common with  $\Delta^9$ -THC, but detailed behavioral testing of synthetic CBD- or CBD-rich cannabis extracts on memory and learning has not been reported. In monkeys, CBD up to 3.2 mg/kg has no effect on the repeated acquisition of conditional discriminations, but  $\Delta^9$ -THC reduced the overall response rate (Winsauer et al., 1999). In  $\Delta^9$ -THC-sensitive working memory paradigms (delayed-matching-to-sample—Heyser et al., 1993; 8-arm radial maze—Lichtman et al., 1995) several groups were unable to induce a spatial memory deficit with doses of 10–30 mg/kg CBD. CBD may thus potentially become an interesting therapeutic agent, as it seems to be devoid of the psychoactive properties of  $\Delta^9$ -THC widely held responsible for the cannabis-induced memory deficits. Therefore, a more detailed analysis of CBD-rich extracts on spatial working memory seems warranted.

Apart from being rich in  $\Delta^9$ -THC, cannabis extracts contain considerable amounts of CBD. Depending on the actual ratio of the two, CBD may alter the effectiveness of  $\Delta^9$ -THC both in terms of therapeutic use, but also in terms of the induction of side effects. It is therefore, important to determine which  $\Delta^9$ -THC/CBD ratio(s) may prove the most suitable with respect to therapeutic efficacy yet reducing psychotropic and/or other negative side effects.

In order to investigate these questions, we developed a working/short-term memory paradigm for the open-field water maze that enabled repeated testing of the same animals in a control and drug state (Von Linstow Roloff et al., 2002a, b). Tolerance to any drug was avoided by long periods of drug-free non-testing. Dose–response relationships were established for both extracts followed by combined treatment in different ratios. Results were compared with synthetic  $\Delta^9$ -THC and additional control experiments determined drug actions on sensorymotor systems. Data confirm previous work with the synthetic cannabinoid WIN55,212-2 (Robinson et al., 2001) in that  $\Delta^9$ -THC-rich extracts impaired spatial working/short-term memory while CBD had no effect.

## 2. Materials and methods

### 2.1. Animals

Ten male hooded Lister rats (Rowett Research Institute, Aberdeen) weighing 250–300 g at the start of the training were housed 3–4 per cage under standard

environmental conditions (artificial light–dark cycle of 12 h: light on at 7 am;  $21 \pm 1$  °C room temperature; 60% relative humidity). Food and water were available ad libitum. All experimental procedures were performed under UK Home Office regulations.

## 2.2. Drugs

Cannabis extracts rich in  $\Delta^9$ -THC and CBD were a gift of GW Pharmaceutical (UK) (50 mg/ml ethanolic solution of  $\Delta^9$ -THC-rich extract contains: 91.5%  $\Delta^9$ -THC, 2.3% CBD, 1.3% cannabiniol (CBN), 4.6% cannabidiolic acid (CBDA), cannabigerol (CBG), cannabichromene (CBC), the *n*-propyl analogue of  $\Delta^9$ -THC (THCV) and  $\Delta^9$ -tetrahydrocannabinolic acid (THCA); 50 mg/ml ethanolic solution of CBD-rich extract contains: 85% CBD, 7.8%  $\Delta^9$ -THC, and 7.2% THCA, CBD, CBG, CBN, and CBDA). Tween 80 (Sigma-Aldrich, UK) was used as vehicle. Drugs and Tween 80 dissolved in ethanol were prepared fresh on each experimental day in a solution of two parts of Tween 80 by weight, ethanol was evaporated under vacuum and the residue re-suspended in saline. Also, we have used synthetic  $\Delta^9$ -THC (National Institute of Drug Abuse—NIDA; 98.4% pure). Doses were estimated for intraperitoneal (i.p.) injection in a volume of 5 ml/kg body weight and administered 30 min prior to the start of each test session. They were calculated to give a final concentration of  $\Delta^9$ -THC in  $\Delta^9$ -THC-rich extract of 0.5, 2, 5 mg/kg, and of CBD in CBD-rich extract: 0.5, 5, 10, and 50 mg/kg. We also co-administered  $\Delta^9$ -THC- and CBD-rich extracts: 2 mg/kg  $\Delta^9$ -THC+0.5 mg/kg CBD, 2 mg/kg  $\Delta^9$ -THC+5 mg/kg CBD, 2 mg/kg  $\Delta^9$ -THC+10 mg/kg CBD.

## 2.3. Behavioral testing in the water maze

### 2.3.1. Apparatus

The water maze consisted of a circular white Perspex pool (150 cm diameter and 50 cm deep) positioned in a room surrounded by several extramaze cues (cupboard, posters, shelves, blinds, books, etc.). It was filled with water at  $25 \pm 2$  °C to a depth of 35 cm. A clear Perspex platform (10 cm diameter) was placed inside the pool at predetermined locations with its top submerged approximately 1 cm below the water surface. All experimental sessions were recorded by an overhead video camera and an automatic tracking system. Data were stored both on video and online using PC-based software (HVS-Image, Hampton, UK) for subsequent analysis.

### 2.3.2. Experimental procedure

**2.3.2.1. Habituation.** Prior to training, animals received a habituation session comprising four trials with curtains drawn around the circumference of the

pool and the platform being randomly placed in the pool. Animals were released from four randomly chosen cardinal points (N, E, S, W) facing the pool wall and an inter-trial interval (ITI) of 30 s was employed.

### 2.3.2.2. Performance in delayed-matching-to-place task.

For training and testing, we used 12 platform positions randomly distributed in the pool; animals experienced these in fully counterbalanced order and were tested on each position first before re-using locations again. The platform position remained constant during each session (one session/day = four trials), but was moved to a new location between days. No curtains were positioned around the pool. Again, four cardinal release sites were used and each animal was given a total of 90 s to locate the submerged platform. If the rat failed to locate the platform within 90 s, it was gently guided to it by the experimenter. It was then left on the platform for 30 s before returning to its home cage.

Training lasted for 6 days and employed ITIs of 30 s and 4 h between trial 1 and trial 2 on 3 days each in random sequence; all other ITIs were 30 s. In trial 1, animals were placed onto the platform for 120 s, but were released into the water on trials 2–4. Performance in trial 2 is of particular interest as it reflects spatial working/short-term memory of the actual platform position. As such, the paradigm tests for latent learning (Whishaw, 1991). It has been proposed that placement may not support as much learning as swimming during trial 1. From a practical view, this is a bonus since one of our aims is to test whether CBD may reverse or even enhance learning. Weaker spatial encoding in a placement trial would allow for memory enhancements to be detected, which would otherwise be overshadowed by floor effects in performance.

DMTP testing involved a within-subject design in which all rats received all drugs and doses and performed all delays. ITIs were 30 s or 4 h; animals were placed onto the platform in trial 1 and swam during trials 2–4. Both injections of drugs (extracts or vehicle) and delays were randomized. Drug administration on 1 day was always followed by vehicle administration on the next day. Animals were tested 4 days/week and given ample time for drugs to wash out between test sessions. We always started with the lowest dose first and increased doses over test sessions.  $\Delta^9$ -THC-rich extracts were tested in the beginning followed by CBD-rich extracts and finally by the combinations of the two drugs.

### 2.3.3. Data acquisition, handling, and analysis

From the swim pattern, we extracted the following measures: (1) pathlength (centimeter—length of the swim path required to find the platform) as an index for spatial knowledge; (2) swim speed (cm/s) as an overall measure for motor activity; (3) thigmotaxis

(percentage trial time wall hugging in the outer 10% of the pool) as index of anxiety. The pathlength has been suggested as the most pertinent measure of spatial memory (Lindner, 1997). Other measures were only considered in case of drug-induced differences in pathlengths. For swim speed, we compared the difference between vehicle and drug state as a change in speed ( $\Delta v$ ) for each individual and each experimental condition (for example, 30 s Tween 80 versus 30 s  $\Delta^9$ -THC-rich extract 0.5 mg/kg, ...). All data were averaged and are expressed as mean  $\pm$  SEM. For statistical analyses, we applied a two-way repeated measures analysis of variance (ANOVA) with drug treatment and delay as factors and concentrated on performance in trial 2. A level of  $p < 0.05$  was considered reliable and followed by further post hoc analyses using either two-way ANOVA (drug by delay) or *t*-test for pairwise comparison at each delay. For the change of swim speed, we used *t*-tests for comparison with baseline (0 = no change).

#### 2.4. Bar test for catalepsy

Catalepsy was measured by means of the bar test (Costall and Olley, 1971). Each animal was placed with both forelegs over a horizontal metal bar (8 mm) fixed 9 cm above the working surface and the descent latency (length of time it retained this position) was recorded for a period of up to 5 min. Animals were injected and tested 30 min, 4 and 24 h after drug administration. These time points were chosen as they coincided with our behavioral testing in the water maze. Drugs and vehicle were alternated and the sequence of drugs was a fully counterbalanced within-subject design in which all animals went through each drug and control condition. Drug doses administered were chosen based on performance in the water maze and included: Tween 80;  $\Delta^9$ -THC-rich extract: 2, 5 mg/kg; CBD-rich extract: 50 mg/kg; co-administration 2 mg/kg  $\Delta^9$ -THC + 10 mg/kg CBD. Descent latencies of all animals were averaged for each drug dose and statistically analyzed in a two-way ANOVA with drug and time after injection as factors.

### 3. Results

#### 3.1. $\Delta^9$ -THC-rich extracts impair spatial working/short-term memory

Three doses of  $\Delta^9$ -THC-rich cannabis extract were tested against Tween 80 in the spatial DMTP task. Results are summarized in Fig. 1. Data are based on three test sessions with each  $\Delta^9$ -THC-rich extract dose recorded against Tween 80. All these Tween 80 data (three replications) were analyzed statistically and

found not to be different ( $F < 1$ ). As a result, we pooled the Tween 80 data of each animal into an overall mean (expressed in Fig. 1). Comparison with performance under  $\Delta^9$ -THC-rich extract revealed a dose-dependent deterioration in working/short-term memory. This is obvious from the swim paths taken during trial 2 (Fig. 1a) and statistical analysis using a  $4 \times 2$  factorial ANOVA (four drug conditions, two delays) supported this impression and yielded a main effect of drug treatment ( $F(3.72) = 5.66$ ;  $p = 0.0015$ ), but neither a main effect of delay nor an interaction ( $F < 1.38$ ). Post hoc analyses comparing individual doses with Tween 80 confirmed no effect in the 0.5 mg/kg  $\Delta^9$ -THC-rich extract condition ( $F < 1.27$ ), but reliable deficits for 2 ( $F(1.36) = 7.88$ ;  $p = 0.008$ ) and 5 ( $F(1.36) = 13.1$ ;  $p = 0.0009$ ) mg/kg  $\Delta^9$ -THC-rich extracts. This deficit was paralleled by an increase in swim speed (Fig. 1c) which reached significance for the 0.5 and 5 mg/kg  $\Delta^9$ -THC-rich extracts, but only at 4 h delay ( $t = 3.8$ ;  $p = 0.005$  and  $t = 2.3$ ;  $p = 0.047$ , respectively, all other  $t < 1.7$ ). Since there was no memory deficit in the 0.5 mg/kg  $\Delta^9$ -THC-rich extract group, the increase in swim speed at 5 mg/kg cannot explain the impairment in performance. Despite spending very little time (<10%) of each trial in the thigmotaxis zone (Fig. 1d), wall hugging behavior was higher after the 4 h delay ( $F(1.72) = 5.7$ ;  $p = 0.02$ ), but was not different for the  $\Delta^9$ -THC-rich extracts relative to Tween 80 ( $F < 1$ ).

Since  $\Delta^9$ -THC-rich extracts also contain other cannabinoids (for details, see Materials and methods) capable of modulating the effect of  $\Delta^9$ -THC, we compared  $\Delta^9$ -THC-rich extracts with synthetic  $\Delta^9$ -THC administered at a dose of 2 mg/kg i.p. When under synthetic  $\Delta^9$ -THC, animals were searching longer for the platform (Fig. 1e) relative to Tween 80 treatment in trial 2 at both delays and this difference was reliable ( $F(3.72) = 5.7$ ;  $p = 0.0015$  for main effect of drug; no interaction, no effect of delay,  $F < 1$ ). However, there was no significant difference in the performance between 2 mg/kg synthetic  $\Delta^9$ -THC and  $\Delta^9$ -THC-rich extracts ( $F < 1.5$ ;  $p > 0.23$ ) suggesting that the memory impairment in the extract was predominantly due to its  $\Delta^9$ -THC content.

#### 3.2. CBD-rich extracts did not affect spatial working/short-term memory

This experiment is a continuation of Exp. 1 after a break of 3 weeks. Animals were tested in four replications and dosed with 0.5–50 mg/kg CBD-rich extracts. Again, all Tween 80 results were pooled and data are presented in Fig. 2. As is obvious there was no memory deficit induced by CBD-rich extracts. Even at a dose of 50 mg/kg, animals readily swam to the new platform location. A  $5 \times 2$  (five drug doses, two delays) factorial

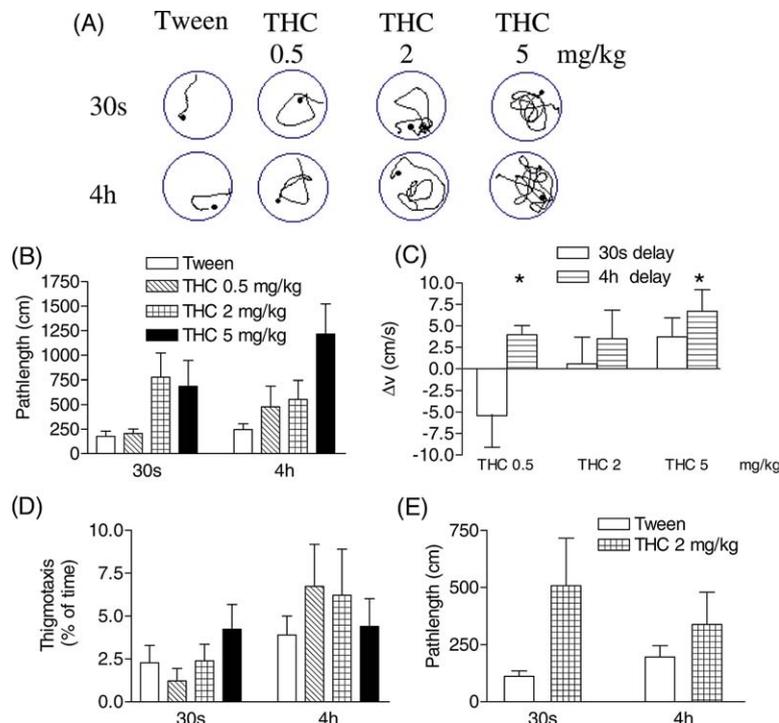


Fig. 1. Dose-dependent impairment of spatial working/short-term memory by  $\Delta^9$ -THC-rich extracts. Drugs were administered i.p. 30 min prior to each test session. Each bar represents the mean  $\pm$  SEM of 10 animals per group. Performance in trial 2 was used as an index of memory. (A) Representative swim traces recorded during trial 2 in each group at both delays (30 s and 4 h). Swim paths are longer at higher doses of  $\Delta^9$ -THC-rich extracts, (B) Pooled data of all drug groups (0–5 mg/kg) measured as overall pathlength in trial 2. A severe deficit appeared for 2 and 5 mg/kg  $\Delta^9$ -THC-rich extracts. Overall two-way ANOVA revealed a main effect of drug treatment with 2 and 5 mg/kg  $\Delta^9$ -THC conditions being significantly impaired ( $p < 0.01$ ), (C) Change in swim speed ( $\Delta v$ ) after treatment with  $\Delta^9$ -THC-rich extracts (0.5–5 mg/kg) in trial 2. Asterisks mark significant differences relative to baseline performance, (D)  $\Delta^9$ -THC-rich extracts (0.5–5 mg/kg) did not affect thigmotaxis (percentage of trial time) in trial 2, (E) Synthetic  $\Delta^9$ -THC (2 mg/kg) increased the pathlength in trial 2 in a manner similar to the same dose of the extracts (see b). This effect was significant in an overall two-way ANOVA ( $p < 0.01$ ).

ANOVA confirmed this impression and yielded no significant effects of drug treatment ( $F < 1.3$ ), but the main effect of delay reached a trend ( $F(1.90) = 3.8$ ;  $p = 0.055$ ). CBD-rich extracts did not affect swim-speed, thigmotaxis or any other parameter analyzed (data not shown).

### 3.3. Co-administration of $\Delta^9$ -THC- and CBD-rich extracts

Given this discordance in effects on working/short-term memory between  $\Delta^9$ -THC- and CBD-rich extracts, we wondered whether they may act differentially in the brain and CBD-rich extracts might be capable of potentiating/antagonizing the effects induced by  $\Delta^9$ -THC-rich extracts. We selected a medium dose of  $\Delta^9$ -THC-rich extract (2 mg/kg) and combined it with 5 or 10 mg/kg CBD-rich extract. A complicated fully counterbalanced drug-administration regime was applied, in which we had numerous Tween 80 sessions. Statistical comparison of these Tween 80 sessions revealed no difference (all  $p > 0.05$ ); to avoid reduction in standard error due to averaging these data, we selected results of the last Tween 80 control session for

further statistical comparison with extract treatment. Pathlengths in trial 2 were longer when animals were under drug-treatment (Fig. 3a,b) and this effect was greater at the 30 s delay than at 4 h. Statistical analysis (four drug treatments–Tween 80,  $\Delta^9$ -THC,  $\Delta^9$ -THC + CBD5,  $\Delta^9$ -THC + CBD10, two delays) confirmed a significant interaction between drug-treatment and delay ( $F(3.72) = 2.8$ ;  $p = 0.047$ ), and there was also a main effect of treatment ( $F(3.72) = 3.05$ ;  $p = 0.034$ ), but the delay failed to attain significance ( $F(1.72) = 3.54$ ;  $p = 0.064$ ). As is clear from Fig. 3b, CBD-rich extracts did not reverse the deficit obtained after  $\Delta^9$ -THC-rich extract exposure. Single drug comparisons supported this impression with all extracts being different from Tween 80 (all  $F > 3.2$ ;  $p < 0.05$ ). While  $\Delta^9$ -THC + CBD 5 mg/kg was not different from  $\Delta^9$ -THC-rich extract alone ( $F < 1$ ), the dose of  $\Delta^9$ -THC + CBD 10 mg/kg was significantly different from  $\Delta^9$ -THC-rich extract (interaction of drug  $\times$  delay:  $F(1.36) = 4.4$ ,  $p = 0.043$ ). The main effect of drug failed to attain significance ( $F(1.36) = 3.4$ ;  $p = 0.074$ ) but the effect of delay was also reliable ( $F(1.36) = 7$ ;  $p = 0.01$ ). This suggests a delay-dependent action of

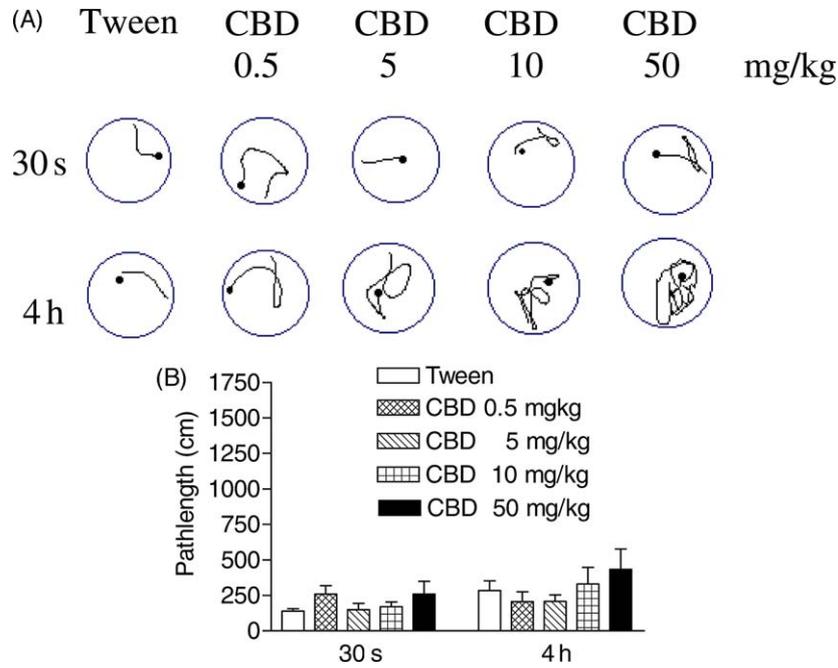


Fig. 2. Effect of CBD-rich extracts (0.5–50 mg/kg) on spatial working/short-term memory. CBD-rich extract were administered i.p. 30 min prior to each test session. (A) Representative swim traces recorded during trial 2 in each group at both delays (30 s and 4 h) show little increase in the length of the swim path, (B) Mean pathlength ( $\pm$ SEM) in trial 2 sorted for delays and drug condition. Overall, CBD-rich extracts (0.5–5 mg/kg) did not significantly increase the pathlength in trial 2.

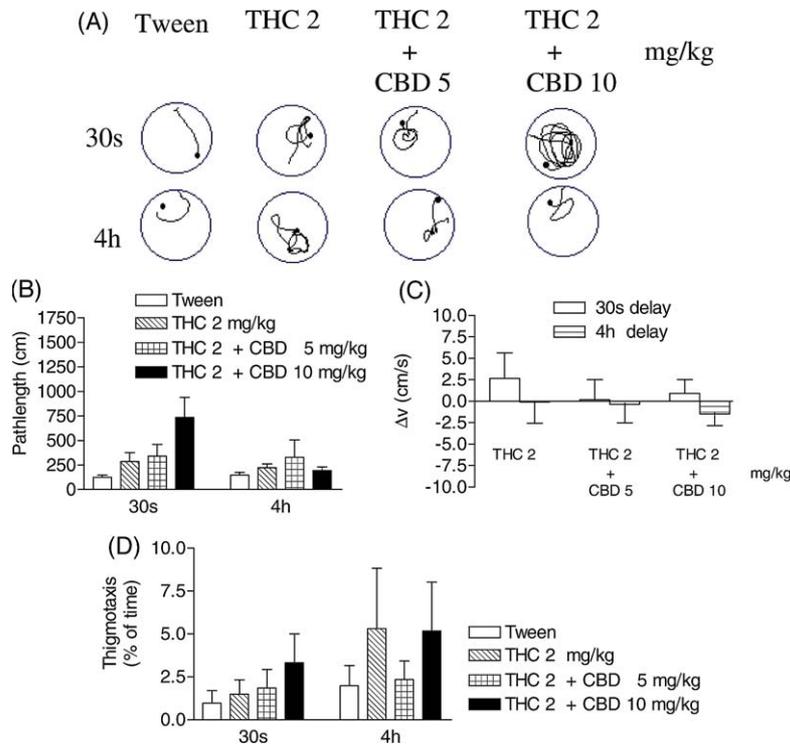


Fig. 3. Effect of co-administration of  $\Delta^9$ -THC and CBD-rich extracts on spatial working/short-term memory.  $\Delta^9$ -THC- and CBD-rich extracts were co-injected i.p. 30 min prior to each test session. Each bar represents the group mean  $\pm$  SEM of 10 animals. (A) Representative swim traces recorded during trial 2 in each group at both delays (30 s and 4 h) reveal significant deficits in the 2 mg/kg  $\Delta^9$ -THC and the 2 mg/kg  $\Delta^9$ -THC + 10 mg/kg CBD-rich extract groups, (B) Pooled data of pathlength for the different drug groups revealed  $\Delta^9$ -THC-rich extract-induced increases in swim path. No reversal by CBD-rich extracts. Overall ANOVA confirmed a significant effect of drug treatment ( $p < 0.05$ ) and comparisons between drugs and Tween 80 yielded significant impairments for all drug combinations ( $p < 0.05$ ).  $\Delta^9$ -THC + CBD10 was different from  $\Delta^9$ -THC-rich extracts (higher at 30 s, lower at 4 h = drug  $\times$  delay interaction:  $p < 0.05$ ), (C) Drugs did not affect swim speed relative to baseline levels, (D) Thigmotaxis was not altered in any of the drug groups.

the high dose of CBD-rich extract on the memory-impairment induced by  $\Delta^9$ -THC, i.e. enhancement of memory deficit at short delays ( $t = 2.02$ ;  $p = 0.05$ ) and no effect at long-delays ( $t < 1$ ). Neither treatment induced significant changes in swim speed (Fig. 3c; all  $t < 1.1$  if compared with baseline) nor had any effect on thigmotaxic behavior (Fig. 3d; all  $F < 1.8$ ,  $p > 0.18$ ).

Since previous work has suggested that CBD can antagonize low-dose effects of  $\Delta^9$ -THC, but enhances high-dose effects of  $\Delta^9$ -THC (Zuardi and Karniol, 1983; Onaivi et al., 1990), the ratio between CBD and  $\Delta^9$ -THC may determine the possible antagonism or synergism of the two compounds. Reversal of  $\Delta^9$ -THC-induced effects (working memory impairment in this case) may be readily achieved by administration of very low doses of CBD. In a final test, we therefore injected 2 mg/kg  $\Delta^9$ -THC-rich extract together with 0.5 mg/kg CBD-rich extract (data not shown). Again, we also tested animals under Tween 80 and  $\Delta^9$ -THC-rich extract alone. Data confirmed our results from Exp. 1 in that there was a working/short-term memory deficit induced by  $\Delta^9$ -THC-rich extract and this was not reversed by 0.5 mg/kg CBD-rich extract. Repeated measures ANOVA confirmed a main effect of drug ( $F(2.54) = 3.5$ ;  $p = 0.038$ ), but no effect of delay or interaction ( $F < 1$ ). Both drug groups differed significantly from Tween 80 ( $F > 5.5$ ;  $p < 0.025$ ), but did not differ from each other ( $F = 0$ ). Consequently, very low doses of CBD-rich extract did not reverse the  $\Delta^9$ -THC-induced working/short-term memory deficit.

### 3.4. Catalepsy measured by bar test

Finally, we investigated the amount of catalepsy induced by the different concentrations of plant extracts. Animals were again tested in a within-subject design such that each drug was infused in random order. Not more than two drugs were tested per week. Data are depicted in Fig. 4 and are summarized for 30 min, 4 and 24 h post-injection. Both 30 min and 4 h reflect time points of testing in the DMTP task, 24 h represents the start of a next test under Tween 80 treatment. Overall, all animals were tested 15 times. Pilot data (not shown) have confirmed that there is no habituation to this paradigm and that rats injected with Tween 80 and exposed to the bar test up to 20 times in 4 weeks do not change descent latencies. By contrast,  $\Delta^9$ -THC-rich extracts increased descent latency in the 5 mg/kg, but not the 2 mg/kg condition. Also, CBD-rich extracts (50 mg/kg) and the combination of 2 mg/kg  $\Delta^9$ -THC- and 10 mg/kg CBD-rich extracts increased the step-down latencies. Although these effects were significant ( $5 \times 3$  repeated measures ANOVA:  $F(4.135) = 5.7$ ;  $p = 0.0003$  for drugs;  $F(2.135) = 12$ ;  $p < 0.0001$  for delay; no interaction) and most prominent for the 4 h delay, a descent

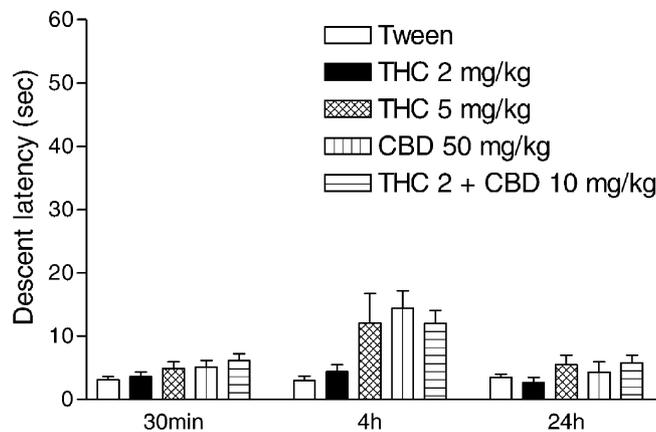


Fig. 4. Effect of  $\Delta^9$ -THC-rich extracts (2 and 5 mg/kg), CBD-rich extracts (50 mg/kg) and their co-administration ( $\Delta^9$ -THC 2 mg/kg and CBD 10 mg/kg) on rat bar test descent latency. Animals were tested at different times after drug administration (30 min, 4 and 24 h). Each bar represents the mean  $\pm$  SEM of 10 animals per group. Despite an overall increase in the descent latency in the drug conditions ( $p < 0.001$ ), the overall time to step down was very short ( $< 20$  s).

latency of 15 s is commonly not perceived as catalepsy since it is below 1 min (Sañudo Peña et al., 2000).

## 4. Discussion

Results of this work reveal that systemic administration of  $\Delta^9$ -THC-rich extracts of the cannabis plant induce a dose-dependent, but delay-independent, spatial working/short-term memory deficit in a DMTP task in the water maze in rats. In contrast, CBD-rich extracts were ineffective. Side effects of both drugs on motor performance, anxiety, and catalepsy were excluded.

Several reports have indicated that commercially available and synthetically generated  $\Delta^9$ -THC can spatially impair spatial working memory when administered systemically. Most prominent are deficits in rats tested in the 8-arm radial maze (Egashira et al., 2002; Hernandez-Tristan et al., 2000; Lichtman and Martin, 1996; Lichtman et al., 1995; Mishima et al., 2001; Molina-Holgado et al., 1995; Nakamura et al., 1991; Stiglick and Kalant, 1982) and T-maze (Jentsch et al., 1997; Nava et al., 2001), and impairments were mimicked by other cannabinoid agonists (for review, see Robinson and Riedel, 2004). However, the authors have not altered task difficulty by employing long delays between sample and choice trials, but kept this ITI constant. Similar to other non-spatial delayed-matching or non-matching-to-sample paradigms (Hampson and Deadwyler, 1999, 2000; Heyser et al., 1993; Mallet and Beninger, 1996, 1998), we extended the ITI between the sample trial (trial 1), in which animals were directly placed onto the platform, and

choice trial (trial 2) in which animals were released into the pool from the perimeter. Although impaired at doses of 2 and 5 mg/kg  $\Delta^9$ -THC-rich extract animals showed no delay-dependent deficit. This observation is in line with work by Hampson and Deadwyler (1998) showing  $\Delta^9$ -THC-induced working memory deficits for all delays longer than 0 s. Although they have used synthetic  $\Delta^9$ -THC, we did not find any qualitative differences between our extract and the synthetic compound. As reported for the delayed-non-match-to-sample task (Hampson and Deadwyler, 1998), 2 mg/kg  $\Delta^9$ -THC-rich extract proved potent for inducing a spatial working memory deficit without severe somatosensory and motor side effects that would compromise learning studies. These data suggest that several if not all types of working/short-term memory are susceptible to disruption by psychoactive cannabinoids.

Work reporting on the actions of CBD on memory is scarce. Only few reports have explored the effects of CBD on memory formation, mainly with a negative outcome (Winsauer et al., 1999; Heyser et al., 1993; Lichtman et al., 1995). This is surprising given that effects of CBD on the central nervous system show some similarities with those of  $\Delta^9$ -THC (for details, see Introduction). We have tested a range of doses of CBD-rich cannabis extracts and results are in line with previous reports. CBD-rich extracts did not affect spatial working/short-term memory. It is therefore safe to argue that the amount of other cannabinoid compounds present in CBD-rich extracts is not sufficient to generate behavioral alterations. This is of particular relevance for the contribution of  $\Delta^9$ -THC, which can have effects on the deterioration of the memory at higher doses.

By contrast, Izquierdo and Nasello (1973) reported a learning impairment in rats treated with 3.5 mg/kg CBD prior to acquisition of an active avoidance task. Thus, the question arises why we did not observe CBD-rich extract induced memory deficits in our paradigm. Firstly, we trained our animals in the working memory paradigm prior to any drug treatment, since we aimed at measurement of spatial performance uncontaminated by any procedural task elements. From this, it is clear that CBD-rich extracts have no effect on spatial working/short-term memory. It remains to be assessed whether CBD-rich extracts may affect long-term memory, but the fact that we did not obtain an acquisition deficit may be taken to argue against such a possibility. Second, Izquierdo's task contains a highly emotional component, foot shock. Cannabinoid agonists and  $\Delta^9$ -THC are well known for their anxiolytic properties (Rodriguez de Fonseca et al., 1997; Berrendero and Maldonado, 2002). CBD also is anxiolytic (Musty, 1984; Guimarões et al., 1990; Zuardi et al., 1982), and this property may be responsible for a reduction in avoidance responses found by Izquierdo and Nasello (1973). Our task, however, is less emotion-

al, especially given the fact that animal's had extensive experience prior to testing with CBD-rich extracts.

Despite some reports suggesting CBD as an antagonist of  $\Delta^9$ -THC-induced actions (Karniol and Carlini, 1973; Welburn et al., 1976; Zuardi and Karniol, 1983), we found CBD-rich extracts did not reverse the spatial memory deficit induced by the  $\Delta^9$ -THC-rich extract. Although this suggests that CBD does not antagonise the actions of  $\Delta^9$ -THC, a careful pharmacological analysis provides some evidence for reversal of  $\Delta^9$ -THC-induced deficits in the CBD-rich extracts alone. Clearly, low doses of CBD-rich extracts had no effect but also had little if any amount of  $\Delta^9$ -THC or other cannabinoids present (see Materials and methods for details). In the dose of 50 mg/kg, CBD-rich extract contained nearly 4 mg/kg of  $\Delta^9$ -THC, a dose that was sufficient to compromise working/short-term memory in our task when given alone (see Fig. 1). This impairment was clearly antagonised in the CBD-rich extract. The fact that additional  $\Delta^9$ -THC from the  $\Delta^9$ -THC-rich extracts was not reversed could be explained in two different ways. First, the CBD-rich extract contained a 12.5-fold higher dose of CBD over  $\Delta^9$ -THC. If additional  $\Delta^9$ -THC-rich extracts are added, CBD:  $\Delta^9$ -THC ratios fall to 0.25-, 2.1- and 3.6-fold more CBD than  $\Delta^9$ -THC for the combinations of 2 mg/kg  $\Delta^9$ -THC + 0.5 mg/kg CBD, 2 mg/kg  $\Delta^9$ -THC + 5 mg/kg CBD, 2 mg/kg  $\Delta^9$ -THC + 10 mg/kg CBD-rich extracts, respectively. It appears therefore that a >10-fold higher dose of CBD over  $\Delta^9$ -THC is necessary to effectively antagonise deficits induced by  $\Delta^9$ -THC, a finding that is in agreement with Zuardi and Karniol (1983). Lower ratios can produce potentiation of the  $\Delta^9$ -THC effect as seen in the study on performance of rats in variable intervals (Zuardi and Karniol, 1983). While there was no potentiation by CBD-rich extracts of the  $\Delta^9$ -THC-mediated spatial working memory deficit, potentiation was observed in the bar test, in which 10 mg/kg CBD-rich extracts also enhanced the cataleptic response in  $\Delta^9$ -THC-rich extract-treated animals. This could reflect a dissociation of CBD-effects on spatial working/short-term memory and motor responses, and it is thus important to address the question of whether  $\Delta^9$ -THC-rich extract-induced memory deficits can be explained in terms of unspecific side effects of the extracts. This is unlikely for the cataleptic response, which was rather weak in our bar test (below 20 s). Furthermore, differences in swim speed have been observed in animals presenting with (5 mg/kg  $\Delta^9$ -THC-rich extract) and without (0.5 mg/kg  $\Delta^9$ -THC-rich extract) working/short-term memory deficits. Such data indicate that  $\Delta^9$ -THC-rich extracts may affect motor responses in general, but these are separate from drug effects on cognition. This may similarly apply to cataleptic responses, which were increased in the pres-

ence of 50 mg/kg CBD-rich extract, but this dose had no effect on spatial working memory.

A second explanation could be that other cannabinoid constituents present in CBD-rich extracts either one alone or several in combination with CBD antagonised the effect of the 4 mg/kg  $\Delta^9$ -THC present in 50 mg/kg CBD-rich extracts. Amounts of these cannabinoids, however, are too small to also block the memory impairment that results from additional administration of  $\Delta^9$ -THC-rich extracts. Identification of the specific cannabinoids effective in antagonising  $\Delta^9$ -THC effects requires further experiments.

Collectively, our data suggest that cannabinoid extracts rich in  $\Delta^9$ -THC or CBD have similar actions to their synthetically generated counterparts. For  $\Delta^9$ -THC-rich extracts, a dose-dependent and delay-independent spatial working/short-term memory impairment was revealed, which is in agreement with previous reports that have applied synthetic  $\Delta^9$ -THC. As for pure CBD, we also found no effect of CBD-rich extracts on spatial working/short-term memory. Pharmacological effects of additional cannabinoids present in our extracts awaits further testing.

### Acknowledgements

This work was supported by grants from the MRC to GR and RGP, NIDA to RGP, and of the Italian M.I.U.R. to the Centre of Excellence on Neurobiology of Dependence—University of Cagliari (WF and PF).

### References

- Berrendero, F., Maldonado, R., 2002. Involvement of the opioid system in the anxiolytic-like effects induced by delta(9)-tetrahydrocannabinol. *Psychopharmacology* 163, 111–117.
- Block, R.I., Ghoneim, M.M., 1993. Effects of chronic marijuana use in human cognition. *Psychopharmacology* 110, 219–228.
- Costall, B., Olley, J.E., 1971. Cholinergic and neuroleptic induced catalepsy: modification by lesions in the caudate-putamen. *Neuropsychopharmacology* 10, 297–306.
- Curran, H.V., Brignell, C., Fletcher, S., Middleton, P., Henry, J., 2002. Cognitive and subjective dose-response effects of acute oral  $\Delta^9$ -tetrahydrocannabinol (THC) in infrequent cannabis users. *Psychopharmacology* 164, 61–70.
- Da Silva, G.E., Takahashi, R.N., 2002. SR141617A prevents  $\Delta^9$ -tetrahydrocannabinol-induced spatial learning deficit in a Morris-type water maze in mice. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 26, 321–325.
- Darley, C.F., Tinklenberg, J.R., Roth, W.T., Atkinson, R.C., 1974. The nature of storage deficits and state-dependent retrieval under marijuana. *Psychopharmacology* 37, 139–149.
- Egashira, N., Mishima, K., Iwasaki, K., Fujiwara, M., 2002. Intracerebral microinjections of  $\Delta^9$ -tetrahydrocannabinol: search for the impairment of spatial memory in the eight-arm radial maze in rats. *Brain Res.* 952, 239–245.
- Evans, A.T., Formukong, E., Evans, F.J., 1987. Actions of cannabis constituents on enzymes of arachidonate metabolism: anti-inflammatory potential. *Biochem. Pharmacol.* 36, 2035–2037.
- Fletcher, J.M., Page, J.B., Francis, D.J., Copeland, K., Naus, M.J., Davis, C.M., Morris, R., Krausskopf, D., Satz, P., 1996. Cognitive correlates of long-term cannabis use in Costa Rican men. *Arch. Gen. Psychiatr.* 53, 1051–1057.
- Guimarães, F.S., Chiaretti, T.M., Graeff, F.G., Guardi, A.W., 1990. Antianxiety effect of cannabidiol in the elevated plus-maze. *Psychopharmacology* 100, 558–559.
- Hampson, A.J., Grimaldi, M., Axelrod, J., Wink, D., 1998. Cannabidiol and  $\Delta^9$ -tetrahydrocannabinol are neuroprotective antioxidants. *Proc. Natl. Acad. Sci. USA* 95, 8268–8273.
- Hampson, R.E., Deadwyler, S.A., 1998. Role of the cannabinoid receptors in memory storage. *Neurobiol. Dis.* 5, 474–482.
- Hampson, R.E., Deadwyler, S.A., 1999. Cannabinoids, hippocampal function and memory. *Life Sci.* 65, 715–723.
- Hampson, R.E., Deadwyler, S.A., 2000. Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. *J. Neurosci.* 20, 8932–8942.
- Hart, C.L., Ward, A.S., Haney, M., Comer, S.D., Foltin, R.W., Fischman, M.W., 2002. Comparison of smoked marijuana and oral  $\Delta^9$ -tetrahydrocannabinol in humans. *Psychopharmacology* 164, 407–415.
- Hernandez-Tristan, R., Arevalo, C., Canals, S., Leret, M.L., 2000. The effects of acute treatment with  $\Delta^9$ -THC on exploratory behaviour and memory in the rat. *J. Physiol. Biochem.* 56, 17–24.
- Heyser, C.J., Hampson, R.E., Deadwyler, S.A., 1993. Effects of  $\Delta^9$ -tetrahydrocannabinol on delayed match to sample performance in rats: alterations in short-term memory associated with changes in task specific firing of hippocampal cells. *J. Pharmacol. Exp. Ther.* 264, 294–307.
- Izquierdo, I., Nasello, A.G., 1973. Effects of cannabidiol and diphenylhydantoin on the hippocampus and learning. *Psychopharmacologia* 31, 167–175.
- Jacobsson, S.O.P., Rongard, E., Stridh, M., Tiger, G., Fowler, C.J., 2000. Serum-dependent effects of tamoxifen and cannabinoids upon C6 glioma cell viability. *Biochem. Pharmacol.* 60, 1807–1813.
- Jentsch, J.D., Andrusiak, E., Tran, A., Bowers, M.B., Roth, R.H., 1997.  $\Delta^9$ -Tetrahydrocannabinol increases prefrontal cortical catecholaminergic utilization and impairs spatial working memory in the rat: blockade of dopaminergic effects by HA966. *Neuropsychopharmacology* 16, 426–432.
- Karler, R., Turkkanis, S.A., 1981. The cannabinoids as potential anti-epileptics. *J. Clin. Pharmacol.* 21, 437S–448S.
- Karniol, I.G., Carlini, E.A., 1973. Pharmacological interaction between cannabidiol and  $\Delta^9$ -tetrahydrocannabinol. *Psychopharmacologia* 33, 53–70.
- Lichtman, A.H., Dimen, K.R., Martin, B.R., 1995. Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. *Psychopharmacology* 119, 282–290.
- Lichtman, A.H., Martin, B.R., 1996.  $\Delta^9$ -Tetrahydrocannabinol impairs spatial memory through cannabinoid receptor mechanism. *Psychopharmacology* 126, 125–131.
- Lichtman, A.H., 2000. SR141716A enhances spatial memory as assessed in a radial-arm maze task in rats. *Eur. J. Pharmacol.* 404, 175–179.
- Lindner, M.D., 1997. Reliability, distribution, and validity of age-related cognitive deficits in the Morris water maze. *Neurobiol. Learn. Mem.* 68, 203–220.
- Mallet, P.E., Beninger, R.J., 1996. The endogenous cannabinoid receptor agonist anandamide impairs memory in rats. *Behav. Pharmacol.* 7, 276–284.
- Mallet, P.E., Beninger, R.J., 1998. The cannabinoid CB<sub>1</sub> receptor antagonist SR141716A attenuates the memory impairment produced by  $\Delta^9$ -tetrahydrocannabinol or anandamide. *Psychopharmacology* 140, 11–19.

- Miller, L.L., Branconnier, R.J., 1983. Cannabis: effects on memory and the cholinergic limbic system. *Psychol. Bull.* 93, 441–456.
- Mishima, K., Egashira, N., Hirose, N., Fujii, M., Matsumoto, Y., Iwasaki, K., Fujiwara, M., 2001. Characteristics of learning and memory impairment induced by  $\Delta^9$ -tetrahydrocannabinol in rats. *Jpn. J. Pharmacol.* 87, 297–308.
- Molina-Holgado, F., Gonzalez, M.I., Leret, M.L., 1995. Effect of  $\Delta^9$ -tetrahydrocannabinol on short-term memory in the rat. *Physiol. Behav.* 57, 177–179.
- Morris, R.G.M., Moser, E.I., Riedel, G., Martin, S.J., Sandin, J., Day, M., O'Carroll, C., 2003. Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. *Philos. Trans. R. Soc. Lond. B: Biol. Sci.* 358, 773–786.
- Musty, R.E., 1984. Possible anxiolytic effects of cannabidiol. In: Agurell, S., Dewey, W.L., Willette, R.E. (Eds.), *The Cannabinoids: Chemical, Pharmacologic and Therapeutic Aspects*. Academic Press, Orlando, pp. 785–813.
- Nakamura, E.M., Da Silva, E.A., Concilio, G.V., Wilkinson, D.A., Masur, J., 1991. Reversible effects of acute and long-term administration of  $\Delta$ -9-tetrahydrocannabinol (THC) on memory in the rat. *Drug Alcohol Depend.* 28, 167–175.
- Nava, F., Carta, G., Colombo, G., Gessa, G.L., 2001. Effects of chronic  $\Delta^9$ -tetrahydrocannabinol treatment on hippocampal extracellular acetylcholine concentration and alteration performance in the T-maze. *Neuropharmacology* 41, 392–399.
- Onaivi, E.S., Green, M.R., Martin, B.R., 1990. Pharmacological characterization of cannabinoids in the elevated plus maze. *J. Pharmacol. Exp. Therap.* 254, 11002–11009.
- Pertwee, R.G., 1988. The central neuropharmacology of psychotropic cannabinoids. *Pharmacol. Ther.* 36, 189–261.
- Pertwee, R.G., 2004. The pharmacology and therapeutic potential of cannabidiol. In: Di Marzo, V. (Ed.), *Cannabinoids*. Kluwer Academic, pp. 32–83.
- Robinson, L., Riedel, G., 2004. Cannabinoid function in spatial learning: an update. *Curr. Neuropharm.* 2, 125–143.
- Robinson, L., Pertwee, R.G., Riedel, G., 2001. Differential effects of CB1 receptor antagonists HU210 and WIN 55,212-2 on working memory in rats. *Behav. Pharmacol.* 12, S84.
- Rodriguez de Fonseca, F., Carrera, M.R.A., Navarro, M., Koob, G.F., Weiss, F., 1997. Activation of corticotrophin-releasing factor in the limbic system during cannabinoid withdrawal. *Science* 276, 2050–2054.
- Sandyk, R., Snider, S.R., Consroe, P., Elias, S.M., 1986. Cannabidiol in dystonic movement disorders. *Psychiatry Res.* 18, 291.
- Sañudo Peña, M.C., Romero, J., Seale, G.E., 2000. Activational role of cannabinoids on movements. *Eur. J. Pharmacol.* 391, 269–274.
- Sofia, R.D., Knobloch, L.C., Vassar, H.B., 1973. The anti-edema activity of various naturally occurring cannabinoids. *Res. Commun. Chem. Pathol. Pharmacol.* 6, 909–918.
- Steele, R.J., Morris, R.G., 1999. Delay-dependent impairment of a matching-to-place task with chronic and intrahippocampal infusion of the NMDA antagonist D-AP5. *Hippocampus* 9, 118–136.
- Stiglick, A., Kalant, H., 1982. Learning impairment in the radial-arm maze following prolonged cannabis treatment in rats. *Psychopharmacology* 77, 117–123.
- Varvel, S.A., Hamm, R.J., Martin, B.R., Lichtman, A.H., 2001. Differential effects of  $\Delta^9$ -THC on spatial reference and working memory in mice. *Psychopharmacology* 157, 142–150.
- Varvel, S.A., Lichtman, A.H., 2002. Evaluation of CB1 receptor knockout mice in the Morris water maze. *J. Pharmacol. Exp. Ther.* 301, 915–924.
- Von Linstow Roloff, E., Platt, B., Riedel, G., 2002a. Long term study of chronic oral aluminium exposure and spatial working memory in rats. *Behav. Neurosci.* 116, 351–356.
- Von Linstow Roloff, E., Platt, B., Riedel, G., 2002b. No spatial working memory deficit in beta-amyloid-exposed rats: a longitudinal study. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 26, 955–970.
- Wallace, M.J., Wiley, J.L., Martin, B.R., DeLorenzo, R.J., 2001. Assessment of the role of CB1 receptors in cannabinoid anticonvulsant effects. *Eur. J. Pharmacol.* 428, 51–57.
- Welburn, P.J., Starter, G.A., Chesher, G.B., Jackson, D.M., 1976. Effect of cannabinoids on the abdominal constriction response in mice: within cannabinoid interactions. *Psychopharmacology* 46, 83–85.
- Whishaw, I.Q., 1991. Latent learning in swimming pool place task in rats: evidence for the use of associative and not cognitive mapping processes. *Q. J. Exp. Psychol.* 43, 83–103.
- Winsauer, P.J., Lambert, P., Moerschbaecher, J.M., 1999. Cannabinoid ligands and their effects on learning and performance in rhesus monkeys. *Behav. Pharmacol.* 10, 497–511.
- Zuardi, A.W., Shirakawa, I., Finkelfarb, E., Karniol, I.G., 1982. Action of cannabidiol on the anxiety and other effects produced by  $\Delta^9$ -THC in normal subjects. *Psychopharmacology* 76, 245–250.
- Zuardi, A.W., Karniol, I.G., 1983. Effect on variable-interval performance in rats of  $\Delta^9$ -tetrahydrocannabinol and cannabidiol, separately and in combination. *Braz. J. Med. Biol. Res.* 16, 141–146.