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ORIGINAL INVESTIGATION

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Disruption of CB_1 receptor signaling impairs extinction of spatial memory in mice

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Abstract *Rationale:* A growing body of in vitro and in vivo evidence indicates that a central endocannabinoid system, consisting of CB_1 receptors and endogenous cannabinoids, modulates specific aspects of mnemonic processes. Previous research has demonstrated that either permanent or drug-induced disruption of CB_1 receptor signaling interferes with the extinction of a conditioned fear response. *Objectives:* In the present study, we evaluated whether the endocannabinoid system also plays a role in extinguishing learned escape behavior in a Morris water maze task. *Methods:* CB_1 (−/−) mice and mice repeatedly treated with 3 mg/kg of the CB_1 receptor antagonist SR 141716 (Rimonabant) were trained to locate a hidden platform in the Morris water maze. Following acquisition, the platform was removed and subjects were assigned to either a massed (i.e., five consecutive sessions consisting of four 2-min trials/session) or a spaced (a single, 1-min trial every 2–4 weeks) extinction protocol. *Results:* Strikingly, both 3 mg/kg SR 141716-treated mice and CB_1 (−/−) mice continued to return to the target location across all five trials in the spaced extinction procedure, while the control mice underwent extinction by the third or fourth trial. In contrast, both the 3-mg/kg SR 141716-treated and CB_1 (−/−) mice exhibited extinction in the massed extinction trial procedure. *Conclusions:* These findings indicate that disruption of CB_1 receptor signaling impairs extinction processes in the Morris water maze, thus lending further support to the hypothesis that the endocannabinoid system plays an integral role in the suppression of non-reinforced learned behaviors.

Keywords Extinction · CB_1 knockouts · SR 141716 (Rimonabant) · Morris water maze · Endocannabinoid · Cannabinoid · Spatial memory

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Introduction

The discovery that CB_1 cannabinoid receptors are heterogeneously expressed throughout the CNS (Herkenham et al. 1991; Matsuda et al. 1993), as well as the subsequent identification of several endogenous ligands that act at these receptors, including anandamide (Devane et al. 1992), 2-AG (Mechoulam et al. 1995; Sugiura et al. 1995), noladin ether (Hanus et al. 2001), and virodhamin (Porter et al. 2002), has sparked a great deal of interest in identifying the physiological roles of this endocannabinoid system. Several functional roles have already been implicated, including the modulation of pain (Calignano et al. 1998; Richardson et al. 1998; Walker et al. 1999), feeding (Di Marzo et al. 2001), neuroexcitoxicity (Marsicano et al. 2003), and cognition (Lichtman 2000; Terranova et al. 1996). It should not be surprising that cognition would be among those systems believed to be influenced by the endocannabinoid system, given that CB_1 receptors (Herkenham et al. 1991) as well as the endocannabinoids anandamide and 2-AG (Di Marzo et al. 2000) are present at high concentrations in the hippocampus and other forebrain areas associated with learning and memory.

A growing body of evidence suggests that the endocannabinoid system modulates several forms of synaptic plasticity that are believed to underlie learning and memory. Specifically, endocannabinoids have been proposed to act as retrograde messengers in which they are released postsynaptically, travel retrogradely across the synapse, and bind to presynaptic CB_1 receptors where they can inhibit the release of inhibitory (e.g., GABA) or excitatory (e.g., glutamate) neurotransmitters. Disruption of CB_1 receptor signaling has been demonstrated to prevent the occurrence of depolarization-induced suppression of inhibition (DSI) in the hippocampus (Ohno-Shosaku et al. 2001; Wilson et al. 2001; Wilson and Nicoll 2001), cerebellum (Diana et al. 2002; Kreitzer and Regehr 2001a; Yoshida et al. 2002), and cortex (Trettel and Levine 2002, 2003). Conversely, endocannabinoids have also been proposed to inhibit depolarization-induced suppression of excitation (DSE) in cerebellum (Kreitzer and Regehr 2001b;

Maejima et al. 2001), ventral tegmental area (Melis et al. 2004), and hippocampus, though DSE is far less prominent in the hippocampus than in the cerebellum (Ohno-Shosaku et al. 2002). Additionally, endocannabinoids may also modulate long-term forms of synaptic plasticity, as in the case of long-term potentiation (LTP) or long-term depression (LTD). CB_1 (−/−) mice have been previously found to exhibit enhanced LTP compared to CB_1 (+/+) mice (Bohme et al. 2000). The aforementioned short-term roles of endocannabinoids may also help explain some of their effects on LTP. For example, the CB_1 antagonist AM251 given during DSI enabled a normally ineffective train of excitatory post-synaptic currents to induce LTP in that cell, but not in neighboring cells (Carlson et al. 2002). These investigators hypothesized that this targeted LTP could underlie some behavioral learning associated with LTP as in the case of "place learning" in maze tasks. Other research has demonstrated that endocannabinoids may also mediate LTD in hippocampus (Chevaleyre and Castillo 2003). Given the extent to which the endocannabinoid system appears to modulate short-term and long-term forms of synaptic plasticity, it should not be surprising that this system plays a tonic role in mnemonic processes.

Indeed, several reports have provided in vivo evidence supporting the notion that the endocannabinoid system modulates specific aspects of learning and memory. Specifically, the disruption of CB_1 receptor signaling through the use of either CB_1 (−/−) mice or CB_1 receptor antagonists has been found to enhance memory in several models of animal cognition. Terranova et al. (1996) were the first to report that SR 141716 dose-dependently improved the social recognition memory of rats, as well as attenuated the deficits displayed by aged mice and rats in the same task. Consistent with these findings is that CB_1 (−/−) mice exhibited better performance than CB_1 (+/+) mice in an object-recognition memory task (Reibaud et al. 1999). Also, rats trained in an extended delay eight-arm radial maze task performed better when treated with SR 141716 than when treated with the vehicle (Lichtman 2000), a dose-related effect that appears to be related to consolidation (Wolff and Leander 2003). However, these apparent memory-enhancing effects of CB_1 receptor blockade are not observed in a variety of operant paradigms that are heavily dependent on working or short-term memory (Brodkin and Moerschbaecher 1997; Hampson and Deadwyler 2000; Mallet and Beninger 1998; Mansbach et al. 1996).

Several recent papers have employed the Morris water maze to investigate the effects of cannabinoids on both acquisition (da Silva and Takahashi 2002; Ferrari et al. 1999) and memory (Varvel et al. 2001). Recently, we have evaluated CB_1 (−/−) and (+/+) mice in this task to elucidate the role that endogenous cannabinoids may play in learning and memory (Varvel and Lichtman 2002). Although performance in the acquisition of a fixed hidden platform task was unaffected by genotype, genotype differences emerged during a reversal task in which the platform was moved to the opposite side of the pool after the mice acquired the task. Whereas the wild-type mice gradually ceased returning to the previous platform location and readily learned the new location, the CB_1 (−/−) mice not only continued to return to the previous location, but also exhibited a significant deficit in learning the new location (Varvel and Lichtman 2002). This perseverance in behavior may have resulted from a resistance to either a time-dependent decrement in performance (i.e., forgetting) or extinction, a process in which learned behaviors are suppressed following non-reinforced trials. In support of the latter possibility, Marsicano et al. (2002) demonstrated that SR 141716-treated wild-type and CB_1 (−/−) mice exhibited extinction deficits in fear-related behavior to a tone that was previously paired with electric foot shock. Similarly, SR 141716 was recently reported to impair the extinction of contextual fear-memory (Suzuki et al. 2004).

The original goals of the present study were to determine whether the endocannabinoid system plays specific roles in extinction and memory duration of a learned escape response in a Morris water maze task. A massed extinction procedure was used in which the platform was removed after acquisition and subjects were given four 2-min trials/day in the maze for a total of 5 days. We initially attempted to assess forgetting by using a within-subject design in which subjects were given a post-acquisition probe trial (i.e., 60 s test with the platform removed) every 2–4 weeks for a total of five tests. However, a control group, which received only a single post-acquisition probe trial that corresponded to the other subjects' final post-acquisition probe trial, demonstrated near-perfect performance, indicating a long-enduring and robust memory of the platform location and precluded our ability to assess forgetting. Instead, the repeated probe trials represented spaced extinction trials. Here, we report that CB_1 (−/−) mice and SR 141716-treated mice exhibit deficits in the spaced extinction procedure, but not in the massed extinction procedure.

Materials and methods

Subjects

Male C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME) as well as CB_1 (−/−) and CB_1 (+/+) mice derived from the Virginia Commonwealth University knockout breeding colony (Varvel and Lichtman 2002) were housed in a temperature-controlled (20–22°C) environment, with a 12-h light/dark cycle. Food and water were available ad libitum in the home cages. All experiments were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

Apparatus

The water maze consisted of a large circular galvanized steel pool (1.8 m diameter, 0.6 m height). A white platform (10 cm diameter) was placed inside, and the tank was filled with water (22°C) until the top of the platform was

submerged 1 cm below the water's surface. A sufficient amount of white paint (Proline-Latex Flat) was added to make the water opaque and render the platform virtually invisible. An automated tracking system (Columbus Instruments, Columbus, OH) analyzed the swim path of each subject and calculated several corresponding dependent measures—escape latencies (the time between being placed in the water and finding the hidden platform), total path lengths, average swim speeds, degree of thigmotaxia (percentage of time spent in periphery of the pool), and the number of entries into specified target areas.

Drugs

SR 141716 was provided by the National Institute on Drug Abuse (Bethesda, MD), and was dissolved in a 1:1 mixture of absolute ethanol and alkamuls-620 (Rhone-Poulenc, Princeton, NJ) and diluted with saline to a final ratio of 1:1:18 (ethanol/alkamuls/saline). Drug injections were administered subcutaneously in an injection volume of 10 μl/g.

Procedure

Prior to acquisition training, each subject was given a single 5-min acclimation session, in which it was placed in the tank with no platform present. Mice then received eight acquisition sessions in which the hidden platform remained in a fixed location, with each session consisting of four trials separated by ~10 min (5 days/week). In addition, a probe trial (60-s duration, no platform present) was given before the first acquisition session and again before the eighth acquisition session. The other training procedures were identical to those previously reported (Varvel et al. 2004; Varvel and Lichtman 2002).

Following acquisition, the hidden platform was removed from the tank and each mouse was subjected to either a massed or spaced extinction procedure. In the massed trial extinction procedure, the mice received five sessions, with each session consisting of four 120 s trials. The initial goal of the spaced-trial experiment was to evaluate whether SR 141716 increases memory duration of the platform location compared to vehicle-treated mice. In an effort to reduce the number of mice used, a within-subject design was employed in which subjects were given a post-acquisition probe trial (i.e., 60 s test with the platform removed) every 2–4 weeks for a total of five tests. The end points consisted of both latency to and path length to where the platform had previously been located. In order to control for the possibility that the repeated probe tests may serve as extinction trials, we included an additional group of control mice (N=6) that were given identical acquisition training as the drug- and vehicle-injected mice, but received only a single post-acquisition probe trial that corresponded to the other two groups' fifth post-acquisition probe trial. In both protocols, one experiment consisted of subjects that were given an injection of either vehicle or SR 141716 (3 mg/kg) 30 min before each acquisition and extinction trial, and second experiment employed CB_1 (+/+) and (−/−) mice. We have previously found that 3 mg/kg was the lowest dose of SR 141716 that significantly antagonized Δ^9-THC-induced impairment in the Morris water maze (Varvel et al. 2001). Sample sizes of each group ranged between ten and 14 mice.

Subsequent experiments were conducted to determine whether the increased swim speeds maintained in SR 141716-treated and CB_1 knockout mice during the massed trials procedure (see Fig. 3) were related to whether their swimming behavior had been contingently associated with escape from the pool. To this end, CB_1 (−/−) and CB_1 (+/+) mice, as well as naïve mice treated daily with 3 mg/kg SR 141716 or vehicle (30 min pre-session; N=8) were placed in the pool with no platform present and allowed to swim for four 120-s trials each day until swim speeds in the control groups began to decline.

Statistical analysis

Data were analyzed using two-factor ANOVAs, examining the effects of session or trial (within-subject) and drug treatment (between-subject). When significant main effects of drug treatment or significant interactions were found, Dunnett's test was used for post-hoc comparisons. In addition, t-tests were used for planned comparisons to examine the effects of drug treatment or genotype at each session. Differences were considered significant at the $p<0.05$ level.

Results

Acquisition

No significant differences in the rate of acquisition were observed between vehicle- and SR 141716-treated mice, or between CB_1 (−/−) and CB_1 (+/+) mice in the standard fixed platform location Morris water maze task. Figures 1a and b show SR 141716 and vehicle led to similar escape latencies and path lengths. There were significant main effects of session for both escape latency, $F(7,211)=32.2$, $p<0.001$, and path length, $F(7,211)=28.4$, $p<0.001$, while no main effect of drug treatment or interaction between session and drug treatment for either measure was observed. Similarly, the CB_1 (−/−) and CB_1 (+/+) mice exhibited nearly identical performance for both measures (Fig. 1c and d). Again, there were significant effects of session [escape latency $F(7,87)=11.8$, $p<0.001$; path length $F(7,87)=5.8$, $p<0.001$], but no main effect of genotype or interaction between session and genotype for either measure was observed.

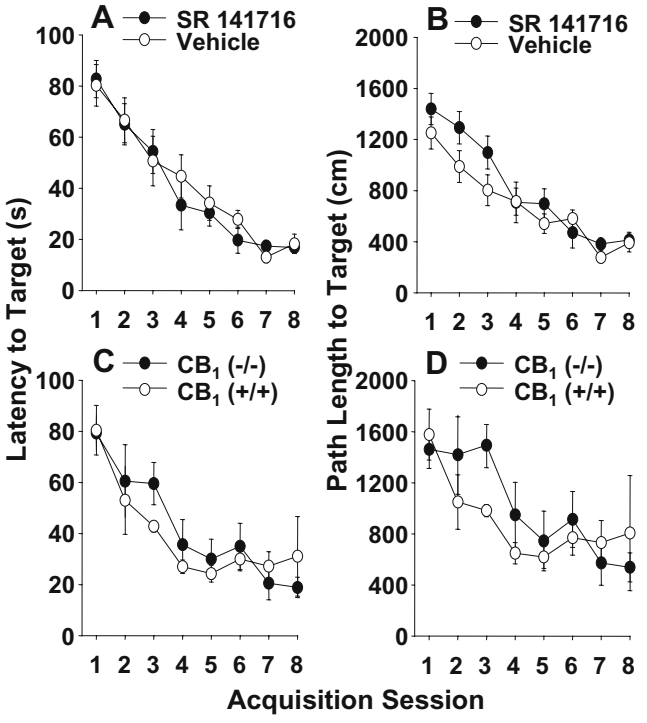

Fig. 1 Acquisition. No differences were found between vehicle-treated and SR 141716-treated mice on either escape latencies (**a**) or path lengths (**b**) during acquisition training in a standard reference memory task. *N*=13 per group. Similarly, no differences were found between CB_1 (−/−), *N*=8, and CB_1 (+/+), *N*=11, on escape latencies (**c**) or path lengths (**d**) during acquisition. Session data represent the average of four daily trials. All data are represented as mean ± SEM

Massed extinction

No differences in extinction rates were detected between SR 141716- and vehicle-treated mice or between CB_1 (−/−) and CB_1 (+/+) mice when the "massed extinction" protocol was used, which consisted of four daily 120 s trials over 5 days, with no platform present. The time it took the subjects to first return to the position where the platform had previously been located (i.e., "latency to target") and the corresponding "path length to target" for SR 141716- and vehicle-treated mice are shown in Fig. 2a and b. Both latencies and path lengths to target significantly increased across sessions, $F(4,74)=9.8$, $p<0.001$ and $F(4,74)=4.5$, $p<0.001$, respectively, though there was no main effect of drug treatment and no interaction between drug treatment and session on either of these measures. As shown in Fig. 2c and d, both CB_1 (−/−) and (+/+) mice exhibited increased latencies and path lengths to target increased across sessions, $F(4,94)=12.5$, $p<0.001$ and $F(4,93)=7.5$, $p<0.001$, respectively (path length data from one mouse on one session was lost due to a technical problem). A significant effect of genotype was observed for the latency to target measure, $F(1,94)=7.7$, $p=0.01$, with the CB_1 (−/−) mice continuing to return to the target location more quickly than CB_1 (+/+) mice. However, this difference is likely an artifact of the increased swim speeds exhibited by the knockout mice (see Fig. 3), as the corresponding path lengths to target failed to differ between the genotypes.

Contingent vs non-contingent swimming

While no effects of compromised CB_1 function on the rate of extinction of the learned spatial bias were apparent when the massed extinction protocol was used, interesting effects on swim speeds were observed. As represented in Fig. 3a, a significant statistical interaction between drug treatment and sessions, in which swim speeds of the vehicle-treated mice decreased across extinction sessions, while those of SR 141716-treated mice did not, $F(4,74)=4.1$, $p<0.001$. Planned comparisons revealed that swim speeds were significantly lower in the vehicle group than in the drug-treated group during the third, fourth, and fifth extinction sessions ($p<0.05$). A similar interaction between genotype and session for swim speed was found, $F(4,93)=4.5$, $p<0.01$ (Fig. 3c). Planned comparisons revealed that swim speeds were significantly lower in CB_1 (+/+) mice compared to the CB_1 (−/−) mice during the fourth and fifth extinction sessions ($p<0.05$). These results raised the intriguing possibility that SR 141716-treated and CB_1 (−/−) mice may have been impaired in their ability to extinguish the generalized conditioned swimming response, separate from their extinction of the spatial bias. In order to test this

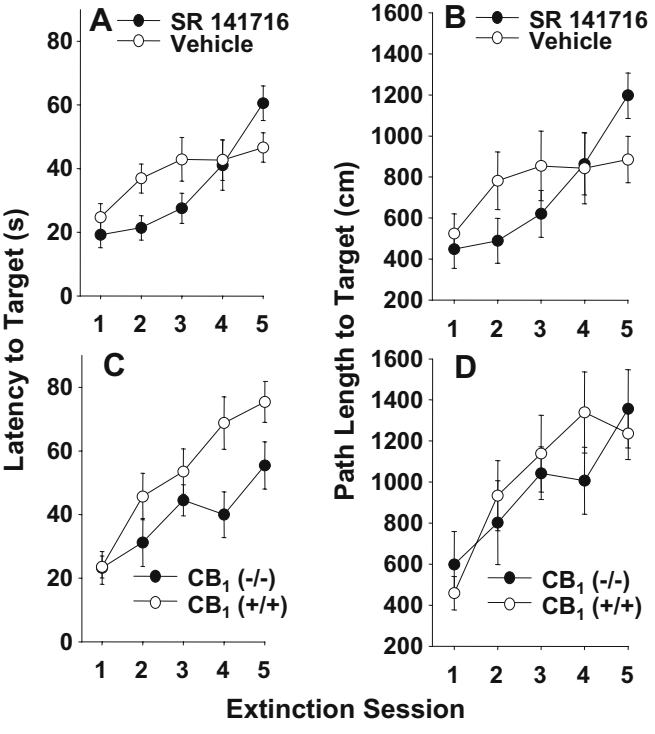

Fig. 2 Massed extinction. No differences were found between SR 141716-treated and vehicle-treated mice in latency to target (**a**) or path length to target (**b**), *N*=13 per group. There was a difference between CB_1 (−/−) and CB_1 (+/+) mice (*N*=8 and 11, respectively) on latency to target (**c**), but not on path length to target (**d**). All data are represented as mean±SEM

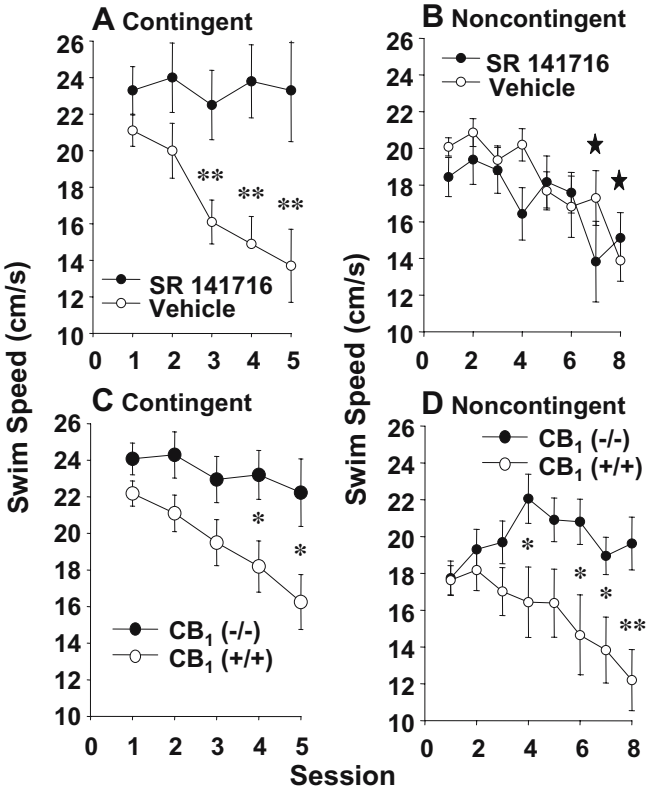

Fig. 3 Swim speeds of vehicle-treated mice and CB_1 (+/+) mice (N=13 and 11, respectively) decreased across extinction sessions when swimming had been contingently associated with escape, while those of SR 141716-treated and CB_1 (−/−) mice (N=13 and 8, respectively) did not (**a**, **c**). In contrast, swim speeds of both vehicle- and SR 141716-treated mice (N=8) decreased in the non-contingent swimming condition (**b**). However, swim speeds of CB_1 (−/−) mice remained high, while those of the CB_1 (+/+) mice (N=6 and 8, respectively) decreased across sessions (**d**). All data are represented as mean±SEM. *p<0.05, **p<0.01 between groups (planned comparisons). *Stars* denote significant differences of both the vehicle- and SR 141716-treated groups combined compared to first session, p<0.05 (Dunnett's test)

possibility, new groups of naïve mice were subjected to a protocol identical to the massed extinction test, except that these mice were never presented with a platform. As shown in Fig. 3b, swim speeds of both vehicle- and SR 141716-treated mice decreased across sessions, $F(7,98)$= 6.3, p<0.001, but there was no significant effect of either drug treatment or interaction between drug treatment and session. The swim speeds of both treatment groups collapsed were significantly lower on sessions 7 and 8 than on session 1 (Dunnett's test). A similar experiment compared CB_1 (−/−) and (+/+) mice under the same conditions. Due to the limited numbers of available knockout mice, both male and female mice were used in this experiment. No significant differences of sex or interactions with sex were found, thus their data were combined. As shown in Fig. 3d, there was a significant interaction between session and genotype, $F(7,111)$=4.5, p<0.001, where swim speeds of CB_1 (+/+) mice decreased across sessions while CB_1 (−/−) maintained high swim speeds throughout the experiment. Planned comparisons showed that swim speeds were lower in CB_1 (+/+) mice than in the CB_1 (−/−) mice during the fourth, sixth, seventh, and eighth sessions. Thus, SR 141716-treated mice exhibited fast swim speeds compared with vehicle-treated mice only when swimming had been contingently associated with escape, while CB_1 (−/−) mice maintained high swim speeds under both conditions.

Spaced extinction trials experiment

Consistent with previous results, no significant differences were found in the acquisition curves between SR 141716- and vehicle-treated mice (data not shown). As shown in Fig. 4a and b, the performance of the control group which was not given a probe trial until 9 weeks after acquisition was indistinguishable from their performance on the last day of acquisition. This remarkable resilience to time-dependent decrements in performance in these mice precluded the assessment of drug-related effects on forgetting with this protocol (our original intent). In contrast, further inspection of Fig. 4 shows that performance of the vehicle-treated mice worsened across the five probe trials, indicating that the use of repeated probe trials constituted a "spaced trials" extinction procedure.

A two-way ANOVA conducted on the post-acquisition probe trial data revealed a significant main effect of drug

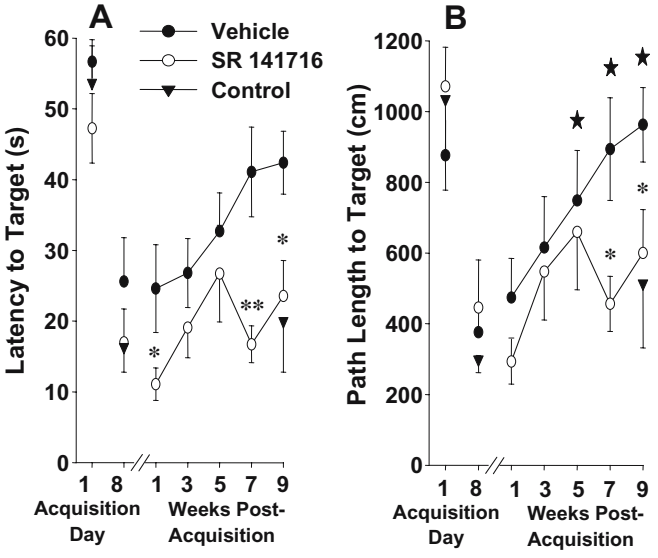

Fig. 4 Spaced extinction in SR 141716- and vehicle-treated mice. Latencies to target (**a**) and path lengths to target (**b**) during probe trials (60 s) given prior to the first and last days of acquisition as well as on five separate extinction trials over the course of several weeks in which the platform was removed. Mice were treated with either 3.0 mg/kg SR 141716 (N=11) or vehicle (N=10) 30 min prior to each session. In addition, a non-injected control condition (N=6) was included that received identical acquisition training as the other two groups, but were given a single probe trial 9 weeks following acquisition, which coincided with the fifth extinction session of the other two groups. *p<0.05, **p<0.01 for drug vs vehicle conditions at a given probe trial (planned comparisons). *Stars* denote significant differences with the first post-acquisition trial, p<0.05 (Dunnett's test). All data are represented as mean±SEM

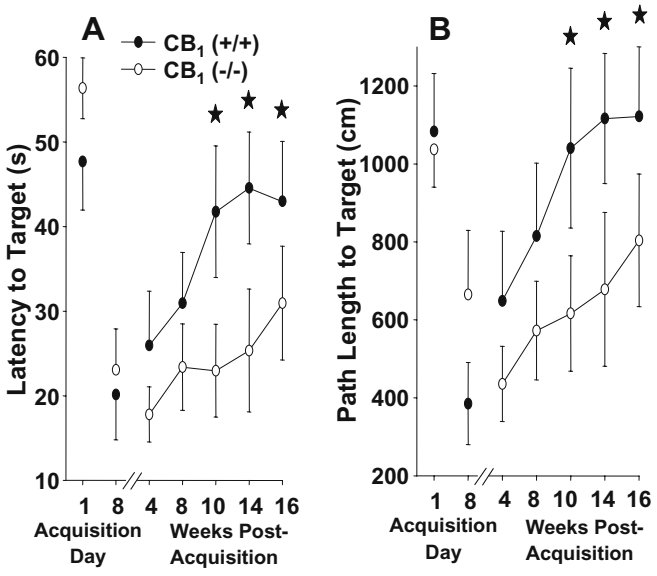

Fig. 5 Spaced extinction in CB_1 (−/−) and (+/+) mice. Latencies to target (**a**) and path lengths to target (**b**) during probe trials (60 s) given prior to the first and last days of acquisition as well as on five separate extinction trials over the course of several weeks in which the platform was removed from the maze in CB_1 (−/−) mice (N=10) and CB_1 (+/+) mice (N=7). *Stars* denote significant differences with the first post-acquisition trial, $p<0.05$ (Dunnett's test). All data are represented as mean ± SEM

treatment for the latency to target data, $F(1,125)=9.2$, $p<0.01$ (Fig. 4a), indicating that SR 141716 disrupted extinction. Planned comparisons conducted at each probe trial revealed that SR 141716-treated mice maintained significantly faster latencies compared to the vehicle-treated mice on the probe trials conducted 1, 7, and 9 weeks after acquisition. Although a two-way ANOVA failed to show a significant effect of SR 141716 for the path length data (p=0.076), planned comparisons revealed that path lengths to target were significantly lower in the SR 141716-treated mice than in the vehicle-treated mice on the fourth and fifth probe trials conducted 7 and 9 weeks post-acquisition, respectfully. Furthermore, results from separate one-way ANOVAs revealed that both latencies and path lengths to target significantly increased across extinction sessions in the vehicle group, $F(5,59)=2.6$, $p<0.05$ and $F(5, 59)=4.3$, $p<0.01$, respectively, but not in the SR 141716-treated group (p=0.10 and p=0.16, respectively). Post-hoc comparisons indicated that the path latencies to target of the vehicle-treated mice were significantly longer on the final three extinction trials than the path length to target on the eighth day of acquisition (Dunnett's test, $p<0.05$).

A similar pattern of results can be seen in Fig. 5, in which CB_1 (−/−) and (+/+) mice were given repeated probe trials following acquisition. Both genotypes exhibited equivalent rates of acquisition. However, extinction was disrupted in the CB_1 (−/−) mice compared to the CB_1 (+/+) mice, as reflected by significant main effects of genotype in both the latency to target measure, $F(1,100)=6.1$, $p<0.05$, and the path length to target measure, $F(1,100)=2.4$, $p<0.05$. Although planned comparisons failed to reveal significant differences between the genotypes at any given probe trial, the significant main effect of genotype was further analyzed by conducting separate one-way ANOVAs for each genotype. The CB_1 (+/+) mice showed a significant trial-dependent increase in latencies, $F(5,41)=3.17$, $p<0.05$, and path lengths, $F(5,41)=3.63$, p=0.01, with post-hoc analyses revealing significantly longer latencies and path lengths on the last three probe trials than each respective measure on the eighth day of acquisition (Dunnett's test, $p<0.05$). These results in the CB_1 (+/+) mice are all consistent with a trial-dependent extinction of the conditioned spatial bias. In stark contrast, the extinction trials had no significant effects in the CB_1 (−/−) mice on latencies (p=0.91) or path lengths (p=0.61), further supporting the concept that the CB_1 receptor plays a role in extinction.

Figure 6 shows swim traces of representative mice of each treatment group following acquisition and during the last extinction probe trial. The SR 141716-treated and CB_1 (−/−) mice continued to swim in the vicinity that the platform was formerly located even after the five extinction trials, while the vehicle-treated and CB_1 (+/+) mice did not.

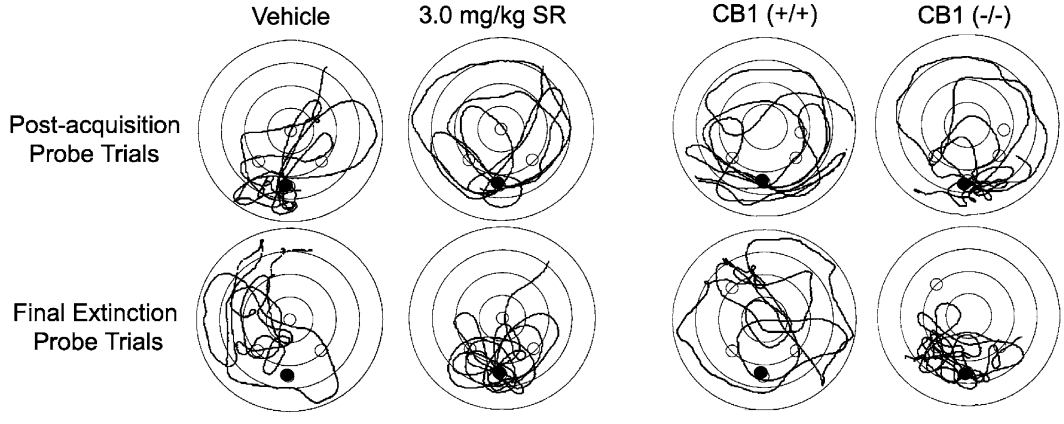

Fig. 6 Representative samples of swim traces for the post-acquisition trials and the last extinction probe trial of vehicle- and SR 141716-treated mice, as well as CB_1 (−/−) and CB_1 (+/+) mice. *Filled circles* represent the location where the platform had previously been located

Discussion

Taken together, the results of the present study suggest that endocannabinoids may play a role in facilitating the processes underlying extinction under specific circumstances. In support of this hypothesis is that compromising CB_1 receptors, by either pharmacological blockade with SR 141716 or genetic deletion, led to extinction deficits in mice that were given repeated probe trials that were spaced apart over several weeks. Specifically, both CB_1 (−/−) mice and SR 141716-treated mice continued to return to where the platform had been located more quickly when compared to their respective controls. It is important to note that the vehicle-treated and CB_1 (+/+) controls clearly extinguished their learned spatial bias across subsequent probe trials. Additionally, the near-perfect performance of the control group, which received only a single post-acquisition probe trial (that coincided with the last probe trial of the other groups) distinguished the trial-dependent nature of the extinction observed here from a simple time-dependent memory decay.

The findings presented here are consistent with our previous report that CB_1 (−/−) mice demonstrated increased perseverance of an acquired spatial memory at the expense of learning a new one (Varvel and Lichtman 2002), and are consistent with the notion that endogenous cannabinoids play a role in extinction. Several other reports have demonstrated that disruption of CB_1 receptor signaling impairs memory in fear conditioning procedures. Previously, SR 141716-treated mice and CB_1 (−/−) mice exhibited impaired extinction of conditioned freezing to a tone that had been paired with foot shock (Marsicano et al. 2002). Interestingly, presentation of the tone (CS) during extinction was sufficient to increase endogenous levels of anandamide and 2-AG in the amygdala. A subsequent study found that SR 141716 also impaired conditioned freezing to the test chamber in which the mice had received the shock (Suzuki et al. 2004). Of consequence, conditioned freezing to a context is believed to involve hippocampal processes, while the hippocampus is not believed to play a role in conditioned freezing to a tone (Phillips and LeDoux 1992).

Although the observation that the SR 141716-treated mice continued to return to the platform location across the spaced probe trials supports a role for endocannabinoids in processes underlying the extinction of non-reinforced behavior, another possible explanation is that the drug did not block the actions of endogenous cannabinoids, but evoked this effect through its own intrinsic activity either at the CB_1 receptor or at a non-cannabinoid site of action. In particular, SR 141716's inverse agonist activity in the [^{35}S]GTPγS binding assay has been well described (Landsman et al. 1997; Pan et al. 1998). Although this drug is 7,000-fold more potent as a CB_1 receptor antagonist than as an inverse agonist in this assay (Sim-Selley et al. 2001), its efficacy as an inverse agonist in vivo is unknown. Alternatively, SR 141716 has also been reported to have a non-CB_1 receptor mechanism of action (Bukoski et al. 2002). Nonetheless, the dose of 3.0 mg/kg SR 141716 used in the present experiments is generally viewed as moderate, sufficient to block the effects of exogenously administered cannabinoids without producing overt behavioral effects. More convincingly, the observation that CB_1 (−/−) mice exhibited a similar phenotype as the SR 141716-treated wild-type mice provides converging evidence supporting the involvement of CB_1 receptors in extinction.

This apparent inhibitory effect on extinction learning is distinguished from the observations that disruption of CB_1 receptor signaling had no effect on the initial acquisition of the water maze task here or in a previous report from our lab in which no differences in Morris water maze acquisition were detected between CB_1 (−/−) and wild-type mice (Varvel and Lichtman 2002). Although it should be noted that our acquisition procedure may be insensitive to detect cognitive enhancement, disruption of CB_1 signaling also failed to affect acquisition of conditioned aversion freezing to a tone that had been paired with foot shock (Marsicano et al. 2002). These observations, taken together, suggest that functioning endocannabinoid systems may not be apparent for some aspects of learning, such as acquisition of spatial learning and conditioned freezing tasks.

In contrast, exogenously applied cannabinoids have been shown to disrupt acquisition of the fixed platform Morris water maze task through a CB_1-dependent receptor mechanism of action (da Silva and Takahashi 2002; Ferrari et al. 1999). Undoubtedly, the concentration of exogenously administered cannabinoids in these studies is likely to be greater at CB_1 receptors than the concentration of the highly labile endogenous cannabinoids, which are likely to also possess vastly different spatial–temporal activation patterns, suggesting that exogenous cannabinoids are likely to do more than simply mimic the function of endocannabinoids. In fact, the very aspects of learning and memory in the Morris water maze that appear to be most sensitive to disruption by exogenous cannabinoids (i.e., acquisition, working memory) seem largely unaffected by blockade of the endogenous system. On the other hand, it will be important to determine whether elevating the concentration of endocannabinoids or administering cannabinoid agonists facilitate extinction. Indeed, a recent report has demonstrated that THC facilitates the extinction of a conditioned place preference to either cocaine or amphetamine (Parker et al. 2004). However, the failure of SR 141716 to block this enhanced extinction and the observation that cannabidiol, an inactive constituent of marijuana, also facilitated extinction in the place preference paradigm suggests a non-CB_1 receptor mechanism.

Curiously, CB_1 receptor deactivation failed to produce a similar attenuation of extinction in the "massed extinction" procedure. Under these relatively intense conditions, both SR 141716-treated and CB_1 (−/−) mice extinguished their spatial biases in a manner indistinguishable from their respective controls. While CB_1 (−/−) mice did display quicker latencies than CB_1 (+/+) mice during these extinction trials,

no such differences in path lengths were observed, suggesting that the significant effect on latencies was secondary to the increased swim speeds observed in these mice, and not due to any differences in extinction. What could account for this apparent discrepancy between the "massed trials" and "spaced trials" protocols? One possibility is that the massed extinction paradigm may have been more stressful to the mice, and this stress may have interfered with their performance, effectively counteracting any possible protection against extinction. Conversely, the extended extinction procedure, which employed single 1-min trials every 2–4 weeks, is likely to be less stressful than the massed extinction paradigm, thereby allowing the effects of endocannabinoid modulation of extinction to be observed. The stress associated with this task may be particularly relevant as a recent report suggests that mice with compromised CB_1 systems may be more reactive to stress, and display a variety of anxiety-like behaviors (Martin et al. 2002). Another possible explanation relates to the distinction between mechanisms mediating short-term extinction learning and those regulating the consolidation of such learning. Suzuki et al. (2004) recently reported that SR 141716 failed to disrupt the within-session (short-term) extinction of a conditioned fear response, but did disrupt extinction when the mice were tested 24 h later. While the timing of the conditioned freezing and water maze extinction experiments cannot be directly compared, the fact that both studies reveal disruptive effects of CB_1 blockade on long-term, but not short-term, extinction learning suggests that there may be similar mechanisms involved in both tasks.

Interestingly, while no attenuation of extinction of the learned spatial basis was observed when the more rigorous extinction protocol was employed, SR 141716-treated and the CB_1 (−/−) mice did appear resistant to the extinction of the swim response itself. Swim speeds of mice in both control groups progressively declined across the massed extinction trials, primarily the result of these mice increasing the amount of time they spent simply floating. In contrast, relatively constant swim speeds were maintained in both the SR 141716-treated and the CB_1 (−/−) mice throughout the extinction protocol. Although high doses of drug have been reported to increase general locomotor activity (Compton et al. 1996), it is unlikely that this explains the present data since the swim speeds of both vehicle- and SR 141716-treated groups gradually decreased after several days of non-contingent swimming in which an escape platform was never available. Thus SR 141716 prevented this decrease in swimming behavior only when swimming had become a conditioned response (i.e., contingent swimming associated with escape via the platform), a phenomenon that seems best understood within the context of an extinction deficit. In contrast, the swim speeds of CB_1 (−/−) mice remained high throughout both the contingent and the non-contingent paradigms, indicating that some other process was involved in maintaining this behavior—possibly related to other consequences of gene disruption throughout ontogeny (Mogil and Grisel 1998). It should be noted that the decrease in swimming behavior observed in the present experiments is somewhat reminiscent of the learned helplessness tasks that model depression (Porsolt et al. 1977), seemingly suggesting that disrupting the function of CB_1 receptors may produce antidepressant-like effects. However, this interpretation does not account for the present results with SR 141716 since no treatment effects were observed when the swimming was an unconditioned response.

As discussed above, stress reactivity may be a particularly important factor given the nature of the procedures used here, and the fact that endocannabinoids have been implicated in mediating emotional responses and reactivity to stress (Martin et al. 2002). It is possible that in the present study disruption of CB_1 signaling increased general levels of anxiety, which led to a concomitant increase in motivation to remember platform location. However, if this were the case, one would expect differences in the initial rates of acquisition or even differences in thigmotaxia, which is often thought to reflect levels of anxiety. No drug or genotype differences were found in the present study for either measure. This hypothesis is also inconsistent with the observation that the extinction deficit was only observed under the less stressful protocol.

The results of the present study support the hypothesis that endocannabinoids may play a specific role in facilitating the extinction of learned behaviors. If the endocannabinoid system was involved in such a process, then disrupting CB_1 receptor signaling could appear in some models as improved memory, while in others inhibition of endocannabinoid signaling may actually interfere with learning tasks that require the suppression of previously learned responses. The observation that disruption of CB_1 receptor signaling does not affect initial learning of the platform location, but CB_1 (−/−) mice exhibit deficits in reversal learning (Varvel and Lichtman 2002) is consistent with this explanation. Conversely, one implication of such a model is that it may be possible to facilitate extinction under certain conditions by administering cannabinoid agonists (Parker et al. 2004) or blocking fatty acid amide hydrolase, which results in elevated levels of endogenous anandamide (Cravatt et al. 2001; Kathuria et al. 2003; Lichtman et al. 2004). It has been suggested that alterations in endocannabinoid signaling may underlie a variety of disorders including cognitive impairment, obsessive–compulsive disorders, and post-traumatic stress syndrome (Marsicano et al. 2002) as well as drug dependence (Parker et al. 2004). Consequently, pharmacotherapies directed at the endocannabinoid system may represent a viable approach to treat a variety of cognitive and behavioral disorders.

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