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Long-term cognitive deficits induced by a single, extremely low dose of tetrahydrocannabinol (THC): Behavioral, pharmacological and biochemical studies in mice

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Abstract

We have previously reported that an injection of a single, extremely low dose (0.001 mg/kg) of Δ^9-tetrahydrocannabinal (THC, the major psychoactive ingredient of marijuana) to mice deteriorated their performance in the Morris water maze test 3 weeks later. In the present study we verify our original findings and show that the long-term cognitive deficits that are induced in mice by a low dose of THC are even more pronounced in another behavioral test—the water T-maze. This effect was abolished by the CB1 receptor antagonist SR141716A, indicating the involvement of CB1 receptors. In an attempt to find a biochemical correlate to these deleterious consequences of such a low dose of THC, we investigated its effect on the activation of extracellular signal-regulated kinase (ERK1/2) in the cerebellum and hippocampus of the mice, two brain regions that were shown to participate in spatial learning. A significant increase in ERK1/2 phosphorylation was found in the cerebellum of mice 24 h following the injection of 0.001 mg/kg THC. These findings lead to further studies into the neuronal mechanisms underlying the long-term deleterious effects of THC and should be taken into consideration when evaluating the therapeutic benefits of cannabinoid drugs.

Keywords: Δ^9-tetrahydrocannabinol (THC); Cannabinoid; CB1 receptor; SR141716A; Cognitive deficits; Water T-maze; Extracellular signal-regulated kinase (ERK)

1. Introduction

Cannabis is the third most commonly used recreational drug after tobacco and alcohol (Baker et al., 2003; Solowij et al., 2002) and is largely regarded as a mild, "soft" drug. While the acute neurobehavioral effects of cannabis are well characterized, there are conflicting reports on the long-term effects of chronic use of cannabis on cognitive functions. Some studies did not find any long-lasting deleterious effects of chronic use of cannabis (Grant et al., 1973; Lyketsos et al., 1999), while other studies reported impairment in specific functions such as attention, memory and executive function (Block 1996; Ehrenreich et al., 1999; Pope, Yurgelun-Todd 1996; Solowij et al., 1995). Due to the limitations of performing intervention studies in human subjects, there is still a debate concerning the validity of these findings, since the results could be attributed to other factors such as drug residues, abstinence effects or methodological drawbacks (Fried et al., 2005; Grant et al., 2003; Solowij et al., 2002). In a previous meta-analysis study, the non-acute neuro-cognitive effects of cannabis use were examined. The study concluded that there might be minor deficits in the ability to learn and remember new information in chronic users, but no other cognitive abilities were affected (Grant et al., 2003). Other studies, that used functional magnetic resonance imaging (fMRI) techniques, have demonstrated that even when no difference was found in the performance of memory or attention tasks between chronic cannabis users and control subjects, there was nonetheless a difference in brain activity. It was found that cannabis users recruited more brain regions compared to control subjects in order to perform similar tasks (Jager et al., 2006; Kanayama et al., 2004), a finding that points to the possibility that subtle cognitive deficits may exist, but are overcome by increasing brain activity.

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Animal studies enable a more direct examination of this issue. Chronic in-vivo exposure to cannabinoid drugs has consistently produced cognitive deficits: chronic treatment of rats with Δ^9-tetrahydrocannabinol (THC), the major psychoactive component of *Cannabis sativa* L., resulted in persistent reduction in maze learning (Fehr et al., 1976; Stiglick, Kalant 1982a; Stiglick, Kalant 1983) and in differential reinforcement responding (Stiglick, Kalant 1982b); repeated exposure to the cannabinoid agonist CP55940 during perinatal, adolescent or early adult ages produced similar long-lasting deficits in working memory and social interaction in rats (O'Shea et al., 2006); and chronic exposure of pregnant rats to a cannabinoid agonist induced a disruption in memory retention in their 40- and 80-day-old offspring (Mereu et al., 2003). These deficits could result from neurotoxic effects in the hippocampus, that were induced by the chronic cannabinoid administration, as they resembled deficits that were found in rats with hippocampal lesions (Morris et al., 1982). This assumption was strengthened by the demonstration of morphological changes in the hippocampus of rats that were chronically treated with cannabinoids, including neuronal death and reduced synaptic density and dendritic length of pyramidal neurons (Landfield et al., 1988; Lawston et al., 2000; Scallet 1991; Scallet et al., 1987). In contrast to these chronic experiments, acute studies suggested that cannabinoids may have neuroprotective properties, and can serve as neuroprotective drugs (for reviews see (Guzman et al., 2002; Mechoulam et al., 2002a; Mechoulam et al., 2002b; Sarne, Mechoulam 2005)): Acute administration of the cannabinoid agonist WIN-55,212-2 was found to protect against global and focal ischemic damage in the hippocampus and cortex (Nagayama et al., 1999); application of THC (van der Stelt et al., 2001a), or of the endocannabinoid anandamide (van der Stelt et al., 2001b), was found to reduce the infarct volume through a CB1-dependent mechanism in an in-vivo model of ouabain-induced excitotoxicity; the endocannabinoid 2-arachidonoyl glycerol (2-AG) was found to reduce brain edema and infarct volume following severe closed head injury (Panikashvili et al., 2001); and the CB1/CB2 agonist BAY 38-7271 demonstrated neuroprotective properties against traumatic brain injury and focal ischemia in rats (Mauler et al., 2003). It was even suggested that the endogenous cannabinoid system may have a physiological role in neuroprotection (Guzman et al., 2001; Marsicano et al., 2003; Mechoulam et al., 2002a).

In order to elucidate these seemingly contradictory effects of cannabinoid drugs, we have presented a hypothesis that offered an explanation to the conflicting findings (Sarne, Keren 2004). The hypothesis was based on our previous in-vitro findings regarding the dual dose-dependent effects of cannabinoids on intracellular calcium (Rubovitch et al., 2002) and on pharmacokinetic in-vivo considerations (Agurell et al., 1986). The hypothesis predicted that high concentrations of cannabinoids will be neuroprotective within a limited timeframe, while very low doses of the drugs will induce neuronal death. According to this hypothesis, an acute dose of a cannabinoid drug results in a high concentration of the drug close to the time of trauma and therefore will protect the brain, while chronic use of cannabinoids exposes the organism to low concentrations of the drugs for long periods of time (due to the slow clearance of the lipophilic cannabinoid drugs) when minor neuronal deficit may accumulate.

In a recent study we have tested the fundamental claim of this hypothesis, namely, that a low dose of THC is expected to induce neuronal damage. We found, indeed, that a single extremely low dose of THC (0.001 mg/kg, a dose that is 3–4 orders of magnitude lower than the dose that produces the known acute effects of the drug in mice) significantly deteriorated the performance of mice in the Morris water maze, an effect that persisted for at least 3 weeks (Tselnicker et al., 2007). The effect of this low dose of THC was reproducible but mild, and required the testing of large groups of mice in order to reach statistical significance. This drawback made the experimental setup impractical for conducting pharmacological studies in order to pursue this initial finding. We therefore attempted to find a more sensitive behavioral test. Furthermore, because that was the first study that showed a long-term deleterious effect of a single low dose of THC on cognitive functions, and due to the extremely important implications of this finding, it was important to examine this effect in another behavioral test.

In the present study we show that the deleterious effect of a single low dose of THC can also be observed using a different behavioral assay — the water T-maze. The effect of THC in the T-maze test was statistically significant even when small groups of mice were used. Thus, this behavioral assay enabled the performance of pharmacological studies on the deteriorating effects of low doses of THC. Hence, we demonstrate here that the ability of a single low dose of THC to induce cognitive deficits was blocked by the CB1 receptor antagonist SR141716A, indicating the involvement of CB1 cannabinoid receptors in this effect. In order to substantiate the biological effect of this extremely low dose of THC, we attempted to identify a biochemical effect that may point to a possible neuronal mechanism. We found that a single injection of 0.001 mg/kg THC to mice induced ERK1/2 activation in the cerebellum (and probably also in the hippocampus) of mice that could be detected 24 h after the treatment.

2. Methods

2.1. Animals

The study was performed on male ICR mice and on male C57BL mice, 8–12 weeks old, weighing 30–40 g.

The animals were housed 6–8 per cage in the Animal Care Facility at a temperature of 21 °C and a 14/10 light/dark cycle, with free access to food and water. All experiments were performed during the light phase. The experimental protocols were approved by the Institutional Animal Care and Use Committee.

2.2. Drug administration

Δ^9-tetrahydrocannabinol (THC) (two different batches donated by NIDA, USA and by Prof. Mechoulam, the Hebrew University, Jerusalem) was dissolved from a stock solution of 100 mg/ml in ethanol, into a vehicle solution consisted of 18:1:1

saline:ethanol:cremophor (Sigma) and injected intraperitoneally (i.p.). Control animals were injected with the vehicle solution. SR141716A (1 mg/kg) was similarly dissolved in the vehicle solution and injected i.p. 30 min before the injection of THC.

2.3. Water T-maze

The T-maze (stem 45×14 cm; arms 53×14 cm; height 37 cm) was immersed in a pool of water at 20–23 °C. A platform (13×13 cm) was placed 0.5 cm below the surface of the water at the end of one of the arms. One to three days before the beginning of the experiment, the mice were introduced individually for 2 min into the pool (without the T-maze), so they will get accustomed to the water.

The experiments comprised of two parts. The first part ("acquisition"), that began 21 days after the injection of THC or vehicle, consisted of 4–5 days, 8 trials per day, approximately 30 min apart. In each trial the mouse was placed into the maze stem with its face to the wall and was required to swim in order to locate the hidden platform or until 120 s had elapsed. If the mouse failed to locate the platform within 120 s, it was guided to it. All mice were allowed then to stay 15 s on the platform before they were removed to their cages and placed under a 60-W lamp to dry and keep warm. For each mouse the location of the platform was kept constant (right or left arm) through all the trials. Following 2 days of rest, the second part ("reversal") of the experiment was conducted. In this part, the location of the hidden platform was changed to the opposite arm for each mouse, and the performance of the mice was recorded for 4–5 days, 8 trails per day. Escape latency (time to get to the platform) and success/failure were recorded for each mouse in each trial. A choice was considered successful only if the mouse turned directly towards the arm where the platform was located. Learning curves (presented as percent of success of all the mice of the same group in each trial, or in each day) were analyzed for the effect of treatment by a two-way analysis of variance (ANOVA) for repeated measures. Post-hoc per-day comparison was done by Student's *t* test using Bonferroni correction for multiple comparisons.

2.4. Biochemical studies

2.4.1. Tissue preparation

Twenty-four hours following the i.p. injection of 0.001 mg/kg THC or vehicle, the mice were killed by cervical dislocation, the brains were removed, the cerebella and hippocampi were obtained and immediately frozen in liquid nitrogen and stored at −80 °C.

2.4.2. Extraction

Each sample was homogenized in ice-cold extraction buffer (400 μl/cerebellum; 200 μl/hippocampus) containing 10 mM potassium phosphate, pH 7.5, 10 mM $MgCl_2$, 5 mM EDTA, 1 mM EGTA, 1 mM sodium orthovandate, 2 mM DTT, 1% Triton X-100, 50 mM β-glycerophosphate, 0.5% protease inhibitor cocktail (Sigma) and 1% phosphatase inhibitor cocktail 1 (Sigma). The samples were homogenized by hand in small glass homogenizers and the homogenates were centrifuged at 15,000 g at 4 °C for 10 min. The supernatants were stored at −80 °C. Protein concentrations were determined with the Bradford Reagent (Sigma).

2.4.3. Western blotting

Equal amounts of total protein (25 μg/sample) were separated on 10% SDS-polyacrylamide gels and then transferred to nitrocellulose membranes (Whatman, Schleicher and Schuell, Dassel, Germany). The membranes were blocked in 10 mM Tris–HCl (pH 7.4), 135 mM NaCl and 0.1% Tween-20 (TTBS) containing 5% fat-free milk powder, for 1 h at room temperature and then incubated either overnight at 4 °C or 90 min at room temperature with a mouse monoclonal antibody against p-ERK1/2 (1: 1000; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). The membranes were then washed and incubated for 60 min at room temperature with horseradish peroxidase-labeled anti-mouse antibody (1: 10,000; Santa Cruz Biothechnology Inc.) and developed using an enhanced chemiluminescence (ECL) reagent. To determine total-ERK1/2 levels, the membranes were stripped by 10 min incubation in 0.1 M NaOH containing 0.2% SDS and reprobed with a rabbit polyclonal antibody raised against total ERK (1:1000; Santa Cruz Biotechnology Inc.). The immunoreactive bands were scanned and analyzed using TINA2.07 software. To allow comparison between different autoradiographic films, the density of the bands was expressed relative to the average control in each film. Results (in terms of Relative O.D.) were compared by Students *t* test.

3. Results

3.1. The long-term effect of a low dose of THC in the water T-maze

The effect of 0.001 mg/kg THC was tested in the water T-maze, where the animal had to learn the side of the hidden platform. The first part of the test ("acquisition"; days 1–5) measured the learning of strategy (looking for a platform at the end of any arm as the only way to escape the water), as well as learning the side where the platform was located (left or right). Within 2–4 days the mice learned the side of the hidden platform and gradually improved their performance. By the fifth day all the mice succeeded in turning to the correct arm of the maze in all the trials (Fig. 1). Concomitantly, the time it took the mice to reach the platform was reduced from an average of 29 s in the first day to less than 8 s by the fifth day of learning. After 2 days of rest the mice still remembered the side where the platform had been, and all of them turned to the previously correct side (where the platform is not present anymore). The second part of the assay (days 8–11 of the experiment, which were days 1–4 of the "reversal phase") measured learning of the new side, while strategy was already acquired. Within these days the mice successfully learned the new location of the platform (Fig. 2).

THC (0.001 mg/kg injected 3 weeks before the test) slowed down the first phase of learning ("acquisition"). This effect, though reproducible in 4 different experiments (each carried out

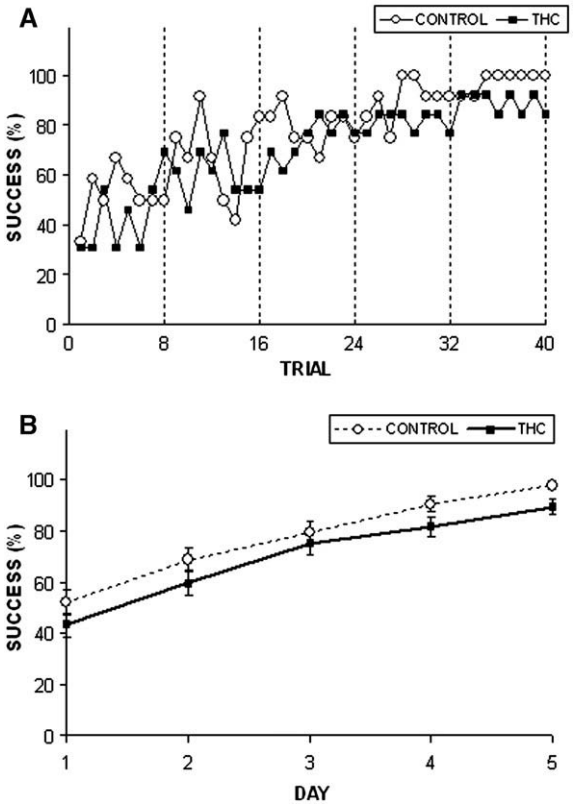

Fig. 1. The effect of a single injection of 0.001 mg/kg THC on the performance of mice in the first phase of learning ("acquisition") of the water T-maze, 3 weeks following the injection. (A) A learning curve depicting the mean percent of success of all the mice in a group per trial, for either THC-injected mice ($n=8$; closed squares) or vehicle-injected mice ($n=8$; open circles). There was a significant difference between trials ($F_{(39,\ 560)}=2.64$, $p<0.05$) but not between groups. (B) Mean percent of success per day for the same mice as in A. There was a significant difference between days ($F_{(4,\ 635)}=17.04$, $p<0.05$) but not between groups. The experiment was repeated 3 more times with similar results. Error bars depict+/− S.E.M.

with 8 THC- vs 8 vehicle-injected mice) failed to reach a level of statistical significance ($p>0.05$) (Fig. 1). THC also slowed down the second phase of learning ("reversal"). This effect was observed in all 4 experiments and was statistically significant in each of them (Fig. 2). THC did not affect the maximal performance, namely, the final rate of success in finding of the relocated platform. A per-day comparison revealed no difference between the two groups on the first and on the last day of learning; nevertheless, on at least two intermediate days, THC-treated mice performed significantly worse than their vehicle-treated controls (Figs. 2B and 3A). THC did not affect the 3-day memory of the correct side, and all the mice, in both groups, remembered the previously learnt side when tested on the first day of the second phase of learning (see first trial in Fig. 2A).

3.2. The effect of THC is mediated by CB1 receptors

The reproducible and significant effect of THC on the second phase of learning enabled us to study the involvement of CB1 receptors in the long-lasting effect of THC. Mice were injected with either the cannabinoid agonist THC (0.001 mg/kg), the CB1 receptors antagonist SR141716A (1 mg/kg), the combination of both drugs, or vehicle. Three weeks later, the mice were introduced to the water T-maze. As in previous experiments, THC significantly slowed down the second phase of learning of the treated mice (Fig. 3). This effect was statistically significant, as was found by ANOVA and by post-hoc comparisons on days 3 and 4. On the other hand, THC failed to slow-down the rate of learning when injected 30 min following the injection of the cannabinoid antagonist: The second phase of learning of the mice that were injected with both SR141716A and THC was similar, or even slightly better, than that of mice injected with SR141716A alone (Fig. 3). These results indicate that the long-term deficit induced by the ultra-low dose of THC is mediated by CB1 receptors.

3.3. The effect of a low dose of THC on phospho-ERK1/2 levels in the cerebellum and hippocampus

The mitogen-activated protein kinases (MAPKs) ERK1/2 have an important role in regulating diverse cellular processes

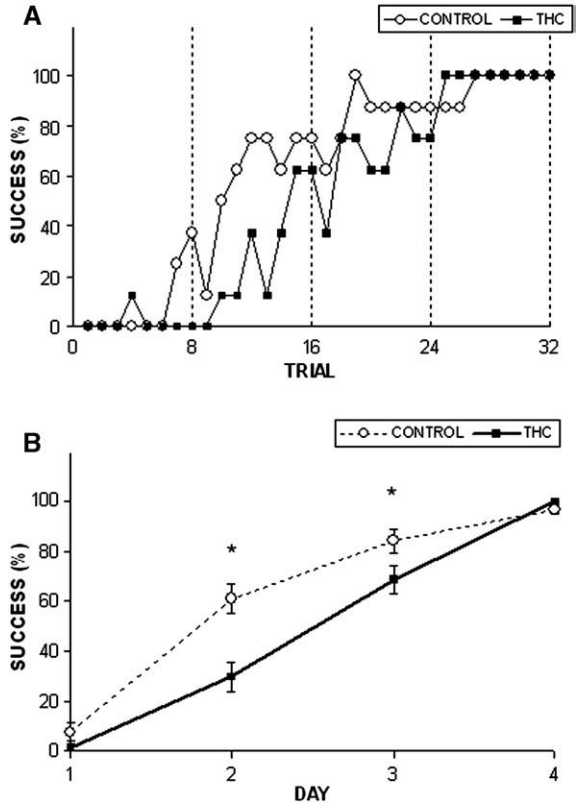

Fig. 2. The effect of a single injection of 0.001 mg/kg THC on the performance of mice in the second phase of learning ("reversal") of the water T-maze, 3 weeks following the injection. (A) A learning curve depicting the mean percent of success of all the mice in a group per trial, for either THC-injected mice ($n=8$; closed squares) or vehicle-injected mice ($n=8$; open circles). There was a significant difference between groups ($F_{(1,\ 448)}=7.51$, $p<0.05$) as well as between trials ($F_{(31,\ 448)}=19.37$, $p<0.05$). (B) Mean percent of success per day for the same mice as in A. There was a significant difference between groups ($F_{(1,\ 508)}=36.88$, $p<0.05$) as well as between days ($F_{(3,\ 508)}=353.8$, $p<0.05$). The experiment was repeated 3 more times with similar results. Error bars depict+/− S.E.M and asterisks indicate $p<0.01$ (Student's t test).

such as growth, differentiation, cell migration, survival and death (reviewed in (Agell et al., 2002)). Cannabinoid agonists were shown to activate ERK signaling pathways in-vitro ((Bouaboula et al., 1995; Derkinderen et al., 2003; Rubovitch et al., 2004; Sanchez et al., 1998) as well as in-vivo when injected at high doses, similar to those that induce the acute behavioral effects of the drugs (Derkinderen et al., 2003; Rubino et al., 2004). In the present work we tested whether a single injection of a 3–4 orders of magnitude lower dose of THC (0.001 mg/kg) induced ERK1/2 phosphorylation in two brain regions that were previously shown to be involved in spatial learning, namely, the hippocampus and the cerebellum ((Morris et al., 1982; Petrosini et al., 1996); see also Discussion). As shown in Fig. 4, 24 h following a single injection of 0.001 mg/kg THC, a significant increase of 65% in p-ERK2 was observed in the cerebellum of 16 THC-injected mice, compared to 16 vehicle-injected control mice ($p<0.05$). A smaller increase of 20% that failed to reach the level of significance, was found in the hippocampus of THC-injected mice. The phosphorylation of ERK in the cerebellum declined within 3 days: 72 h after the injection of 0.001 mg/kg of THC only a slight, insignificant increase of 8% in pERK was observed (8 THC- vs 8 vehicle-injected mice; data not shown). It should be noted that both ERK1 and ERK2 were detected in our preparations, but the signal of pERK2 was much stronger. However, when calculated, the effect on ERK1 activation was similar to that observed for ERK2. There was no effect on total ERK (tERK), hence pERK/tERK was significantly elevated (Fig. 4), suggesting the activation of ERK in the cerebellum.

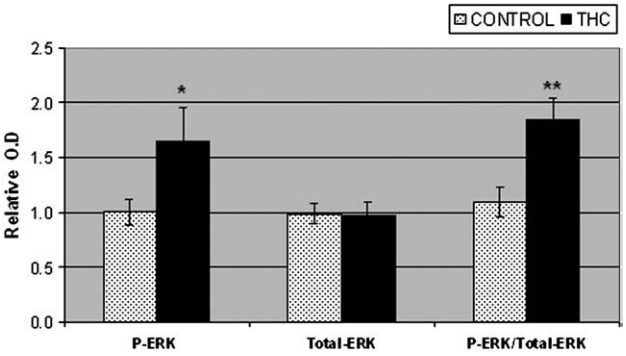

Fig. 4. The effect of a single injection of 0.001 mg/kg THC to mice on ERK2 phosphorylation in the cerebellum, 24 h following the treatment. Densitometric analysis (in terms of Relative O.D.) of pERK and total ERK in the cerebellum of either THC-injected ($n=16$; dark columns) or vehicle-injected ($n=16$; light columns) mice. There was a significant elevation in pERK levels in THC-injected mice (*$p<0.05$) compared to controls, while total-ERK levels did not change, hence pERK/total ERK significantly increased in THC-injected compared to control, vehicle-injected mice(**$p<0.01$). The experiment was repeated three times (each experiment consisting of 3–7 mice in each group) with similar results, and the combined results are presented as O.D. relative to the average O.D. of controls in each film.

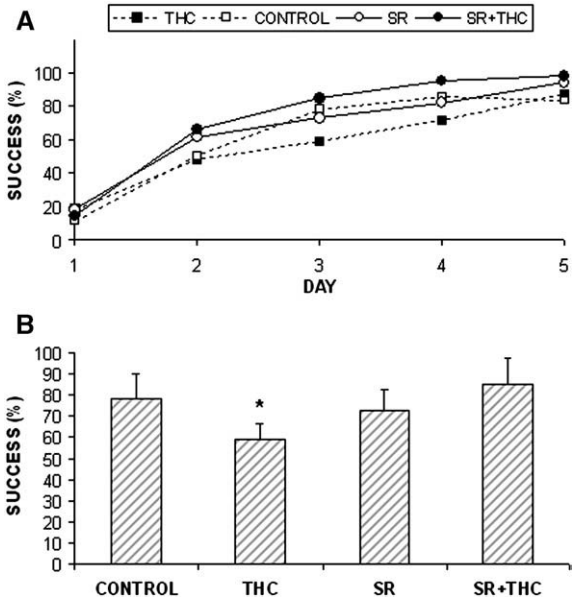

Fig. 3. Inhibition of the effect of 0.001 mg/kg THC on learning in the second phase ("reversal") of the water T-maze by the CB1 receptor antagonist SR141716A. (A) A learning curve depicting the mean percent of success of all the mice in a group per day. Four groups of mice were injected 3 weeks before the T-maze test with either vehicle ($n=12$; open squares), THC ($n=12$; closed squares), SR141716A (1 mg/kg, $n=12$; open circles) or SR141716A and THC ($n=12$; closed circles). THC significantly slowed down learning when injected alone ($F_{(1,\ 886)}=9.29$, $p<0.05$), but not when injected 30 min following the antagonist. (B) Analysis of the mean percent of success on day 3 of the same mice as in A (*$p<0.01$, Student's t test). The experiment was repeated twice (6 mice×4 groups in each experiment) with similar results, and the combined results were analyzed and presented.

4. Discussion

Cannabis is one of the most widely used drugs of abuse in the world (Adams and Martin 1996). It is commonly used as a recreational substance and is generally considered a safe drug. Moreover, cannabinoid drugs are used, or considered to be used, therapeutically for several clinical conditions, including treatment of pain and inflammation, against vomiting and nausea in cancer patients undergoing chemotherapy, for appetite stimulation in AIDS and anorexia patients and for treatment of muscle spasms in multiple sclerosis patients (Di Marzo et al., 2004). On the other hand, there is accumulating evidence of deleterious effects of long-term use of cannabinoids, which have been demonstrated both in humans and in animal models (see Introduction). In view of the widespread use of cannabis for recreational purposes and the potential benefit of the use of cannabinoid drugs in the clinic, it is important to study and determine the circumstances under which these drugs may be harmful.

We have presented a hypothesis (Sarne and Keren 2004) predicting that administration of low doses of cannabinoid drugs will induce neuronal death, while administration of a high dose of the drugs close to the time of trauma will protect against neuronal damage. Indeed, we have recently reported that an injection of a single ultra-low dose of THC (1000–10,000 times lower than the dose required to induce acute behavioral effects) to mice induced long-term cognitive deficits that were detected in the Morris water maze test (Tselnicker et al., 2007). The effect of the low dose of THC was mild, though reproducible, and required large numbers of animals in order to reach statistical

significance, therefore rendering this experimental paradigm impractical for further studies into the mechanism of the deteriorating effect of THC.

In the present study we examined the long-term effect of the single ultra-low dose of THC in another behavioral test, in order to substantiate our original finding in a different assay and to find a more sensitive test, where smaller groups of animals may yield a significant effect. We showed that 0.001 mg/kg THC slowed down learning in the water T-maze test 3 weeks following its injection. The effect of THC was more pronounced during the second phase of the test ("reversal") when the mice had to find the relocated platform. It is worth mentioning that the endocannabinoid system was recently suggested to be involved in reversal learning but not in task acquisition (Hill et al., 2005). Since the effect of THC on the second phase of learning ("reversal") was statistically significant even when small groups of mice (8 THC- and 8 vehicle-injected) were used (Fig. 2), it was therefore chosen as a suitable assay for conducting further studies in order to asses the mechanism underlying the neurocognitive deficits that are induced by a low dose of THC.

Cannabinoids exert most of their effects in the central nervous system through the cannabinoid CB1 receptor, but there is evidence for the presence of CB2 receptors in neurons as well (Onaivi et al., 2006; Skaper et al., 1996; Van Sickle et al., 2005). Moreover, some of the effects of cannabinoids are not mediated by cannabinoid receptors at all (for example (Hampson et al., 1998; Marsicano et al., 2002; Nagayama et al., 1999)). We have therefore examined the involvement of the cannabinoid CB1 receptor in mediating the deleterious effect of the low dose of THC, by the use of SR141716A, a specific CB1 antagonist. Our finding that SR141716A prevented the effect of THC (Fig. 3) demonstrates that this deleterious process is mediated by CB1 receptors, a fact that points to the specificity of the effect of THC.

The next step was to start searching for a biochemical pathway that may mediate the deteriorating long-term effect of this ultra-low dose of THC. The extracellular-regulated kinases (ERK1/2) have an important role in regulating cell homeostasis, including the processes of cell survival and cell death (reviewed in (Agell et al., 2002)). Many reports have demonstrated an increase in ERK1/2 phosphorylation after brain injury in-vivo, and in cell culture models for cellular damage in-vitro (reviewed in (Liou et al., 2003)). Moreover, inhibitors of ERK1/2 phosphorylation were shown to attenuate neuronal death due to ischemia, trauma, NMDA-mediated glutamate excitotoxicity and other cytotoxic stimuli (Liou et al., 2003). Furthermore, cannabinoid agonists were shown to activate ERK in-vitro (Bouaboula et al., 1995; Derkinderen et al., 2003; Rubovitch et al., 2004; Sanchez et al., 1998) as well as in-vivo, when used at high doses, similar to those that induce the acute behavioral effects of the drugs (Derkinderen et al., 2003; Rubino et al., 2004). Similarly, chronic administration of cannabinoids was shown to induce ERK-dependent adaptive processes (Rubino et al., 2004; Tonini et al., 2006). We have therefore chosen to test the involvement of the ERK signaling pathway in the long-term effect of THC.

Since many reports suggest that induction of either apoptotic or necrotic cell death involves sustained, rather than transient activation of ERK pathways (Agell et al., 2002; Galve-Roperh et al., 2002; Murray et al., 1998; Runden et al., 1998; Stanciu et al., 2000), we examined the effect of 0.001 mg/kg THC on ERK1/2 phosphorylation 24 h following its injection. Indeed, we have found a significant increase in ERK1/2 activation in the cerebellum of THC-injected mice 24 h after the treatment (Fig. 4). A smaller increase, that did not reach the level of significance, was also detected in the hippocampus. These two brain regions are known to be involved in spatial learning (Federico et al., 2006; Morris et al., 1982; Peinado-Manzano 1990; Petrosini et al., 1996). Especially of interest is the finding that rats with cerebellar lesions showed a stronger impairment in the T-maze than in the Morris water maze (Petrosini et al., 1996). This fits our own findings that THC induces a stronger effect in the water T-maze (the present study) compared to its effect in the Morris water maze (Tselnicker et al., 2007). These findings point to the possible involvement of ERK signaling cascades in the deteriorating effect of the low dose of THC. The exact chain of events that leads from ERK activation (which terminates within 3 days after the injection of THC) to the induction of a cognitive deficit (which lasts for at least 4 weeks) remains to be studied. One may even suggest that the activation of ERK signaling does not contribute at all to the deleterious effect of THC but rather that it is a part of a rescuing process which is mobilized in parallel, as activation of ERK was also shown to support survival of neurons following brain injury (Agell et al., 2002).

In summary, we have shown that administration of a single extremely low dose of THC to mice induces long-term cognitive deficits that can be detected in the water T-maze test. This long-term deleterious effect of THC is mediated by cannabinoid CB1 receptors. We have also shown that this ultra-low dose of THC induced the activation of ERK in the cerebellum (and possibly in the hippocampus) 24 h after its injection to mice. This finding will enable further studies into the neuronal mechanisms that are involved in the long-term deleterious cognitive effects of cannabinoid drugs.

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