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# POST-TRAINING CB1 CANNABINOID RECEPTOR AGONIST ACTIVATION DISRUPTS LONG-TERM CONSOLIDATION OF SPATIAL MEMORIES IN THE HIPPOCAMPUS

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**Abstract—Cannabinoids have long been associated with mnemonic deficits. However, existing evidence has generally focused on the effect of cannabinoids when they are delivered prior to task-training, and such findings are confounded by possible drug effects on sensory, motor, and/or motivational systems that support the acquisition and the expression of learning. The present study investigated the effects of the $CB_1$-receptor agonist WIN 55,212-2 (WIN) on memory consolidation in the Morris water maze. In experiment 1, systemic injections of either WIN or DMSO vehicle were given daily following each training day (post-training), and rats were probe-tested 1 week or 4 weeks later. Rats injected with 1 mg/kg and 3 mg/kg of WIN spent significantly less time in the target quadrant compared with controls 4 weeks later, while no difference was observed at 1-week retention. In experiment 2, intrahippocampal injections of WIN were administered to the dorsal hippocampus following each training day and rats were again probe-tested 1 week or 4 weeks later. Rats bilaterally infused with WIN at 2.5 $\mu$g and 5 $\mu$g (per side) during training spent significantly less time in the target quadrant than vehicle controls on probe trial 4 weeks later, while no difference was seen at 1-week retention. Taken together, our results showed that post-training activation of $CB_1$ receptors in the hippocampus disrupts long-term memory consolidation but has no effect on acquisition and short-term retention. Plausible pharmacological interactions between cannabinoids and other neurotransmitter systems and associated plasticity mechanisms are discussed. © 2008 Published by Elsevier Ltd on behalf of IBRO.**

**Key words: cannabinoids, CB1 receptor, consolidation, hippocampus, spatial memory, WIN 55,212-2.**

The plant *Cannabis sativa*, more commonly known as marijuana, has long been used for its psychoactive and medicinal properties. Like many psychoactive substances, marijuana delivers both positive and negative effects. Beneficial effects of marijuana include euphoria, analgesia, lowering of intraocular pressure, appetite enhancements, as well as anti-emesis (Grotenhermen, 2003). Negative effects of marijuana-use include attention and memory deficits, hallucination, and respiratory diseases as a result of smoking the drug. The major psychoactive cannabinoid in marijuana plants has been identified as delta-9-tetrahydrocannabinol ($\Delta^9$-THC) (Gaoni and Mechoulam, 1964). In mammals, $\Delta^9$-THC has equal affinity to the two known cannabinoid receptor subtypes (cannabinoid receptor 1, $CB_1R$; and cannabinoid receptor 2, $CB_2R$) and possibly a third receptor subtype (G-protein coupled receptor GPR55) recently discovered by Baker and colleagues (2006).

Internally, endogenous cannabinoid agonists or endocannabinoids have been known to activate cannabinoid receptors. In mammals, $CB_2Rs$ are mainly found in the periphery, whereas $CB_1Rs$ are predominantly found in the CNS. In both humans and rats, $CB_1Rs$ are strongly expressed in the hippocampus, basal ganglia, and substantia nigra, among various neural and visceral regions (Mailleux and Vanderhaeghen, 1992; Mailleux et al., 1992). Physiological and cognitive effects of cannabinoids are generally attributed to $CB_1R$ activation (for review, see Grotenhermen, 2005).

A number of studies have established the influence of $CB_1R$ on learning and memory (Misner and Sullivan, 1999; Nava et al., 2001; Pamplona and Takahashi, 2006). In humans, marijuana use is associated with cognitive decline such as reduced alertness, impaired working memory, and impaired acquisition of psychomotor tasks (Wadsworth et al., 2006; Papafotiou et al., 2005). In rodents, cannabinoid activation has been observed to impair memory (Varvel and Lichtman, 2002), as well cannabinoid blockade has been consistently found to facilitate memory (Castellano et al., 2003). Specifically, $CB_1R$ activation in the dorsal hippocampus is known to produce impairments of spatial memory as well as nonspatial deficits; the mnemonic impairments produced by $CB_1R$ agonists are reversible by $CB_1R$ blockade (Pamplona and Takahashi, 2006; Egashira et al., 2002; Mallet and Beninger, 1998).

While the effects of $CB_1R$ activation on learning and memory are well-established, the specific role of cannabinoids in the different stages of memory is unclear. The majority of published findings are focused on the systemic effects of cannabinoids delivered prior to training and/or during acquisition. It is not apparent from these studies whether cannabinoids affect the acquisition, consolidation, or the retrieval of memory. Moreover, memory performances are often confounded by state-dependent effects of cannabinoids on sensorimotor functions and motivation, as well as the physiological effects produced by $CB_2R$

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*Abbreviations:* $CB_1R$, cannabinoid receptor 1; $CB_2R$, cannabinoid receptor 2; DMSO, dimethylsulfoxide; LTP, long term potentiation; MWM, Morris water maze; NMDAR, *N*-methyl-D-aspartate receptor; WIN, WIN 55,212-2; $\Delta^9$-THC, delta-9-tetrahydrocannabinol.

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activation in the periphery. Thus, the effects of cannabinoids during various stages of memory processing are unclear.

The goal of the present study is to determine if cannabinoid activation affects memory consolidation. We propose to dissociate the effects of cannabinoids on consolidation by activating $CB_1R$ after daily training in the Morris water maze (MWM)—a time when memory consolidation is most likely to occur—and assess spatial recall at various retention intervals after acquisition. We administered the synthetic $CB_1$ agonist WIN 55,212-2 (WIN) systemically and intracranially to assess both methods of drug delivery on spatial recall. We chose the dorsal hippocampus as our intracranial site of administration as this region is strongly implicated in spatial memory (Ferbinteanu and McDonald, 2000, 2003). We chose the MWM task as our behavioral assay for several reasons. Firstly, performance in the MWM is hippocampal-dependent; thus the task is well suited to assess our selective targeting of $CB_1Rs$ in the dorsal hippocampus (Morris et al., 1982; Sutherland et al., 1982; McDonald and Hong, 2000). Secondly, spatial acquisition in the MWM is gradual; drug treatments can be administered in between days of training when memory consolidation is most likely to occur. Thirdly, learning in the MWM is robust; memory for the task is retained for weeks, thus allowing assessments of cannabinoid effects in both long-term and short-term memories.

## EXPERIMENTAL PROCEDURES

### Subjects

One hundred eleven Long-Evans male rats (weight 225–275 g) were obtained from Charles River Laboratories (Saint-Constant, Québec, Canada). Of these, 64 animals were allocated to experiment 1 and 47 animals to experiment 2. Rats were housed in pairs within clear plastic bins, with food and water available *ad libitum*. Rats were maintained on a 12-h light/dark cycle, and the colony was maintained at 20–21 °C. Testing took place during the light period of the cycle.

### Apparatus

Subjects were trained for six consecutive days in a standard MWM with invisible platform. The pool measured 1.5 m in diameter and 0.5 m deep. A stationary platform was positioned in the northeast quadrant of the pool, 28 cm from the pool perimeter. A round white cylindrical pedestal (12 cm in diameter and 28 cm tall) served as the platform. Skim milk powder was added to the pool water to render it opaque. The water level was kept approximately 2 cm above the platform surface, to render the platform invisible. The test room was 3.1×6.1 m, with the pool raised 48 cm above the floor in the center of the room. The walls of the pool room had multiple black and white as well as colored posters, which served as distal cues. Water temperature in the pool was maintained at 20–22 °C.

### Behavioral training

*Place learning.* Rats were trained in the MWM navigation task for spatial memory of an invisible platform in the northeast quadrant. For six consecutive days, subjects performed eight distributed swimming trials per day with two trials beginning in each of the four start positions (N, E, S, W). The order of start positions was randomized for each day, and across days, for all animals. Latency and swim patterns were digitally tracked by HVS Image Water 2020™ (©1985–2002). Animals were trained in groups of eight; each animal was removed from the holding cage individually and placed in the water facing the pool wall. Each training trial began when the rat was released in the water; trials terminated when the rat reached the platform or after 60 s had elapsed. If the animal did not find the platform after 60 s, it was guided to the platform location by the experimenter. Following escape or aided placement onto the platform, the animal was required to remain there for 10 s before returning to its holding cage by the experimenter.

*Probe test.* During the probe test trial, the invisible platform was removed from the water maze. Rats were transported in their respective groups to the water maze room, and were left there for 20 min to habituate to the room. Rats were taken one at a time from the holding cage, and placed in the water facing the pool wall from due west, which was deemed to be the farthest point from the platform location. The trial began when each rat was released in the water, and terminated after 30 s had elapsed; each rat was then returned to its home cage.

### Experiment 1: Systemic injections of WIN on spatial consolidation

Fig. 1 provides the brief protocol of experiments 1 and 2. In experiment 1, 64 rats were randomly assigned to one of the four treatment groups: 0.5 mg/kg, 1 mg/kg, 3 mg/kg, or DMSO vehicle. The dose of 0.5 mg/kg was included in our experiment at a later time in an attempt to find an ineffective dose of the drug. WIN was dissolved in concentrations of 0.5 mg/ml, 1 mg/ml or 3 mg/ml in 100% dimethylsulfoxide (DMSO) in preparations for the i.p. injections of 0.5 mg/kg, 1 mg/kg or 3 mg/kg, respectively. During spatial acquisition, rats received six consecutive days of training in the MWM and were injected with WIN daily after training. Injections were made within 5 min of the last swim of the day. A probe test for spatial memory recall was given at the two different retention periods (i.e. 1 week or 4 weeks post-training). Thus, four groups (i.e. the four doses) and two retention periods were tested in the present experiment. An average of eight animals was assigned to each dose at each delay. Body-weights were obtained every other day before the start of water maze training to prepare for the amount to be injected. Following their injections, rats were returned to their home cages.

### Experiment 2: Intrahippocampal infusions of WIN on spatial consolidation

To examine the effects of intracranial $CB_1R$ activation on spatial memory consolidation, WIN was microinfused into the dorsal hip-

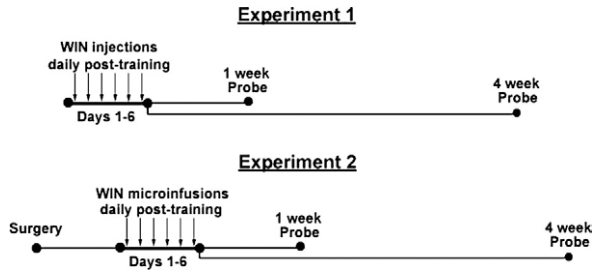


**Fig. 1.** Summary of test protocols for experiments 1 and 2. In experiment 1, rats were trained to a hidden platform location in the MWM on six consecutive days and received systemic injections of WIN daily post-training. Rats were either probe-tested 1 week or 4 weeks later. In experiment 2, rats received dorsal hippocampal cannulae surgical implants were trained to a hidden platform location as in experiment 1. After each day's training, rats received intrahippocampal infusions of WIN. Rats were either probe-tested 1 week or 4 weeks later.

pocampus daily post-training. In preparation for the microinfusions, all 47 rats received bilateral cannulae implantation to the dorsal hippocampus prior to behavioral training.

*Surgery.* Surgical operations were done according to guidelines provided by the Canadian Council on Animal Care (CCAC) on the ethical use of animals. The minimal number of rats required for data collection was used and measures were taken to minimize their suffering. Surgery was done under isoflurane anesthesia in a standard stereotaxic apparatus. During surgery ophthalmic liquid gel was applied to the animal's eyes for protection, the hair was removed from the top of the animal's head with an electric shaver, and the scalp was cleaned with alcohol and Hibitane. An incision was made down the midline with a scalpel blade from above the ears to below the eyes. The fascia (periosteum) was cut laterally across the top of the skull and pushed to the edges of the skull with a sterile gauze swab. The skin was retracted with four mosquito forceps to expose the skull surface, and trephining holes were drilled into the skull using a 2 mm drill bit and a high speed drill. Rats were bilaterally implanted with 26 gauge guide cannulae into the dorsal hippocampus. The tip coordinates for the cannulae were AP: −4.0; ML: ±2.5; DV: −1.9; the coordinates were in millimeters relative to the skull surface at bregma. Three to four jeweler's screws were secured into the skull and dental acrylic was applied to hold the cannulae in place. Obturators (made from 32 gauge wires) were inserted flush with the tip of the cannulae to block foreign materials from entering the brain. Following surgery, animals were s.c. injected with 0.07 ml of 0.3 mg/ml buprenorphine (Temgesic) for analgesia. Animals were monitored after surgery until they became active. Rats were then housed individually for 3 days, to allow for recovery and were group-housed and handled daily thereafter. The rats were given a total of 1 week to recover from surgery before behavioral testing commenced.

Upon recovery from surgery, rats were trained in the MWM for acquisition. Rats were randomly assigned to three groups that included a low dose (5 $\mu$g/$\mu$l), high dose (10 $\mu$g/$\mu$l), or control condition and were trained with their respective groups (Martin et al., 1999).

*Microinfusions.* After the last swim on each training day, rats were individually transported to an adjacent room to receive intrahippocampal microinfusions. WIN was bilaterally infused to the predetermined coordinates in the dorsal hippocampus within 3–5 min of the rat's last swim. In preparation for the infusions, rats were individually wrapped in a small towel to keep them immobilized, and obturators were removed from the guide cannulae. To introduce WIN or the vehicle into the brain parenchyma, a 32 gauge injector needle, attached to a Harvard mini-pump via polyethylene tubing (PE20), was passed through each cannula to extend 1.0 mm beyond the cannula tip. Rats were bilaterally infused with low dose, high dose, or vehicle at a rate of 0.5 $\mu$l/min for 1 min. Injector needles were left in place for an additional 2 min to allow for drug diffusion. The extent of diffusion was estimated to be 1 mm in diameter from the injector tips.

WIN drug was prepared for microinfusions at the following concentrations: 5 $\mu$g/$\mu$l (low dose) and 10 $\mu$g/$\mu$l (high dose) in vehicle solution. The vehicle consists of 1:1:18 of ethanol:alkamuls:saline. Powdered WIN was added to a 1:1 mixture of ethanol and alkamuls EL-620 (Rhodia Pharma Solutions, Cranbury, NJ, USA) to vortex until dissolved, after which it was diluted with 18 parts of saline (for methods, see Lichtman et al., 1995).

*Histology.* Upon completion of the experiment, animals were killed with an overdose of euthansol (0.4 ml per animal administered i.p.). Animals were transcardially perfused with 60 ml of 0.9% saline, followed by 60 ml of 4% formalin. Brains were removed and were post-fixed in a fixative solution of 30% sucrose in 4% formalin. Brains were frozen on a cryostat at −21 °C, and 40 $\mu$m coronal sections were cut. Sections near the target coordinates were mounted and stained in Cresyl Violet (0.1%).

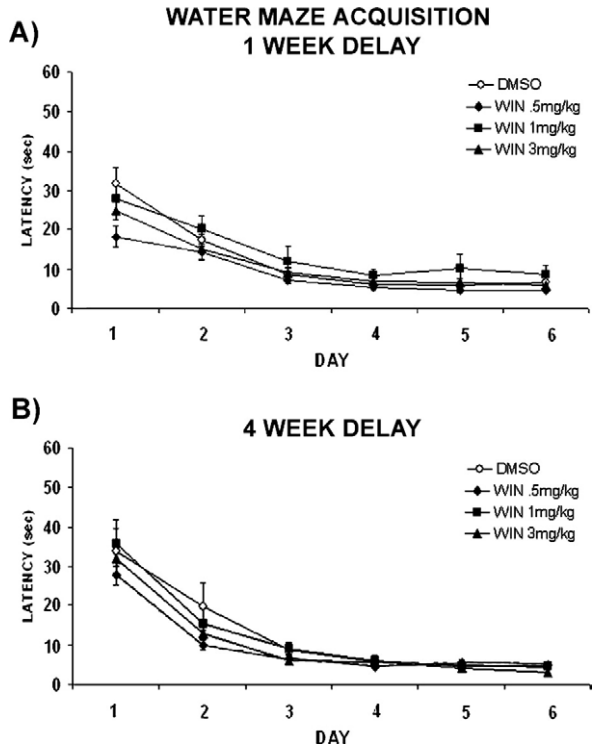


**Fig. 2.** Acquisition of the hidden platform location in the MWM as expressed by group mean escape latencies (seconds) over 6 days of training for rats in experiment 1. The plots indicate spatial learning of the hidden platform location by all doses for rats probe-tested 1 week (A) or 4 weeks (B) later. Daily injection of WIN and DMSO post-training has no effect on spatial acquisition as there was no significant difference in mean escape latencies among the doses. Data expressed as mean±S.E.M.

## RESULTS

### Experiment 1

*Place learning for 1-week delay.* Fig. 2A displays the learning curve for the four groups of animals that underwent water maze training for the 1-week delay. Although the figure shows latency data for the 6 days of training, the analysis was only carried out on days 2 through 6 because the first post-training manipulation occurred after day 1. Latency was defined as the amount of time from the start of each trial to when the animal reached the platform. A two-way ANOVA with repeated measures performed on the behavioral data confirmed our impressions that there were no differences among the various doses of WIN [$F(3, 28)=1.71$, $P=0.19$] as their latency to locate the platform decreased similarly over the training sessions [$F(4, 112)=59.52$, $P<0.000$].

*Probe test at 1-week delay.* Fig. 3 displays the representative probe swim paths as well as the performance on the probe test for rats across the two delays in experiment 1. For data analysis on the probe test, the percentage of time spent in the target quadrant (i.e. the northeast

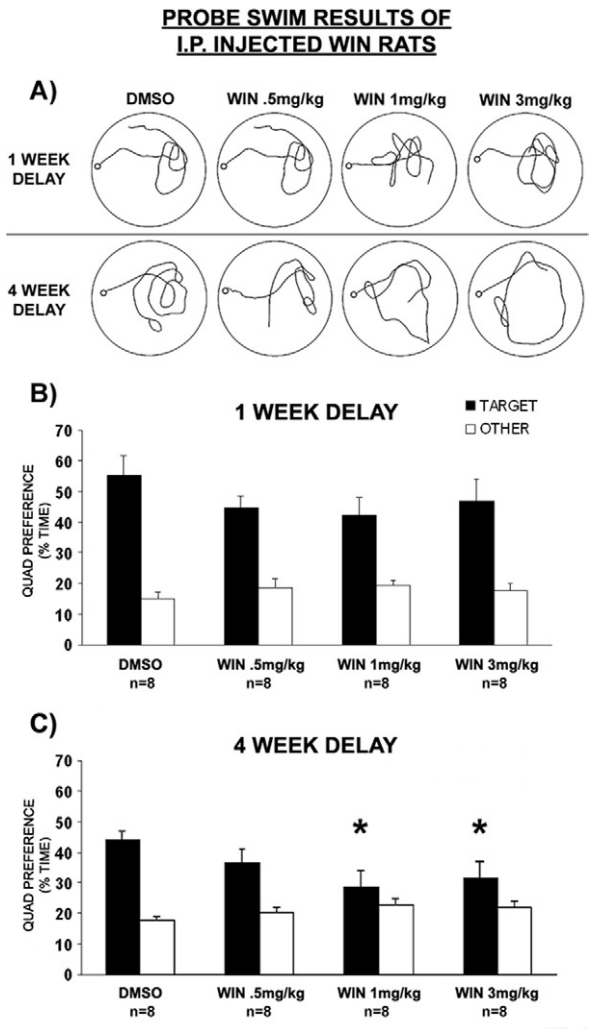


**Fig. 3.** Data from experiment 1 showing representative swim paths and results of the probe trial (0–30 s) at 1 or 4 weeks after the last day of MWM acquisition. For the swim paths (A), all probe trials began from due west (denoted by the small circles); the northeast quadrant is the target quadrant. Probe test at 1 week post-training (B) showed no significant difference in the preference for target quadrant. Probe test at 4 weeks post-training (C) showed significantly less preference for the target quadrant for WIN 1 mg/kg group and WIN 3 mg/kg group compared with controls. * $P<0.05$ compared with the mean target preference for controls. Data expressed as mean±S.E.M.

quadrant) was compared with the average percentage of time spent in the three non-target quadrants for each delay. Fig. 3B displays the results of the probe test given 1 week after the last training session. As depicted, all groups of animals displayed good retention of the platform location; they spent significantly greater amount of time in the target quadrant relative to the other quadrants. This observation was supported by a two-way ANOVA with fixed effects that indicated no effect of group [$F(3, 28)=.97$, $P=0.42$], and a significant effect of quadrant [$F(1, 28)=60.69$, $P<0.000$].

*Place learning for 4-week delay.* The performance of a second set of rats given place training is presented in Fig. 2B. As shown, there was no difference in platform acquisition among the groups, as all latency scores decreased similarly over the training days. This impression was verified by a two-way ANOVA with repeated measures that showed a highly significant effect across training days [$F(4, 112)=26.22$, $P<0.000$], but no group effect [$F(3, 28)=1.17$, $P=0.34$].

*Probe test at 4-week delay.* Fig. 3C displays the results of the probe test given after a 4-week delay. A two-way ANOVA with fixed effects showed a significant effect of quadrant [$F(1, 28)=25.03$, $P<0.000$], but no group interaction [$F(3, 28)=2.48$, $P=0.08$]. Post hoc comparisons (LSD) revealed a significant difference between the controls and the WIN 1 mg/kg group [$P<0.02$], and between the controls and the WIN 3 mg/kg group [$P<0.05$]. No differences were observed between the controls and WIN 0.5 mg/kg group [$P=0.24$].

Taken together, the results of experiment 1 indicate that post-training systemic injections of WIN at both 1 mg/kg and 3 mg/kg during acquisition phase disrupt spatial recall 4 weeks later. No effect on spatial recall was evident at 1-week retention across doses. A sub-dose of 0.5 mg/kg of WIN tested at both 1-week and 4-week retentions did not disrupt recall. The WIN drug did not appear to alter memory at every dose and retention; a minimal dose of 1 mg/kg appears to be required to produce spatial memory impairments 4 weeks post-training.

## Experiment 2

*Histology.* The locations of the cannulae or injector tips were determined under light microscope viewing with the aid of Paxinos and Watson's atlas (1998). Data from two animals from the 1-week retention were excluded due to infected cannulae ($n=1$, control rat) and faulty cannula placements outside the target region ($n=1$, high dose rat); three animals in the 4-week retention were excluded due to infected cannulae ($n=2$, one control and one low dose rat) and faulty cannula placements ($n=1$, control rat). The final data analysis included data from 42 rats; 22 rats were probe-tested following 1-week retention and 20 rats following 4-week retention. Fig. 4 depicts the cannula placements of all animals included in the analysis.

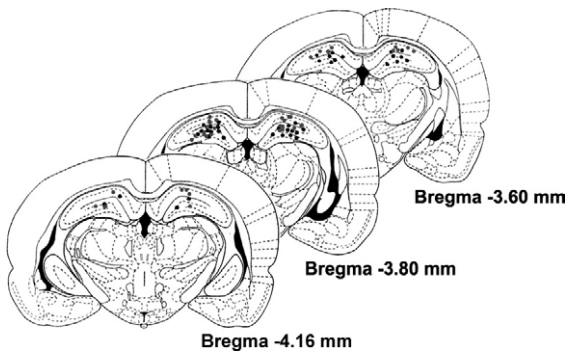


**Fig. 4.** Location of the injector sites in the dorsal hippocampus in experiment 1 (gray dots) and experiment 2 (black dots). Placements are shown from all animals included in the behavioral analyses (figure adapted from Paxinos and Watson, 1998).

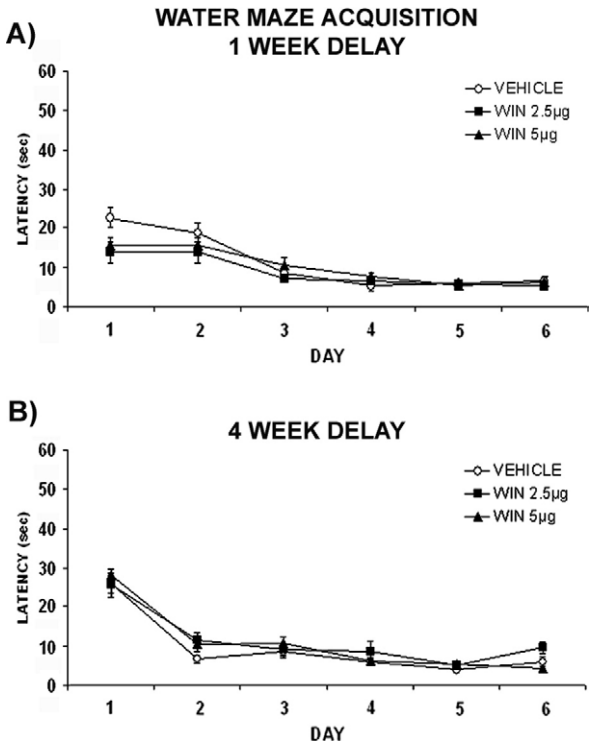


**Fig. 5.** Acquisition of the hidden platform location in the MWM over 6 days of training as expressed by group mean escape latencies for rats in experiment 2. Rats who received bilateral microinfusion of the vehicle, 2.5 $\mu$g of WIN, or 5 $\mu$g of WIN to the dorsal hippocampus daily post-training were probe-tested at either 1 week (A) or 4 weeks (B) later. The plots showed robust learning of the platform location by all doses of intracranial WIN. No significant difference in mean escape latencies among the treatment groups was found. Data expressed as mean±S.E.M.

*Place learning for 1-week delay.* The acquisition of the hidden platform for the intracranially injected animals with 1-week retention ($n$=22) is shown in Fig. 5A. As illustrated, acquisition was unaffected by the dose of daily intracranial infusion of WIN post-training as all groups reached the platform location more readily as training progressed. This was supported by a two-way ANOVA with repeated measures indicating a main effect of latencies across days 2–6 [$F$(4, 76)=49.72, $P$<0.000], but no significant effects of group [$F$(2, 19)=.72, $P$=0.50] or interaction [$F$(8, 76)=1.60, $P$=0.20].

*Probe test at 1-week delay.* Fig. 6 displays the representative probe swim paths as well as the performance on the probe test for rats across the two delays in experiment 2. As shown in Fig. 6B, all groups at 1-week delay exhibited a preference for the target quadrant. A two-way ANOVA with fixed effects was performed on the percentage of time spent in the target quadrant and the average percentage of time spent in the three non-target quadrant. For rats probe-tested 1 week after the end of training, a main effect of quadrant [$F$(1, 19)=25.50, $P$<0.000] was found; all rats showed a preference toward the target quadrant regardless of group. No main effect of group [$F$(2, 19)=.36, $P$=0.70] or an interaction of group and quadrant preference [$F$(2, 19)=.38, $P$=0.69] was observed.

*Place learning for 4-week delay.* Fig. 5B shows the latencies for the intracranially injected animals ($n$=21) across 6 days of place learning. A two-way ANOVA with repeated measures found a main effect of latency across training days 2–6 [$F$(4, 68)=7.184, $P$<0.000], confirming acquisition of the platform location. There was no significant difference among the three groups during acquisition [$F$(2, 17)=1.347, $P$=0.286], and no interaction was evident between group and latencies across days [$F$(8, 68)=1.784, $P$=0.095].

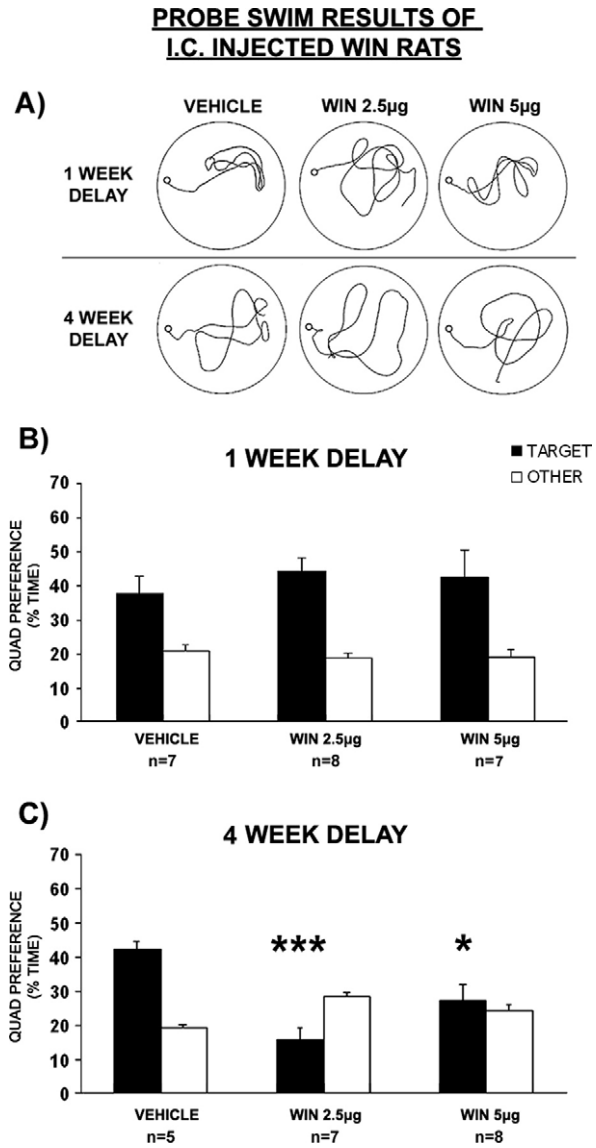


**Fig. 6.** Data from experiment 2 showing representative swim paths and results on the probe trial (0–30 s) at 1 or 4 weeks after the last day of MWM acquisition. For the swim paths (A), all probe trials began from due west (denoted by the small circles) as with experiment 1; the northeast quadrant is the target quadrant. Probe test results at 1 week post-training (B) showed no significant difference among the groups of rats. At 4 weeks post-training (C), both WIN groups showed significantly less preference for the target quadrant compared with vehicle controls. * $P$<0.05, *** $P$<0.01, compared with the mean target preference of vehicle rats. Data expressed as mean±S.E.M.

*Probe test at 4-week delay.* A probe test was conducted 4 weeks after the last acquisition training day. As can be seen from Fig. 6C, the vehicle group spent more time in the target versus non-target quadrants, while the WIN groups did not show the same preference. A two-way ANOVA with fixed effects performed on the probe trial supported this observation indicating a main effect of group [$F(2, 17)=9.639$, $P=0.002$] and an interaction between group and quadrant [$F(2, 17)=9.616$, $P=0.002$]). Post hoc comparisons (LSD) revealed significant differences among all treatment groups in the amount of time spent in target and non-target quadrants. Target quadrant preference was significantly different between vehicle and WIN 2.5 $\mu$g (low dose) [$P<0.000$], vehicle and WIN 5 $\mu$g (high dose) [$P=0.02$], and between WIN 2.5 $\mu$g and 5 $\mu$g groups [$P=0.047$]. Non-target quadrant preferences were significantly different between vehicle and WIN 2.5 $\mu$g [$P<0.000$], vehicle and WIN 5 $\mu$g [$P=0.021$], and WIN 2.5 $\mu$g and 5 $\mu$g groups [$P=0.047$].

In summary, the results of experiment 2 show that post-training intracranial injections of WIN to the dorsal hippocampus selectively disrupt long-term spatial recall. Injections of both 2.5 $\mu$g and 5 $\mu$g of WIN during acquisition spared spatial recall 1 week post-training, while disrupted spatial recall 4 weeks later. The greatest impairment at 4 weeks' retention was observed in the group of subjects that received the 2.5 $\mu$g dose of WIN.

## DISCUSSION

Using a post-training injection paradigm, the present study demonstrated that the cannabinoid agonist WIN impairs the consolidation of long-term spatial memory while sparing spatial acquisition and short-term retention. In both experiments 1 and 2, WIN had no effect on the acquisition of the hidden platform location in the Morris water task. In experiment 1, systemic injections of the drug after daily training produced no impairment of recall 1 week later. However, when retention was tested at 4 weeks, spatial recall was impaired. Similarly in experiment 2, intrahippocampal infusions of WIN did not disrupt acquisition of the hidden platform location; it spared spatial recall at 1 week, while disrupting recall 4 weeks later. Our results from experiment 2 also confirmed that the observed impairment was due to $CB_1R$ activation localized in the dorsal hippocampus. In summary, we conclude that post-training activation of $CB_1R$ in hippocampus disrupts spatial memory consolidation for long-term but not short-term spatial memory.

Interestingly, the consolidation impairment we observed in experiment 2 appears to be related to the dose of WIN microinfused in a biphasic fashion. The high dose treatment group showed a moderate but significant impairment while the low dose treatment group showed greater impairment than both the high-dose and the vehicle-infused groups.

Although we did not explore the effects of cannabinoid antagonist in our study, recent evidence from Takahashi and colleagues (2005) has shown that the $CB_1R$ antagonist SR 141716A administered post-training facilitates consolidation in mice in an inhibitory avoidance task. Their findings parallel ours in which cannabinoid agonist WIN impairs consolidation, thus providing indirect support that our effect was due to $CB_1R$ targeting. In addition, we observed similar long-term memory impairments in both systemic injections and intrahippocampal infusions of WIN. As $CB_2R$ is not expressed in the hippocampus, the observed impairment is likely due to $CB_1R$ activation.

### Selective targeting of memory consolidation

In contrast to the majority of cannabinoid memory research in which cannabinoids are typically administered prior to acquisition or training, the present study was able to dissociate the extraneous effects of cannabinoid exposure on acquisition by selectively targeting post-training memory consolidation. In our study, the cannabinoid agonist WIN was administered daily post-training when memory consolidation is most likely to occur. It should be noted that in our design, while there is a possibility that post-training administration of WIN may affect acquisition on the next day, we found no such evidence. Spatial acquisition of WIN rats was comparable to controls. Retrieval was also not targeted, as rats were drug free at the time of recall. Thus, our interpretation only pertains to the effects of cannabinoids on memory consolidation.

### Short-term versus long-term memory consolidation

In experiments 1 and 2, post-training daily administrations of WIN during acquisition produced a significant impairment in spatial retention 4 weeks later. However, no impairment was observed after retention of 1 week. One possible explanation for this effect is that relatively short term spatial memory (as in our 1-week retention paradigm) utilizes a different consolidation mechanism than that used for long-term memory. Previous work from our group has shown that NMDAR (*N*-methyl-D-aspartate receptor) blockade in either the dorsal hippocampus or the mediodorsal striatum impairs long-term but not short-term spatial retention (McDonald et al., 2005; Holahan et al., 2005). Using third generation knockout mice with reversible CA1-specific NMDAR functions, Shimizu and colleagues (2000) found that spatial recall was impaired when CA1-NMDARs were switched off during the early, but not the late, consolidation period (i.e. after training had ended); thus indicating that NMDAR plasticity is critical for early phases of memory consolidation. This evidence implicates NMDAR-mediated plasticity as the mechanism for long-term memory consolidation in the hippocampus.

Accumulating evidences suggest a link between NMDAR plasticity and cannabinoid activities (Davies et al., 2002; Terranova et al., 1995). By and large, cannabinoid receptor activation has an inhibitory effect on long term potentiation (LTP) induction; activation of $CB_1R$ is known to suppress NMDAR-mediated LTP by inhibiting voltage-gated $Ca^{2+}$ channels and activating $K^+$ channels (Melis et al., 2004). Thus, post-training administration of WIN in our current paradigm may dis-

rupt consolidation of long-term memory in the hippocampus via a suppression of NMDAR activity.

### NMDAR-mediated plasticity and acetylcholine on memory consolidation

Our findings from the intrahippocampal infusions of WIN in experiment 2 confirmed the long-term mnemonic impairment produced by systemic injections of the same drug in experiment 1 and showed that the cause of the impairment is localized in the dorsal hippocampus. This is consistent with previous research which implicates the dorsal hippocampus in spatial learning and memory (Moser et al., 1993; Ferbinteanu and McDonald, 2000, 2003; Hannesson et al., 2004; Cimadevilla et al., 2005). In experiment 2, daily post-training intrahippocampal infusions of WIN produced impairments in spatial recall which appear to be biphasic and dose-dependent, with the greatest magnitude of impairment produced at low dose infusion of the drug.

Although it is purely conjecture at this point, it is interesting to note a relationship between $CB_1R$ activation and hippocampal cholinergic release that could provide a plausible mechanism for the biphasic and dose-dependent effect of intrahippocampal WIN we found. As mentioned above, $CB_1R$ activation inhibits NMDAR plasticity in the hippocampus. Blockade of NMDAR has been shown to increase extracellular levels of acetylcholine in the hippocampus (Giovannini et al., 1994) and there is evidence that suggests a dose-dependent, biphasic relationship between $CB_1R$ activity and hippocampal cholinergic release (Tzavara et al., 2003). At low dose, systemic injection of WIN was found to enhance cholinergic efflux, while at high dose, it inhibited cholinergic activity. Functionally, acetylcholine tone has been implicated in memory consolidation. Findings from clinical studies have implicated better memory performances with low levels of acetylcholine during post-training slow-wave sleep, a stage of sleep that is critical for declarative memory consolidation (Gais and Born, 2004).

Various studies have reported that $CB_1R$ activation can result in biphasic (Sulcova et al., 1997; Darmani et al., 2002; Drews et al., 2005) or triphasic (Sanudo-Pena et al., 2000) changes in motoric function. Biphasic changes in neural responses have also been reported to occur after $CB_1R$ activation of neurons in the retina (Fan and Yazulla, 2003) and in the basolateral amygdala (Pistis et al., 2004). One possible explanation for the biphasic results in this study, therefore, is that microinfusion of WIN may have disrupted memory consolidation by enhancing hippocampal cholinergic release at the low dose thereby impairing consolidation, while only mildly affecting cholinergic release at higher dose of the drug. This process is likely to have occurred as a result of $CB_1R$-induced inhibition of NMDAR, since both $CB_1R$ and acetylcholine activities have been empirically linked with NMDAR.

The present findings have shown that systemic $CB_1R$ activation negatively impacts spatial memory consolidation and long-term retention of such memories. Moreover, we have shown that the impairment is caused by $CB_1R$ activation localized in the dorsal hippocampus. The process by which spatial memory is consolidated in the hippocampus is likely due to the complex interplay between cannabinoids, NMDAR and cholinergic receptors in the hippocampus. Further studies are needed to examine the interaction between NMDA and acetylcholine on cannabinoid-mediated plasticity and behavior.

### Implications

The present study provide evidence that long-term retention of information acquired prior to the use of cannabinoids is compromised, echoing previous findings that cannabinoids have negative effects on memory. Our findings underscore the adverse effects of cannabis on the long-term consolidation of memory and have important implications for both medicinal and recreational users of marijuana.

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