Research

Opposing actions of chronic Δ^9 -tetrahydrocannabinol and cannabinoid antagonists on hippocampal long-term potentiation

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Memory deficits produced by marijuana arise partly via interaction of the psychoactive component, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), with cannabinoid receptors in the hippocampus. Although cannabinoids acutely reduce glutamate release and block hippocampal long-term potentiation (LTP), a potential substrate for learning and memory, the consequences of prolonged exposure to Δ^9 -THC for hippocampal function are poorly understood. Rats were injected with Δ^9 -THC (10 mg/kg, i.p., q.d.) for 1, 3, or 7 d, and electrophysiological recordings were performed in hippocampal slices 1 d after the final injection. At this time, Δ^9 -THC was undetectable in hippocampus using liquid chromatography–mass spectrometry (LC-MS). Hippocampal LTP generated using high-frequency (HFS) or theta burst stimulation was not observed in brain slices from the 7-d Δ^9 -THC-treated animals. Δ^9 -THC also blocked HFS-LTP after 3 d, but not 1 d of treatment. The complete blockade of LTP persisted for 3 d after the last Δ^9 -THC injection, and full reversal of the LTP deficit was not observed up to 14 d following Δ^9 -THC withdrawal. The cannabinoid antagonist AM251 (2 mg/kg), administered before each Δ^9 -THC injection prevented the blockade of LTP, and 7-d treatment with AM251 alone significantly increased the level of LTP. Chronic Δ^9 -THC also produced tolerance to the inhibition of synaptic GABA, but not glutamate release by the agonist WIN55,212-2. These data define consequences of repeated Δ^9 -THC exposure for synaptic plasticity in the hippocampus that may help explain memory impairments in humans following chronic marijuana use.

Memory deficits following marijuana use are major adverse effects of this widely used illicit drug. Thus, acute exposure to the major psychoactive component of marijuana, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), or synthetic cannabinoid compounds disrupts spatial and cognitive performance tasks in animals (Lichtman et al. 1995; Hampson and Deadwyler 1999) and humans (Heishman et al. 1997). It is likely that this is a consequence of the activation of a dense population of cannabinoid receptors located in the hippocampus (Katona et al. 2000), a brain region known to be involved in the processing of both spatial and episodic memory (Burgess et al. 2002). A ubiquitous cellular effect of cannabinoids is the presynaptic inhibition of both GABA and glutamate release throughout the brain (Wilson and Nicoll 2002; Hoffman and Lupica 2005). In the hippocampus, this appears to represent the primary means through which cannabinoids acutely disrupt neuronal network activity (Hajos et al. 2000). Whereas only a few animal studies have examined the effects of long-term cannabinoid exposure on hippocampal function (Lawston et al. 2000; Hampson et al. 2003; Hill et al. 2004), clinical studies have suggested either that the repeated use of marijuana produces persistent cognitive deficits in humans (Pope and Yurgelun-Todd 1996; Bolla et al. 2002), or that these deleterious cognitive effects are reversible (Harrison et al. 2002; Pope et al. 2002).

One mechanism through which repeated cannabinoid exposure might alter cognition and memory is through the disruption

of synaptic plasticity that is thought to initiate relatively long-term changes in synaptic efficacy. Because long-term potentiation (LTP) represents an experimentally induced long-lived change in synaptic strength following the activation of central excitatory synapses, it is posited that the mechanisms supporting this phenomenon may be similar or identical to those supporting naturally occurring long-term synaptic changes in the brain (Bliss and Collingridge 1993; Lynch 2004). In the CA1 region of the hippocampus, LTP can be produced by a variety of stimulus patterns that activate glutamatergic Schaffer collateral/commissural (sc) inputs to the pyramidal neurons. Acute exposure to Δ^9 -THC or other synthetic and endogenous cannabinoid agonists blocks LTP in the CA1 region in vitro (Nowicky et al. 1987; Stella et al. 1997; Misner and Sullivan 1999), and this can be prevented by the cannabinoid receptor antagonist SR141716A (rimonabant) (Collins et al. 1995). This cannabinoid-induced impairment of hippocampal LTP was reversed via the facilitation of NMDA receptor function by either the depolarization of CA1 pyramidal neurons or by removing Mg^{2+} from the extracellular space (Misner and Sullivan 1999). This suggested that the blockade of LTP by cannabinoid agonists resulted from a decrease in the probability of glutamate release via presynaptic receptors (Misner and Sullivan 1999). Whereas these experiments clearly demonstrated that acute exposure to cannabinoids impaired LTP, the consequences of long-term exposure to Δ^9 -THC remain largely unknown, despite the obvious importance of this information for understanding the consequences of chronic marijuana use in humans. In the present study, we define the consequences of long-term exposure to Δ^9 -THC on synaptic processes and LTP in the hippocampus, and explore the relationship be-

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tween these effects and the development of cannabinoid receptor tolerance.

Results

Chronic Δ^9 -THC blocks hippocampal LTP

Twenty-four hours following the final drug or vehicle injection, field EPSPs (fEPSPs) elicited by Schaffer collateral (sc) stimulation were recorded in the stratum radiatum of the CA1 region of the hippocampus. Brief, high-frequency stimulation (HFS) of sc axons residing in the stratum radiatum initiated stable, robust LTP of the synaptic fEPSP response that persisted for ≥ 60 min in hippocampal slices obtained from animals treated with the Δ^9 -THC vehicle solution for 7 d ($n = 7$ slices/5 rats). In contrast, HFS-LTP was not observed in hippocampal brain slices obtained from rats 24 h after the final injection of a 7-d treatment with Δ^9 -THC ($n = 19$ slices/9 rats, $P < 0.001$; ANOVA) (Fig. 1A).

Figure 1. Blockade of LTP following repeated daily treatment with Δ^9 -THC. (A, top panel) Averaged (5 sweeps) fEPSPs obtained prior to (Pre) and 60 min following (Post) delivery of high-frequency LTP-inducing stimulation (HFS) recorded in hippocampal brain slices 24 h after a 7-d treatment with either vehicle (left) or 10 mg/kg Δ^9 -THC (right). (Bottom panel) Mean time course of fEPSP slope measured in brain slices obtained from vehicle (VEH) and chronic Δ^9 -THC-treated rats. The arrow indicates the timing of HFS. (B) Blockade of LTP induced by theta-burst stimulation (TBS, θ). In slices from vehicle-treated rats, TBS-LTP persisted for >60 min following stimulation. TBS-LTP was not observed in slices from Δ^9 -THC-treated rats. (C) Mean time course of HFS-LTP observed in brain slices obtained from animals treated with vehicle, or with Δ^9 -THC for 1, 3, or 7 d.

Chronic Δ^9 -THC blocks hippocampal LTP induced by theta-burst stimulation

Theta-burst stimulation (TBS) mimics a hippocampal synaptic rhythm that occurs naturally during exploratory behavior in vivo, and is known to initiate LTP (Larson et al. 1986). Theta-burst LTP is also thought to rely more on the transient, frequency-dependent suppression of GABAergic inhibition than is LTP observed following HFS (Chapman et al. 1998). Furthermore, TBS-LTP is inhibited by cannabinoid agonists in vivo (Hill et al. 2004). In slices obtained from 7-d vehicle-treated rats ($n = 4$ slices/3 rats), TBS induced stable, although smaller LTP than that produced by HFS (Fig. 1), and similar to HFS-LTP, TBS-LTP persisted for ≥ 60 min following the last stimulus train in these brain slices. However, in brain slices obtained from animals chronically treated with Δ^9 -THC, significant TBS-LTP was not observed ($P < 0.001$, ANOVA; $n = 10$ slices/6 rats) (Fig. 1B). Thus, LTP produced by both HFS and TBS was blocked 24 h after the 7-d exposure to Δ^9 -THC.

Temporal requirements for the blockade of LTP by Δ^9 -THC

A previous study demonstrated that a single exposure to Δ^9 -THC in vivo impaired another form of synaptic plasticity known as long-term depression (LTD) in both the nucleus accumbens and hippocampus (Mato et al. 2004). Therefore, we examined the possibility that hippocampal LTP was similarly disrupted by a single Δ^9 -THC exposure, and more generally we sought to define the temporal requirements for the LTP impairment in the hippocampus. HFS-LTP was initiated in hippocampal slices obtained either 1 d following a single injection of 10 mg/kg of Δ^9 -THC, or following a 3-d treatment with the agonist. Whereas the single treatment with Δ^9 -THC did not significantly alter HFS-LTP ($P > 0.05$ vs. vehicle, $n = 7$ slices/3 rats) (Fig. 1C), the 3-d treatment ($n = 14$ slices/5 rats) caused a significant impairment of LTP at this time point ($P < 0.001$ vs. 7-d vehicle or 1-d Δ^9 -THC treatment; ANOVA) (Fig. 1C). Thus, it appears that unlike LTD of excitatory synaptic transmission in the nucleus accumbens (Mato et al. 2004) and inhibitory transmission (I-LTD) (Chevalleyre and Castillo 2003) in the hippocampus, repeated exposure to Δ^9 -THC was required to observe the impairment of HFS-LTP in the rat hippocampus.

Accumulation of Δ^9 -THC in hippocampus does not explain the physiological consequences of chronic exposure to the drug

One potential explanation for the ability of repeated, but not single injections of Δ^9 -THC to block hippocampal LTP might be through the accumulation of this highly lipophilic molecule in brain tissue. Thus, high local concentrations of Δ^9 -THC might then occupy cannabinoid receptors and block LTP through the well-known acute reduction in glutamatergic synaptic transmission (Misner and Sullivan 1999). Therefore, additional experiments were performed to evaluate this possibility.

First, the relationship between presynaptic axon fiber volley and fEPSP amplitudes in slices obtained from 7-d vehicle- and 7-d Δ^9 -THC-treated rats was examined. There was no effect of chronic Δ^9 -THC on this relationship (Fig. 2A), suggesting that synaptic strength was not altered by the chronic treatment.

Second, the effect of the cannabinoid receptor antagonist rimonabant (SR141716A, 1 μ M) was evaluated on fEPSPs in slices obtained from Δ^9 -THC-treated rats ($n = 10$ slices/5 rats). Rimonabant did not alter the fEPSP amplitudes, suggesting that cannabinoid receptors were not occupied, and that baseline responses

Figure 2. The blockade of HFS-LTP measured at 24 h after 7-d treatment with chronic Δ^9 -THC does not result from the accumulation of the drug in brain tissue, nor from altered brain slice viability. (A) Plot of input-output relationship of presynaptic fiber volley to fEPSP amplitude in hippocampal slices from 7-d vehicle (VEH) and 7-d Δ^9 -THC-treated (THC) rats. No differences were observed between the two groups. (B) Bath application of the CB1R antagonist rimonabant (SR141716A) did not alter fEPSPs in slices from 7-d Δ^9 -THC-treated rats, suggesting that receptors were not tonically activated by the drug. (C) Detection of Δ^9 -THC in rodent brain tissue using LC-MS, at varying times after its acute or chronic injection. Δ^9 -THC levels were measured in the cerebellum (CB), hippocampus (Hp), or striatum (Str) at either 0.5 or 24 h following a single injection of the drug (Δ^9 -THC + 0.5hr, Δ^9 -THC + 24hr, respectively), or 24 h following a once daily, 7-d treatment with the drug (Δ^9 -THC $\times$ 7 + 24hr). Meaningful concentrations of Δ^9 -THC were only measured in brain tissue from animals in the Δ^9 -THC + 0.5 h group, suggesting that at the time point used in most of our physiological studies of LTP (i.e., Δ^9 -THC $\times$ 7 + 24hr), the drug was no longer present at pharmacologically relevant concentrations in the hippocampus. (ND) Nondetectable.

were not inhibited by residual Δ^9 -THC 24 h following the last injection (Fig. 2B).

Finally, LC-MS was used to directly measure Δ^9 -THC in the hippocampi of rats treated with the agonist for 7 d or 1 d, followed by a 24-h withdrawal, or in rats treated with Δ^9 -THC 30 min before dissection of brain areas ($n = 6$ animals per group). Δ^9 -THC was not detected in the hippocampi, striata, or cerebella from rats treated for 7 d with Δ^9 -THC (Fig. 2C, Δ^9 -THC $\times$ 7 + 24hr) and was found in very low concentrations in the 1-d treatment group (Fig. 2C, Δ^9 -THC + 24hr). In contrast, Δ^9 -THC was detected at high concentrations in all measured brain regions of rats given a single injection (10 mg/kg) 30 min before dissection (Fig. 2C, Δ^9 -THC + 0.5hr). Thus, Δ^9 -THC was not detected in brain tissue at the time point used throughout most of our studies (i.e., the Δ^9 -THC $\times$ 7 + 24hr group). Additionally, these data strongly suggest that the consequences of chronic Δ^9 -THC exposure were not mediated by ongoing pharmacological actions of this cannabinoid agonist following its accumulation in tissue, but rather through more enduring neurobiological changes in hippocampal function brought about through repeated exposure to the drug.

Temporal requirements for LTP recovery following chronic Δ^9 -THC treatment

The preceding studies demonstrated that the effects of chronic Δ^9 -THC on hippocampal function were mediated by a neurobiological change that required repeated exposure to Δ^9 -THC. Therefore, we next examined the potential reversal of the blockade of LTP caused by this drug. Hippocampal slices were obtained from rats sacrificed at 1 d, 3 d, 7 d, and 14 d following the last of seven daily Δ^9 -THC injections, and HFS-LTP was examined. As described above, HFS-LTP was not observed in hippocampi from rats 1 d following the last Δ^9 -THC injection (Fig. 3B). However, statistically significant LTP was observed, relative to slices taken 1 d following the last Δ^9 -THC injection, at 3 d ($n = 12$ slices/5 rats), 7 d ($n = 13$ slices/5 rats), and at 14 d ($n = 17$ slices, 6 rats) after the cessation of this Δ^9 -THC treatment ($P < 0.0001$ vs. baseline; ANOVA) (Fig. 3B). However, despite the significant levels of HFS-LTP seen at 3–14 d after withdrawal from chronic Δ^9 -THC, the degree of synaptic plasticity observed in the hippocampal slices from vehicle-treated animals remained significantly larger than that observed in any of the withdrawal groups ($P < 0.001$ vs. 7-d vehicle treatment; ANOVA) (Fig. 3B). Therefore, although the impairment of LTP by chronic Δ^9 -THC was partially reversed following withdrawal of this drug for 3–14 d, there appeared to be a relatively long-lasting deficit in this form of plasticity.

Cannabinoid receptor antagonism prevents the Δ^9 -THC blockade of LTP

To determine whether the effects of chronic Δ^9 -THC on hippocampal LTP were due to its interaction with cannabinoid receptors, the antagonist AM251 (2 mg/kg) was injected 30 min prior to each injection of Δ^9 -THC for 7 d, and HFS-LTP in the hippocampus was measured. One day following the final injections of

Figure 3. Partial recovery of HFS-LTP during withdrawal from 7 d of treatment with Δ^9 -THC. (A) A single experiment that was conducted 7 d following a 7-d exposure to Δ^9 -THC. The averaged fEPSP waveforms ($n = 5$ sweeps) were obtained at the points indicated in the time course below. The arrow indicates the timing of HFS. Note the robust HFS-LTP seen in this representative example. (B) Summary of HFS-LTP recovery in hippocampal slices following either 1 d, 3 d, or 7 d of withdrawal from Δ^9 -THC. The level of HFS-LTP obtained in the 3-d, 7-d, and 14-d (data not shown) withdrawal groups all differed significantly from both the 1-d and the vehicle-injected control.

Figure 4. The CB1R antagonist AM251 reverses the blockade of HFS-LTP by Δ^9 -THC, and increases LTP when administered chronically. (A) Mean effect of chronic treatment (7 d) with Δ^9 -THC (10 mg/kg), Δ^9 -THC + AM251, or vehicle on HFS-LTP (arrow) in hippocampal slices. AM251 (2 mg/kg) was administered 30 min prior to each of seven daily Δ^9 -THC injections. (B) Chronic AM251 enhancement of HFS-LTP. Animals were given i.p. injections of AM251, alone, for 7 d (2 mg/kg). Hippocampal HFS-LTP was measured 24 h following the last AM251 injection. HFS-LTP was significantly enhanced compared to vehicle-treated controls. (C) Time course from a single hippocampal slice experiment in which HFS-LTP was induced during acute AM251 (1 μ M) treatment. HFS was delivered at the time indicated by the arrow, and averaged fEPSPs were obtained at the indicated times. (D) Mean effects of acute AM251 exposure on HFS-LTP. No significant differences in HFS-LTP were observed between untreated and AM251-treated slices obtained from drug-naïve animals.

AM251 and Δ^9 -THC ($n = 6$ slices/3 rats), HFS-LTP was observed at levels that were not significantly different from those in slices obtained from animals that received only vehicle injections ($P > 0.05$; ANOVA) (Fig. 4A). This suggested that the antagonist blocked the effects of chronic Δ^9 -THC by acting at the same pharmacological site as Δ^9 -THC. Interestingly, slices taken from animals that were treated for 7 d with the antagonist alone showed a significant enhancement of HFS-LTP, relative to vehicle-treated controls ($n = 15$ slices/5 rats, $P < 0.001$; ANOVA) (Fig. 4B). To determine whether this ability of AM251 to enhance LTP might

result from an ongoing acute effect of the drug following its accumulation in brain tissue during the chronic treatment (e.g., via the blockade of endogenous cannabinoid activity), its effects were measured on HFS-LTP in brain slices obtained from drug-naïve rats. To ensure equilibrium concentrations of the lipophilic antagonist, slices were incubated for ≥ 1 h in a saturating concentration of AM251 (1 μ M) prior to initiating LTP, and this AM251 concentration was maintained throughout the remainder of the recording. AM251 did not affect fEPSP slope values (AM251 treatment, $n = 6$ slices/4 rats; 0.61 ± 0.07 mV/msec; no treatment, $n = 5$ slices/4 rats; 0.63 ± 0.04 mV/msec), and HFS-LTP was unaffected by the acute exposure to this drug ($P > 0.05$; ANOVA) (Fig. 4C,D). Furthermore, in similar experiments conducted with the cannabinoid antagonist rimonabant (1 μ M), HFS-LTP was unaltered ($n = 9$ slices/4 rats) (data not shown). This suggests that cannabinoid receptor antagonists do not acutely alter HFS-LTP, and that endogenous cannabinoids released during HFS (Stella et al. 1997) are not likely involved in the initiation or maintenance of HFS-LTP.

At what site does Δ^9 -THC act to block LTP in the hippocampus?

Recent studies have implicated cannabinoid CB1 receptors (CB1Rs) as mediating the actions of both endogenous and exogenous cannabinoids in the rodent hippocampus (Hajos et al. 2000; Hoffman and Lupica 2000; Kawamura et al. 2006; Takahashi and Castillo 2006). Thus, it has been demonstrated that activation of presynaptic cannabinoid receptors can inhibit the release of glutamate from sc nerve terminals, or GABA from the axon terminals of cholecystokinin (CCK)-immunoreactive interneurons in the hippocampus. Therefore, we investigated the effects of chronic Δ^9 -THC treatment on these synaptic pathways.

Chronic Δ^9 -THC-induced LTP impairment: Glutamatergic mechanisms

The initiation of hippocampal CA1 LTP depends on the activation of postsynaptic NMDA receptors (Morris et al. 1986). Therefore, we examined NMDA receptor function following the 7-d Δ^9 -THC treatment by measuring input-output relationships of synaptically evoked NMDA receptor-dependent currents in CA1 pyramidal cells. Neurons were voltage clamped at +40 mV, during blockade of GABA_A and AMPA-mediated currents by picrotoxin (100 μ M) and DNQX (10 μ M), respectively. Under these conditions, pyramidal neurons demonstrated robust outward synaptic NMDA currents whose amplitude was dependent on the strength of electrical stimulation of sc axons (Fig. 5A), and were completely blocked by application of the NMDA receptor antagonist APV (40 μ M). However, the synaptic NMDA currents did not differ significantly between the vehicle-treated ($n = 9$ neurons/7 rats) and the Δ^9 -THC-treated ($n = 12$ neurons/9 rats) groups ($P > 0.05$) (Fig. 5A). These data therefore argue against the possibility of chronic Δ^9 -THC affecting NMDA receptor function, as an explanation of the impaired HFS-LTP following exposure to this drug.

It is well known that acutely cannabinoid agonists reduce glutamate release probability at the CA3–CA1 sc synapse, and thereby alter the relationship between closely spaced pairs of evoked glutamatergic EPSCs (Misner and Sullivan 1999; Hajos et al. 2001). It has also recently been demonstrated that the glutamatergic parallel fiber input to cerebellar Purkinje cells exhibits changes in the relationship between paired EPSCs following chronic Δ^9 -THC (Tonini et al. 2006). Therefore, we examined paired-pulse facilitation of AMPA-receptor-mediated EPSCs at several interstimulus intervals, using whole-cell recordings from CA1 pyramidal neurons in slices of hippocampus obtained from

Figure 5. Chronic exposure to Δ^9 -THC does not alter NMDA receptor function, but reduces paired pulse facilitation of AMPA-mediated EPSCs. (A) Input-output relationship between stimulus intensity and whole-cell NMDA-mediated synaptic EPSC charge (area = nA $\times$ msec) recorded from CA1 pyramidal cells in brain slices obtained from animals treated with Δ^9 -THC or vehicle for 7 d. Outward NMDA currents were elicited via electrical stimulation of the sc fibers in neurons voltage-clamped at +40 mV, during application of picrotoxin (100 μ M) and DNQX (40 μ M). Representative mean traces of NMDA currents at varying stimulus intensities are shown for both a vehicle-treated and a Δ^9 -THC-treated neuron. No significant differences were observed. (B) Paired-pulse facilitation of evoked AMPA-mediated EPSCs at varying interstimulus intervals. Representative traces from slices obtained from a vehicle-treated (left) and THC-treated (right) rat, using a 20-msec paired pulse interval. Synaptic facilitation was significantly reduced in THC-treated slices at shorter inter-pulse intervals, relative to vehicle controls.

chronic vehicle- or Δ^9 -THC-treated rats. Similar to the results of Tonini et al. (2006) in cerebellar slices, we observed a significant reduction of the paired EPSC ratio (EPSC2/EPSC1) at short interstimulus intervals in the neurons obtained from the chronic Δ^9 -THC-treated rats, suggesting an increase in the probability of glutamate release ($P < 0.001$, ANOVA; vehicle: $n = 11$ neurons/8 rats; Δ^9 -THC: $n = 14$ neurons/8 rats) (Fig. 5B).

Another measure of glutamatergic synaptic activity is derived from the analysis of spontaneous, quantal EPSCs (sEPSCs) in CA1 pyramidal neurons during whole-cell recordings in which GABA_A and NMDA receptors are blocked with picrotoxin (100 μ M) and APV (40 μ M), respectively. This analysis revealed that the 7-d Δ^9 -THC treatment was associated with a significant ($P < 0.001$) increase in mean sEPSC amplitude (16.2 ± 1.1 pA,

$n = 15$ neurons/9 rats), as compared to that observed in neurons from vehicle-injected control rats (10.5 ± 0.6 pA, $n = 13$ neurons/8 rats) (Fig. 6A,B). In contrast, the frequency of sEPSCs did not differ ($P > 0.05$) between the two chronic treatment conditions (Fig. 6D). The mean frequency of sEPSCs in slices from vehicle-treated rats was 0.46 ± 0.07 Hz, whereas that from Δ^9 -THC-treated animals was 0.49 ± 0.17 Hz (Fig. 6D, inset). Additionally, the sEPSC rise and decay time constants were unaltered by the chronic drug treatment (Fig. 6E). An additional analysis of the sEPSC amplitude distribution revealed that the increase in sEPSC amplitude in neurons from Δ^9 -THC-treated animals appeared to be due to an increase in the amplitude of events that were larger than ~ 15 pA, as well as a decrease in the probability of events smaller than ~ 7 pA (Fig. 6C).

Evaluation of cannabinoid receptor tolerance following chronic Δ^9 -THC: Glutamatergic transmission

Previous results from our laboratory have shown that CB1Rs located on glutamatergic afferents to the nucleus accumbens demonstrate tolerance following the same 7-d Δ^9 -THC treatment used in the present study (Hoffman et al. 2003). To determine whether tolerance developed at glutamatergic sc axon terminals in the present study, and whether this might help explain the impaired LTP following chronic Δ^9 -THC, concentration-response curves for the inhibition of fEPSPs by WIN55,212-2 were constructed in vehicle- and Δ^9 -THC-treated rat hippocampi ($n = 4$ –9 slices per concentration, 6 rats) (Fig. 7A). However, neither the maximal inhibition of the fEPSP nor the half-maximal concentrations (EC_{50}) of WIN55,212-2 for this effect were altered by Δ^9 -THC treatment (Fig. 7A). The EC_{50} for the inhibition of the fEPSP in vehicle-treated rats was 465 nM (95% C.I. = 313 nM to 693 nM), whereas in the hippocampi from Δ^9 -THC-treated rats, it was 576 nM (95% C.I. = 353 nM to 944 nM). Therefore, we conclude that tolerance to the presynaptic actions of cannabinoids at receptors located on sc axon terminals did not occur following chronic Δ^9 -THC treatment.

Chronic Δ^9 -THC-induced LTP impairment: GABAergic mechanisms

Although recent studies have demonstrated CB1R expression on sc axon terminals (Kawamura et al. 2006; Takahashi and Castillo 2006), these receptors are more abundantly expressed on the axon terminals of a subpopulation of GABAergic interneurons that colocalize the neuropeptide CCK in the hippocampus (Katonaka et al. 1999; Hoffman and Lupica 2000). When these receptors are activated by WIN55,212-2, GABA release is inhibited via G-protein $\beta\gamma$ subunit interactions with voltage-dependent (N-type) Ca^{2+} channels (Hoffman and Lupica 2000; Wilson and Nicoll 2002). GABAergic mechanisms can also influence the initiation and expression of LTP in the hippocampus. For example, CA1 LTP is facilitated by GABA_A receptor antagonists (Wigstrom and Gustafsson 1983), and it can be prevented by GABA_B auto-receptor blockade (Davies et al. 1991). For these reasons, and because the activation of hippocampal CB1Rs on interneurons can acutely disrupt intrinsic rhythms that may play a role in the effects of Δ^9 -THC on memory (Hajos et al. 2000), we examined the consequences of chronic exposure to Δ^9 -THC on GABAergic signaling in the hippocampus.

Whole-cell recordings of CB1R-sensitive spontaneous GABA IPSCs (sIPSCs) (Hoffman and Lupica 2000) were performed in CA1 pyramidal neurons in hippocampal slices obtained from chronic Δ^9 -THC- or vehicle-treated animals. However, neither the average amplitude (control: 18.9 ± 4.1 pA, $n = 6$ neurons/3 rats; Δ^9 -THC: 23.2 ± 2.5 pA, $n = 9$ neurons/5 rats) nor the average frequency (control: 3.5 ± 1.1 Hz; Δ^9 -THC: 4.0 ± 0.8 Hz) of

Figure 6. Analysis of spontaneous EPSCs (sEPSCs) in hippocampal CA1 pyramidal neurons from vehicle and 7-d Δ^9 -THC-treated rats. (A) Sample traces of sEPSCs obtained in whole-cell recordings (Vhold = 70 mV) single neurons under each condition. Note the larger amplitude of events in the traces on the right. (B) Mean cumulative probability plot and mean amplitude from all neurons ($n = 13$ neurons/8 vehicle-treated rats; $n = 15$ neurons/9 rats Δ^9 -THC-treated animals). A significant increase in the mean sEPSC amplitude was observed during Δ^9 -THC withdrawal. (C) Mean probability distribution of sEPSCs obtained in each group of neurons. Note the rightward shift in the sEPSC amplitude distribution, and the decrease in smaller sEPSCs (<7 pA) in the neurons obtained from the Δ^9 -THC-treated group. (D) Mean cumulative interevent interval distribution and mean frequency of sEPSCs from the same cells. No differences in sEPSC frequency were observed. (E) Mean rise and decay times of sEPSCs. No significant differences were observed in either parameter.

the sIPSCs was significantly altered following the 7-d exposure to Δ^9 -THC ($P > 0.05$; ANOVA) (Fig. 8). This suggested that basal GABAergic synaptic transmission was not altered by this treatment.

Evaluation of cannabinoid receptor tolerance following chronic Δ^9 -THC: GABAergic transmission

To determine the extent to which functional tolerance at GABAergic synapses occurred following repeated Δ^9 -THC treatment, we evaluated the effect of several concentrations of WIN55,212-2 (0.1 μ M, 1 μ M, and 5 μ M) on electrically evoked IPSCs in CA1 pyramidal cells (Fig. 7B). As described previously in hippocampal slices from naive rats (Hoffman and Lupica 2000), neurons obtained from vehicle-treated animals ($n = 23$ neurons/12 rats) demonstrated evoked GABAergic IPSCs that were inhibited by WIN55,212-2 in a concentration-dependent manner (Fig. 7B). In contrast, the effect of WIN55,212-2 on evoked IPSCs recorded from neurons obtained from rats treated for 7 d with Δ^9 -THC was significantly smaller at each concentration of the agonist ($n = 19$ neurons/10 rats, $P < 0.001$ vs. vehicle; ANOVA) (Fig. 7B). This suggested that whereas baseline spontaneous synaptic GABA release was unaffected by 7-d Δ^9 -THC, tolerance was observed at CB1Rs located on hippocampal GABAergic terminals. Taken together these data suggest that tolerance to WIN55,212-2 was observed at GABAergic, but not glutamatergic synapses onto

CA1 pyramidal neurons following chronic Δ^9 -THC exposure (Fig. 7A vs. Fig. 7B).

Discussion

The major finding of this study was that daily exposure to Δ^9 -THC, the primary psychoactive constituent of marijuana, blocked hippocampal LTP initiated at a time when the drug was not detected by LC-MS in brain tissue. This demonstrates that chronic Δ^9 -THC can cause relatively enduring changes in brain function that interfere with the mechanisms supporting LTP, a putative cellular model of memory. This effect was not observed 24 h following a single treatment with Δ^9 -THC, and LTP significantly recovered within 3 d following cessation of Δ^9 -THC exposure, suggesting a transient and at least partially reversible change in the mechanisms underlying this form of synaptic plasticity. In addition, the blockade of LTP by chronic Δ^9 -THC was completely prevented by coadministration of the cannabinoid receptor antagonist AM251, suggesting the involvement of cannabinoid receptors in the effects of Δ^9 -THC.

Another novel finding of the present study was that AM251 alone significantly increased the level of LTP. This observation is consistent with previous reports that acute exposure to the CB1 antagonist rimonabant can facilitate memory in rats (Lichtman 2000; Takahashi et al. 2005), and suggests that long-term treatment with cannabinoid receptor antagonists can also act to enhance the mechanisms that support memory and may represent a beneficial action of this class of drugs (Boyd and Fremming 2005).

Alternatively, this effect of AM251 may result from its reported inverse agonist properties (Landsman et al. 1997; Sim-Selley et al. 2001; Pertwee and Ross 2002). However, it is important to note that inverse agonism has not been observed with AM251 or rimonabant in all systems (Savinainen et al. 2003), and particularly not on measures of synaptic neurotransmitter release (Hoffman and Lupica 2000; Wilson and Nicoll 2001; Chevalere and Castillo 2003). The increased LTP observed following 7 d of treatment with AM251 may also be clinically important because the cannabinoid receptor antagonist rimonabant will soon likely be approved in the USA for long-term use in humans (Boyd and Fremming 2005; Van Gaal et al. 2005).

The memory-disruptive effects of acute cannabinoids are well-established in vivo (Hampson and Deadwyler 1999; Varvel et al. 2001; Hill et al. 2004), and their effects on animal models of synaptic plasticity, such as LTP, have been extensively examined in detail in vitro (Nowicky et al. 1987; Collins et al. 1995; Teranova et al. 1995; Stella et al. 1997; Misner and Sullivan 1999). These studies demonstrated that LTP was blocked in hippocampal slices by the acute application of Δ^9 -THC (Nowicky et al. 1987) and other cannabinoid agonists (Collins et al. 1995; Teranova et al. 1995; Stella et al. 1997; Misner and Sullivan 1999), and that this likely reflected the inhibition of glutamate release, and the consequential reduction of NMDA receptor activation,

Figure 7. Differential tolerance to WIN 55,212-2 at GABAergic and glutamatergic synapses following withdrawal from repeated Δ^9 -THC. (A) Concentration-response curves for the effect of WIN55,212-2 on fEPSPs in brain slices recorded 24 h after a 7-d treatment with either Δ^9 -THC or vehicle. No significant differences between groups were observed (vehicle EC₅₀ = 465 nM; Δ^9 -THC-treated EC₅₀ = 576 nM). (B) Whole-cell recordings of the concentration-dependent effects of WIN55,212-2 on electrically evoked GABA_A IPSCs in hippocampal slices obtained from 7-d vehicle- and 7-d Δ^9 -THC-treated rats. The inhibition of the evoked IPSCs by WIN55,212-2 was significantly (** P < 0.01; ANOVA) reduced at each concentration following Δ^9 -THC treatment, indicating the development of tolerance. The number of neurons used at each concentration, under each chronic treatment condition, is shown in parentheses. Note that the fEPSP and the evoked IPSC concentration-response curves are plotted on the same abscissa.

which is obligatory for hippocampal CA1 LTP (Morris et al. 1986; Madison et al. 1991; Lynch 2004). It has also been demonstrated that long-term treatment with the synthetic cannabinoid HU-210 impairs memory in the Morris water maze, and reduces TBS-LTP in vivo (Hill et al. 2004). Although these studies provide evidence of the negative consequences of cannabinoids on memory during either acute or persistent exposure, we chose to examine the effects of withdrawal from Δ^9 -THC on LTP to determine whether there were enduring neurobiological consequences after chronic exposure to the drug. In addition, we wished to avoid potential confounds associated with long-term treatment with lipophilic ligands, and the possibility that the effects observed after long-term treatment were simply the result of ongoing drug action following its buildup in brain tissue. Therefore, unlike our study, the disruption of hippocampal-dependent memory and TBS-LTP seen during extended treatment with HU-210 in vivo may have reflected ongoing acute pharmacological effects of the agonist at tolerant cannabinoid receptors (Hill et al. 2004). Furthermore, it was not determined whether the effects of chronic cannabinoid treatment on LTP were antagonist-reversible (Hill et al. 2004). Therefore, the possibility that noncannabinoid sites were involved could not be discounted in this prior study. Our study demonstrated using analytical chemistry (LC-MS) that the continued action of Δ^9 -THC at cannabinoid receptors was not necessary to account for the blockade of TBS-, or HFS-LTP during withdrawal from this drug, and that the antagonist AM251 eliminated the blockade of hippocampal LTP caused by chronic Δ^9 -THC when it was administered prior to each Δ^9 -THC injection in vivo. This provides

strong evidence that the actions of Δ^9 -THC were mediated by long-term interaction with cannabinoid receptors.

Potential synaptic mechanisms of Δ^9 -THC blockade of LTP

The most frequently observed physiological consequence of cannabinoid receptor activation in the CNS is the inhibition of neurotransmitter release from GABAergic or glutamatergic axon terminals (Wilson and Nicoll 2002; Freund et al. 2003; Hoffman and Lupica 2005). Therefore, the inhibition of ongoing synaptic activity in the hippocampus by Δ^9 -THC represents a mechanism through which cannabinoids might act to disrupt cognitive processing and memory (Heishman et al. 1997; Hampson and Deadwyler 1999; Varvel et al. 2001; Bolla et al. 2002; Pope et al. 2002). In an effort to identify the neural substrates that were affected following chronic Δ^9 -THC, we assessed baseline measures of synaptic transmission, as well as the cannabinoid receptor-mediated modulation of synaptic neurotransmitter release.

Chronic Δ^9 -THC effects on glutamatergic synapses

Since hippocampal LTP is dependent on the activation of both AMPA and NMDA receptors (Madison et al. 1991), and cannabinoids are known to reduce the probability of glutamate release from sc axon terminals (Sullivan 2000), several experiments were performed to assess changes in this pathway. First, the input-output relationship between the presynaptic fiber volley and the glutamate-mediated fEPSP was unchanged by chronic Δ^9 -THC treatment, suggesting a lack of general impairment in slice physiology, and that there was no ongoing activation of presynaptic cannabinoid receptors. Second, synaptic NMDA receptor currents were unchanged following the 7-d treatment with Δ^9 -THC, suggesting that the blockade of LTP was not a consequence of reduced NMDA receptor function. Third, since it was possible that the function of cannabinoid receptors, and their putative role in either the facilitation (Carlson et al. 2002; Chevaleyre and Castillo 2004) or inhibition of LTP (Bohme et al. 2000; Slanina et al. 2005), was altered by chronic Δ^9 -THC, we examined the possibility that tolerance had developed using the full agonist, WIN55,212-2. These studies demonstrated that, in contrast to GABAergic synapses (see below), the inhibitory effect of WIN55,212-2 on glutamatergic fEPSPs was not altered by chronic Δ^9 -THC. Fourth, in order to determine whether endogenous cannabinoid molecules, known to be released by HFS (e.g., 2-arachidonylglycerol) (Stella et al. 1997), played a role in the establishment of HFS-LTP, we examined the effects of the CB1R antagonists AM251 and rimonabant in naive hippocampal slices. We found that acute blockade of these receptors during HFS did not affect LTP, confirming that endogenous cannabinoids were not involved in this form of hippocampal plasticity in vitro (Slanina et al. 2005).

In contrast to these negative results, whole-cell experiments demonstrated that the average quantal AMPA sEPSC amplitude was increased in the Δ^9 -THC-withdrawn neurons, whereas the frequency and kinetics of these events were unchanged. Analysis of the distribution of the sEPSC amplitudes revealed an increase in the probability of larger events (>15 pA), and a reduction in the probability of smaller events (<7 pA), which is similar to that observed in CA1 pyramidal neurons during withdrawal of the benzodiazepine flurazepam (Van Sickel and Tietz 2002). Therefore, this may represent a neuroadaptive change to drug withdrawal, or may be secondary to the chronic perturbation of GABAergic circuitry in the hippocampus (Van Sickel and Tietz 2002). In addition to the increase in sEPSC amplitude, we also observed a decrease in the paired evoked EPSC ratio at shorter

Figure 8. Effects of chronic Δ^9 -THC treatment on GABAergic synaptic function. (A) Spontaneous, GABA_A IPSCs (sIPSCs) from a 7-d vehicle-treated, and a 7-d Δ^9 -THC-treated rat were recorded in the presence of ionotropic glutamate receptor antagonists. (B) Mean cumulative probability plot and mean amplitudes of sIPSCs for all neurons recorded under each condition (vehicle: $n = 6$ neurons/3 rats; Δ^9 -THC: $n = 9$ neurons/5 rats). (C) Average cumulative probability plot of the interevent interval and mean frequency of sIPSCs from the same cells. No differences ($K-S > 0.05$ for cumulative plots, and $P > 0.05$ for mean values) in either the amplitude or frequency of the events were observed between these two groups, indicating that basal GABAergic synaptic function was unaltered following repeated Δ^9 -THC.

interstimulus intervals following Δ^9 -THC treatment. This is consistent with an increased probability of neurotransmitter release (Stuart and Redman 1991), and is in agreement with a recent study that examined paired glutamatergic synaptic currents in the cerebellum following chronic Δ^9 -THC treatment (Tonini et al. 2006). Interestingly, LTP was also blocked by chronic Δ^9 -THC in this study (Tonini et al. 2006), suggesting that the increase in glutamate release probability and the blockade of this form of synaptic plasticity may be linked through unknown mechanisms and may be a general consequence of prolonged cannabinoid receptor occupation. Although the changes we describe in glutamatergic transmission may in part explain the LTP deficit during Δ^9 -THC withdrawal in the hippocampus, adaptations in other neurobiological substrates linked to both LTP and CB1R function, such as adenylate cyclase/cAMP (Howlett and Fleming 1984; Blitzer et al. 1995; Otmakhova et al. 2000) or extracellular-signal-regulated kinase (ERK) (English and Sweatt 1996; Derkinderen et al. 2003; Tonini et al. 2006), are known to occur, and also must be considered. Additional studies at a finer level of analysis will be necessary to more thoroughly examine these possibilities.

Chronic Δ^9 -THC effects on GABAergic synapses

The activation of CB1 receptors located on inhibitory axon terminals inhibits GABA release in the hippocampus (Hoffman and Lupica 2000; Wilson and Nicoll 2001). Therefore, we examined the effects of chronic Δ^9 -THC on baseline GABAergic synaptic transmission by measuring quantal sIPSCs that are sensitive to CB1R activation (Hoffman and Lupica 2000; Wilson et al. 2001). However, chronic Δ^9 -THC treatment did not alter these sIPSCs, suggesting that basal synaptic GABAergic transmission was unaffected by this treatment. In contrast, CB1R tolerance was observed at these GABAergic synapses following chronic Δ^9 -THC

treatment, as indicated by a decrease in the sensitivity of stimulus-evoked IPSCs to WIN55,212-2.

The differential Δ^9 -THC-induced tolerance at GABAergic and glutamatergic axon terminals in the present study might be explained by the controversial proposal of distinct cannabinoid receptors mediating the effects of agonists at these sites in the hippocampus (Breivogel et al. 2001; Hajos et al. 2001; Hoffman et al. 2005). However, recent reports suggested that CB1Rs were located on both glutamatergic sc terminals and GABAergic axon terminals and were responsible for the cannabinoid-mediated inhibition of the release of these neurotransmitters in area CA1 of the hippocampus (Kawamura et al. 2006; Takahashi and Castillo 2006). Nevertheless, the present data indicate that the receptor mediating the inhibition of glutamate release from sc terminals was considerably less sensitive ($EC_{50} = 465$ nM) to WIN55,212-2 than the CB1R mediating the inhibition of GABA release ($EC_{50} = 138$ nM) (Hoffman and Lupica 2000). Therefore, despite evidence localizing CB1Rs to sc axon terminals, pharmacological evidence from several independent laboratories (Hajos et al. 2001; Hajos and Freund 2002; Ohno-Shosaku

et al. 2002; Hoffman et al. 2005) implies either that the CB1R located on sc terminals exhibits distinct pharmacological properties (i.e., lower affinity for WIN55,212-2 and differential sensitivity to chronic Δ^9 -THC), or that another binding site is available to interact with Δ^9 -THC and WIN55,212-2.

Similar to the changes observed in baseline glutamatergic transmission described above, it is unclear whether Δ^9 -THC-induced tolerance at CB1Rs at GABAergic synapses played a role in the blockade of LTP. Furthermore, it might reasonably be asked how this might contribute to the blockade of a form of synaptic plasticity that relies on the activation of glutamatergic synapses? Whereas additional data will be needed to address this point more directly, the importance of the GABAergic system and CB1Rs in hippocampal-dependent memory can be seen in a study in which acute Δ^9 -THC disrupted performance in the Morris water- and T-maze tasks in wild-type, but not CB1R^{-/-} mice (Varvel and Lichtman 2002). The disruption of working memory by Δ^9 -THC was reversed in the wild-type mice by the GABA_A receptor antagonist bicuculline (Varvel et al. 2004), implicating both the GABAergic system and CB1Rs in the memory-disruptive effects of this drug. It has also been established that GABAergic function plays a critical role in determining the direction of hippocampal synaptic plasticity (Yu et al. 2001), and that hippocampal interneuron activity is necessary to drive rhythmic cellular networks at θ or γ frequencies, which are permissive to memory formation and the expression of LTP (Traub et al. 1998). Moreover, these intrinsic hippocampal rhythms can be disrupted by cannabinoids acting on CB1Rs on GABAergic terminals (Hajos et al. 2000). These studies, together with our observation of tolerance to cannabinoid agonists acting at confirmed CB1Rs on GABAergic axon terminals (Katona et al. 1999; Hoffman and Lupica 2000), and the known interaction of endogenous cannabinoids with these receptors (Wilson and Nicoll 2001), lead us to hypothesize that the down-regulation of CB1R function at

GABAergic terminals (Romero et al. 1997; Breivogel et al. 1999) may contribute to the disruption of LTP during withdrawal from long-term Δ^9 -THC. This further implies that the Δ^9 -THC-induced deficit in hippocampal LTP may result from the disruption of the ongoing regulation of GABAergic function by endogenous cannabinoids (Freund et al. 2003), which may modulate GABAergic tone, and act to establish a suitable cellular environment for LTP to occur. Conversely, chronic treatment with antagonists for CB1Rs may augment memory and LTP, perhaps by blocking the ongoing endocannabinoid modulation of hippocampal activity.

Implications for human marijuana use

The extent to which repeated marijuana exposure produces long-term disruptions in cognitive processing in humans is controversial (Pope et al. 1995; Lundqvist 2005). Although a study by Bolla et al. (2002) found that memory deficits persisted in heavy marijuana users, even after 28 d of abstinence, others found that cognitive deficits normalized 7 d following cessation of marijuana use (Pope et al. 2001), and that even heavy marijuana users may recover cognitive function following 3 mo of abstinence (Fried et al. 2005). Our results indicate that the blockade of LTP following chronic Δ^9 -THC exposure is significantly recovered at 3 d following chronic Δ^9 -THC withdrawal, but was only partially reversed at this time, and was still significantly impaired at 2 wk of withdrawal. Thus, it is possible that human studies demonstrating prolonged cognitive deficits following chronic marijuana use may have utilized measures that are more sensitive to these enduring changes in synaptic plasticity, as compared to those identifying more complete cognitive recovery. In either case, our study implies that relatively long-lived neurophysiological changes are possible, and that additional studies are required in both animals and humans to more completely understand the effects of marijuana and Δ^9 -THC on cognitive function and synaptic plasticity.

Materials and Methods

Drug treatment and animal subjects

Animal protocols were conducted in strict accordance with NIH guidelines, were designed to minimize both the number of animals used and animal suffering, and were approved by the Animal Care and Use Committee of the NIDA Intramural Research Program. Young (2–4 wk) male Sprague-Dawley rats were given a single, daily intraperitoneal injection of a solution consisting of either a Tween 80, DMSO, 0.9% NaCl vehicle solution (1:2:7 ratio) or Δ^9 -THC (2 mg/mL dissolved in the vehicle solution), at a dose of 10 mg/kg. A total of 69 Δ^9 -THC-treated rats and 34 vehicle-treated rats were used in the electrophysiological studies. On average, two to three brain slices from each animal were used in the extracellular electrophysiological experiments, and two neurons were studied per animal in the whole-cell electrophysiological experiments. AM251 (0.4 mg/mL dissolved in the vehicle) was administered at a dose of 2 mg/kg, either alone or 30 min prior to THC administration. The 10-mg/kg dose of Δ^9 -THC was chosen because it is known to rapidly cause behavioral tolerance and the uncoupling of the CB1R from G proteins and adenylyl cyclase (Romero et al. 1997; Breivogel et al. 1999). In addition, our studies using LC-MS indicated that levels of Δ^9 -THC in rat brain 30 min after a single i.p. injection (~200 ng/g) (Fig. 2C) were similar to those found in mice following a 1-mg/kg i.v. injection that corresponded to a blood level of ~100 ng/mL Δ^9 -THC (Varvel et al. 2005). Furthermore, comparable blood levels of ~85–150 ng/mL Δ^9 -THC are observed in humans following eight to 10 “puffs” from a standard marijuana cigarette containing 3.55% Δ^9 -THC, which is sufficient to cause a self-reported marijuana “high” (Heishman et al. 1990).

Slice preparation

Hippocampal brain slices were prepared as previously described (Hoffman and Lupica 2000). At 24 h (± 1 h) following the final injection, animals were decapitated and the brains rapidly removed and immersed in cold (4°C), oxygenated high-sucrose, low- Ca^{2+} -containing artificial cerebrospinal fluid (aCSF) of the following composition: 87 mM NaCl, 2.5 mM KCl, 7 mM MgCl_2 , 0.5 mM CaCl_2 , 1.25 mM NaH_2PO_4 , 25 mM glucose, 75 mM sucrose, and 25 mM NaHCO_3 . Coronal slices were cut at 300–350- μm thickness using a vibrating tissue slicer (VT1000S; Leica Instruments). Hemisectioned brain slices containing the hippocampus were then incubated in normal aCSF consisting of 126 mM NaCl, 3.0 mM KCl, 1.5 mM MgCl_2 , 2.4 mM CaCl_2 , 1.2 mM NaH_2PO_4 , 11.0 mM glucose, and 25 mM NaHCO_3 , saturated with 95% O_2 and 5% CO_2 , at room temperature for ≥ 90 min before recordings. Individual brain slices were transferred to a recording chamber and continuously superfused with normal aCSF (2 mL/min), and were maintained at 30°–32°C using a temperature-controlled solution heater (TC-324B; Warner Instruments). Drugs were delivered via superfusion using a syringe pump (Razel).

Electrophysiological recordings

All recordings were performed in the CA1 region of the hippocampus. Extracellular field EPSPs (fEPSPs) were obtained using 3 M NaCl-filled electrodes and an AC amplifier (A-M Systems Model 1800), and the signals were high- (10 Hz) and low-pass (10 kHz) filtered. Data were acquired to a personal computer at 4 kHz via an A/D board (National Instruments PCI 6024E, or Digidata 1320A; Axon Instruments), using Windows-based software (WCP, courtesy of John Dempster, University of Strathclyde, Glasgow, UK; <http://www.strath.ac.uk/sipbs/>; or pCLAMP 9.0, Axon Instruments). Responses were elicited by electrical stimulation of the stratum radiatum at a frequency of 0.033 Hz using single, 0.1-msec pulses, delivered through a bipolar electrode constructed with formvar-insulated nichrome wire. The stimulus intensity was adjusted to produce fEPSPs with peak amplitudes of 0.5–1 mV (30%–40% of the maximal response). At least 10 min of stable baseline recordings were obtained prior to the delivery of drugs or LTP stimulation. The peak amplitude and slope of the initial (1–2 msec) rising phase of the fEPSP were calculated offline using the acquisition software, and changes in the synaptic response were normalized to the baseline period. High-frequency stimulation (HFS) consisted of three 1-sec trains of 100 Hz, delivered at 10-sec intervals, at twice the stimulation intensity used to evoke the baseline response. Following the HFS trains, the stimulation intensity was then re-set at the level used to evoke the baseline fEPSP. Theta-burst stimulation (TBS) was delivered according to a previously published protocol (Chevalayre and Castillo 2003), and consisted of four episodes of stimulation, at 5-sec intervals. Each episode consisted of 10 bursts of five pulses, at 100 Hz, with the bursts delivered at 200-msec intervals.

Whole-cell electrophysiological recordings were performed using an Axopatch 200B amplifier (Axon Instruments) and electrodes pulled from borosilicate glass (1.5 mm O.D., 0.86 mm I.D.; Sutter Instruments). Electrodes were filled with solutions containing either 125.0 mM CsCl, 10.0 mM HEPES, 1.0 mM EGTA, 0.1 mM CaCl_2 , 2.0 mM Mg^{2+} -ATP, and 0.2 mM Na^+ -GTP; or 100 mM CsCH_3SO_3 , 60 mM CsCl, 0.2 mM EGTA, 10 mM HEPES, 1.0 mM MgCl_2 , 1.0 mM Mg^{2+} -ATP, and 0.3 mM Na^+ -GTP. All intracellular solutions also contained QX-314 (Sigma; 1 mg/mL) and were adjusted to pH 7.2–7.4. Series resistance was monitored with a -10 mV voltage step (200 msec), and only cells maintaining stable access ($<10\%$ change in resistance over the duration of the recording) were included in analyses. Synaptic NMDA-receptor-mediated EPSCs were isolated at +40 mV in the presence of the GABA_A receptor/ Cl^- channel blocker, picrotoxin (100 μM), and the ionotropic glutamate receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 μM) using the CsCH_3SO_3 -based intracellular solution. AMPA-receptor-mediated EPSCs and sEPSCs were isolated at -70 to -80 mV in the presence of D-(-)-2-amino-5-phosphonopentanoic acid (APV; 40 μM).

and picrotoxin (100 μ M), also using the CsCH₃SO₃-based solution. GABA_A-receptor-mediated evoked IPSCs and sIPSCs were isolated at -80 mV in the presence of 40 μ M APV and 10 μ M DNQX using the CsCl-based intracellular solution. sEPSCs and sIPSCs from 2–3-min recording epochs were detected and analyzed off-line using Windows-based software (MiniAnalysis v.6.0; <http://www.synaptosoft.com>).

Measurement of Δ^9 -THC levels in brain

Methods for extraction and LC-MS quantification of Δ^9 -THC from whole blood and brain tissue were modified from a previously described procedure (Lichtman et al. 2001). Calibration standards were prepared from mouse whole blood and homogenized brain (2:1, water/brain, [v/w]) obtained from individual untreated animals. Fifty nanograms of deuterated Δ^9 -THC (Radian Corporation) was added as an internal standard to the blood samples and brain homogenates that contained varying quantities of Δ^9 -THC. The same quantity of deuterated Δ^9 -THC was added to samples of hippocampus, striatum, and cerebellum, obtained from treated individual animals (i.e., tissues from individual animals were not pooled). Following an equilibration period, 2.5 mL of cold acetonitrile (HPLC-grade; Fisher Scientific Co.) was added dropwise while mixing. The samples were then centrifuged (Precision, Vari-Hi-Speed Centricone; Precision Scientific) at 2500 rpm for 15 min to precipitate solids and then stored in a freezer (-20°C) overnight to permit the acetonitrile layer to separate from aqueous layers. The next day the acetonitrile layer was evaporated under nitrogen. Finally, the Δ^9 -THC/deuterated Δ^9 -THC was resolubilized in 0.1 mL of methanol (HPLC-grade; Fisher Scientific Co.). LC-MS (Micromass Quattro II Triple Stage Quadrupole Mass Spectrometer) identification was used for quantification of Δ^9 -THC and deuterated Δ^9 -THC in blood and individual brain region brain matrices using an 85:15 methanol/1% glacial acetic acid (0.1% formic acid) mobile phase. A guard column was used inline with the standard reverse-phase C18 column. The mass spectrometer was run in APCI Δ mode. Ions analyzed in single-ion monitoring mode were 315 for Δ^9 -THC and 318 for deuterated Δ^9 -THC. A calibration curve was constructed for each assay based on linear regression using the peak area ratios of Δ^9 -THC to deuterated Δ^9 -THC of the extracted calibration samples. No peaks were detected above background in the blank control samples, blank blood samples, or blank brain samples. The extracted standard curves ranged from 25 to 5000 ng Δ^9 -THC/g sample and were included in each run for the determination of Δ^9 -THC concentrations in blood and brain. Samples containing concentrations falling outside of this range were excluded from the analysis. Standard curves were almost always linear ($r = 0.99$), but in the rare cases that they were not linear, the experimental samples were excluded from analysis.

Drugs

WIN55,212-2 and AM251 were purchased from Tocris-Cookson. DNQX, APV, and picrotoxin were obtained from Sigma. SR141716A (rimonabant) and Δ^9 -THC (200 mg/mL in EtOH) were obtained from the National Institute on Drug Abuse (Bethesda, MD). The ethanol was evaporated under a stream of nitrogen gas, and the remaining Δ^9 -THC resin was suspended in an equivalent volume of DMSO. Δ^9 -THC was then diluted to 2 mg/mL in Tween-80 (10%), DMSO (20%), and saline (70%).

Statistics

Group data are presented as mean $\pm$ SEM. In all cases, both the number of animals and the number of brain slices used are indicated. The data were obtained from multiple subjects in each group to ensure that results were replicable. LTP data were analyzed using a repeated measures ANOVA design. All statistical tests were performed using a critical probability (α) of $P < 0.05$ (GB-Stat, Dynamic Microsystems; or Prism v4.0, GraphPad Software). Post hoc analyses (Tukey-Kramer or Newman-Keuls) were performed only when the ANOVA yielded a significant main effect. Concentration-response curves were generated in Prism v4.0, using a sigmoidal-dose response equation: $Y = \text{Maximum}$

minimum/(1 + EC₅₀/X); where X is the concentration, Y is the response amplitude, and EC₅₀ is the drug concentration at which the half-maximal effect is observed.

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