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Acute Cannabinoids Impair Working Memory through Astroglial CB_1 Receptor Modulation of Hippocampal LTD

Jing Han,[1,13] Philip Kesner,[2,13] Mathilde Metna-Laurent,[3,4,13] Tingting Duan,[5,6] Lin Xu,[5] Francois Georges,[4,7] Muriel Koehl,[3,4] Djoher Nora Abrous,[3,4] Juan Mendizabal-Zubiaga,[8] Pedro Grandes,[8] Qingsong Liu,[9] Guang Bai,[10] Wei Wang,[11] Lize Xiong,[12] Wei Ren,[1] Giovanni Marsicano,[3,4,14,*] and Xia Zhang[2,14,*]

[1]College of Life Sciences and Key Laboratory of Modern Teaching Technology, Shaanxi Normal University, Xian 710062, China
[2]University of Ottawa Institute of Mental Health Research at The Royal, Department of Psychiatry, and Department of Cellular and Molecular Medicine, Ottawa K1Z 7K4, Canada
[3]INSERM, Neurocentre Magendie
[4]University of Bordeaux, Neurocentre Magendie
Physiopathologie de la Plasticité Neuronale, U862, F-33000 Bordeaux, France
[5]Key Lab of Animal Models and Human Disease Mechanisms, Kunming Institute of Zoology, Chinese Academy of Science, Kunming 650223, China
[6]School of Life Sciences, University of Science and Technology of China, Hefei 230027, China
[7]CNRS, Interdisciplinary Institute for Neuroscience, UMR 5297, Bordeaux 33000, France
[8]Department of Neurosciences, Faculty of Medicine and Dentistry, Basque Country University, Leioa 48940, Spain
[9]Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226, USA
[10]Department of Neural and Pain Sciences, Dental School, Program in Neuroscience, University of Maryland, Baltimore, MD 21201, USA
[11]Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubai 430030, China
[12]Department of Anesthesiology, Xijing Hospital, Fourth Military Medical University, Xian 710032, China
[13]These authors contributed equally to this work
[14]These authors contributed equally to this work
*Correspondence: giovanni.marsicano@inserm.fr (G.M.), xia.zhang@rohcg.on.ca (X.Z.)
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SUMMARY

Impairment of working memory is one of the most important deleterious effects of marijuana intoxication in humans, but its underlying mechanisms are presently unknown. Here, we demonstrate that the impairment of spatial working memory (SWM) and in vivo long-term depression (LTD) of synaptic strength at hippocampal CA3-CA1 synapses, induced by an acute exposure of exogenous cannabinoids, is fully abolished in conditional mutant mice lacking type-1 cannabinoid receptors (CB_1R) in brain astroglial cells but is conserved in mice lacking CB_1R in glutamatergic or GABAergic neurons. Blockade of neuronal glutamate *N*-methyl-D-aspartate receptors (NMDAR) and of synaptic trafficking of glutamate α-amino-3-hydroxy-5-methyl-isoxazole propionic acid receptors (AMPAR) also abolishes cannabinoid effects on SWM and LTD induction and expression. We conclude that the impairment of working memory by marijuana and cannabinoids is due to the activation of astroglial CB_1R and is associated with astroglia-dependent hippocampal LTD in vivo.

INTRODUCTION

The treatments of pain, nausea, seizures, ischemia, cerebral trauma and tumors in humans and/or animals are some of the potential therapeutic applications of derivatives of the plant *Cannabis sativa* (marijuana) or synthetic cannabinoids (Lemberger, 1980; Robson, 2001; Brooks, 2002; Carlini, 2004; Hall et al., 2005). However, the potential therapeutic use of cannabis is limited by important side-effects associated with its use (Pacher et al., 2006). One of the major side effects of marijuana intoxication is the impairment of working memory in humans (Ranganathan and D'Souza, 2006) and animals (Lichtman and Martin, 1996; Hampson and Deadwyler, 2000; Nava et al., 2001; Varvel and Lichtman, 2002; Fadda et al., 2004; Hill et al., 2004; Wise et al., 2009), but the cellular mechanisms of this effect are presently not known.

Working memory is the ability to transiently hold and process information for reasoning, comprehension and learning, such as active thinking. Baddeley introduced a multicomponent model of human working memory with a central executive system responsible for information integration and coordination of two subsystems (Baddeley, 2003). One subsystem, the phonological loop, stores the sound of language while the other subsystem, the visuo-spatial sketch pad, stores visual (e.g., color) and spatial information (i.e., location). This theory suggests a key role of spatial processing in working memory

performance. Spatial working memory (SWM) in humans and animals requires online processing of information within many brain regions including the hippocampus (Hassabis et al., 2007; Kesner, 2007). The hippocampal excitatory CA3-CA1 synapses, which connect glutamatergic axons of CA3 pyramidal neurons, including the ipsilateral Schaffer collaterals and contralateral commissural fibers, with dendrites of CA1 pyramidal neurons (Witter and Amaral, 2004), have been proposed to play a key role in SWM (Rolls and Kesner, 2006).

Multiple forms of memory are likely subserved by activity- or experience-dependent long-term potentiation (LTP) and depression (LTD) of synaptic strength (Malenka and Bear, 2004). Chronic exposure of rats to cannabinoids impairs both LTP induction at CA3-CA1 synapses and hippocampal-dependent SWM (Hill et al., 2004), suggesting a link between LTP impairment and SWM impairment. This idea is supported by recent data that knockout of the AMPAR GluR1 subunit impairs both LTP induction at CA3-CA1 synapses and SWM (Sanderson et al., 2008). If LTP at CA3-CA1 synapses indeed contributes to SWM, LTD at these synapses may play a role in SWM impairment, because LTD could counteract LTP at the same synapses (Han et al., 2011).

Cannabinoid type-1 receptor (CB_1R), one of the most abundant G protein-coupled receptors in the brain (Herkenham et al., 1990), is found in both GABAergic and glutamatergic neurons in the hippocampal CA1 region (Herkenham et al., 1990; Kawamura et al., 2006; Marsicano and Lutz, 2006). Its main neuronal action is to inhibit presynaptic neurotransmitter release (Kano et al., 2009; Marsicano and Lutz, 2006). Indeed, cannabinoids can depress excitatory transmission at CA3-CA1 synapses in brain slices via activation of CB_1R (Misner and Sullivan, 1999; Hajos et al., 2001; Kawamura et al., 2006; Marsicano and Lutz, 2006; Takahashi and Castillo, 2006; Bajo et al., 2009; Serpa et al., 2009; Hoffman et al., 2010). Thus, cannabinoid-induced decrease of excitatory transmission might be related to SWM impairment. It is entirely unknown, however, whether cannabinoids are able to induce LTD at CA3-CA1 synapses in living animals and whether such in vivo LTD might contribute to SWM impairment induced by exogenous cannabinoids. In addition to the presence in neurons, CB_1R is also found in hippocampal astroglial cells and its activation, by stimulating Ca^{2+}-dependent release of glutamate, potentiates synaptic transmission at CA3-CA1 synapses in brain slices (Navarrete and Araque, 2010). However, the roles of astroglial CB_1R in the modulation of behavior and synaptic plasticity in living animals are not known.

In this study, we employed conditional mutagenesis, in vivo electrophysiology and behavioral tests to study the mechanism underlying the effect of cannabinoids on hippocampal-dependent SWM. Surprisingly, we found that activation of astroglial CB_1R, but not neuronal CB_1R, by exogenous cannabinoids mediates SWM impairment and LTD induction at CA3-CA1 synapses in vivo. Our data reveal an unanticipated hippocampal pathway linking astroglial activity, synaptic plasticity and memory processing, and define the specific mechanisms likely underlying cannabinoid-induced impairment of SWM in living animals.

RESULTS

Cannabinoids Induce In Vivo LTD at CA3-CA1 Synapses

In vivo recordings of field excitatory postsynaptic potentials (fEPSP) from CA3-CA1 synapses in anesthetized rats revealed that an i.p. injection of HU210 (0.05 or 0.1 mg/kg), a potent synthetic cannabinoid, or Δ^9-tetrahydrocannabinol (THC, 5 mg/kg), the major psychoactive ingredient of marijuana, decreased fEPSP amplitude to approximately 40% of the baseline levels (Figures 1A and 1G). Similar results were obtained after an intra-CA1 infusion of HU210 (Figures S1A and S1C). In studies hereafter, animals received an i.p. injection of 0.05 mg/kg of HU210 or 5 mg/kg of THC if not otherwise stated.

Cannabinoid-induced depression of synaptic transmission at CA3-CA1 synapses in brain slices is not defined as LTD, because it is fully reversed by application of CB_1R antagonists 10 min after cannabinoid application (Chevaleyre et al., 2006; Hajos et al., 2001; Kawamura et al., 2006). This indicates the requirement of a continuous activation of CB_1R for cannabinoid depression of transmission at CA3-CA1 synapses, a characteristic of transient synaptic depression but not of LTD (Chevaleyre et al., 2006). However, we observed that the decreased EPSP amplitude was blocked by injection of the selective CB_1R antagonist AM281 (3 mg/kg, i.p.) (Cui et al., 2001) 10 min before, but not 10 min after HU210 administration (Figures 1B and 1G), thus indicating LTD induction by cannabinoid exposure in vivo (hereafter referred to as CB-LTD). This idea is further supported by two lines of evidence. First, while synaptic transmission depression can be transient (in min) or long-lasting (i.e., LTD lasting > 24 h), a HU210 injection (0.1 mg/kg, i.p.) induced CB-LTD at CA3-CA1 synapses for > 24 hr in freely moving rats (Figures 1E and 1G), at a time where the acute effects of the drug should be decreased. Second, while the maintenance of late-phase LTD, but not early-phase LTD or transient synaptic transmission depression, requires new protein synthesis (Kelleher et al., 2004), administration of inhibitors of protein translation (anisomycin, 18 mg/kg, i.p.) (Puighermanal et al., 2009) or RNA transcription (actinomycin-D, 72 μg/12 μl, i.c.v.) (Manahan-Vaughan et al., 2000) 2 hr before HU210 injection selectively reversed the late-phase expression of CB-LTD (Figures 1C and 1G).

To identify if CB_1R expressed in the CA1 area contributes to CB-LTD at CA3-CA1 synapses, we applied adenoviral vectors-containing shRNA against CB_1R into the CA1 region 4 days prior to HU210 injection. shRNA CB_1R specifically knocked down CA1 expression of CB_1R (Figure 1F) and suppressed CB-LTD at CA3-CA1 synapses (Figures 1D and 1G). Interestingly, the cannabinoid effect seems to be specific for the CA3-CA1 pathway, because systemic HU210 did not induce CB-LTD at synapses of the perforant path onto dentate gyrus neurons (Figures S1B and S1C). Thus, in vivo cannabinoid exposure induces an in vivo LTD at CA3-CA1 synapses.

Neuronal CB_1R Is Dispensable for CB-LTD at CA3-CA1 Synapses

Glutamatergic presynaptic membranes of CA3-CA1 synapses contain CB_1R (Kawamura et al., 2006). To test whether CB-LTD

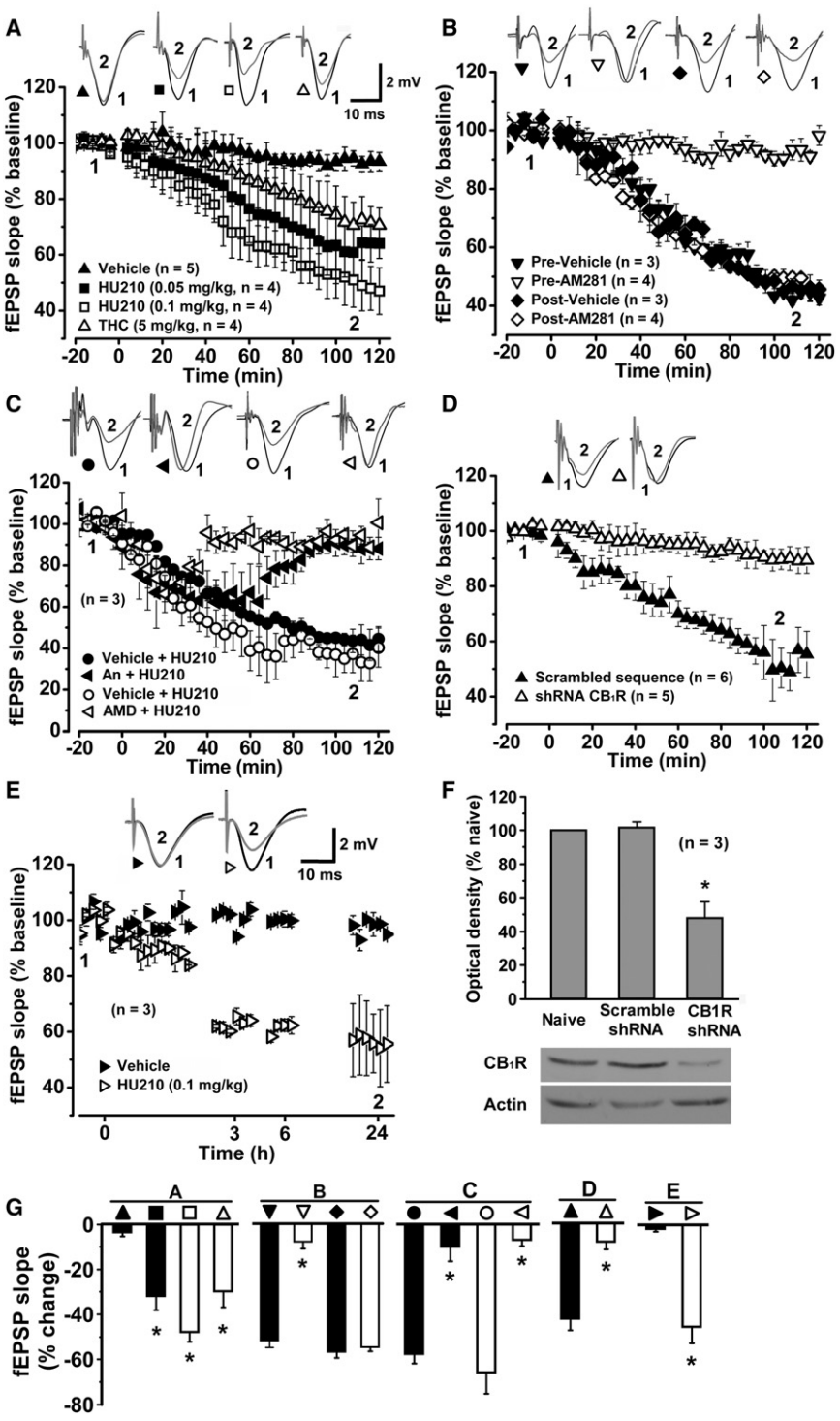

Figure 1. Cannabinoids Induce In Vivo LTD at CA3-CA1 Synapses

(A–E) Plots of normalized fEPSP slopes in anesthetized rats (A–D) or freely moving rats (E) show that cannabinoid injection at 0 min elicits CA1 LTD lasting for > 2 hr (A–D) or > 24 hr (E), which is blocked by AM281 administration 10 min before, but not 10 min after, HU210 injection (B), or by intra-CA1 infusion of shRNA CB_1R (D), and that anisomycin (An) and actinomycin-D (AMD) selectively reverse the late-phase expression of HU210-elicited LTD (C). Representative fEPSP traces before (1) and after (2) vehicle or cannabinoid injection are shown above each plot.

(F) Graph (top) and immunoblotting photos (bottom) show a reduction of CA1 CB_1R expression by shRNA CB_1R.

(G) Histogram summarizes the average percent change of fEPSP slope before (1) and after (2) vehicle or cannabinoid injection as depicted in panels (A)–(E).

All summary graphs show means ± standard error of the mean (SEM); n = numbers of animals recorded in each group (A–E) or numbers of experiments conducted (F) in each group. *p < 0.01 versus vehicle control, Bonferronni post-hoc test after one-way ANOVA (A: $F_{3,13} = 56.560$, $p < 0.01$; B: $F_{3,10} = 39.001$, $p < 0.01$; C: $F_{3,8} = 47.210$, $p < 0.01$; F: $F_{2,6} = 34.990$, $p < 0.01$) or t test.

See also Figure S1.

depends on "glutamatergic" CB_1R, we examined mutant mice carrying a selective deletion of the *CB_1R* gene in cortical and hippocampal glutamatergic principal neurons (Glu-*CB_1R*-KO) (Monory et al., 2006; Bellocchio et al., 2010). Surprisingly, THC induced a CB-LTD at CA3-CA1 synapses that was indistinguishable between wild-type mice and Glu-*CB_1R*-KO littermates (Figures 2A and 2C). We then determined the induction of CB-LTD in mutant mice carrying a selective deletion of the *CB_1R* gene in brain GABAergic neurons (GABA-*CB_1R*-KO), including CA1 GABAergic neurons (Monory et al., 2006; Bellocchio et al., 2010). Again, THC induced a CB-LTD at CA3-CA1 synapses that was indistinguishable between wild-type mice and GABA-*CB_1R*-KO littermates (Figures 2A and 2C). Thus, CB_1R expressed in glutamatergic or GABAergic neurons does not participate in this in vivo form of CB-LTD in the hippocampal CA1 region.

Astroglial CB_1R Mediates CB-LTD at CA3-CA1 Synapses

CB_1R is also functionally expressed in CA1 astrocytes (Navarrete and Araque, 2008). Therefore, astroglial CB_1R might play a role in CB-LTD at CA3-CA1 synapses. To directly address this issue, we generated tamoxifen-inducible conditional mutant mice specifically lacking CB_1R expression in astrocytes. "Floxed" *CB_1R* mutant mice (Marsicano et al., 2003) were crossed with transgenic mice expressing the inducible version of the Cre recombinase CreERT2 under the control of the promoter of the human glial fibrillary acidic protein (GFAP-CreERT2 mice,

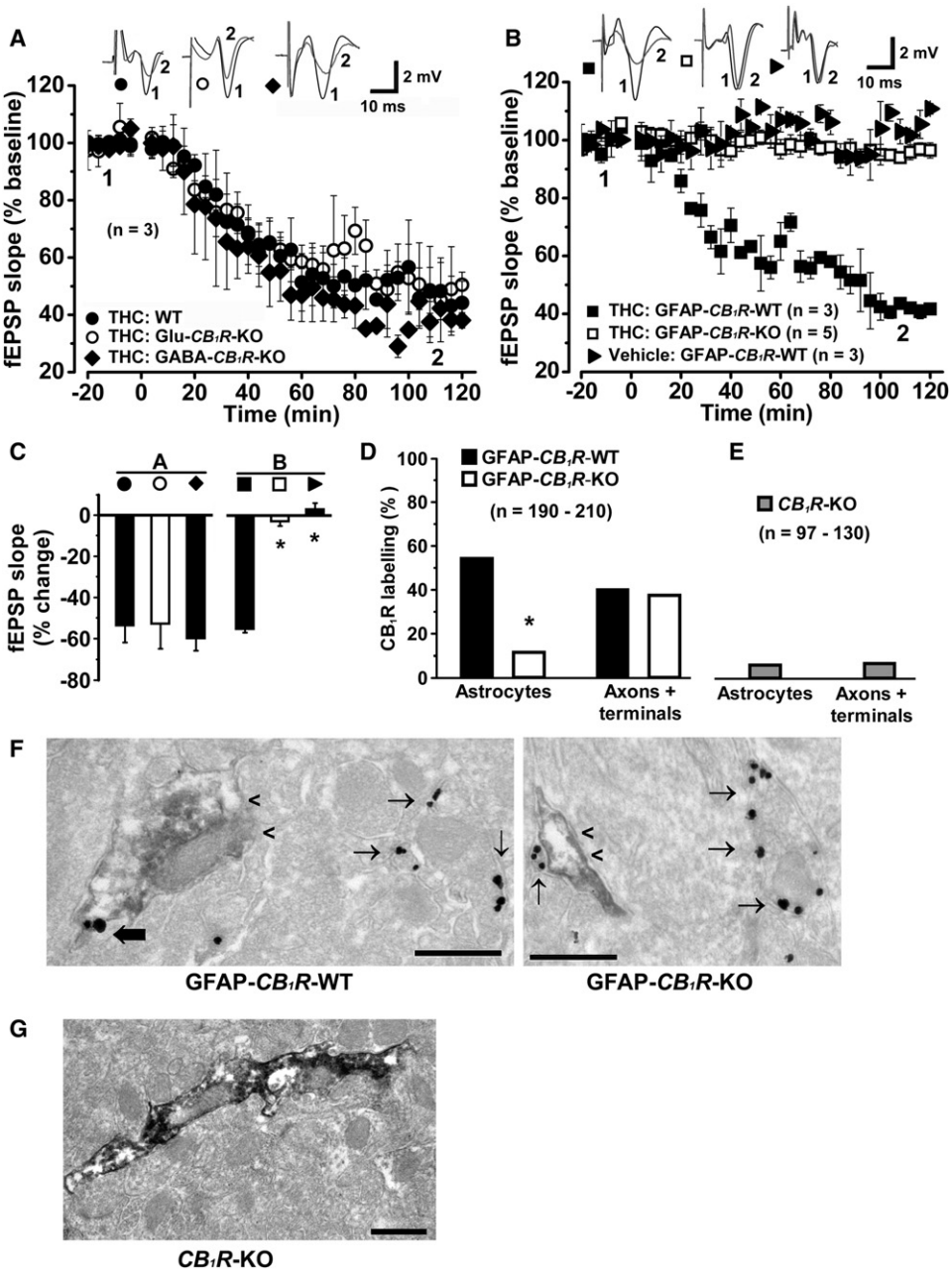

Figure 2. Cannabinoids Elicit CA1 LTD via Astroglial CB_1R but Not Neuronal CB_1R

(A and B) Plots of normalized fEPSP slopes in anesthetized mice show that THC injection at 0 min elicits CA1 LTD in wild-type (WT), Glu-*CB_1R*-KO and GABA-*CB_1R*-KO mice (A), but not in GFAP-*CB_1R*-KO mice (B). Representative fEPSP traces before (1) and after (2) treatment are shown above each plot.

(C) Histogram summarizes the average percent changes of fEPSP slope before (1) and after (2) treatment.

(D and E) Histograms summarize the percentage of CB_1R-labeled astrocytes and axons/terminals in GFAP-*CB_1R*-WT mice, GFAP-*CB_1R*-KO mice and *CB_1R*-KO mice.

(F) Electron microscopic images show a high density of CB_1R immunopositive silver grains (small arrows) in axons/terminals of both tamoxifen-treated GFAP-*CB_1R*-WT and GFAP-*CB_1R*-KO mice, and a low density of silver grains (large arrow) in DAB-stained astrocytes (arrowheads) of GFAP-*CB_1R*-WT mice but not of GFAP-*CB_1R*-KO littermates. The scale bar represents 500 nm.

(G) An electron microscopic image shows an absence of CB_1R immunopositive silver grains in astrocytes stained with peroxidase/DAB and axons. The scale bar represents 500 nm.

All summary graphs show means ± SEM; n = numbers of animals recorded (A, B) or numbers of positive immunoreactive profiles counted (D, E) in each group. *$p < 0.01$ versus control, Bonferronni post-hoc test after one-way ANOVA (A: $F_{2,6} = 68.603$, $p = 0.884$; B: $F_{2,8} = 42.009$, $p < 0.01$) or square Chi test (D).

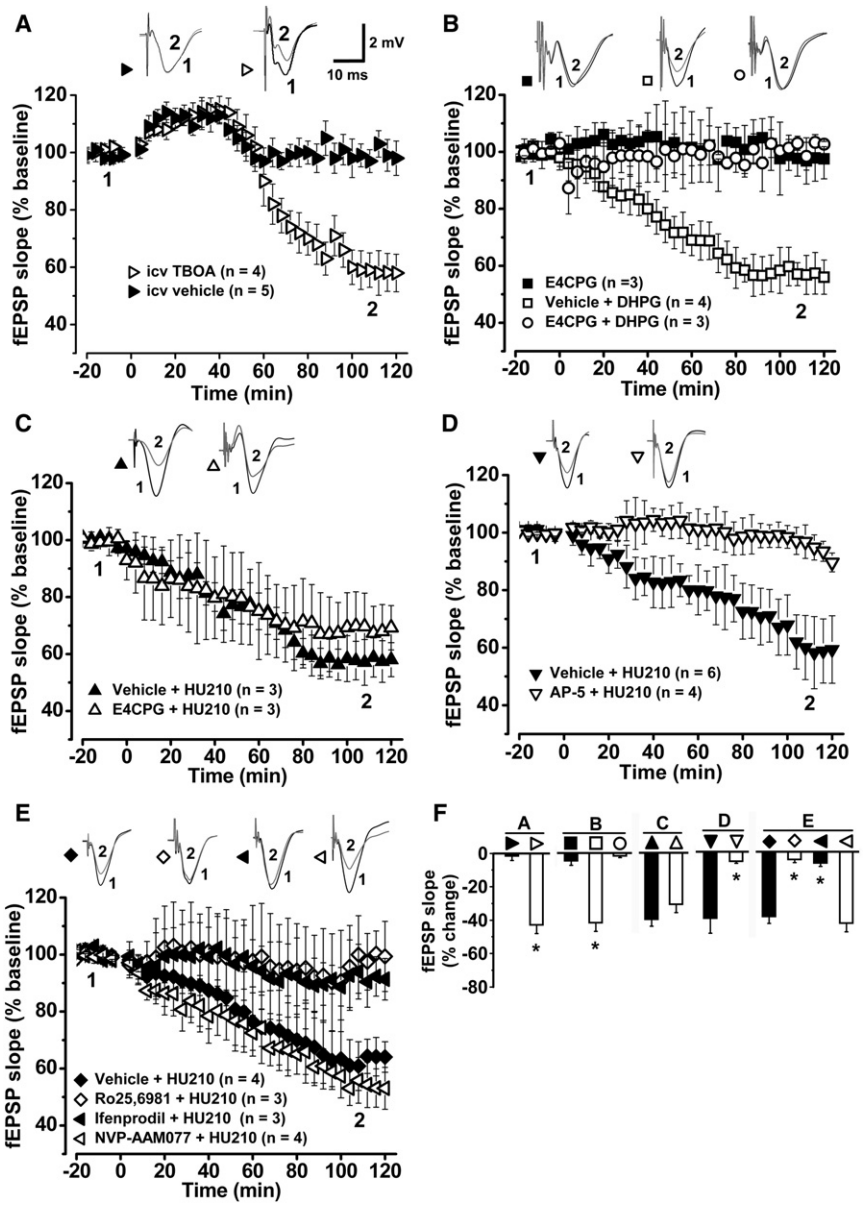

Figure 3. Cannabinoids Induce NMDAR-Dependent LTD at CA3-CA1 Synapses

(A–E) Plots of normalized fEPSP slopes in anesthetized rats are presented with representative fEPSP traces (above plots) before (1) and after (2) vehicle or drug injection. An i.c.v. injection of TBOA induces LTD (A). E4CPG, but not vehicle, blocks LTD induced by DHPG injection at 0 min (B) without significant effects on LTD induced by HU210 (C). Intra-CA1 application of AP-5 suppresses HU210-induced LTD (D). Systemic administration of Ro25-6981 and ifenprodil, but not NVP-AAM077, prevents HU210-induced LTD (E).

(F) Histogram summarizes the average percent change of fEPSP slope before (1) and after (2) drug or vehicle injection.

All summary graphs show means ± SEM; n = numbers of animals recorded in each group. *$p < 0.01$ versus control, Bonferronni post-hoc test after one-way ANOVA (B: $F_{2,7} = 36.090$, $p < 0.01$; E: $F_{3,10} = 40.409$, $p < 0.01$) or t test.

Hirrlinger et al., 2006) to eventually obtain the GFAP-*CB$_1$R*-KO mouse line. As compared to tamoxifen-treated wild-type littermate controls (GFAP-*CB$_1$R*-WT), GFAP-*CB$_1$R*-KO mice displayed a 79% reduction ($p < 0.01$) in the number of CA1 astrocytes labeled with a CB$_1$R antibody (Figures 2D and 2F), whereas only background levels were observed in constitutive *CB$_1$R*-KO mice (Figures 2E and 2G). Conversely, no difference ($p = 0.2293$) was observed between GFAP-*CB$_1$R*-WT and GFAP-*CB$_1$R*-KO mice in the number of CB$_1$R-labeled CA1 neuronal axons/terminals (Figures 2D and 2F). THC elicited CB-LTD at CA3-CA1 synapses in tamoxifen-treated wild-type mice but not in GFAP-*CB$_1$R*-KO mutant littermates (Figures 2B and 2C). Therefore, cannabinoid exposure in vivo elicits CB-LTD at CA3-CA1 synapses through CB$_1$R expressed in astroglial cells.

Mechanisms of CB-LTD at CA3-CA1 Synapses

Cannabinoids are able to activate hippocampal astroglial CB$_1$R to increase extracellular glutamate levels (Navarrete and Araque, 2008). If a similar mechanism is involved in CB-LTD, LTD should be induced by the glutamate-uptake inhibitor DL-threo-β-benzyloxyaspartate (TBOA). Indeed, an i.c.v. injection of TBOA (10 nmol) (Wong et al., 2007) induced in vivo LTD at CA3-CA1 synapses (Figures 3A and 3F). If increased extracellular levels of glutamate induce LTD at CA3-CA1 synapse, postsynaptic metabotropic glutamate receptor (mGluR) may be responsible for this LTD induction, because postsynaptic mGluR activation produces LTD (Chevaleyre et al., 2006; Lovinger, 2008). However, the selective group I/group II mGluR antagonist ethyl-4-carboxyphenylglycine (E4CPG, 35 nM/3.5 μl, i.c.v.) completely blocked in vivo LTD induced by the group I mGluR agonist dihydroxyphenylglycine (DHPG, 100 nM/5 μl, i.c.v.), but did not alter CB-LTD (Figures 3B, 3C, and 3F). Surprisingly, CB-LTD was fully blocked by the selective NMDAR antagonist AP-5 (50 mM, intra-CA1 iontophoretic ejection at −20 nA for 10 min) (Maalouf et al., 1998) (Figures 3D and 3F), and by the NR2B-preferring NMDAR antagonists Ro25-6981 (6 mg/kg, i.p.) (Fox et al., 2006) and ifenprodil (5 mg/kg, i.p.) (Higgins et al., 2005) (Figures 3E and 3F). However, the NR2A-preferring NMDAR antagonist NVP-AAM077 (1.2 mg/kg, i.p.) (Fox et al., 2006) did not alter CB-LTD in the same conditions (Figures 3E and 3F). Thus, in vivo cannabinoid exposure induces CB-LTD at CA3-CA1 synapses via activation of NR2B-containing NMDAR.

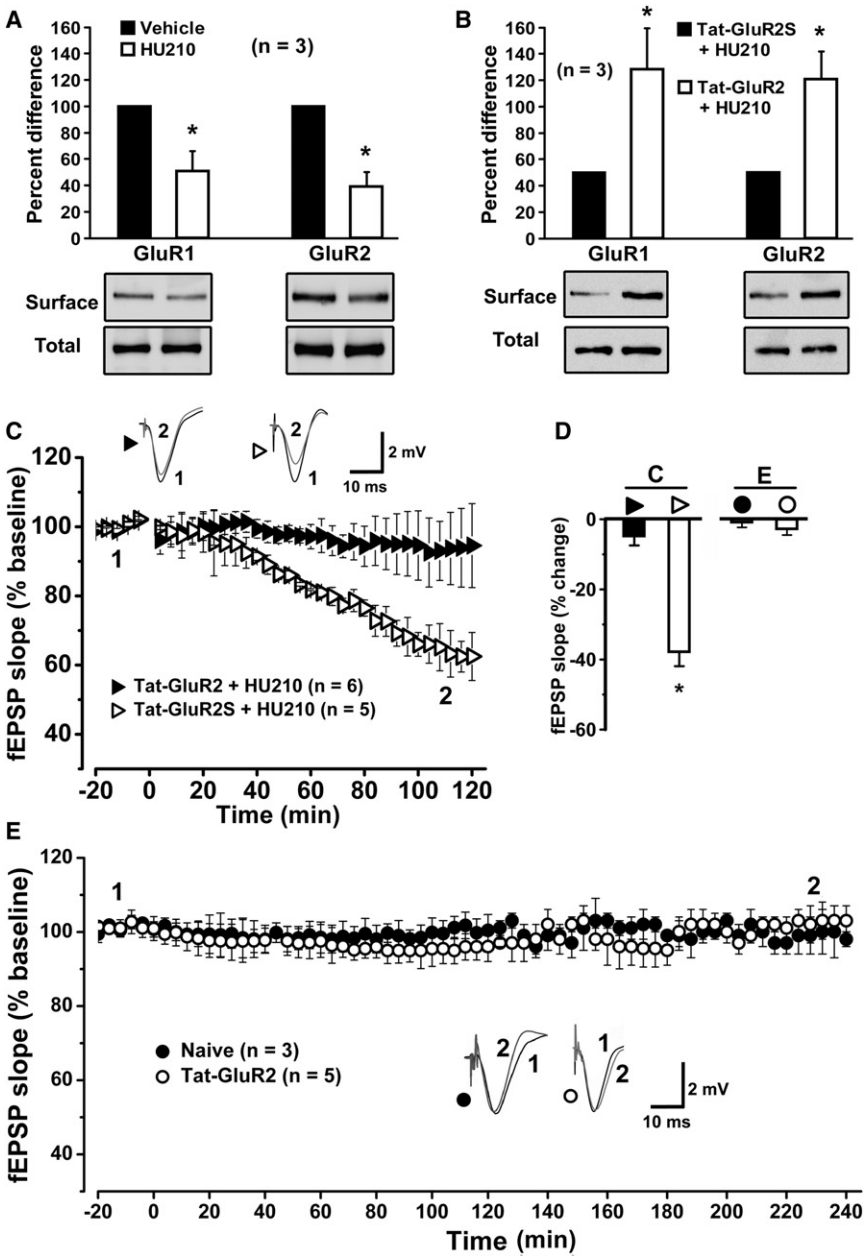

Figure 4. Cannabinoids Induce AMPAR Endocytosis-Dependent Expression of CA1 LTD

(A and B) Graphs and immunoblotting (bottom photos) show a decrease of GluR1 and GluR2 at the synaptic surface of CA1 neurons after HU210 injection, which is blocked by pretreatment with Tat-GluR2 but not Tat-GluR2S.

(C) Plot of normalized fEPSP slopes in anesthetized rats shows that injection of Tat-GluR2, but not Tat-GluR2S, 2 hr before HU210 injection at 0 min blocks HU210-induced LTD. Representative fEPSP traces before (1) and after (2) HU210 injection are shown above the plot.

(D) Histogram summarizes the average percent change of fEPSP slope before (1) and after (2) HU210 injection (C) or Tat-GluR2 injection (E).

(E) Plot of normalized slopes of fEPSPs in anesthetized rats shows both naive rats and rats receiving Tat-GluR2 injection at 0 min display similar fEPSPs at CA3-CA1 synapses for 4 hr. Representative fEPSP traces recorded during −10–0 min (1) and 230–240 min (2) are shown below the slopes.

All summary graphs show means ± SEM; n = numbers of experiments conducted (A and B) or numbers of animals recorded (C and E) in each group. *$p < 0.05$ versus control, t test.

The expression of NMDAR-mediated LTD requires facilitated endocytosis of postsynaptic AMPAR (Collingridge et al., 2010). AMPAR in CA1 pyramidal cells consists of 81% of GluR1/GluR2 at synaptic membranes (Lu et al., 2009). The surface levels of GluR1/GluR2 in synaptosomes isolated from the CA1 region significantly decreased after HU210 injection (Figure 4A), suggesting endocytosis of AMPAR in postsynaptic CA1 pyramidal cells following cannabinoid exposure in vivo. The administration of the brain-penetrating version of a peptide able to block GluR2 endocytosis ("Tat-GluR2" peptide, 1.5 μmol/kg, i.p.), but not of its scrambled analog (Tat-GluR2S) (Brebner et al., 2005; Wong et al., 2007; Collingridge et al., 2010), specifically blocked both HU210-induced GluR1/GluR2 endocytosis (Figure 4B) in the CA1 and CB-LTD (Figures 4C and 4D). Tat-GluR2 (1.5 μmol/kg, i.p.) did not significantly change the fEPSP amplitude at CA3-CA1 synapses for 4 hr after injection (Figures 4D and 4E). Altogether, these data strongly suggest that postsynaptic endocytosis of GluR1/GluR2 mediates the expression of CB-LTD at CA3-CA1 synapses.

Cannabinoid Impairment of Working Memory Shares the Same Mechanisms of CB-LTD

CB-LTD is characterized by (1) activation of astroglial CB_1R, (2) activation of NMDAR, and (3) internalization of AMPAR. These mechanisms were assessed in different behavioral models of cannabinoid impairment of spatial working memory (SWM).

The role of astroglial CB_1R in cannabinoid impairment of SWM was assessed by examining SWM performance of tamoxifen-treated GFAP-*CB_1R*-WT and GFAP-*CB_1R*-KO littermates with a delayed-matching-to place (DMTP) version of the Morris water maze test (Steele and Morris, 1999). No significant differences were observed between wild-type and mutant littermates during training (Figure S2A). In agreement with a previous study (Varvel and Lichtman, 2002), THC impaired SWM performance in GFAP-*CB_1R*-WT mice, as evidenced by a significant decrease of both latency saving ratios (Figure 5A) and path saving ratios (Figure 5B). In contrast, THC did not

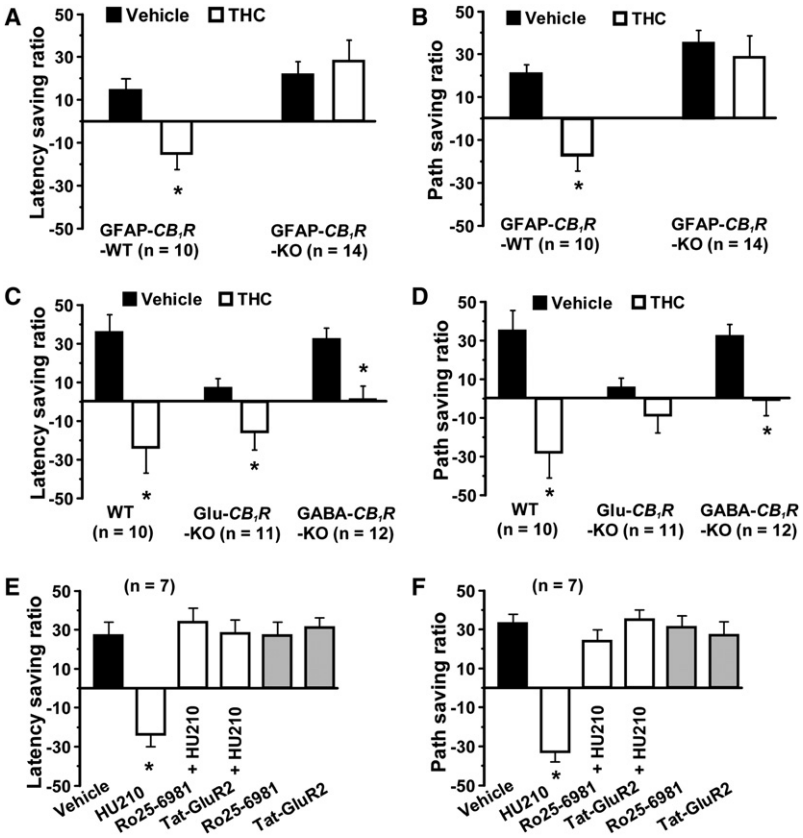

Figure 5. Astroglial CB_1R, NMDAR, and AMPAR Mediate Cannabinoid Impairment of SWM

(A–D) Mouse DMTP version of the Morris water maze test. THC reduces both latency saving ratio (A) and path saving ratio (B) in wild-type mice (A – D) and GABA-*CB_1R*-KO littermates but not in GFAP-*CB_1R*-KO littermates. While vehicle-treated Glu-*CB_1R*-KO littermates show a significant decrease of both latency saving ratio and path saving ratio relative to vehicle-treated wild-type mice (C and D), THC reduces latency saving ratio (C) but not path saving ratio (D) in Glu-*CB_1R*-KO littermates.

(E and F) Rat DMTP version of the Morris water maze test. HU210 reduces both path saving ratio (E) and latency saving ratio (F), which are prevented by i.p. pretreatment with Ro25-6981 or Tat-GluR2, while neither Ro25-6981 nor Tat-GluR2 significantly affects the ratio in the absence of HU210.

All summary graphs show means ± SEM; n = numbers of animals tested in each group. *$p < 0.05$ versus control, Bonferronni post-hoc test after repeated-measure two-way ANOVA ([A] $F_{1,22} = 13.010$, $p < 0.01$; [B] $F_{1,22} = 7.999$, $p < 0.01$; [C] treatment: $F_{1,30} = 37.28$, $p < 0.001$; genotype x treatment $F_{2,30} = 2.92$, $p > 0.05$; [D] treatment: $F_{1,30} = 30.01$, $p < 0.001$; genotype x treatment $F_{2,30} = 4.25$, $p < 0.05$) or one-way ANOVA ([E] $F_{5,36} = 19.307$, $p < 0.01$; [F] $F_{5,36} = 13.110$, $p < 0.01$).

See also Figures S2 and S5.

produce significant effects on GFAP-*CB_1R*-KO littermates (Figures 5A and 5B). While Glu-*CB_1R*-KO littermates showed a significant impairment of the acquisition of SWM (Figure S2B) and subsequent poor performance of SWM in comparison with wild-type mice (Figures 5C and 5D), THC impaired SWM performance (Figure 5C). Both GABA-*CB_1R*-KO littermates and control wild-type mice showed similar acquisition of SWM (Figure S2B), and THC impaired SWM performance (Figures 5C and 5D). THC treatment did not alter swim speed of GFAP-*CB_1R*-WT and GFAP-*CB_1R*-KO mice (Figure S2C), but slightly decreased this parameter in Glu- and GABA-*CB_1R*-KO mice and WT littermates (Figure S2D). However, this slight effect was equal for all genotypes (Figure S2D) and was equally distributed among different trials (data not shown), thereby excluding its involvement in the altered SWM performance of the mice. Thus, CB_1R in glutamatergic neurons, but not CB_1R in GABAergic neurons or astroglial cells, is necessary for mice to acquire SWM. Notably, however, astroglial CB_1R, but not glutamatergic or GABAergic neuronal CB_1R, is necessary to produce the detrimental effects of THC on SWM.

To test if NMDAR activation plays a role in cannabinoid impairment of SWM, rats were tested in a T-maze using a delayed nonmatching to sample protocol (DNMTST) (Kelsey and Vargas, 1993). After 6 daily training sessions to ensure that the task was mastered (>80% correct choices, Figure S3A), rats received 2 daily test sessions 30 min after injection of HU210 or vehicle. Ten min before HU210 injection, rats were pretreated with Ro25-6981 or ifenprodil, two NR2B-preferring NMDAR antagonists, or NVP-AAM077, a NR2A-prefering NMDAR antagonist. The results show that NR2B- but not NR2A-preferring NMDAR antagonists abrogated HU210-induced impairment of SWM performance (Figure 6A). Thus, activation of NR2B-containing NMDAR is necessary for the cannabinoid-induced impairment of SWM.

The effects of the blockade of AMPAR internalization on cannabinoid-induced SWM impairment was also tested in the DNMTST paradigm. After 6 daily training sessions (Figure S3B), rats received Tat-GluR2 or Tat-GluR2S (1.5 μmol/kg, i.p.) (Brebner et al., 2005; Wong et al., 2007) 2 hr before HU210 injection on each of the two testing days. Tat-GluR2, but not Tat-GluR2S, abolished HU210 impairment of SWM performance (Figure 6B). To determine the specific role of the CA1 region, after 6 daily training sessions (Figure S3C), Tat-GluR2 or Tat-GluR2S was infused bilaterally within the dorsal CA1 region (15 pmol/per injection) (Brebner et al., 2005; Wong et al., 2007) (Figure 6C) 60 min before each HU210 injection on each testing day. Intra-CA1 infusion of Tat-GluR2, but not Tat-GluR2S, blocked HU210 impairment of SWM performance (Figure 6D). Neither systemic nor intra-CA1 administration of Tat-GluR2 significantly affected basal locomotor activity, anxiety level or motor balance (Figures S4A–S4E). Thus, AMPAR internalization in the CA1 hippocampal region is necessary for cannabinoid-induced alteration of SWM.

If intra-CA1 infusion of HU210 is able to induce CB-LTD at CA3-CA1 synapses (Figures S1A and S1C), a bilateral intra-CA1 infusion of HU210 should impair SWM. As expected, after six daily training sessions (Figure S3D), HU210 (0.1 μg/0.5 μl/side) impaired rat SWM performance (Figure 6E).

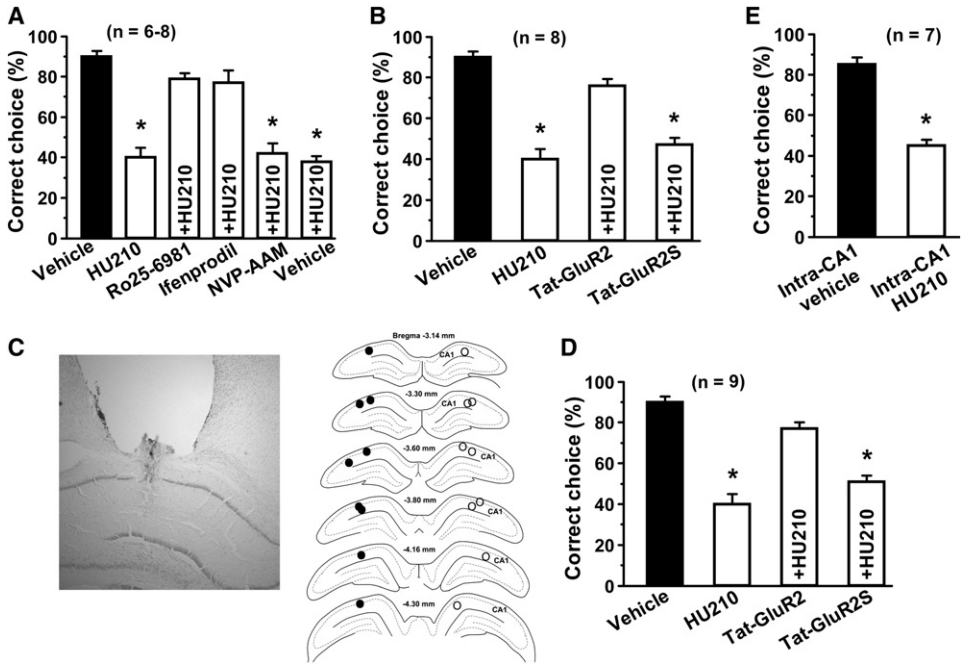

Figure 6. NMDAR and AMPAR Mediate Cannabinoid Impairment of SWM

(A) Rat DNMTS T-maze. HU210 suppresses SWM performances, which is prevented by i.p. pretreatment with Ro25-6981 and ifenprodil, but not with NVP-AAM077.

(B and D) Rat DNMTS T-maze. Systemic (B) and intra-CA1 administration (D) of Tat-GluR2, but not Tat-GluR2S, blocks HU210 impairment of SWM performance.

(C) Photograph (left) shows location of an intra-CA1 cannula, and histograms (right) show reconstructions of histology sections illustrating CA1 injection sites of Tat-GluR2 (solid circle) and Tat-GluR2S (open circle).

(E) Intra-CA1 injection of HU210, but not vehicle, impairs SWM performance.

All summary graphs show means ± SEM; n = numbers of animals tested in each group. *$p < 0.01$ versus control, Bonferronni post-hoc test after one-way ANOVA (A: $F_{5,36} = 59.070$, $p < 0.01$; B: $F_{3,28} = 54.220$, $p < 0.01$; D: $F_{3,32} = 41.562$, $p < 0.01$; E: $F_{1,12} = 36.090$, $p < 0.01$).

See also Figures S3 and S4.

Finally, we tested if the results obtained with the DNMTST paradigm were reproducible with the DMTP water maze paradigm. One day after five daily training sessions to establish the baseline levels of SWM (Figure S5A), rats received a test session of four trials. HU210 treatment before the test session impaired SWM performance, which was blocked by pretreatment with Ro25-6981 or Tat-GluR2 (Figures 5E and 5F). Neither Ro25-6981 nor Tat-GluR2 administration alone significantly changed saving ratios (Figures 5E and 5F), suggesting that neither NR2B-preferring NMDAR antagonists nor Tat-GluR2 interferes with basal SWM performance. Swim speeds during the SWM task were not influenced by different treatments (Figure S5B). Thus, cannabinoid administration alters SWM performance in different behavioral tasks through the same mechanisms.

Altogether, these data show that the same mechanisms underlying CB-LTD at hippocampal CA3-CA1 synapses (activation of astroglial CB_1R, activation of NMDAR and removal of AMPAR from the synaptic surface) also mediate cannabinoid-induced alterations of hippocampal-dependent SWM.

DISCUSSION

This study shows that one of the most common effects of cannabinoid intoxication in humans and animals, the impairment of SWM, is due to activation of astroglial CB_1R. Furthermore, a novel form of cannabinoid-induced long-term synaptic plasticity in the hippocampus appears to mechanistically underlie this effect of cannabinoids in vivo. Our results are consistent with a scenario (Figure 7), in which cannabinoid exposure in vivo activates astroglial CB_1R to increase ambient glutamate, which in turn activates NR2B-containing NMDAR to trigger AMPAR internalization at CA3-CA1 synapses. These events ultimately induce CB-LTD at these synapses, altering the function of hippocampal circuits that likely become unable to process SWM (Figure 7).

Early studies demonstrate that CB_1R is expressed at high levels by neurons throughout the whole brain (Herkenham et al., 1990; Matsuda et al., 1993; Tsou et al., 1998). More recent studies show that CB_1R is more abundant in GABAergic interneurons than in glutamatergic principal neurons (Kawamura et al., 2006). In the hippocampal CA1 area, CB_1R density on GABAergic presynaptic membranes is at least 10–20 times higher than that on glutamatergic presynaptic membranes (Kawamura et al., 2006; Bellocchio et al., 2010). Cannabinoid depression of in vitro excitatory or inhibitory synaptic transmission has been consistently shown to require CB_1R in either glutamatergic or GABAergic presynaptic terminals, respectively (Misner and Sullivan, 1999; Chevaleyre et al., 2006; Kawamura

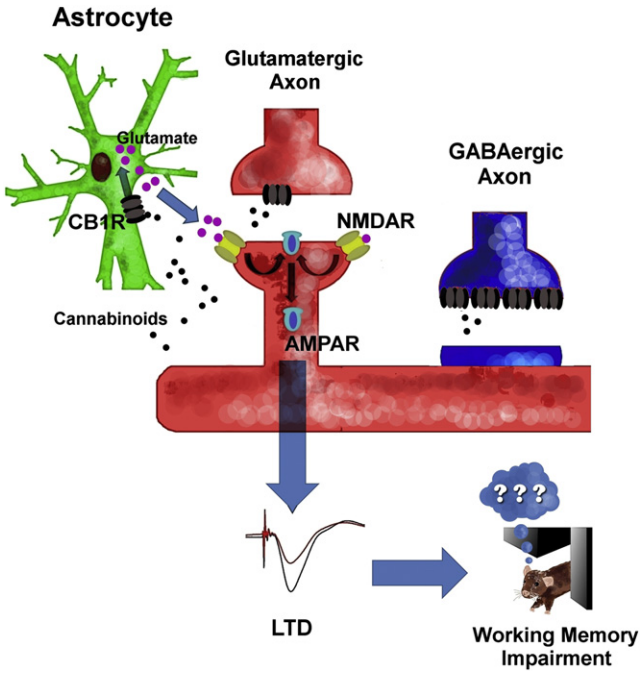

Figure 7. Proposed Model for In Vivo LTD Production at CA3-CA1 Synapses and Subsequent Working Memory Impairment

CB_1R exists in CA1 astrocytes (Figures 2D–2G) and presynaptic membranes with 10- to 20-fold of CB_1R density in GABAergic membranes than glutamatergic membranes (Kawamura et al., 2006). GABAergic and glutamatergic terminals containing CB_1R synapse with dendrites and spines of CA1 pyramidal cells, respectively (Kawamura et al., 2006). In vitro activation of presynaptic CB_1R by cannabinoids reduces the release of glutamate and GABA from glutamatergic and GABAergic membranes, respectively. However, cannabinoid exposure in vivo sequentially activates astroglial CB_1R and postsynaptic NR2B-containing NMDAR, which elicits AMPAR endocytosis-mediated expression of in vivo LTD at CA3-CA1 synapses, resulting in working memory impairment.

et al., 2006; Takahashi and Castillo, 2006; Navarrete and Araque, 2008, 2010; Bajo et al., 2009). Indeed, cannabinoids fail to reduce excitatory or inhibitory synaptic transmission in hippocampal slices of conditional mutant mice lacking CB_1R expression in either glutamatergic or GABAergic hippocampal neurons, respectively (Domenici et al., 2006; Monory et al., 2006). Unexpectedly, we observed here that in vivo exposure to exogenous cannabinoids induced full CB-LTD at excitatory CA3-CA1 synapses in both wild-type mice and mutant littermates lacking CB_1R in either CA1 glutamatergic or GABAergic neurons. These data do not support an involvement of glutamatergic or GABAergic CB_1R in in vivo CB-LTD at CA3-CA1 synapses.

The presence of CB_1R has also been suggested in brain astrocytes (Moldrich and Wenger, 2000; Rodriguez et al., 2001; Salio et al., 2002), but the extremely low levels of CB_1R expression in this cell population did not allow reaching the same conclusive evidence of functional data (Navarrete and Araque, 2008, 2010). The use of double immunostaining applied to wild-type and conditional or constitutive *CB_1R* mutant mice allowed us to provide conclusive electron microscopic evidence that CB_1R is expressed and quantifiable in hippocampal astrocytes. We have further showed here that in vivo CB-LTD at CA3-CA1 synapses was not detectable in tamoxifen-inducible conditional mutant mice specifically lacking CB_1R expression in astrocytes (i.e., GFAP-*CB_1R*-KO littermates). Our results strongly suggest a requirement of astroglial CB_1R for CB-LTD at CA3-CA1 synapses in living animals.

However, we also found that THC exposure in vivo did not significantly alter basal synaptic transmission in GFAP-*CB_1R*-KO littermates. These data, together with the finding that the density of presynaptic CB_1R at CA3-CA1 synapses is just above the background levels (Kawamura et al., 2006), suggest a negligible role of presynaptic CB_1R in excitatory transmission in vivo at CA3-CA1 synapses in response to exogenous cannabinoid exposure. Thus, in vitro cannabinoid application decreases excitatory synaptic transmission at CA3-CA1 synapses via activation of "glutamatergic" CB_1R, whereas in vivo cannabinoid administration induces CB-LTD via astroglial CB_1R without significant effects on presynaptic CB_1R. The exact reason for this apparent mechanistic discrepancy between in vitro and in vivo effects of cannabinoids on synaptic transmission and plasticity is not known. Nevertheless, it is important to note that intact astroglial networks play prominent roles in brain functioning (Giaume et al., 2010). Indeed, astrocytes are more associated in networks than neurons due to the presence of high levels of gap junctions and direct intercellular communications (Giaume et al., 2010). It is therefore possible that the unavoidable disruption of these networks by slicing procedures might alter the impact of astroglial CB_1R signaling in vitro. Meanwhile, slicing procedures might also upregulate the number or function of presynaptic CB_1R, leading to a decrease of glutamatergic transmission upon its activation by exogenous cannabinoids. This idea is supported by the evidence that although CB_1R density is at least 10-20 times higher on inhibitory than excitatory terminals in the CA1 region (Kawamura et al., 2006; Bellocchio et al., 2010), application of a saturating concentration of WIN22,212-2 (2 μM) to hippocampal slices produced similar depression (~50%) of EPSC (Kawamura et al., 2006) and IPSC (Hajos and Freund, 2002) in the CA1 area. Because brain slice preparations are extensively used for studying alterations of synaptic strength following in vitro application of other drugs of abuse, it is worthwhile to explore whether astrocytes play a key role in the in vivo effects of these drugs of abuse that are different from their in vitro effects.

Recent studies with brain slices show that endocannabinoids activate CA1 astroglial CB_1R to increase extracellular glutamate levels, which in turn activate presynaptic mGluR to induce LTP at CA3-CA1 synapses (Navarrete and Araque, 2008, 2010). However, we show here that cannabinoids activate astroglial cells to induce in vivo LTD at CA3-CA1 synapses. It is currently unknown why activation of astroglial CB_1R by in vitro endocannabinoid and in vivo cannabinoid induces, respectively, in vitro LTP and in vivo LTD at CA3-CA1 synapses. It is possible that activation of astroglial CB_1R in brain slices with disrupted astroglial networks might produce lower levels of interstitial glutamate than those produced in living animals with intact astroglial networks, which then activate presynaptic mGluR in vitro and

postsynaptic NMDAR in vivo, respectively, to induce in vitro LTP and in vivo LTD at CA3-CA1 synapses.

This study confirmed the consistent finding that HU210 and THC impair SWM in rodents (Lichtman and Martin, 1996; Hampson and Deadwyler, 2000; Nava et al., 2001; Varvel and Lichtman, 2002; Fadda et al., 2004; Hill et al., 2004; Wise et al., 2009). Although a recent study claimed the inability of systemic HU210 injection to impair SWM tested with the DMTP water maze paradigm (Robinson et al., 2007), this study failed to use the 'saving ratio' analysis as we and others (Varvel and Lichtman, 2002) have successfully used to identify the detrimental effects of HU210 and THC on rodent SWM performance.

While glutamatergic axonal CB_1R is in part responsible for cannabinoid-elicited locomotor suppression, catalepsy and hypothermia (Monory et al., 2007), hippocampal GABAergic axonal CB_1R likely plays a key role in cannabinoid impairment of long-term memory (Puighermanal et al., 2009). Our data using GABA-*CB_1R*-KO mice clearly show that "GABAergic" CB_1R is fully dispensable both for basal performance of the SWM task and, most importantly in this context, for the acute effect of exogenous cannabinoids. By showing that Glu-*CB_1R*-KO mice are impaired in basal performance of the SWM task, our data suggest that CB_1R expressed in cortical glutamatergic neurons participates in the endogenous control of SWM. This control might be exerted acutely by endogenous mobilization of endocannabinoids during the task or can also be due to developmental effects of *CB_1R* deletion in this cell population (Mulder et al., 2008). However, exogenous THC treatment of Glu-*CB_1R*-KO mice is still able to further reduce their poor performance, strongly suggesting the dispensable role of "glutamatergic" CB_1R in the acute effects of exogenous cannabinoids on SWM performance.

Conversely, by showing that GFAP-*CB_1R*-KO mice display normal learning of SWM, but totally fail to respond to THC, the present study provides striking evidence for the necessary role of astroglial CB_1R in SWM impairment induced by exogenous cannabinoids.

Cannabinoid-induced LTD and impairment of SWM share not only the dependency on astroglial CB_1R but also a whole series of well-defined molecular mechanisms. Thus, the pharmacological blockade of NR2B-containing NMDAR, but not NR2A-containing NMDAR, prevented both CB-LTD at CA3-CA1 synapses and cannabinoid impairment of SWM. Moreover, the Tat-GluR2 peptide can selectively block the facilitated endocytosis of AMPAR (Collingridge et al., 2010), the final step of the expression of NMDAR-dependent LTD (Collingridge et al., 2010), without significant effects on LTP induction or basal synaptic transmission (Collingridge et al., 2010). Both systemic and intra-CA1 application of the Tat-GluR2 peptide not only disrupted the expression of CB-LTD at CA3-CA1 synapses but also cannabinoid impairment of SWM, as assessed with both the DMTP version of the Morris water maze test and the DNMTS T-maze test.

Collectively, at least three key molecular mechanisms are shared by CB-LTD and cannabinoid-induced impairment of SWM: (1) activation of astroglial CB_1R by the exogenous cannabinoid; (2) increase of local glutamate and activation of NR2B-containing NMDAR; (3) endocytosis of AMPAR (Figure 7). These findings strongly suggest a causative role of CB-LTD at CA3-CA1 synapses in cannabinoid-induced impairment of SWM and reveal novel mechanistic views of the role of astrocytes in learning and memory processes and of the memory-disruptive effects of marijuana intoxication.

EXPERIMENTAL PROCEDURES

Generation of Mutant Mice

Constitutive *CB_1R*-KO mice and conditional Glu-*CB_1R*-KO and GABA-*CB_1R*-KO mice were generated and genotyped as described (Marsicano et al., 2002; Monory et al., 2006). GFAP-*CB_1R*-KO mice were generated using the Cre/loxP system. Mice carrying the "floxed" *CB_1R* gene (*$CB_1^{f/f}$*) (Marsicano et al., 2003) were crossed with GFAP-CreERT2 mice (Hirrlinger et al., 2006), using a three-step backcrossing procedure to obtain *$CB_1R^{f/f;GFAP\text{-}CreERT2}$* and *$CB_1R^{f/f}$* littermates, called GFAP-*CB_1R*-KO and GFAP-*CB_1R*-WT, respectively.

Immunohistochemistry for Electron Microscopy

Animals were transcardially fixed with 0.1% glutaraldehyde, 4% formaldehyde and 0.2% picric acid or with 2% formaldehyde and 8% picric acid. Hippocampal vibrosections were cut for double preembedding staining of CB_1R and GFAP with silver-intensified immunogold method and immunoperoxidase method. Tissue preparations were photographed for quantification of positive immunoreactive profiles. Detailed procedures are described in the extended methods in the SOMs.

Adenovirus Preparation and Administration

Recombinant adenoviruses were prepared as described (Liu et al., 2010). After intra-CA1 infusion of adenoviral vectors (10^{10} plaque-forming units/μl/injection), the CA1 area surrounding the injection tract was dissected 4 days later for quantification of CB_1R protein with procedures as described (Ji et al., 2006; Liu et al., 2010).

Synaptosomal Surface AMPAR Measurement

Biotinylation experiments for the CA1 area on hippocampal slices were performed as described (Kim et al., 2007). Protein fractions were transferred onto nitrocellulose membranes, which were probed with primary antibodies to GluR1 (1:250, Millipore, Billerica, MA) or GluR2 (1:500, Millipore, Billerica, MA) overnight at 4°C. Bands were analyzed by densitometry, and receptor ratios for AMPAR subunits were determined by dividing the surface intensity by the total intensity.

Electrophysiology Analysis

Under anesthesia, rats or mice received implantation of stimulating and recording electrodes into the CA1 region. fEPSPs were evoked by applying single pulses of stimulation at 0.067 Hz. Stimulus pulse intensities were 20-60 nA with a duration of 500 μs. Spike2 software was utilized to record data. Procedures for fEPSP recordings from freely moving rats were generally similar to those from anaesthetized rats with the exception of allowing rats to recover for 2 weeks after surgery for electrode implantation. Detailed procedures are described in the extended methods in the SOMs.

Behavioral Tests

Water Maze Test

Mice were tested in a DMTP version of the Morris water maze paradigm (Steele and Morris, 1999). Briefly, after a habituation session of 3 trials without spatial cues, mice received daily training sessions of 4 trials each with the maximal escape latency of 60 s, and 30 min before each of the sessions 6 through 12 and before the 13th session, mice were treated with vehicle and THC (5 mg/kg, i.p.), respectively. Performances of individual SWMs were calculated using the "saving ratio" procedure (Varvel and Lichtman, 2002) and calculated as follows: *path saving ratio = (path-length $trial_1$ - path-length $trial_4$) / (path-length $trial_1$ + path-length $trial_4$); and latency saving ratio = (escape latency $trial_1$ - escape latency $trial_4$) / (escape latency $trial_1$ + escape latency $trial_4$)*. Procedures for rat water maze test were generally similar to mouse water maze test with the exception that rats received 5 daily sessions of SWM

training 1 day before a testing session of 4 trials with the maximal escape latency of 90 s. Detailed procedures are described in the extended methods in the SOMs.

Other Behavioral Tests

Rats were examined with the DNMTS T-maze test (Kelsey and Vargas, 1993), locomotor activity test (Ji et al., 2006), elevated-plus-maze test (Ji et al., 2006) and motor balance tests (Ji et al., 2006).

Statistical Analysis

Results were reported as mean ± SEM. Statistical analysis of the data was performed using a student t test, square Chi test, one-way ANOVA, or one-way or two-way ANOVA for repeated-measures, followed by Bonferronni post-hoc test. Statistical significance was set at $p < 0.05$.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures and five figures and can be found with this article online at doi:10.1016/j.cell.2012.01.037.

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REFERENCES

Baddeley, A. (2003). Working memory: looking back and looking forward. Nat. Rev. Neurosci. *4*, 829–839.

Bajo, M., Roberto, M., and Schweitzer, P.J. (2009). Differential alteration of hippocampal excitatory synaptic transmission by cannabinoid ligands. Neurosci. Res. *87*, 766–775.

Bellocchio, L., Lafenêtre, P., Cannich, A., Cota, D., Puente, N., Grandes, P., Chaouloff, F., Piazza, P.V., and Marsicano, G. (2010). Bimodal control of stimulated food intake by the endocannabinoid system. Nat. Neurosci. *13*, 281–283.

Brebner, K., Wong, T.P., Liu, L., Liu, Y., Campsall, P., Gray, S., Phelps, L., Phillips, A.G., and Wang, Y.T. (2005). Nucleus accumbens long-term depression and the expression of behavioral sensitization. Science *310*, 1340–1343.

Brooks, J.W. (2002). Cannabinoids and analgesia. Curr. Anaesth. Crit. Care *13*, 215–220.

Carlini, E.A. (2004). The good and the bad effects of (-) trans-delta-9-tetrahydrocannabinol (Delta 9-THC) on humans. Toxicon *44*, 461–467.

Chevaleyre, V., Takahashi, K.A., and Castillo, P.E. (2006). Endocannabinoid-mediated synaptic plasticity in the CNS. Annu. Rev. Neurosci. *29*, 37–76.

Collingridge, G.L., Peineau, S., Howland, J.G., and Wang, Y.T. (2010). Long-term depression in the CNS. Nat. Rev. Neurosci. *11*, 459–473.

Cui, S.S., Bowen, R.C., Gu, G.B., Hannesson, D.K., Yu, P.H., and Zhang, X. (2001). Prevention of cannabinoid withdrawal syndrome by lithium: involvement of oxytocinergic neuronal activation. J. Neurosci. *21*, 9867–9876.

Domenici, M.R., Azad, S.C., Marsicano, G., Schierloh, A., Wotjak, C.T., Dodt, H.U., Zieglgänsberger, W., Lutz, B., and Rammes, G. (2006). Cannabinoid receptor type 1 located on presynaptic terminals of principal neurons in the forebrain controls glutamatergic synaptic transmission. J. Neurosci. *26*, 5794–5799.

Fadda, P., Robinson, L., Fratta, W., Pertwee, R.G., and Riedel, G. (2004). Differential effects of THC- or CBD-rich cannabis extracts on working memory in rats. Neuropharm *47*, 1170–1179.

Fox, C.J., Russell, K.I., Wang, Y.T., and Christie, B.R. (2006). Contribution of NR2A and NR2B NMDA subunits to bidirectional synaptic plasticity in the hippocampus in vivo. Hippocampus *16*, 907–915.

Giaume, C., Koulakoff, A., Roux, L., Holcman, D., and Rouach, N. (2010). Astroglial networks: a step further in neuroglial and gliovascular interactions. Nat. Rev. Neurosci. *11*, 87–99.

Hajos, N., and Freund, T.F. (2002). Pharmacological separation of cannabinoid sensitive receptors on hippocampal excitatory and inhibitory fibers. Neuropharm. *43*, 503–510.

Hajos, N., Ledent, C., and Freund, T.F. (2001). Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. Neuroscience *106*, 1–4.

Hall, W., Christie, M., and Currow, D. (2005). Cannabinoids and cancer: causation, remediation, and palliation. Lancet Oncol. *6*, 35–42.

Hampson, R.E., and Deadwyler, S.A. (2000). Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. J. Neurosci. *20*, 8932–8942.

Han, J., Liu, Z., Ren, W., and Zhang, X. (2011). Counteractive effects of cannabinoid and nicotine addictive behavior. Neuroreport *22*, 181–184.

Hassabis, D., Kumaran, D., Vann, S.D., and Maguire, E.A. (2007). Patients with hippocampal amnesia cannot imagine new experiences. Proc. Natl. Acad. Sci. USA *104*, 1726–1731.

Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R., and Rice, K.C. (1990). Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. USA *87*, 1932–1936.

Higgins, G.A., Ballard, T.M., Enderlin, M., Haman, M., and Kemp, J.A. (2005). Evidence for improved performance in cognitive tasks following selective NR2B NMDA receptor antagonist pre-treatment in the rat. Psychopharm. *179*, 85–98.

Hill, M.N., Froc, D.J., Fox, C.J., Gorzalka, B.B., and Christie, B.R. (2004). Prolonged cannabinoid treatment results in spatial working memory deficits and impaired long-term potentiation in the CA1 region of the hippocampus in vivo. Eur. J. Neurosci. *20*, 859–863.

Hirrlinger, P.G., Scheller, A., Braun, C., Hirrlinger, J., and Kirchhoff, F. (2006). Temporal control of gene recombination in astrocytes by transgenic expression of the tamoxifen-inducible DNA recombinase variant CreERT2. Glia *54*, 11–20.

Hoffman, A.F., Laaris, N., Kawamura, M., Masino, S.A., and Lupica, C.R. (2010). Control of cannabinoid CB1 receptor function on glutamate axon terminals by endogenous adenosine acting at A1 receptors. J. Neurosci. *30*, 545–555.

Ji, S.P., Zhang, Y., Van Cleemput, J., Jiang, W., Liao, M., Li, L., Wan, Q., Backstrom, J.R., and Zhang, X. (2006). Disruption of PTEN coupling with 5-HT2C receptors suppresses behavioral responses induced by drugs of abuse. Nat. Med. *12*, 324–329.

Kano, M., Ohno-Shosaku, T., Hashimotodani, Y., Uchigashima, M., and Watanabe, M. (2009). Endocannabinoid-mediated control of synaptic transmission. Physiol. Rev. *89*, 309–380.

Kawamura, Y., Fukaya, M., Maejima, T., Yoshida, T., Miura, E., Watanabe, M., Ohno-Shosaku, T., and Kano, M. (2006). The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. J. Neurosci. *26*, 2991–3001.

Kelleher, R.J., III, Govindarajan, A., and Tonegawa, S. (2004). Translational regulatory mechanisms in persistent forms of synaptic plasticity. Neuron *44*, 59–73.

Kelsey, J.E., and Vargas, H. (1993). Medial septal lesions disrupt spatial, but not nonspatial, working memory in rats. Behav. Neurosci. *107*, 565–574.

Kesner, R.P. (2007). Behavioral functions of the CA3 subregion of the hippocampus. Learn. Mem. *14*, 771–781.

Kim, J., Lee, S., Park, K., Hong, I., Song, B., Son, G., Park, H., Kim, W.R., Park, E., Choe, H.K., et al. (2007). Amygdala depotentiation and fear extinction. Proc. Natl. Acad. Sci. USA *104*, 20955–20960.

Lemberger, L. (1980). Potential therapeutic usefulness of marijuana. Annu. Rev. Pharmacol. Toxicol. *20*, 151–172.

Lichtman, A.H., and Martin, B.R. (1996). Δ^9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. Psychopharm. *126*, 125–131.

Liu, Z., Han, J., Jia, L., Maillet, J.C., Bai, G., Xu, L., Jia, Z., Zheng, Q., Zhang, W., Monette, R., et al. (2010). Synaptic neurotransmission depression in ventral tegmental dopamine neurons and cannabinoid-associated addictive learning. PLoS ONE *5*, e15634.

Lovinger, D.M. (2008). Presynaptic modulation by endocannabinoids. Handbook Exp. Pharmacol. *184*, 435–477.

Lu, W., Shi, Y., Jackson, A.C., Bjorgan, K., During, M.J., Sprengel, R., Seeburg, P.H., and Nicoll, R.A. (2009). Subunit composition of synaptic AMPA receptors revealed by a single-cell genetic approach. Neuron *62*, 254–268.

Maalouf, M., Dykes, R.W., and Miasnikov, A.A. (1998). Effects of D-AP5 and NMDA microiontophoresis on associative learning in the barrel cortex of awake rats. Brain Res. *793*, 149–168.

Malenka, R.C., and Bear, M.F. (2004). LTP and LTD: an embarrassment of riches. Neuron *44*, 5–21.

Manahan-Vaughan, D., Kulla, A., and Frey, J.U. (2000). Requirement of translation but not transcription for the maintenance of long-term depression in the CA1 region of freely moving rats. J. Neurosci. *20*, 8572–8576.

Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., Azad, S.C., Cascio, M.G., Gutiérrez, S.O., van der Stelt, M., et al. (2003). CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science *302*, 84–88.

Marsicano, G., and Lutz, B. (2006). Neuromodulatory functions of the endocannabinoid system. J. Endocrinol. Invest. *29*, 27–46.

Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., Hermann, H., Tang, J., Hofmann, C., Zieglgänsberger, W., et al. (2002). The endogenous cannabinoid system controls extinction of aversive memories. Nature *418*, 530–534.

Matsuda, L.A., Bonner, T.I., and Lolait, S.J. (1993). Localization of cannabinoid receptor mRNA in rat brain. J. Comp. Neurol. *327*, 535–550.

Misner, D.L., and Sullivan, J.M. (1999). Mechanism of cannabinoid effects on long-term potentiation and depression in hippocampal CA1 neurons. J. Neurosci. *19*, 6795–6805.

Moldrich, G., and Wenger, T. (2000). Localization of the CB 1 cannabinoid receptor in the rat brain. An immunohistochemical study. Peptides *21*, 1735–1742.

Monory, K., Blaudzun, H., Massa, F., Kaiser, N., Lemberger, T., Schütz, G., Wotjak, C.T., Lutz, B., and Marsicano, G. (2007). Genetic dissection of behavioral and automatic effects of Δ9-tetrahydrocannabinol in mice. PLoS Biol. *5*, e269.

Monory, K., Massa, F., Egertová, M., Eder, M., Blaudzun, H., Westenbroek, R., Kelsch, W., Jacob, W., Marsch, R., Ekker, M., et al. (2006). The endocannabinoid system controls key epileptogenic circuits in the hippocampus. Neuron *51*, 455–466.

Mulder, J., Aguado, T., Keimpema, E., Barabás, K., Ballester Rosado, C.J., Nguyen, L., Monory, K., Marsicano, G., Di Marzo, V., Hurd, Y.L., et al. (2008). Endocannabinoid signaling controls pyramidal cell specification and long-range axon patterning. Proc. Natl. Acad. Sci. USA *105*, 8760–8765.

Nava, F., Carta, G., Colombo, G., and Gessa, G.L. (2001). Effects of chronic Delta(9)-tetrahydrocannabinol treatment on hippocampal extracellular acetylcholine concentration and alternation performance in the T-maze. Neuropharm. *41*, 392–399.

Navarrete, M., and Araque, A. (2008). Endocannabinoids mediate neuron-astrocyte communication. Neuron *57*, 883–893.

Navarrete, M., and Araque, A. (2010). Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. Neuron *68*, 113–126.

Pacher, P., Bátkai, S., and Kunos, G. (2006). The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol. Rev. *58*, 389–462.

Puighermanal, E., Marsicano, G., Busquets-Garcia, A., Lutz, B., Maldonado, R., and Ozaita, A. (2009). Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. Nat. Neurosci. *12*, 1152–1158.

Ranganathan, M., and D'Souza, D.C. (2006). The acute effects of cannabinoids on memory in humans: a review. Psychopharm. *188*, 425–444.

Robinson, L., Goonawardena, A.V., Pertwee, R.G., Hampson, R.E., and Riedel, G. (2007). The synthetic cannabinoid HU210 induces spatial memory deficits and suppresses hippocampal firing rate in rats. Br. J. Pharmacol. *151*, 688–700.

Robson, P. (2001). Therapeutic aspects of cannabis and cannabinoids. Br. J. Psychiatry *178*, 107–115.

Rodriguez, J.J., Mackie, K., and Pickel, V.M. (2001). Ultrastructural localization of the CB1 cannabinoid receptor in μ-opioid receptor patches of the rat caudate putamen nucleus. J. Neurosci. *21*, 823–833.

Rolls, E.T., and Kesner, R.P. (2006). A computational theory of hippocampal function, and empirical tests of the theory. Prog. Neurobiol. *79*, 1–48.

Salio, C., Doly, S., Fischer, J., Franzoni, M.F., and Conrath, M. (2002). Neuronal and astrocytic localization of the cannabinoid receptor-1 in the dorsal horn of the rat spinal cord. Neurosci. Lett. *329*, 13–16.

Sanderson, D.J., Good, M.A., Seeburg, P.H., Sprengel, R., Rawlins, J.N., and Bannerman, D.M. (2008). The role of the GluR-A (GluR1) AMPA receptor subunit in learning and memory. Prog. Brain Res. *169*, 159–178.

Serpa, A., Ribeiro, J.A., and Sebastião, A.M. (2009). Cannabinoid CB1 and adenosine A1 receptors independently inhibit hippocampal synaptic transmission. Eur. J. Pharmacol. *623*, 41–46.

Steele, R.J., and Morris, R.G. (1999). Delay-dependent impairment of a matching-to-place task with chronic and intrahippocampal infusion of the NMDA-antagonist D-AP5. Hippocampus *9*, 118–136.

Takahashi, K.A., and Castillo, P.E. (2006). The CB1 cannabinoid receptor mediates glutamatergic synaptic suppression in the hippocampus. Neuroscience *139*, 795–802.

Tsou, K., Brown, S., Sanudo-Pena, M.C., Mackie, K., and Walker, J.M. (1998). Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience *83*, 393–411.

Varvel, S.A., and Lichtman, A.H. (2002). Evaluation of CB1 receptor knockout mice in the Morris water maze. J. Pharmacol. Exp. Ther. *301*, 915–924.

Wise, L.E., Thorpe, A.J., and Lichtman, A.H. (2009). Hippocampal CB(1) receptors mediate the memory impairing effects of Delta(9)-tetrahydrocannabinol. Neuropsychopharmacology *34*, 2072–2080.

Witter, M.P., and Amaral, D.G. (2004). In The Rat Nervous System, G. Paxinos (Academic Press, Elsevier, ed. 3), chap. 21., 637–704.

Wong, T.P., Howland, J.G., Robillard, J.M., Ge, Y., Yu, W., Titterness, A.K., Brebner, K., Liu, L., Weinberg, J., Christie, B.R., et al. (2007). Hippocampal long-term depression mediates acute stress-induced spatial memory retrieval impairment. Proc. Natl. Acad. Sci. USA *104*, 11471–11476.