Psychopharmacology (1995) 119 : 282–290 © Springer-Verlag 1995

ORIGINAL INVESTIGATION

Aron H. Lichtman · Katherine R. Dimen
Billy R. Martin

Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats

Received: 11 June 1994 / Final version: 31 August 1994

Abstract The purpose of the present study was to investigate the disruptive effects of cannabinoids on working memory as assessed in the eight-arm radial-maze. Systemic administration of Δ^9-THC, WIN-55,212-2, and CP-55,940 increased the number of errors committed in the radial-maze. CP-55,940 was the most potent cannabinoid in impairing memory (ED_{50} = 0.13 mg/kg). Δ^9-THC and WIN-55,212-2 disrupted maze-choice accuracy at equipotent doses (ED_{50} values = 2.1 and 2.2 mg/kg, respectively). In addition, systemic administration of each of these agents retarded completion time. Whereas the doses of Δ^9-THC and CP-55,940 required to retard maze performance were higher than those needed to increase error numbers, WIN-55,212-2 was equipotent in both of these measures. On the other hand, neither anandamide, the putative endogenous cannabinoid ligand, nor cannabidiol, an inactive naturally occurring cannabinoid, had any apparent effects on memory. A second aim of this study was to elucidate the neuroanatomical substrates mediating the disruptive effects of cannabinoids on memory. Intrahippocampal injections of CP-55,940 impaired maze performance in a dose-dependent manner (ED_{50} = 8 μg/rat), but did not retard the amount of time required to complete the maze. The effects of intrahippocampal CP-55,940 were apparently specific to cognition because no other cannabinoid pharmacological effects (e.g., antinociception, hypothermia, and catalepsy) were detected. This dissociation between choice accuracy in the radial-maze and other cannabinoid pharmacological effects suggests that the working memory deficits produced by cannabinoids may be mediated by cannabinoid receptors in the hippocampus.

A. H. Lichtman · K. R. Dimen · B. R. Martin
Department of Pharmacology and Toxicology
MCV Station, Box 613, Medical College of Virginia/
Virginia Commonwealth University, Richmond, VA 23298, USA

Key words Radial-arm maze · Δ^9-tetrahydrocannabinol (Δ^9-THC) · CP-55,940 · WIN-55,212-2 · Anandamide · Cannabidiol · Hippocampus · Antinociception · Catalepsy · Rectal temperature

Introduction

The naturally occurring cannabinoids, such as Δ^9-THC, and synthetic compounds have been demonstrated to produced a multitude of motor and sensory alterations in laboratory animals including antinociception, catalepsy, hypothermia, and decreases in spontaneous locomotion (Dewey 1986; Martin 1986). These compounds are also known to impair learning and memory in rodents (Carlini et al. 1970), nonhuman primates (Ferraro and Grilly 1973; Evans 1992), and humans (Abel 1971). Δ^9-THC has been found to disrupt memory as assessed in the delayed match to sample (DMTS) task (Heyser et al. 1993), Lashley III maze (Carlini et al. 1970), and the eight-arm radial-maze (Nakamura et al. 1991). The eight-arm radial-maze task has been particularly useful in assessing the effects of drugs on spatial memory (Olton 1987). The standard procedure is designed to assess working memory by requiring the subject to enter each of the eight runway arms in order to obtain food reinforcement. Drugs that impair memory, such as the anticholinergics (Levin 1988), increase the number of errors committed, (i.e., entries into previously visited arms). Few studies have evaluated the effects of cannabinoid intoxication on memory as assessed in the radial-maze. Although acute administration of Δ^9-THC has been reported to produce modest deficits in maze performance (Nakamura et al. 1991), a limitation of that study was that only one drug dose was employed.

The hippocampus and its related structures appear to be pivotal for working memory as its damage by surgical or chemical methods severely impairs

performance in a variety of tasks including the radial-maze (Olton and Werz 1978; Olton 1987; McLamb et al. 1988). It is of consequence that the hippocampus contains one of the highest concentrations of cannabinoid receptors in the brain (Herkenham et al. 1991; Jansen et al. 1992; Mailleux et al. 1992; Thomas et al. 1992). Several observations are consistent with the notion that the hippocampus mediates the disruptive effects of the cannabinoids on memory. In particular, Δ^9-THC-induced impairment in the DMTS task was associated with a specific decrease in hippocampal cell discharge (Heyser et al. 1993). In addition Δ^9-THC has been shown to alter cerebral metabolism in several brain regions including the hippocampus (Margulies and Hammer 1991). The hippocampus has also been found to be sensitive to cannabinoid treatment in vitro. Δ^9-THC applied to hippocampal tissue affects long-term potentiation (LTP) (Norwicky and Teyler 1987), a phenomenon proposed to be a neural mechanism for information storage in the brain, in a biphasic fashion. Finally, chronic administration of Δ^9-THC reduces the concentration of synapses in the CA3 region of the hippocampus (Scallet et al. 1987).

The purpose of the present study was to investigate the disruptive effects of cannabinoids on working memory as assessed in the eight-arm radial maze. In addition to assessing whether Δ^9-THC-induced memory impairment was dose-related, the following structurally diverse cannabinoids were evaluated (see Fig. 1): CP-55,940, a potent bicyclic analog that has been used to characterize the receptor (Devane et al. 1988); WIN-55,212-2, an aminoalkylindole analog (Compton et al. 1992a, b); cannabidiol, an inactive cannabinoid present in marijuana (Compton et al. 1990); and anandamide, a putative endogenous cannabinoid ligand (Devane et al. 1992). A second objective of this study was to investigate whether cannabinoids can impair working memory by direct action on the hippocampus. This hypothesis was tested by examining the impact of intrahippocampal administration of CP-55,940 on radial-maze performance. These animals were also evaluated for antinociception, catalepsy, and hypothermia in order to determine whether these other behavioral effects of cannabinoids are mediated by separate neuronatomical structures.

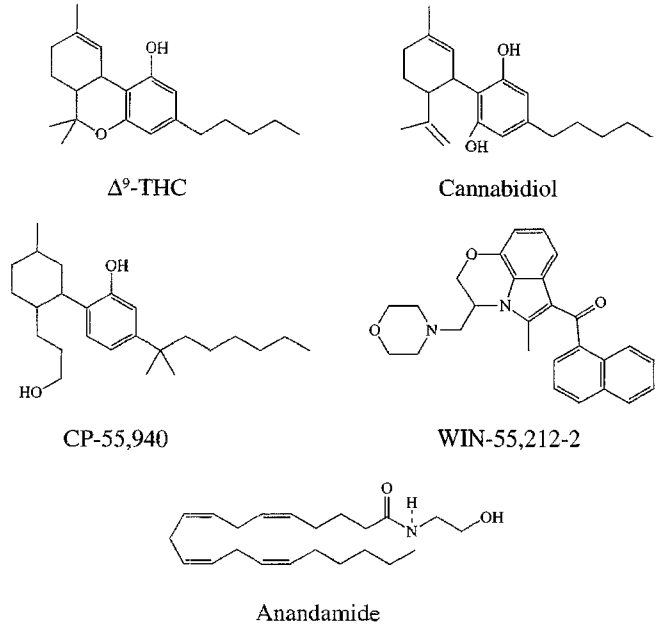

Fig. 1 Chemical structures of naturally occurring and synthetic cannabinoids that were evaluated for activity in the eight-arm radial-maze

Materials and methods

Subjects

Sprague-Dawley (Harlan, Ind.) male rats served as subjects and were individually housed in a temperature controlled (20–22 °C) environment with a 12-h light/dark cycle. Initially, 12 rats were trained in the maze and tested with Δ^9-THC; however, one of the animals was dropped from the study and not tested with any other cannabinoids due to respiratory virus. Subjects were placed on a food-restricted diet and maintained at a weight between 290 g and 300 g, approximately 85% of their free-feeding weight, for the duration of the experiment. Daily food rations (12–16 g, Prolab, Agway) were given immediately after each experimental session. Water was available ad libitum.

Drugs

Δ^9-THC and cannabidiol were provided by the National Institute on Drug Abuse, CP-55,940 was provided by Pfizer (Groton, Conn.) WIN-55,212-2 was obtained from (Sterling Winthrop, Albany, N.Y.), and anandamide was supplied by Organix (Woburn, Mass.). Each drug was dissolved in a 1:1 mixture of absolute ethanol and Emulphor-620 (Rhone-Poulenc, Princeton, N.J.) and diluted with saline to form a final vehicle mixture of ethanol:emulphor:saline (1:1:18). Intraperitoneal (IP) injections were given in a volume of 1 ml/kg and intrahippocampal injections were given bilaterally in a volume of 1 µl. In an initial experiment dimethylsulfoxide (DMSO) was employed as the vehicle for intrahippocampal injections. Because the DMSO vehicle alone severely disrupted maze performance, ethanol:emulphor:saline served as the vehicle for all intrahippocampal injections.

Surgery

Each animal was anesthetized with sodium pentobarbital (60 mg/kg) and two 23-gauge stainless-steel guide cannulae were implanted bilaterally into the hippocampus using a stereotaxic technique and bregma as the reference point . The coordinates were A/P–3.3 mm, L (+) and (–) 1.5 mm, and D/V –2.0 mm (Paxinos and Watson 1986) which resulted in the tip of the cannula being dorsal to the hippocampus. The subjects were given a 1-week recovery after cannulae implantation prior to maze training. For all intracerebral injection, 29-gauge stainless steel needles were inserted simultaneously into the bilateral cannulae and advanced 1.5 mm beyond the tip to sites within the hippocampus. The injection was of 1 min duration and the needles were removed 2 min after infusion. Each cannula was kept patent with a stainless steel obturator. At the conclusion of the study all animals were given a lethal dose of sodium barbital (100 mg/kg). A 1-µl atiquot of absolute

blue was microinjected through the cannulae to label the injection sites, and the brain was removed for histological examination. The bilateral placement sites were identified from 20-μm serial coronal sections. A total of six naive subjects were implanted with bilateral cannulae aimed at the dorsal hippocampus and tested with CP-55,940.

Apparatus

Radial arm maze

The description of equipment and training procedures were similar to that previously described (Olton 1987). The apparatus was made from plywood and consisted of a round central platform (34 cm in diameter) with eight radiating arms attached to the platform at equal distances from each other. The maze surface was coated with polyurethane and Whistle all purpose cleaner with ammonia was used to clean the apparatus prior to each subject's test. Each arm was 86 cm long, 9 cm wide, and surrounded by a wall 10 cm wall high. A guillotine door was attached to each run-way entrance. Two light sources from opposite corners of the room were used for illumination and visual cues. In addition, several other distinctive visual cues were located around the room.

Antinociception

The tail flick response to radiant heat (D'Amour and Smith 1941; Dewey et al. 1970) was used to assess antinociception. The intensity of the radiant heat source was set to elicit baseline latencies of 3–4 s, and an automatic 8-s cut-off was used.

Rectal temperature

Core temperature, to the nearest 0.1°C, was recorded by inserting a rectal probe connected to telethermometer (Yellow Spring Industries, Yellow Springs, Ohio) to a depth of 4.5 cm.

Ring immobility

A ring test procedure previously validated in mice (Pertwee 1972) was modified and automated to assess catalepsy in rats (Martin et al. 1992). Each subject was placed on a ring (13 cm diameter) which was elevated 38 cm from a table top. A CCD camera (Panasonic, BLV-200) was focused on the rat for a 5-mi n recording session. The videotape was transmitted to a Macintosh II microcomputer via a Scion Image-Capture 2 board at the speed of 30 frames/s in 256 shades of gray. The captured image was divided into 56 000 individual picture elements (pixels) which were then assigned value of either 0 (white) or 256 (black). An image was assessed approximately one frame per second. Each pixel from any given image was subtracted from the corresponding pixel from the previous frame and recorded by the computer to determine whether the animal was immobile. This objective measure of ring immobility has been demonstrated to have a 0.94 reliability coefficient with trained human observers (Martin et al. 1992).

Behavioral testing

During acquisition, the subjects had access to all eight-arms and a 45 mg Noyes pellet was placed 5 cm from the end. Training with guillotine doors began once subjects reliably obtained all food pellets within the 10-min test period. Each session began with the subject placed on the center platform with all doors down. Five seconds later all of the doors were raised. After a subject crossed the threshold into a run-way, all of the arms except the selected arm were blocked with the guillotine doors. The door to the recently visited arm was lowered once the subject returned to the center platform. Subjects were given additional trials until either all eight-arms were visited or 10 min had elapsed, whichever came first. The experimenter recorded which arm was visited and whether or not the pellet was consumed for each arm entrance, as well as the duration of the session. Subjects were defined as failing if two or more arms were revisited or an error of omission was committed (i.e., failure to visit all eight arms). Subjects became proficient in the task within 15–20 training sessions. Each rat was placed in the maze 20 min after drug or vehicle administration, with the exception of the anandamide study. A 10-min pretreatment period was used for anandamide because of its short duration of action (Deutsch and Chin 1993). For each drug, the order of dose administration was counterbalanced among subjects in a quasi Latin-square design to control for any order effects. Subjects were tested with either drug or vehicle after successfully completing the maze on 2 consecutive days. Consequently, the minimum interdose interval was never less than 72 h and generally was 1 week.

Preinjection tail-flick latencies and core temperature was first assessed in subjects given intrahippocampal infusions.Each subject was tested in the radial-maze 20 min after the intracerebral injection, tail-flick latencies and rectal temperature were assessed at 30 min, and catalepsy at 40 min.

Data analysis

Radial arm maze dependent measures included the number of errors committed, the total amount of time required to complete the maze, the total number of run-way arms entered, and the trial at which the first error occurred. The trial at which the first reentry occurred was determined by the following formula: 8 – number of arms remaining unvisited when the first error occurred; for example, an 8 indicated that no errors were committed, a7 indicated that the error was committed on the eighth selection, and a 1 meant that the first error was made on the second choice. A reciprocal transformation was performed on mean number of errors committed in the hippocampal study in order to reduce the heterogeneity of the variance (Winer 1971). Each of these indices was analyzed using ANOVA, and the Tukey test was used for post-hoc analysis when appropriate. Differences were considered significant at the $P < 0.05$.

The potency of cannabinoids in disrupting radialmaze performance was calculated by first converting the data to a nominal scale in which subjects were defined as passing if they visited all eight-arms with either one or no error. Conversely, subjects were designated as failing if they either reentered two or more arms, or committed an error of omission. The percent of animals that failed the test under each condition was determined and analyzed by the Cochran Q test (Siegel 1956); differences were considered significant at the $P < 0.05$ level. The ED_{50} values were then calculated (Litchfield and Wilcoxon 1949).

In order to assess the impact of cannabinoids on locomotor behavior, completion time for each drug that retarded maze completion time was first transformed to maximum possible effect by the following formula:

$$\%MPE = (\text{drug maze time} - \text{vehicle maze time}) \div (10 \text{ min} - \text{vehicle maze time}) \times 100$$

The ED_{50} values were then calculated for the graded data (Tallarida and Murray 1987).

Separate ANOVAs were conducted on tail-flick, rectal temperature, and immobility data. Rectal temperature data were expressed as the difference between post- and pre-injection values (°C). Catalepsy data were expressed as the mean ring immobility

time (s). Tail-flick response latencies were expressed as %MPE by the following equation:

$$\%MPE = (\text{test latency} - \text{control latency}) \div (\text{cut-off-time} - \text{control latency}) \times 100$$

Results

The percent of animals that failed to meet criteria in the maze after systemic administration of Δ^9-THC, CP-55,940, or WIN-55,212-2 or intrahippocampal CP-55,940 is depicted in Table 1. The effects of all drug treatments were reversible as no residual effects were observed in training sessions following a test day. None of the subjects failed after IP injections of the vehicle. The ED_{50} values of each of the treatments in impairing memory and retarding maze completion time are shown in Table 2. Systemic administration of Δ^9-THC, CP-55,940, and WIN-55,212-2 increased both failure rate and the time required to complete the maze. In contrast, intrahippocampal CP-55,940 increased failure rate, but had no impact on maze completion time.

Table 1 Percent of rats that failed to reach criteria in the eight-arm radial-maze after IP or intrahippocampal administration of various cannabinoids

Drug	Route of administration	Percent failed
Δ^9-THC (mg/kg), n = 12	IP	
0		0
1		25
3		50
5.6		92
CP-55,940 (mg/kg), n = 11	IP	
vehicle		0
0.125		27
0.15		73
0.18		82
0.25		82
WIN-55,212-2 (mg/kg), n = 11	IP	
vehicle		0
1		27
3		55
5.6		82
10		82
CP-55,940 (μg/rat), n = 6	intrahippocampal	
vehicle		17
5		33
10		50
20		100

Table 2 Cannabinoid potency in eliciting maze failure and retarding the time required to complete the maze

Drug	Route of administration	Failing maze ED_{50} values	Retarding maze completion ED_{50} values
Δ^9-THC	IP	2.1 mg/kg	5.3 mg/kg
CP-55,940	IP	0.13 mg/kg	0.20 mg/kg
WIN-55,212-2	IP	2.2 mg/kg	2.1 mg/kg
CP-55,940	intrahippocampal	8 μg/rat	no effect

Systemic administration

Δ^9-THC significantly increased the percent of animals that failed the maze [$Q(3) = 15.8$, $P < 0.05$]. Similarly, it significantly increased the number of arms revisited [$F(3,33) = 5.1$, $P < 0.05$; Fig. 2a]. Subjects committed significantly more errors when treated with 3 mg/kg or 5.6 mg/kg Δ^9-THC than the vehicle ($P < 0.05$). Δ^9-THC also reduced the number of arms visited before the first error was committed [$F(3,33) = 8.3$, $P < 0.05$; Fig. 2b]. The two highest doses resulted in the first reentry occurring significantly earlier than after vehicle treatment ($P < 0.05$). In addition, the 5.6 and 1 mg/kg doses significantly differed from each other ($P < 0.05$). Δ^9-THC significantly increased the amount of time required to complete the task [$F(3,33) = 7.0$, $P < 0.05$; see Fig. 2c]. Subjects required significantly more time to complete the maze after treatment with 5.6 mg/kg than after treatment with either vehicle or 1 mg/kg Δ^9-THC ($P < 0.05$). Finally, Δ^9-THC had no impact on the total number of arms visited during the 10-min test [$F(3,33) = 1.5$, > 0.20; see Fig. 2d].

A significant increase in the percent of animals that failed the maze occurred after administration of CP-55,940 [$Q(4) = 21.5$, $P < 0.05$]. As shown in Fig. 2a, CP-55,940 significantly increased the number of errors committed [$F(4,40) = 5.0$, $P < 0.05$]. Subjects

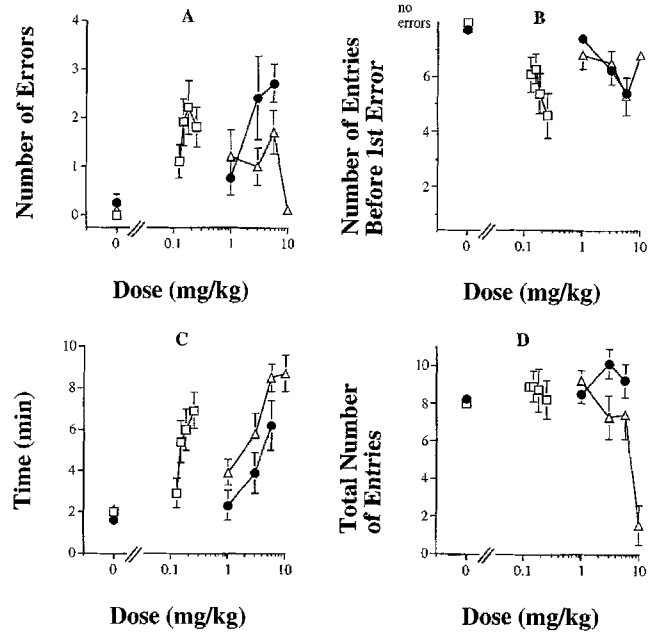

Fig. 2 The effects of IP administered Δ^9-THC (—•—), CP-55,940 (—□—), and WIN-55,212-2 (—Δ—) on eight arm radial-maze performance. All results are presented as means ± SEM **a** The number of errors committed during testing. **b** The trial at which the first error occurred. **c** The amount of time required to complete the maze. **d** The total number of arms visited during each test

committed significantly more errors after treatment with 0.15, 0.18, and 0.25 mg/kg CP-55,940 than the vehicle treatment ($P < 0.05$). CP-55,940 also reduced the number of arms visited before the first error occurred [$F(4,40) = 4.6$, $P < 0.05$; Fig. 2b]. The first error was committed significantly earlier after treatment with either 0.18 or 0.25 mg/kg CP-55,940 than the vehicle condition ($P < 0.05$). CP-55,940 significantly increased the amount of time required to complete the maze [$F(4,40) = 7.7$, $P < 0.05$; Fig. 2c]. Subjects required significantly more time to complete the maze after treatment with either 0.15, 0.18, or 0.25 mg/kg of drug than vehicle ($P < 0.05$). In addition, the 0.18 and 0.25 mg/kg doses significantly retarded test time duration when compared to the 0.125 mg/kg dose ($P < 0.05$). The total number of arms visited was not affected by CP-55,940 [$F(4,40) = 0.4$, $P > 0.20$; Fig. 2d].

WIN-55,212-2 significantly increased the percent of animals that failed the maze [$Q(4) = 20.4$, $P < 0.05$]. Illustrated in Fig. 2a–d are the effects of WIN-55,212-2 on maze performance. The drug had a significant effect on the total number of reentries committed [$F(4,40) = 4.4$, $P < 0.05$]. Subjects committed more errors after an injection with 5.6 mg/kg WIN-55,212-2 than either vehicle treatment or an injection of 10 mg/kg ($P < 0.05$). The drug also had a significant effect on the number of arms that was visited before the first error was committed [$F(4,40) = 5.7$, $P < 0.05$]. The mean trial at which a reentry occured after treatment with 5.6 mg/kg was 6.3 ± 0.7, significantly earlier than the vehicle, 1 mg/kg, and 10 mg/kg values ($P < 0.05$). In addition, the amount of time required to complete the maze was increased in a dose-related manner by the drug [$F(4,40) = 17.86$, $P < 0.05$]. Subjects spent significantly more time in the maze with 3, 5.6 and 10 mg/kg doses of WIN-55,212-2 tested than vehicle ($P < 0.05$). Also, 10 mg/kg WIN-55,212-2 significantly differed from the 3 mg/kg dose. Finally, the total number of arms entered was significantly effected by the drug [$F(4,40) = 12.5$, $P < 0.05$]. Rats treated with 10 mg/kg WIN-55,212-2 entered only 1.5±1 arms, significantly fewer arms than after treatment with each of the other conditions ($P < 0.05$). However, after a 10 mg/kg injection of WIN-55,212-2 subjects exhibited virtually no activity throughout the duration of the test and thus failed to make criteria because of errors of omission. Consequently, it is difficult to distinguish between memory deficits and generalized decreases in performance after treatment with high doses of WIN-55,212-2.

As can be seen in Table 3, neither anadamide nor cannabidol increased the percent of animals failing the task ($P > 0.60$ for each). Cannabidiol failed to alter maze performance ($P > 0.50$ for each measure). Similarly, anandamide, had few significant effects on maze performance. It had no impact on the number of errors committed, the trial at which the first error was committed, and the total number of arms entered ($P > 0.50$ for each measure). Anandamide did, however, significantly increase the amount of time required to complete the task [$F(2,20) = 3.9$, $P < 0.05$]. The time to complete the maze was significantly longer after an injection of 30 mg/kg anandamide than vehicle ($P < 0.05$).

Table 3 The effects of cannabidiol and anandamide on eight-arm radial maze performance, $n = 11$

Drug	Percent fail	Number of choices[a]	Time(min)[a]
Cannabidiol			
10 mg/kg	0	8.0 + 0.0	2.2 ± 0.1
30 mg/kg	0	8.1 ± 0.1	2.2 ± 0.1
Anandamide			
0 mg/kg	0	8.1 ± 0.1	2.3 ± 0.1
10 mg/kg	9.1	8.0 ± 0.1	4.1 ± 0.7
30 mg/kg	9.1	7.8 ± 0.4	4.7 ± 0.8*

* Significantly different from vehicle, $P < 0.05$
[a] Values presented as mean ± SEM

Intrahippocampal CP-55940

The intrahippocampal injection sites were located in the various fields of Ammon's horn as well as in the dentate gyrus (Fig. 3a). Figure 3b depicts a representative brain section containing the bilateral cannulae tracts from one of the subjects in the study. CP-55,940 microinjected directly into the hippocampus significantly increased the percent of subjects failing the maze in a dose-related fashion [$Q(3) = 8.4$, $P < 0.05$; Table 1]. There was also a significant effect on the number of errors committed during testing [$F(3,15) = 4.6$, $P < 0.05$; Fig. 4a]. More reentries occurred after the highest dose of CP-55,940 (20 µg) than the vehicle ($P < 0.05$). CP-55,940 significantly decreased the trial at which the first error occurred [$F(3,15) = 5.2$, $P < 0.05$; Fig. 4b]. Subjects committed the first error at an earlier trial after treatment with 20 µg CP-55,940 than vehicle ($P < 0.05$). As shown in Fig. 4c, however, intracerebral administration of CP-55,940 had no impact on either the amount of time to complete the maze (left ordinate) or the number of arms visited (right ordinate) [$F(3,15) = 0.6$, $P > 0.20$ and $F(3,15) = 1.6$, $P > 0.20$, respectively]. Finally, intrahippocampal CP-55,940 (see Table 4) also failed to

Table 4 Intrahippocampal CP-55,940 failed to produce antinociception, catalepsy, or hypothermia. All values are presented as mean ± SEM $n = 6$

CP-55,940 (µg/rat)	Tail-flick test (%MPE)	Ring immobility (sec)	Change in rectal temperature (°C)
0	3 ± 9	12 ± 4	0.8 ± 0.2
5	0 ± 3	35 ± 17	0.6 ± 0.0
10	5 ± 2	8 ± 5	0.6 ± 0.1
20	8 ± 5	15 ± 5	0.7 ± 0.1

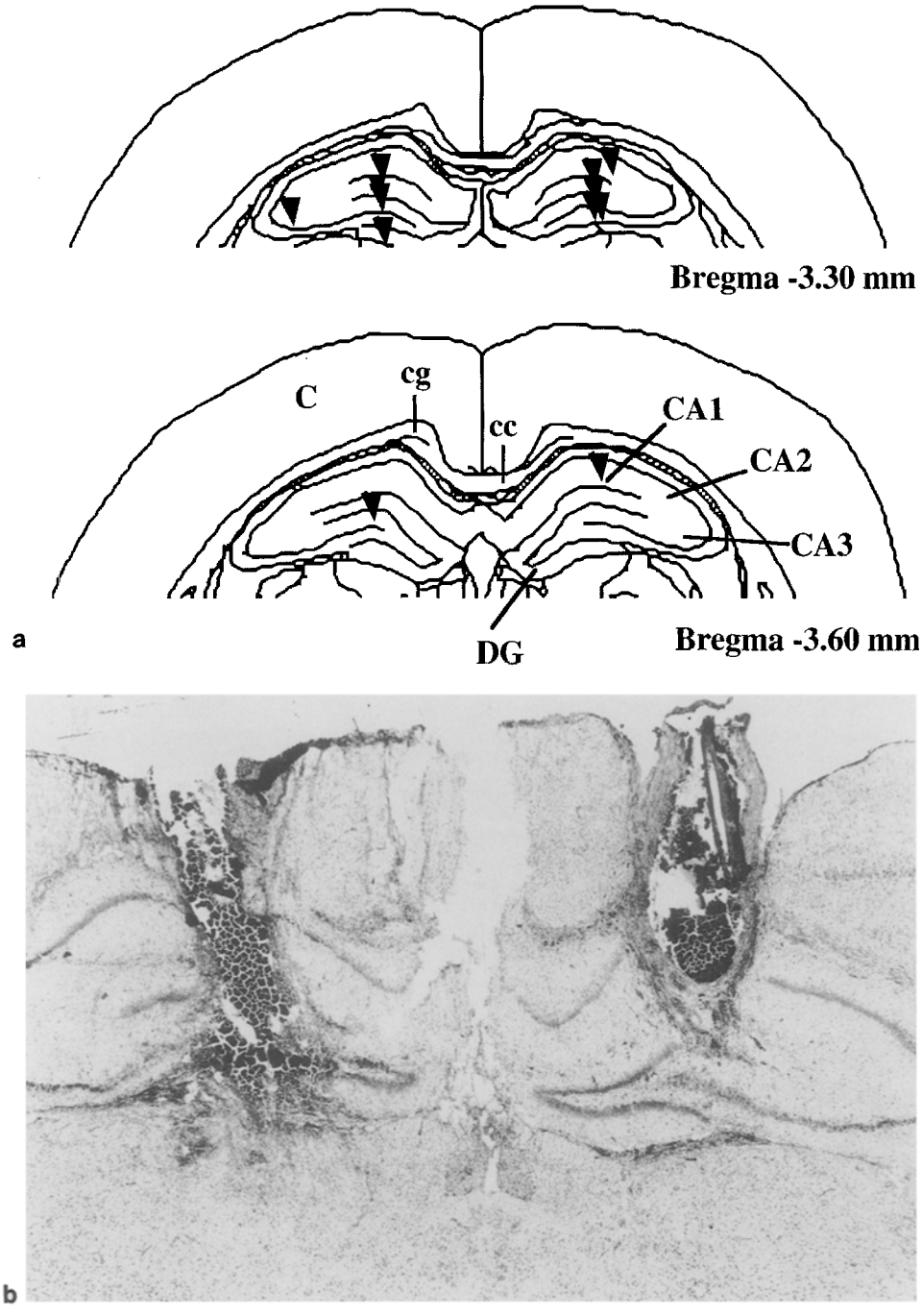

Fig. 3a Schematic representation of the intrahippocampal injection sites (▼). *C* cortex, *CA1* fields *CA1-3* of Ammon's horn, *cc* corpus callosum, *cg* cingulum, *DG* dentate gyrus (Bloom et al. 1989). **b** Photomicrograph of a representative intrahippocampal bilateral cannulae placement. The tissue was stained in cresyl violet

produce antinociception [$F(3,15) = 0.40$, $P > 0.50$], catalepsy [$F(3,15) = 2.1$, $P = 0.14$], or hypothermia [$F(3,15) = 0.2$, $P > 0.5$].

Discussion

Acute administration of a single dose of Δ^9-THC has been previously reported to produce a small, but significant increase in the number of errors committed in the radial-maze (Nakamura et al. 1991). In addition to confirming this finding, we report that systemic administration of Δ^9-THC as well as the synthetic analogs CP-55,940 and WIN-55,212-2 disrupted maze performance in a dose-dependent fashion. In contrast, neither anandamide, the putative endogenous cannabinoid ligand, nor cannabidiol affected maze performance. Finally, intracerebral administration of CP-55,940 into the dorsal hippocampus also impaired choice accuracy in the maze. Cannabinoid-induced disruption of maze performance was reversible, as no residual effects of drug treatment were observed in subsequent training sessions. These results suggest that systemic administration as well as intrahippocampal infusion of cannabinoids acutely impair working memory.

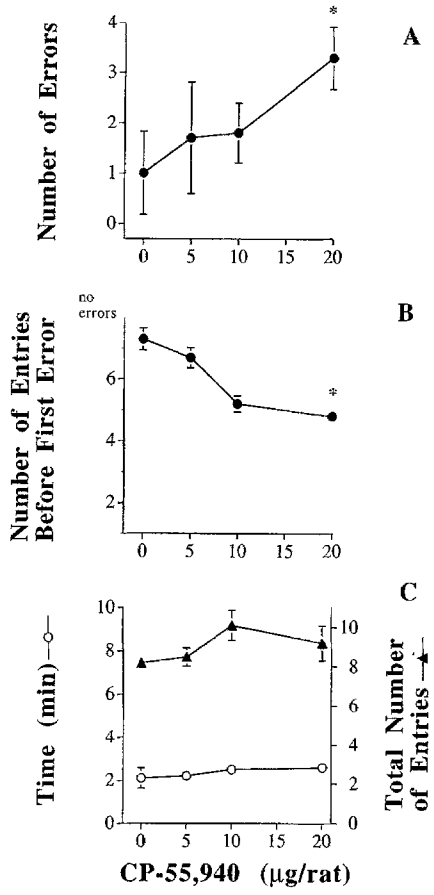

Fig. 4 The effects of intracerebral administration of CP-55,940 into the dorsal hippocampus on eight arm radial-maze performance. All results are presented as means ± SEM, $n = 6$. **a** Number of errors committed during testing. **b** The trial at which the first error occurred. **c** *Left ordinate*: the amount of time required to complete the maze. *Right ordinate*: the total number of arms visited during each test. * Significantly different from vehicle ($P < 0.05$)

Alternatively, the effects of cannabinoids in the radial-arm maze may not reflect memory impairment directly, but may have resulted from deficits in either locomotor activity or motivation. For example, the known effect of cannabinoids on locomotor activity may have had an impact on the rats' ability to run the maze. On the other hand, the number of reentries may have increased because the drugs may have reduced either the salience of the food reinforcement or hunger. Although it is difficult to rule out motivational factors, the food pellet at the end of each of the runway arms was always consumed whenever an arm was selected regardless of drug treatment. In addition, food rations were consumed after drug treatment and body weight was unaffected by drug treatment. A third alternative explanation is that cannabinoids impaired choice accuracy and hence memory indirectly by increasing the amount of time between choices. In other words, the locomotor suppressive effects of cannabinoids may have increased the difficulty of the task independently of direct drug effects on memory by delaying the animal's choice of arm entry. However, several observations indicate that cannabinoid impairment of maze performance can be dissociated from the inhibition of locomotor activity. Most notably, intracerebral administration of CP-55,940 into the hippocampus impaired choice accuracy without retarding the time required to complete the maze. In addition, although WIN-55,212-2 elicited maze failure and retarded the time required to complete the maze at equivalent ED_{50} values, Δ^9-THC and CP-55,940 were approximately twice as potent in eliciting maze failure than locomotor suppression. Conversely, anandamide had no apparent effects on memory even though it significantly increased the amount of time required to complete the maze. Similarly, using a low dose of Δ^9-THC (1.25 mg/kg) Nakamura et al. (1991) found a small but significant increase in errors with no apparent effects on locomotor activity. Although both locomotor and motivational effects undoubtedly can influence radial-maze performance, the most parsimonious explanation for the results in the present study is that cannabinoids impaired working memory independently of any effects on motor abilities.

Many of the pharmacological effects produced by cannabinoids appear to be mediated by cannabinoid receptors in the CNS (Thomas et al. 1991; Compton et al. 1993). The results of the present study in which cannabinoids impaired choice accuracy in the eight-arm radial-maze are generally consistent with a receptor mechanism of action. As indicated by their ED_{50} values (Table 2), CP-55,940 was approximately 16–fold more potent than both Δ^9-THC and WIN-55,212-2. Similarly, CP-55,940 has been shown to be considerably more potent than Δ^9-THC in binding affinity (Compton et al. 1993), drug discrimination in rats (Gold et al. 1992), and in a combination of unconditioned behaviors in mice, including antinociception, locomotor activity, catalepsy, and hypothermia (Compton et al. 1992a, b). The failure of the naturally occurring cannabidiol to produce any effects in the radial-maze is consistent with its inactivity in in vitro and in vivo tests. It neither binds to the cannabinoid receptor (Compton et al. 1993) nor inhibits forskolin-stimulated adenylyl cyclase (Howlett and Fleming 1984). Similarly, it is inactive in a variety of behavioral tests and does not generalize to Δ^9-THC in the drug discrimination paradigm (Jarbe et al. 1986). On the other hand, WIN-55,212-2 (Jansen et al. 1992) has been found to be more potent than Δ^9-THC (Compton et al. 1993) in relative binding affinity as well as in some, but not all, behavioral tests (Compton et al. 1992a, b).

The virtual lack of activity after anandamide administration was surprising. The effects of anandamide in both in vitro and in vivo assays suggest that it produces cannabinoid effects. Anandamide binds to the cannabinoid receptor (Devane et al. 1992), inhibits forskolin-stimulated adenylyl cyclase (Vogel et al. 1993), and produces similar behavioral effects as other

cannabinoids in mice (Fride and Mechoulam 1993). However, anandamide was also inactive in the delayed nonmatch to sample memory task in rats (Crawley et al. 1993). Thus, the activity of anandamide may be species specific; Mechoulam has also found it to lack cannabinoid properties in rats (personal communication). Furthermore, the rapid degradation of anandamide in cells and tissues (Deutsch and Chin 1993) may have accounted for its lack of activity in the present study, as well.

Cannabinoids are known to produce a wide range of pharmacological effects, some of which suggest potential therapeutic value (Dewey 1986; Martin 1986). Although the cannabinoids possess analgesic (Noyes et al. 1975), antiemetic (Gralla et al. 1984), antiglaucoma (McLaughlin and Chiou 1985), and anticonvulsive (Martin et al. 1987) properties, their disruptive effects on information processing and storage may limit their clinical utility. The radial-maze could be a useful tool in identifying and characterizing the untoward effects (e.g., cognitive dysfunction) of potential clinical agents such as the cannabinoids. For example, the data from the present study suggest that anandamide or other naturally occurring cannabinoids (Hanus et al. 1993) may be useful agents if they possess therapeutically beneficial characteristics without impairing memory.

The results of the present study suggest that cannabinoids can act directly in the hippocampus to impair working memory. It is unknown, however, whether cannabinoids act solely in the hippocampus to disrupt maze performance or multiple brain areas contribute to this effect. Another issue which merits further investigation is the relationship between cannabinoid systems and other neurochemical systems on memory. For example, there is strong evidence suggesting the importance of central cholinergic systems in radial-maze performance. Cholingeric antagonists have long been known to impair memory as assessed in the radial-maze (Olton 1987; Levin 1988). Moreover, the profound disruption in maze performance caused by cholinergic denervation of the hippocampus can be ameliorated by the administration of muscarinic agonists (Murray and Fibiger 1985; Tilson et al. 1988; McGurk et al. 1991).

In conclusion, the results of the present study support previous observations in which cannabinoids were found to impair memory in rats (Carlini et al. 1970; Nakamura et al. 1991; Heyser et al. 1993). The dose-related disruption in choice accuracy elicited by intrahippocampal CP-55,940 is the first evidence implicating direct hippocampal involvement in cannabinoid-induced memory impairment. The effect appears to be specific to choice accuracy because the amount of time required to complete the maze was unaffected. Conversely, intrahippocampal injections of CP-55,940 failed to alter tail-flick responding, rectal temperature, or ring immobility. This dissociation of choice accuracy in the radial-maze from other cannabinoid pharmacological effects suggests that the hippocampus may play an important role in the cognitive alterations produced by cannabinoids independent of other cannabinoid effects.

Acknowledgements The authors thank Dr. Colleen R. McLaughlin for her constructive comments on an earlier version of this manuscript. The authors also appreciate the assistance of Dr. Linda L. Phillips and Ted Velardo for their efforts in preparing the photomicrograph. This research was supported by NIDA grants DA-03672 and DA-08387.

References

Abel EL (1971) Retrieval of information after use of marihuana. Nature 231:58

Bloom FE, Young WG, Kim YM (1989) Brain Browser™. Academic Press, San Diego

Carlini EA, Hamaoui A, Bieniek D, Korte F (1970) Effects of (–)Δ^9-trans-tetrahydrocannabinol and a synthetic derivative on maze performance of rats. Pharmacology 4:359–368

Compton DR, Little PJ, Martin BR, Gilman JW, Saha JK, Jorapur VS, Sard HP, Razdan RK (1990) Synthesis and pharmacological evaluation of amino, azido, and nitrogen mustard analogues of 10-substituted cannabidiol and 11- or 12-substituted Δ^8-tetrahydrocannabinol. J Med Chem 33:1437–1443

Compton DR, Gold LH, Ward SJ, Balster RL, Martin BR (1992a) Aminoalkylindole analogs: cannabimimetic activity of a class of compounds structurally distinct from Δ^9-tetrahydrocannabinol. J Pharmacol Exp Ther 263: 1118–1126

Compton DR, Johnson MR, Melvin LS, Martin BR (1992b) Pharmacological profile of a series of bicyclic cannabinoid analogs—classification as cannabimimetic agents. J Pharmacol Exp Ther 260: 201–209

Compton DR, Rice KC, Costa BRD, Razdan RK, Melvin LS, Johnson MR, Martin BR (1993) Cannabinoid structure-activity relationships: correlation of receptor binding and in vivo activities. J Pharmacol Exp Ther 265:218–226

Crawley JN, Corwin RL, Robinson JK, Felder CC, Devane WA, Axelrod J (1993) Anandamide, an endogenous ligand of the cannabinoid receptor, induces hypomotility and hypothermia in vivo in rodents. Pharmacol Biochem Behav 46:967–972

D'Amour FE, Smith DL (1941) A method for determining loss of pain sensation. J Pharmacol Exp Ther 72:74–79

Deutsch DG, Chin SA (1993) Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. Biochem Pharmacol 46:791–796

Devane WA, Dysarz IFA, Johnson MR, Melvin LS, Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 34:605–613

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258:1946–1949

Dewey WL (1986) Cannabinoid pharmacology. Pharmacol Rev 38:151–178

Dewey WL, Harris LS, Howes JF, Nuite JA (1970) The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. J Pharmacol Exp Ther 175: 435–442

Evans (1992) Effects of drugs of abuse on acquisition of behavioral chains in squirrel monkeys. Psychopharmacology 107:55–60

Ferraro DP, Grilly DM (1973) Lack of tolerance to Δ^9-tetrahydrocannabinol in chimpanzees. Science 179:490–492

Fride E, Mechoulam R (1993) Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. Eur J Pharmacol 231:313–314

Gold LH, Balster RL, Barrett RL, Britt DT, Martin BR (1992) A comparison of the discriminative stimululs properties of Δ^9-THC and CP-55,940 in rats and rhesus monkeys. J Pharmacol Exp Ther 262:479–486

Gralla RJ, Tyson LB, Bordin LA, Clark RA, Kelsen DP, Kris MG, Kalman LB, Groshen S (1984) Antiemetic therapy:a review of recent studies and a report of a random assignment trial comparing metoclopramide with Δ^9-tetrahydrocannabinol. Cancer Res Rep 68:163–172

Hanus L, Gopher A, Almog S, Mechoulam R (1993) Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. J Med Chem 36:3032–3034

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC (1991) Characterization and localization of cannabinoid receptors in rat brain: A quantative in vitro autoradiographic study. J Neurosci 11:563–583

Heyser CJ, Hampson RE, Deadwyler SA (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

Howlett AC, Fleming RM (1984) Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. Mol Pharmacol 26:532–538

Jansen EM, Haycock DA, Ward SJ, Seybold VS (1992) Distribution of cannabinoid receptors in rat brain determined with aminoalkylindoles. Brain Res 575:93–102

Jarbe TU, Hiltunen AJ, Lander N, Mechoulam R (1986) Cannabimimetic activity (delta 1-THC cue) of cannabidiol monomethyl ether and two stereoisomeric hexahydrocannabinols in rats and pigeons. Pharmacol Biochem Behav 25:393–399

Levin ED (1988) Psychopharmacological effects in the radial-arm maze. Neurosci Biobehav Rev 12:168–175

Litchfield JT, Wilcoxon F (1949) A simplified method of evaluating dose-effect experiments J Pharmacol Exp Ther 96:99–113

Mailleux P, Verslijpe M, Vanderhaeghen JJ (1992) Initial observations on the distribution of cannabinoid receptor binding sites in the human adult basal ganglia using autoradiography. Neurosci Lett 139:7–9

Margulies JE, Hammer RP (1991) Δ^9-tetrahydrocannabinol alters cerebral metabolism in a biphasic, dose-dependent manner in rat brain. Eur J Pharmacol 202:373–378

Martin AR, Consroe P, Kane VV, Shah V, Singh V, Lander N, Mechoulam R, Srebnik M (1987) Structure-anticonvulsant activity relationships of cannabidiol analogs. In:Rapaka RA, Makriyannis A (eds) Structure-activity relationships of the cannabinoids. NIDA Res Monogr pp 48–58

Martin BR (1986) Cellular effects of cannabinoids. Pharmacol Rev 38: 45–74

Martin BR, Prescott WR, Zhu M (1992) Quantitation of rodent catalepsy by a computer-imaging technique. Pharmacol Biochem Behav 43:381–386

McGurk SR, Levin ED, Butcher LL (1991) Impairment of radial-arm maze performance in rats following lesions involving the cholinergic medial pathway:reversal by arecoline and differential effecs of muscarinic and nicotinic antagonists. Neuroscience 44:137–147

McLamb RL, Mundy WR, Tilson HA (1988) Intradentate colchicine disrupts the acquisition and performance of a working memory task in the radial-arm maze. Neurotoxicology 9:521–528

McLaughlin MA, Chiou GC (1985) A synopsis of recent developments in antiglaucoma drugs. J Ocul Pharmacol 1:101–121

Murray CL, Fibiger BC (1985) Learning and memory defecits after lesions of the nucleus basalis magnocellularis:reversal by physostigmine. Neuroscience 14:1025–1032

Nakamura EM, da Silva EA, Concilio GV, Wilkinson DA, Masur J (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

Norwicky AV, Teyler TJ (1987) The modulation of long-term potentiation by delta-9-tetrahydrocannabinol in the rat hippocampus, in vitro. Brain Res Bull 19:663–672

Noyes JR, Brunk SF, Avery DH, Canter A (1975) The analgesic properties of delta-9-tetrahydrocannabinol and codeine. Clin Pharmacol Ther 18:84–89

Olton DS (1987) The radial arm maze as a tool in behavioral pharmacology. Physiol Behav 40:793–797

Olton DS, Werz MA (1978) Hippocampal function and behavior:spatial discrimination and response inhibition. Physiol Behav 20:597–605

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates

Pertwee RG (1972) The ring test:a quantitative method for assessing the "cataleptic" effect of cannabis in mice. Br J Pharmacol 46:753–763

Scallet AC, Uemura E, Andrews A, Ali SF, McMillan DE, Paule MG, Brown RM, Slikker W (1987) Morphometric studies of the rat hippocampus following chronic delta-9-tetrahydrocannabinol (THC). Brain Res 436:193–198

Siegel S (1956) Nonparametric statistics for the behavioral sciences 159–166

Tallarida RJ, Murray RB (1987) Graded dose-response. Manual of Pharmacologic Calculations 26–31

Thomas BF, Compton DR, Martin BR, Semus SF (1991) Modeling the cannabinoid receptor:a three-dimensional quantitative structure-activity analysis. Mol Pharmacol 40:656–665

Thomas BF, Wei X, Martin BR (1992) Characterization and autoradiographic localization of the cannabinoid binding site in rat brain using [^{3}H]11-OH-Δ^9-THC-DMH. J Pharmacol Exp Ther 263:1383–1390

Tilson HA, McLamb RL, Shaw S, Rogers BC, Pediatitakis P, Cook L (1988) Radial-arm maze defecitis produced by colchisine administered into the area of the nucleus basalis are ameliorated by cholinergic agents. Brain Res 438:83–94

Vogel Z, Berg J, Levy R, Saya D, Heldman E, Mechoulam R (1993) Anandamide, a brain endogenous compound, interacts specifically with cannabinoid receptors and inhibits adenylate cyclase. J Neurochem 61:352–355

Winer BJ (1971) Choice of scale and transformations. In:(eds.) Statistical principles in experimental design. McGraw-Hill, NY, pp 397–402