Brief Communication

Functional Tolerance and Blockade of Long-Term Depression at Synapses in the Nucleus Accumbens after Chronic Cannabinoid Exposure

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The rewarding properties of the psychoactive constituents of marijuana, termed "cannabinoids," may reflect actions on synaptic transmission in the nucleus accumbens (NAc). Furthermore, long-term changes in these synapses may support the addictive process. Excitatory and inhibitory synapses are acutely inhibited by cannabinoids in the NAc, and endogenous cannabinoids (endocannabinoids) play a critical role in the expression of long-term depression (LTD) of excitatory cortical afferents in this structure. Because humans often use marijuana for prolonged periods, we examined the impact of long-term cannabinoid exposure on synaptic processes in an animal model. Electrophysiological recordings in rat brain slices containing the NAc were performed after chronic exposure to vehicle solution, Δ^9-tetrahydrocannabinol (THC), or the cannabinoid agonist *R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-(1-naphthalenyl)methanone mesylate (WIN55,212-2). Extracellular glutamatergic postsynaptic potentials and whole-cell GABAergic IPSCs were concentration-dependently inhibited by WIN55,212-2 in slices from naive or vehicle-treated animals. However, the sensitivity to WIN55,212-2 was diminished in chronic agonist-treated animals. In addition, cross-tolerance to the inhibitory effect of the μ-opioid agonist Tyr-D-Ala^2,*N*-CH_3-Phe^4,Gly-ol-enkephalin was observed. Endocannabinoid-mediated LTD was initiated via electrical stimulation (5 min, 10 Hz) of glutamatergic afferents to the NAc and was completely blocked by the cannabinoid receptor antagonist SR141716A [*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide] in vehicle-treated animals. LTD was not observed in brain slices from rats chronically treated with Δ^9-THC or WIN55,212-2. These data demonstrate that long-term exposure to the active ingredient of marijuana blocks synaptic plasticity in the NAc and reduces the sensitivity of GABAergic and glutamatergic synapses to both cannabinoids and opioids.

Key words: **marijuana; Δ^9-THC; nucleus accumbens; synaptic plasticity; drug abuse; tolerance**

Introduction

Marijuana and its pharmacologically active constituents, termed "cannabinoids," affect a variety of behaviors, including nociception, memory, and locomotion (Abood and Martin, 1992). The acute neurobiological actions of cannabinoids have been well characterized. Through the activation of specific receptors (termed "CB1") in various brain regions, cannabinoids can inhibit the release of glutamate (Gerdeman and Lovinger, 2001; Robbe et al., 2001) and GABA (Hoffman and Lupica, 2000, 2001). Although this presynaptic effect represents a likely mechanism to explain the behavioral actions of cannabinoids in animals and humans, few studies have addressed the effects of repeated cannabinoid administration on synaptic processes. Such studies are important because of the widespread and often long-term use of marijuana by humans for medicinal and illicit purposes. Behavioral and biochemical studies in animals have demonstrated the rapid development of tolerance to many of the effects of cannabinoids during chronic administration (Rodriguez et al., 1994; Breivogel et al., 1999; Bass and Martin, 2000; Maldonado, 2002). However, the effects of repeated cannabinoid administration on synaptic physiology have not been evaluated.

The nucleus accumbens (NAc) is thought to play a critical role in motivation and the rewarding effects of virtually all abused drugs, including marijuana (Gardner and Lowinson, 1991). At least two mechanisms have been proposed to explain the acute rewarding effects of marijuana. First, in common with other abused drugs (Wise, 1996), cannabinoids enhance dopamine release in the NAc (Chen et al., 1990). Second, the presence of CB1 receptors in the NAc (Herkenham et al., 1991; Robbe et al., 2001) prompted the hypothesis that cannabinoids may be rewarding by directly modulating neuronal activity in this brain region (Hoffman and Lupica, 2001). In support of this hypothesis, cannabinoid agonists were found to inhibit synaptic transmission in the dorsal striatum (Gerdeman and Lovinger, 2001) and the NAc (Hoffman and Lupica, 2001; Robbe et al., 2001). More recently, endocannabinoids have been shown to play a role in the estab-

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lishment of long-term depression (LTD) of glutamatergic synaptic transmission in the striatum (Gerdeman et al., 2002) and the NAc (Robbe et al., 2002b). Although the role of LTD in the NAc is unknown, it is hypothesized that altered synaptic plasticity after repeated drug exposure may underlie the addictive potential of several abused drugs, including marijuana (Gerdeman et al., 2003). In the present study, we evaluated the development of tolerance at synapses in the NAc, as well as changes in synaptic plasticity after chronic cannabinoid exposure.

Materials and Methods

Drug treatment. All animal protocols were performed in accordance with National Institutes of Health guidelines and were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse (NIDA) Intramural Research Program. Male Sprague Dawley rats (2–4 weeks of age) were given a single, daily intraperitoneal injection of a vehicle solution consisting of Tween 80, DMSO, 0.9% NaCl (1:2:7), *R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-(1-naphthalenyl)methanone mesylate (WIN55,212-2) (2 mg/ml, 10 mg/kg), or Δ^9-tetrahydrocannabinol (THC; 2 mg/ml, 10 mg/kg) for 7 consecutive days. Bradykinesia and/or catalepsy were routinely observed during the initial cannabinoid exposure.

Slice preparation. Brain slices were prepared as described previously (Hoffman and Lupica, 2001). Rats were decapitated 1 d after the final injection, and brains were rapidly removed and chilled in ice-cold artificial CSF (aCSF) consisting of (in mM): 126 NaCl, 3 KCl, 1.5 $MgCl_2$, 2.4 $CaCl_2$, 1.2 NaH_2PO_4, 11 glucose, and 26 $NaHCO_3$, saturated with 95% O_2 and 5% CO_2. Coronal slices were cut at 300–350 μm thickness using a microtome (VT1000S; Leica Instruments, Nussloch, Germany). Slices were incubated in aCSF at room temperature for ≥90 min before recordings. During recordings, slices were continuously superfused with aCSF (2 ml/min). Drugs were delivered via superfusion using a syringe pump (Razel, Stamford, CT).

Electrophysiological recordings. All recordings were performed in the area immediately surrounding the anterior commissure, designated as the core of the NAc (Paxinos and Watson, 1986). Synaptic responses were evoked at 0.033 Hz by a single 0.1 msec pulse, via a bipolar stimulating electrode placed 100–200 μm from the recording electrode. Stimulus intensity was adjusted to evoke a response that was 30–40% of maximum. Whole-cell voltage-clamp recordings of IPSCs were performed (Hoffman and Lupica, 2001) using an Axopatch 200B amplifier and stored directly to a personal computer using pClamp software and a Digidata 1320A analog-to-digital (A/D) board (Axon Instruments, Burlingame, CA). Electrodes were filled with a solution containing (in mM): 125 CsCl, 10 HEPES, 1 EGTA, 0.1 $CaCl_2$, 2 Mg^{2+}-ATP, 0.2 Na^+-GTP, and 2 *N*-(2,6-dimethylphenylcarbamoylmethyl)triethylammonium chloride, pH 7.2–7.4. IPSCs were isolated by adding the glutamate receptor antagonists DNQX (10 μM) and APV (40 μM) to the aCSF. Series resistance was monitored using −10 mV voltage steps (200 msec). Recordings of extracellular, glutamate-driven population spikes (PSs) were performed using a differential amplifier (A-M Systems) and electrodes filled with 3 M NaCl. To isolate PSs, the Cl^- channel-blocker picrotoxin (100 μM) was added to the aCSF. The LTD protocol consisted of 10 Hz stimulation applied for 5 min. Extracellular data were acquired and stored on a personal computer via an A/D board (PCI 6024E; National Instruments, Austin, TX) and Windows-based software (Whole-Cell Program; courtesy of Dr. John Dempster, University of Strathclyde, Glasgow, UK).

Drugs. WIN55,212-2, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251), and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) were purchased from Tocris-Cookson (Ballwin, MO). DNQX, APV, picrotoxin, N^6-cyclopentyladenosine (N^6-CPA), and Tyr-D-Ala^2,*N*-CH_3-Phe^4,Gly-ol-enkephalin (DAMGO) were obtained from Sigma (St. Louis, MO). *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (SR141716A) and Δ^9-THC (200 mg/ml EtOH) were obtained through the NIDA supply system (Bethesda, MD). 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 the mean ± SEM. Statistical tests were performed using a critical probability of $p < 0.05$ [Prism, version 3.03 (GraphPad Software, San Diego CA) or GB-Stat, version 5.0 (Dynamic Microsystems, Silver Spring, MD)]. *Post hoc* analyses were performed only when an ANOVA yielded a significant main effect. Concentration-response curves were obtained using a sigmoidal dose–response equation: $Y = \text{Maximum} - \text{minimum}/(1 + EC_{50}/X)$, where X is the concentration, Y is the response amplitude, and EC_{50} is the concentration at which the half-maximal effect is observed.

Results

Tolerance to the synaptic effects of cannabinoids

Cannabinoids acutely inhibit extracellular field responses in the NAc core via presynaptic inhibition of glutamate release (Robbe et al., 2001) and have been shown to have no effect on medium spiny neuron passive membrane properties (Hoffman and Lupica, 2001). The glutamatergic nature of the extracellular PS was confirmed through application of the non-NMDA ionotropic glutamate receptor antagonist DNQX (10 μM), which completely blocked the N2 component (Fig. 1*A*) of the field potential (latency, 4 ± 0.2 msec) without altering the nonsynaptic N1 component (latency, 1.4 ± 0.1 msec; $n = 20$). Similarly, in slices from naive animals, WIN55,212-2 (1 μM) reduced the N2 component (67 ± 8% of control; $n = 10$) without affecting the N1 component (96 ± 2%; $n = 10$). To determine whether chronic cannabinoid treatment altered the sensitivity of glutamatergic synapses to cannabinoid-mediated inhibition, extracellular recordings were performed in brain slices prepared from rats chronically treated with either WIN55,212-2 or Δ^9-THC (10 mg/kg, i.p.). The CB1 antagonist SR141716A (1 μM) did not significantly alter baseline extracellular responses after chronic WIN55,212-2 treatment ($n = 5$; 91 ± 4% of control), and the antagonist AM251 (1 μM) did not alter PS amplitude (99 ± 3%; $n = 3$) 24 hr after a single injection of WIN55,212-2, suggesting that residual cannabinoid accumulation in the brain slices was not a confounding factor in these experiments.

WIN55,212-2 inhibited only the N2 component of the PS responses in slices from both chronic vehicle-treated and cannabinoid-treated animals in a concentration-dependent manner (Fig. 1*A*). However, responses recorded in slices from chronically treated animals were significantly less sensitive to WIN55,212-2, as demonstrated by a rightward shift in the dose–response curve (Fig. 1*A*). The EC_{50} for the WIN55,212-2-mediated inhibition of the responses was 143 nM [95% confidence interval (CI) = 58–345 nM] in control slices and 1.18 μM (95% CI = 492 nM to 2.83 μM) in slices obtained from chronic WIN55,212-2-treated animals. NAc slices from animals chronically treated with Δ^9-THC also showed reduced sensitivity to WIN55,212-2 (EC_{50} = 1.22 μM; 95% CI = 59 nM to 26 μM).

In addition to inhibiting glutamate release in the NAc, cannabinoids, including WIN55,212-2 (EC_{50} = 123 nM), presynaptically inhibit GABAergic IPSCs recorded from NAc medium spiny neurons in a concentration-dependent manner (Hoffman and Lupica, 2001). To determine whether the presynaptic effects of cannabinoids on GABAergic transmission also demonstrated tolerance, evoked IPSCs (eIPSCs) were measured in slices from vehicle-treated, Δ^9-THC-treated, and WIN55,212-2-treated rats. WIN55,212-2 (500 nM) produced similar inhibition in slices taken from naive and vehicle-treated rats ($p > 0.05$; $n = 5$; unpaired *t* test). However, the inhibition of eIPSCs by either 500 nM or 1 μM WIN55,212-2 was significantly reduced in slices obtained from rats chronically treated with either WIN55,212-2 or Δ^9-THC (Fig. 1*B*).

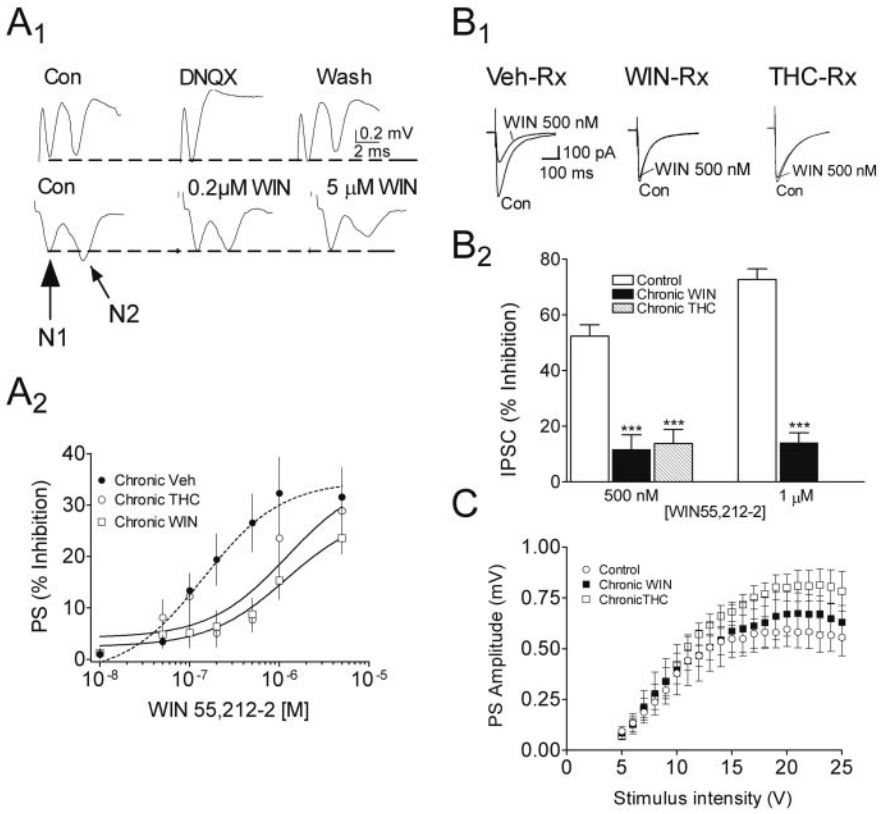

Figure 1. Tolerance to WIN55,212-2 (WIN)-mediated inhibition of glutamate and GABA release in the NAc. A_1, The N2 component (small arrow) of the extracellular response is reversibly eliminated by DNQX (10 μM) and is concentration-dependently reduced by WIN55,212-2 in control (Con) slices. Note that neither compound altered the nonsynaptic N1 component (large arrow). Dashed horizontal lines in *A* define the baseline response. A_2, Concentration-response curve for PS inhibition in slices from rats after chronic vehicle (●; EC_{50} = 143 nM), WIN55,212-2 (□; EC_{50} = 1.18 μM), or Δ^9-THC (○; EC_{50} = 1.22 μM). Each point represents the mean ± SEM of three to nine slices. B_1, Traces of eIPSCs and inhibition by 500 nM WIN55,212-2 in slices from a vehicle (Veh)-treated, WIN55,212-2-treated, and Δ^9-THC-treated animals. B_2, Summary of the inhibition of eIPSCs by WIN55,212-2 in slices from animals treated with vehicle (500 nM, $n = 4$; 1 μM, $n = 3$), WIN55,212-2 (500 nM, $n = 5$; 1 μM, $n = 3$), and Δ^9-THC (500 nM, $n = 5$) (***$p < 0.001$ versus control; one-way ANOVA and *post hoc* comparison). *C*, Stimulus intensity versus PS amplitude relationship in slices from vehicle-treated (control), WIN55,212-2-treated, and Δ^9-THC-treated rats. No differences among the groups were observed.

To exclude the possibility that the observed tolerance to the cannabinoid agonists was attributable to compromised slice viability after chronic drug treatment, we measured spontaneous IPSCs (sIPSCs) and the relationship between stimulus intensity and PS amplitude (input–output) in slices obtained from chronic vehicle and cannabinoid-treated rats. Neither the input–output relationship of the PS response (Fig. 1*C*) nor the sIPSC amplitudes or frequency (control: 25.4 ± 3.9 pA, 1.9 ± 0.3 Hz, $n = 7$; chronic WIN55,212-2: 24.4 ± 6.2 pA, 2.1 ± 0.5 Hz, $n = 5$; chronic Δ^9-THC: 23 ± 2.7 pA, 2.1 ± 0.5 Hz, $n = 6$) were significantly altered after chronic treatment, suggesting that brain slices obtained from animals chronically exposed to cannabinoid agonists were not physiologically compromised or altered in a nonspecific manner.

Effects of chronic cannabinoid treatment on inhibition by other neuromodulators

To determine the specificity of the chronic cannabinoid treatment, we measured the effects of other G-protein-coupled receptor agonists on PS responses in the NAc of chronically treated animals. Adenosine produced a concentration-dependent inhibition in control slices that was blocked by the adenosine A1 receptor antagonist DPCPX (200 nM; 105 ± 4% of control; $n = 5$). Chronic treatment with WIN55,212-2 did not alter the inhibition of the PS response by adenosine (EC_{50} = 41 and 47 μM for control and chronic WIN55,212-2 treatment, respectively) (Fig. 2*A*). Similarly, this treatment did not affect the inhibition of the response by the selective adenosine A1 receptor agonist N^6-CPA (Fig. 2*A*). In contrast, the inhibition of the PS response produced by the selective μ-opioid agonist DAMGO (400 nM) was significantly reduced in slices from animals chronically treated with WIN55,212-2 or Δ^9-THC (Fig. 2*B*). These results suggest that cross-tolerance is observed between CB1 and μ-opioid receptors in the NAc after chronic cannabinoid administration.

Chronic cannabinoid exposure blocks LTD in the NAc

Repetitive stimulation of cortical afferents at a wide range of frequencies results in the LTD of glutamatergic inputs to medium spiny neurons in the NAc and the dorsal striatum that is critically dependent on endocannabinoids (Gerdeman et al., 2002; Robbe et al., 2002b). Because this form of LTD may be involved in mediating some of the relatively long-term changes in NAc function after chronic drug exposure (Thomas et al., 2001), we examined whether chronic cannabinoid treatment would alter synaptic plasticity. We found that LTD of the PSs was reliably produced by stimulating glutamatergic axons in the NAc at 10 Hz for 5 min. In slices from untreated animals, this stimulation produced a significant, long-lasting (>30 min) reduction in PS response amplitude that was blocked by pretreatment of the slices with the CB1 receptor antagonist SR141716A (1 μM; $p > 0.05$; repeated-measures ANOVA and Tukey–Kramer *post hoc* comparison) (Fig. 3*A*). As shown previously (Robbe et al., 2002b), LTD of evoked EPSCs was also observed in medium spiny neurons using whole-cell voltage-clamp recordings in NAc slices obtained from naive rats (66 ± 3% of control; $n = 4$), and this was completely blocked by SR141716A ($n = 2$). Next, we investigated the effects of chronic cannabinoid treatment on LTD by comparing slices from chronic vehicle-treated or chronic cannabinoid-treated rats. Thirty minutes after the 10 Hz stimulus, control slices demonstrated a significant reduction in PS response amplitude (i.e., LTD) relative to the baseline response ($p < 0.05$). In contrast, PS response amplitudes in NAc slices from rats chronically treated with WIN55,212-2 or Δ^9-THC were not significantly reduced compared with the baseline response 30 min after the 10 Hz stimulation. Thus, endocannabinoid-dependent LTD was eliminated by chronic cannabinoid treatment.

Discussion

We report several novel findings in the present study regarding the effects of chronic cannabinoid exposure on synaptic transmission in the NAc. First, chronic treatment with Δ^9-THC or

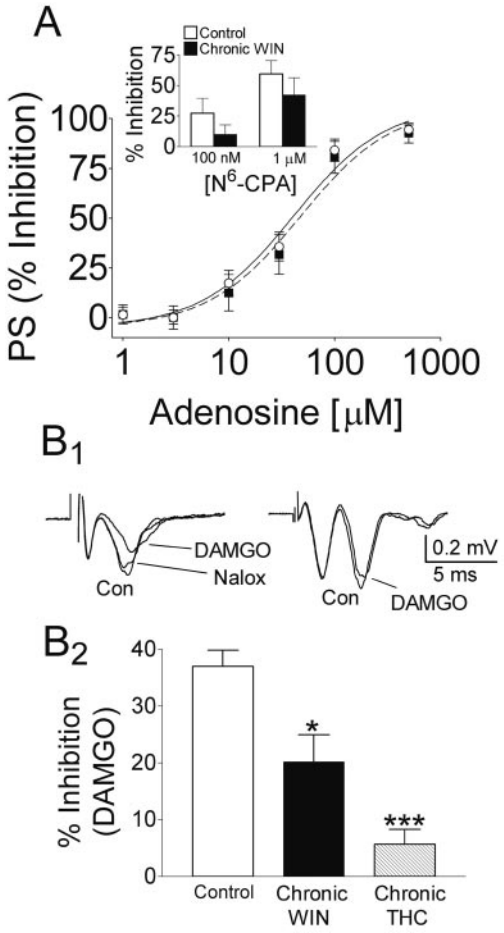

Figure 2. Cross-tolerance between CB1 and μ-opioid receptors in the NAc after chronic cannabinoid treatment. *A*, Concentration-response curves for PS inhibition by adenosine in slices from control (○; $EC_{50} = 41$ μM) and WIN55,212-2 (WIN)-treated (■; $EC_{50} = 47$ μM) animals. Four to seven slices were used to generate each data point. Inset, Inhibition by the A1 adenosine receptor agonist N^6-CPA (100 nM and 1 μM) in slices from control ($n = 5$) and WIN55,212-2-treated ($n = 8$) animals. No significant differences between groups were observed ($p > 0.05$; unpaired *t* test). B_1, left, PS recording in a control (Con) slice shows the inhibition produced by DAMGO (400 nM) and reversal by naloxone (Nalox; 5 μM). Right, Traces obtained in a slice from an animal chronically treated with Δ^9-THC. B_2, Summary of the effect of DAMGO (400 nM) in slices from animals treated with vehicle (control; $n = 4$), chronic WIN55,212-2 ($n = 6$), or chronic Δ^9-THC ($n = 6$) (*$p < 0.05$ and ***$p < 0.001$ versus control; one-way ANOVA and *post hoc* comparison).

WIN55,212-2 resulted in decreased sensitivity of glutamatergic and GABAergic synapses to the inhibitory effects of the cannabinoid agonist WIN55,212-2. Second, chronic treatment also reduced the inhibitory effect of the μ-opioid agonist DAMGO at glutamatergic synapses. Third, we found that endocannabinoid-mediated LTD was blocked in the NAc after chronic Δ^9-THC or WIN55,212-2 exposure. Together, these findings suggest that long-term marijuana use can alter the modulatory effect of endogenous and exogenous cannabinoids on synaptic transmission in this motivationally relevant brain structure.

The observation that reductions in cannabinoid sensitivity were observed at GABAergic and glutamatergic synapses in the NAc suggests that CB1 receptors located on these axon terminals exhibited functional tolerance after chronic cannabinoid exposure. Possible mechanisms to explain this tolerance include a decrease in the number of CB1 receptors on GABAergic and glutamatergic terminals or an uncoupling of the CB1 receptor from its intracellular effectors. A decrease in CB1 binding in both the dorsal and ventral striatum was reported after 7 d of Δ^9-THC treatment, corresponding to the development of behavioral tolerance (Rodriguez et al., 1994). However, others have reported either no change in CB1 binding (Abood et al., 1993) or only marginal decreases in striatal [^{3}H]-WIN55,212-2 binding and WIN55,212-2-stimulated [^{35}S]-GTPγS incorporation after chronic Δ^9-THC (Breivogel et al., 1999). A decrease in specific G-protein subunit mRNA after chronic cannabinoid administration suggests that the development of tolerance may also reflect disrupted G-protein signaling (Rubino et al., 1997). Because the acute inhibition of glutamate release by cannabinoids is thought to reflect either the modulation of voltage-dependent K^+ or Ca^{2+} channels in the NAc (Robbe et al., 2001), it is also possible that chronic treatment uncouples the CB1 receptor from these ion channels.

Tolerance to many of the behavioral effects of cannabinoids is well established in animals (Maldonado, 2002). Although different treatment schedules have been used, pronounced tolerance to the behavioral effects of cannabinoids typically develops in <1 week using protocols similar or identical to ours (Abood et al., 1993; Bass and Martin, 2000). Our data suggest that tolerance did not result from general physiological deficits in slices obtained from chronically treated rats, because baseline synaptic transmission was not altered between chronic vehicle-treated and chronic THC-treated rats, as shown by the unaltered input–output relationships of the PS responses and normal sIPSC parameters. In addition, the continued presence of agonist in brain tissue does not appear to explain our results, because PS responses were not changed by antagonists in slices obtained from chronically treated animals or from animals given a single injection of WIN55,212-2, 24 hr before recording. Additionally, the lack of response to the antagonist under these conditions implies that endocannabinoids were either not tonically released in the NAc or were cleared before CB1 receptor activation could occur.

Another intriguing observation in the present study was that chronic cannabinoid treatment reduced the sensitivity of glutamatergic synapses to μ-opioid receptor-mediated inhibition in the NAc. This is of interest because numerous studies have also demonstrated functional interactions between opioid and cannabinoid systems in the brain reward circuitry. For example, the operant reinforcing effects of morphine and morphine withdrawal syndrome are attenuated in CB1 receptor knock-out mice (Ledent et al., 1999). Conversely, tolerance to Δ^9-THC, as well as CB1 antagonist-precipitated withdrawal, is greatly reduced in preproenkephalin knock-out mice (Valverde et al., 2000). An interaction between opioid and cannabinoid systems is also demonstrated by the ability of naloxone to block cannabinoid-induced dopamine release in the NAc (Chen et al., 1990). In the present study, cross-tolerance to μ-opioid receptors appeared specific, rather than a general property of all G-protein-coupled receptors on glutamate axon terminals in the NAc, because the sensitivity of these synapses to adenosine A1 receptor activation was unaltered. In this respect, chronic cannabinoid treatment differs from chronic morphine treatment in the NAc, because the latter enhances adenosine sensitivity, probably by altering adenosine transport (Brundege and Williams, 2002). The mechanism of the μ and CB1 receptor cross-tolerance is unknown. However, a previous study found no changes in the number of μ-opioid receptor-binding sites in the mouse brain, despite the development of cross-tolerance to morphine after chronic Δ^9-THC treatment (Thorat and Bhargava, 1994). On the basis of these results, we hypothesize that the functional cross-tolerance may result from the altered coupling of G-proteins or other effectors

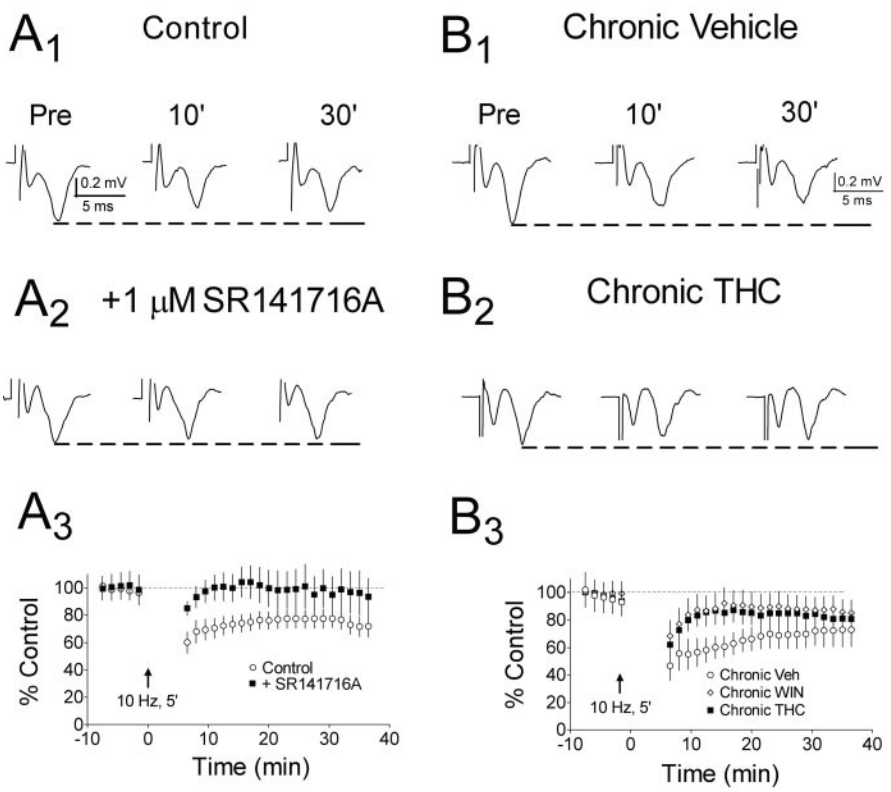

Figure 3. LTD in NAc slices. A_1, Traces from a control slice before (Pre), 10 min after, and 30 min after delivery of a 5 min, 10 Hz stimulus. A_2, Traces from a slice pretreated with the CB1 antagonist SR141716A (1 μM). Note the lack of LTD in the response. A_3, Group summary showing LTD in the absence (○; $n = 9$) and presence (■; $n = 6$) of SR141716A. B_1, Traces from a slice from a rat treated chronically with vehicle before, 10 min after, and 30 min after the LTD stimulus. B_2, A slice from a rat chronically treated with Δ^9-THC. B_3, Group summary showing LTD in slices from rats treated with vehicle (Veh) (○; $n = 7$), WIN55,212-2 (WIN) (◇; $n = 9$), and Δ^9-THC (■; $n = 10$). At both the 10 and 30 min time points, only slices from vehicle-treated animals showed significant LTD ($p < 0.05$; repeated-measures ANOVA and Tukey–Kramer *post hoc* comparison). Note that the N1 component in all responses is unaltered after the LTD stimulation. Dashed horizontal lines define baseline responses.

common to both CB1 and μ-opioid receptors (Manzanares et al., 1999).

Alterations in synaptic activity by abused drugs may represent a means through which repeated exposure results in compulsive, addictive behavior. One currently popular hypothesis is that addiction may involve the recruitment of critical cellular processes that are normally required for learning to occur (for review, see Gerdeman et al., 2003). Because LTD and long-term potentiation (LTP) represent synaptic mechanisms through which relatively long-lived changes in neurobiological function can occur, several recent studies have examined changes in these forms of synaptic plasticity after repeated exposure to abused drugs. These studies have demonstrated that chronic cocaine occludes LTD in the NAc through the inhibition of excitatory synapses (Thomas et al., 2001), and that chronic ethanol treatment decreases hippocampal LTP (Roberto et al., 2002). Moreover, there is evidence that endogenous cannabinoids play an essential role in the induction of LTD at GABAergic synapses in the amygdala (Marsicano et al., 2002) and at excitatory synapses in the striatum (Gerdeman and Lovinger, 2001) and in the NAc (Robbe et al., 2002b). Because of these findings, and because humans often use marijuana for prolonged periods of time (Millman and Sbriglio, 1986), we examined whether long-term cannabinoid exposure could alter synaptic plasticity in the NAc. Our studies using SR141716A to occlude NAc LTD confirmed the role of CB1 receptors in this form of plasticity (Gerdeman et al., 2002), and we found that LTD was completely blocked in the NAc after chronic treatment with Δ^9-THC or the synthetic cannabinoid agonist WIN55,212-2. This finding, and our observation of tolerance to exogenously applied WIN55,212-2, suggests that chronic cannabinoid administration reduces the sensitivity of CB1 receptors at NAc synapses to both exogenous and endogenous cannabinoids. From these observations, we hypothesize that chronic marijuana use can reduce the likelihood of endocannabinoid-mediated synaptic plasticity in the NAc, thereby disrupting information processing in this motivationally relevant brain structure. Although identical mechanisms producing tolerance to Δ^9-THC might also explain changes in endocannabinoid-dependent LTD, our results do not preclude the possibility that repeated administration of cannabinoids may alter other systems involved in the LTD process, such as metabotropic glutamate receptor sensitivity (Robbe et al., 2002a) or the formation and release of endocannabinoids (Di Marzo et al., 2000). However, our tolerance data suggest that this may explain the blockade of LTD, and, collectively, this study implies that chronic cannabinoid exposure can block synaptic plasticity resulting from the activation of behaviorally relevant inputs to NAc medium spiny neurons.

In summary, the present work reveals for the first time that chronic administration of either the synthetic cannabinoid agonist WIN55,212-2 or a psychoactive constituent of marijuana, Δ^9-THC, produces functional tolerance to the effects of both CB1 and μ-opioid receptor activation at NAc synapses. Furthermore, this CB1 receptor tolerance appears to prevent endocannabinoids from participating in the establishment of LTD at excitatory synapses in the NAc. The extent to which these changes are linked to the addictive liability of marijuana remains to be determined. However, we speculate that long-term marijuana use will result in a decreased ability to generate LTD in the NAc and that this alteration in the reward circuitry plays a role in compulsive marijuana use.

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