Synaptic Targets of Δ^9 -Tetrahydrocannabinol in the Central Nervous System

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The availability of potent synthetic agonists for cannabinoid receptors has facilitated our understanding of cannabinoid actions on synaptic transmission in the central nervous system. Moreover, the ability of these compounds to inhibit neurotransmitter release at many central synapses is thought to underlie most of the behavioral effects of cannabinoid agonists. However, despite the widespread use and misuse of marijuana, and recognition of its potential adverse psychological effects in humans, comparatively few studies have examined the actions of its primary psychoactive constituent, Δ^9 -tetrahydrocannabinol (THC), at well-defined synaptic pathways. Here we examine the recent literature describing the effects of acute and repeated THC exposure on synaptic function in several brain regions and explore the importance of these neurobiological actions of THC in drug addiction.

Marijuana (*Cannabis sativa*) is the most commonly used illicit drug in the United States, and its prevalence and abuse potential among adolescents continues to increase (Substance Abuse and Mental Health Services Administration 2002). Cannabis dependence is currently recognized in the Diagnostic and Statistical Manual of Mental Health Disorders (American Psychiatric Association 2000) and is essentially defined by three major criteria: (1) the expenditure of considerable time and resources to acquire cannabis, (2) continued use of cannabis despite significant use-related negative physical and/or psychological consequences, and (3) the need for increasing amounts of cannabis to maintain the desired level of intoxication. Additionally, there is now strong evidence for cannabis withdrawal symptoms in humans that

include insomnia, cognitive impairment, emotional lability, psychiatric depression, irritability, and anger. However, despite its widespread use compared with other drugs and its growing availability to larger segments of the population, our knowledge of the effects of acute and long-term use of cannabis on the brain remains relatively poor.

The major psychoactive component of marijuana, Δ^9 -tetrahydrocannabinol (THC), was isolated nearly 50 years ago by Mechoulam and colleagues (Gaoni and Mechoulam 1964; Mechoulam and Gaoni 1965). Many subsequent studies have established that most of the psychoactive effects of cannabis, such as euphoria, relaxation, anxiety, confusion, memory loss, and paranoia, are mediated by THC (Adams and Martin 1996; Wachtel et al. 2002; Hall and

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Degenhardt 2009). These actions of THC, and those of synthetic and endogenous cannabinoid molecules, occur through the activation of distinct cannabinoid receptors (Devane et al. 1988; Howlett et al. 1990), and it is through specific antagonism of cannabinoid receptors that the CB1 receptor subtype has been identified as mediating the subjective “high” described by human marijuana users (Huestis et al. 2001). Although it is well established that THC mediates most of the psychoactive actions of cannabis, there are also a large number of additional cannabinoid molecules present in the marijuana plant (Mechoulam 1970; Adams and Martin 1996). However, our understanding of the pharmacological actions of most of these compounds is limited, particularly with regard to putative synaptic function. Therefore, our discussion here will necessarily remain confined to the actions of THC.

Whereas the behavioral effects of THC are well-established by many studies, comparatively few physiological investigations have examined the actions of this drug on synaptic function (Foy et al. 1982; Nowicky et al. 1987)—studies that are best conducted in vitro. The relative paucity of studies examining synaptic effects of THC may reflect the extremely poor aqueous solubility of this compound and the limited extent to which this lipophilic molecule can access synaptic cannabinoid receptors in brain-slice preparations (Laaris et al. 2010). Consequently, much of the mechanistic information regarding the effects of cannabinoids on synaptic function has been obtained using more soluble synthetic agonists, rather than THC (Mackie and Hille 1992; Sullivan 1999; Hoffman and Lupica 2000, 2001; Gerdeman and Lovinger 2001). These studies have been critical in defining the primary physiological role of cannabinoid receptors as presynaptic modulators of excitatory and inhibitory neurotransmitter release in the central nervous system (CNS) (Fig. 1), and have identified these same sites as mediating the effects of endogenous cannabinoids that are produced by neurons during physiological activation (Wilson and Nicoll 2001; Alger 2002). However, given the widespread use of THC in humans, its proposed involvement in addiction

Figure 1. Generalized scheme of the sites of THC action throughout the mammalian central nervous system. CB1 receptors are shown on both excitatory glutamatergic (Glut.) and inhibitory (GABA) axon terminals, and in many cases these synapses are formed with neurons providing output (Output neuron) of central nuclei (e.g., hippocampal pyramidal neurons and MSNs in the NAc and dorsal striatum). These presynaptic CB1 receptors can be activated by the release of endocannabinoids (eCB) on depolarization of postsynaptic neurons or by exogenous agonists such as THC. Also shown is the chemical structure of THC (*inset*).

and mental illness (Leweke and Koethe 2008), the potential expansion of its use in human therapeutic contexts, and the experimental evidence that it can act at non-cannabinoid receptor sites (Hejazi et al. 2006; De Petrocellis and Di Marzo 2010; Xiong et al. 2011), we believe that it is important to define its capacity for altering brain activity, with a particular emphasis on synaptic function. Therefore, our intention here is to review the recent literature to examine the neurobiological substrates acted on by THC, to highlight its effects on specific synaptic processes following acute and long-term exposure, and to examine its potential roles in drug addiction.

LOCALIZATION AND FUNCTIONAL PROPERTIES OF CANNABINOID RECEPTORS IN THE CNS

Two receptor subtypes are known to mediate the effects of synthetic and natural cannabinoid agonists in the CNS. Both CB1 and CB2 cannabinoid receptors belong to the larger class of

G-protein-coupled receptors (GPCRs) and have been designated as either “central” or “peripheral,” based on their localization in the CNS or in peripheral nonneuronal tissues, respectively (Pertwee 1997). This designation has recently been challenged by several reports demonstrating CB2 receptors in the brain, albeit at much lower levels than CB1, and these receptors may mediate some of the central actions of THC as well as synthetic and endogenous CB agonists (Van Sickle et al. 2005; Atwood and Mackie 2010; Xi et al. 2011). However, all physiological studies to date using CB1 selective antagonists and CB1 receptor “knockout” mice in intact neural circuits have shown that the CB1 receptor mediates the inhibition of inhibitory (Hoffman and Lupica 2000; Manzoni and Bockaert 2001; Wilson et al. 2001; Kreitzer et al. 2002; Chevalere and Castillo 2003) and excitatory (Sullivan 1999; Gerdeman and Lovinger 2001; Robbe et al. 2002; Melis et al. 2004; Straiker and Mackie 2005; Takahashi and Castillo 2006) neurotransmitter release from axon terminals (Fig. 1). Thus, although it is possible that CB2 receptors may influence synaptic transmission in the CNS, the existing literature supports a predominant, if not exclusive, role for CB1 receptors as the major target for endogenous and exogenous cannabinoids at axon terminals.

The activation of synaptic CB1 receptors by endogenous and exogenous agonists inhibits neurotransmitter release directly, via inhibitory coupling to voltage-dependent calcium channels (Mackie and Hille 1992; Sullivan 1999; Hoffman and Lupica 2000), or through activation of potassium channels, which shortens action potential duration and lessens the amount of neurotransmitter release per action potential (Mu et al. 1999; Schweitzer 2000; Robbe et al. 2001). Both CB1-dependent mechanisms appear to occur through the direct interaction of $\beta\gamma$ G-protein subunits with the ion channels, rather than through a second messenger (Hoffman and Lupica 2000; Robbe et al. 2001), although other effectors have been shown to be involved in some of the presynaptic effects of cannabinoids (Pan et al. 2008; Benard et al. 2012). Endocannabinoids that are released by neurons during heightened neuronal activity

also interact with axon terminal CB1 receptors to inhibit the release of GABA or glutamate (Alger 2002; Melis et al. 2004; Riegel and Lupica 2004). These transient endocannabinoid-dependent phenomena have been labeled depolarization-induced suppression of inhibition or excitation (DSI/DSE), respectively. The activation of CB1 receptors by endocannabinoids can also initiate long-term forms of synaptic plasticity, including long-term depression (LTD), and can modify the strength of long-term potentiation (LTP) (Carlson et al. 2002; Kortleven et al. 2011). The reader is referred to several reviews on endocannabinoid-mediated plasticity for further detail (Alger 2002; Gerdeman et al. 2003; Chevalere et al. 2006; Kano et al. 2009; Alger and Kim 2011).

ACUTE ACTIONS OF THC ON SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS

Excitatory Synaptic Transmission

The well-described actions of marijuana on cognitive function and memory (Abel 1970; Drew and Miller 1974), together with the established role of the hippocampus in various forms of learning and memory (Squire 1992; Burgess et al. 2002; Bird and Burgess 2008), led investigators to evaluate the effects of THC in hippocampal brain slices. Early studies described relatively complex bimodal effects on extracellularly recorded field potentials in the hippocampus (Foy et al. 1982; Nowicky et al. 1987). However, these studies were performed before identification of CB1 receptors as mediators of cannabinoid actions and before the development of cannabinoid receptor antagonists and transgenic animals. Additionally, the effective THC concentrations in these studies are not consistent with more contemporary studies in which the aqueous insolubility of THC in brain slices is specifically addressed (Hoffman et al. 2010; Laaris et al. 2010). Several studies have circumvented the difficulty of low levels of passive diffusion of THC into brain slices through the use of synaptically coupled hippocampal neurons in culture. One of these studies showed that THC inhibited glutamatergic

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synaptic responses onto neighboring cultured hippocampal pyramidal neurons with a 50% effective concentration (EC_{50}) of 20 nM (Shen and Thayer 1999). Because the efficacy of THC was less than that of the synthetic aminoalkylindole cannabinoid agonist, WIN55,212-2, the investigators concluded that THC was a partial agonist at CB1 receptors located on excitatory axon terminals in the hippocampus (Shen and Thayer 1999). We have also found that THC can act as a partial agonist at presynaptic CB1 receptors to inhibit glutamate-mediated synaptic responses in hippocampal slices (Hoffman et al. 2010). However, the effects of THC on glutamate release were only observed when THC was solubilized in β -cyclodextrin (Hoffman et al. 2010), which greatly improves the penetration of the drug into brain slices (Hazekamp and Verpoorte 2006). In contrast to these studies, other work in hippocampal cultures has showed that, whereas THC had no effect on glutamatergic synaptic transmission, it blocked the effects of both synthetic and endogenous cannabinoid agonists at these same synapses (Straiker and Mackie 2005). However, prolonged (~ 19 h) application of THC to cultured hippocampal neurons desensitized CB1 receptors on glutamate terminals, suggesting that THC can antagonize the effects of full agonists at CB1 receptors acutely, but can also effectively down-regulate CB1 receptor function following longer exposure. This observation led to the provocative hypothesis that some of the psychoactive effects of THC may be mediated by antagonism of endocannabinoid actions, rather than through direct activation of CB1 receptors in the CNS (Straiker and Mackie 2005).

Inhibitory Synaptic Transmission

Together, the above studies examining the effects of THC on glutamatergic synaptic transmission showed that the phytocannabinoid acts either as a partial agonist at CB1 receptors or as an antagonist of exogenous and endogenous cannabinoids at these receptors (Shen and Thayer 1999; Straiker and Mackie 2005). However, in contrast to these studies, much less attention has been given to the effects of THC on GABAergic synaptic transmission in the CNS,

despite the much higher density of CB1 receptors on these axon terminals compared with glutamate terminals in the hippocampus (Marsicano and Lutz 1999; Kawamura et al. 2006). To address this we investigated the effects of THC on GABAergic synaptic transmission in hippocampal brain slices using THC solubilized in β -cyclodextrin, as described previously. We found, similar to studies with synthetic and endogenous cannabinoids, that THC inhibits synaptic GABA release via activation of presynaptic CB1 receptors. However, in contrast to the partial agonist properties of THC at excitatory synapses, we found that synaptic GABA currents were inhibited with an efficacy that was not distinguishable from the full agonist, WIN55,212-2 (Fig. 2) (Laaris et al. 2010). This difference in agonist efficacy at excitatory versus inhibitory synapses in the hippocampus likely reflects the lower level of CB1 receptor expression on glutamatergic axon terminals compared with GABAergic terminals (Kawamura et al. 2006), because partial agonist activity is often observed where “receptor reserve” is low (Hoyer and Boddeke 1993). The differential efficacy of THC at excitatory versus inhibitory hippocampal synapses is likely important for the modulation of hippocampal circuits and for the pronounced effects of marijuana on cognitive function and memory in humans. Thus, inhibitory axon terminals would likely be more sensitive to, and more completely inhibited by, THC, leading to a disruption of hippocampal network activity that is largely controlled by synchronized GABA neuron activity and is profoundly disrupted by cannabinoids (Hajos et al. 2000; Robbe et al. 2006). In support of this hypothesis, targeted deletion of the CB1 receptor from GABAergic neurons prevented the disruption of hippocampal-dependent behavior by THC, whereas deletion of this receptor from glutamatergic neurons was ineffective in abolishing the effects of THC (Puighermanal et al. 2009). These data, together with those demonstrating full agonist effects of THC at inhibitory synapses in the hippocampus, suggest that the well-known effects of this drug on memory and cognition in humans likely occur through the presynaptic inhibition of GABA release.

Figure 2. Inhibition of synaptic GABA release from inhibitory interneuron axon terminals in the mouse hippocampus by THC. (A) Illustration of a whole-cell voltage clamp configuration to record GABA-mediated synaptic currents evoked by electrical stimulation of GABA neuron axon terminals, and the recording of non-synaptic GABA_A currents activated by ultraviolet (UV) light uncaging of GABA in the same hippocampal pyramidal neurons. UV light was focused into a $\sim 45\text{-}\mu\text{m}$ spot near the pyramidal neuron soma to release free GABA from α -carboxy-2-nitrobenzyl (CNB)-caged GABA. (B) Synaptic and nonsynaptic GABA_A receptor currents are unaffected by THC (10 μM), suspended in β -cyclodextrin (see text) when applied to hippocampal slices from CB1^{-/-} mice. (C) THC inhibition of synaptic GABA_A receptor-mediated responses evoked by electrical stimulation (squares) and absence of THC effects on nonsynaptic GABA_A currents activated by UV uncaging of GABA (black circles) in hippocampal slices from CB1^{+/+} mice. (D) Comparison of concentration–response curves for the aminoalkylindole synthetic cannabinoid agonist WIN55,212-2 and THC. Note that whereas THC is less potent than WIN55,212-2, the maximal inhibition of IPSCs by both cannabinoids is similar on GABAergic synaptic responses. (Adapted from Laaris et al. 2010; reprinted, with permission, from the author.)

ACUTE EFFECTS OF THC: NON-CANNABINOID-RECEPTOR SITES OF ACTION

To date, there have been few studies examining the direct effects of THC on synaptic transmission in other brain areas. Brown and colleagues reported that THC (10 μM) and WIN55,212-2

(3 μM) produce similar effects on striatal glutamate release, although a full dose–response analysis was not performed (Brown et al. 2003). Interestingly, these investigators also report that THC acts through CB1 receptors to inhibit glutamate uptake, which then activated presynaptic metabotropic glutamate receptors (Brown et al. 2003). Effects of THC on neurotransmitter

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uptake have been observed previously (Maneuf et al. 1996; Coull et al. 1997). However, the kinetics of directly activated nonsynaptic GABA_A receptor currents by GABA released with ultraviolet (UV) laser uncaging (Fig. 2) were insensitive to THC, suggesting that GABA uptake is not affected by THC in the hippocampus (Laaris et al. 2010). More recently, it has been shown that THC can impair cellular metabolism through activation of CB1 receptors located on mitochondria, and that these receptors regulate hippocampal DSI (Benard et al. 2012). Another potential target that has been described for THC is the glycine receptor-chloride channel (Hejazi et al. 2006). This group showed that THC potentiates glycine currents in cultured ventral tegmental area (VTA) neurons, with an EC₅₀ of 115 nM (Hejazi et al. 2006). This occurred independently of CB1 receptor activation through interaction with specific sites on the glycine receptor (Xiong et al. 2011). Thus, although further study is needed, it appears that both CB1 receptor-dependent and -independent actions of THC may exist in the CNS.

ENDURING SYNAPTIC EFFECTS OF ACUTE IN VIVO THC

Numerous studies have evaluated the effects of acute in vivo exposure to addictive drugs, such as cocaine, nicotine, and alcohol, on synaptic function, using an “ex vivo” strategy in which electrophysiological recordings are made from neurons in brain slices obtained from drug exposed animals (Ungless et al. 2001; Saal et al. 2003; Niehaus et al. 2010). However, comparatively few studies have used this strategy with THC exposure. Mato and colleagues examined the effects of a single, acute injection of THC (3 mg/kg, i.p.) in mice (Mato et al. 2004) and found a CB1-dependent impairment of LTD of synaptic transmission at both inhibitory synapses in the hippocampus and at excitatory synapses on medium spiny neurons (MSNs) in the nucleus accumbens (NAc). Each form of LTD is dependent on the release of endogenous cannabinoids during conditioning trains of electrical stimulation of the brain slices, as

well as the activation of presynaptic CB1 receptors (Robbe et al. 2002; Chevalere and Castillo 2003). Although there was no change in CB1 receptor expression or G-protein coupling to these receptors following single injections of THC, a reduction in cannabinoid agonist-mediated inhibition of both GABA and glutamate release suggested the development of rapid tolerance. Whereas behavioral studies have shown rapid tolerance to the behavioral effects of THC (Hutcheson et al. 1998), this was the first study to show synaptic changes following an acute dosing paradigm. It is not clear how such a rapid loss of CB1 receptor sensitivity develops, although there is evidence to suggest that CB1 receptors are highly mobile within the plasma membrane of cortical neurons and that this mobility can be dramatically reduced by short-term agonist treatment (Mikasova et al. 2008).

A single exposure to THC can also cause the selective remodeling of a population of glutamatergic synaptic inputs to midbrain dopamine (DA) neurons in the ventral tegmental area (VTA) (Good and Lupica 2010). In this study, a single in vivo exposure to THC (10 mg/kg, i.p.) rapidly increased the expression of AMPA receptors containing the GluA1 subunit at subcortical pedunculopontine nucleus (PPN) glutamatergic inputs to VTA DA neurons in rats (Good and Lupica 2010). Furthermore, this synaptic remodeling was prevented by pretreatment with the CB1 antagonist AM251. Because the GluA1 subunit confers calcium permeability and increases the single-channel conductance of AMPA receptors, this THC-induced modification is thought to result in long-term potentiation (LTP) of these synapses and thereby increase the subcortical drive of VTA DA neurons by the PPN (Argilli et al. 2008; Good and Lupica 2010). This modification in AMPA receptor function also renders these glutamate synapses susceptible to subsequent LTD, which occurs through the insertion of calcium-impermeable GluA2 AMPA receptor subunits and the removal of GluA1 (Good and Lupica 2010). Thus, a single exposure to THC can produce an enduring increase in the sensitivity of DA neurons to subcortical excitatory drive at a specific set of synapses, and this can be modified

by subsequent activation of this circuit. This suggests that one of the early neurobiological responses to THC might be a change in VTA DA neuron sensitivity to glutamatergic synaptic inputs, and this may subsequently play a role in the long-term modification in mesolimbocortical DA output. In contrast, to the effects of a single exposure to THC, a single exposure to cocaine increased GluA1 function at both PPN and non-PPN glutamatergic synapses onto VTA DA neurons (Good and Lupica 2010). Although previous data have suggested that many commonly abused drugs affect synaptic plasticity in VTA DA neurons in a similar fashion (Saal et al. 2003), it would appear that THC may have somewhat unique, pathway-dependent effects in this brain reward circuit. This further suggests that different abused drugs may have the capacity to alter distinct excitatory inputs to midbrain DA neurons.

ENDURING SYNAPTIC EFFECTS OF LONG-TERM IN VIVO THC

In contrast to the limited number of studies examining the ability of acute THC to affect synaptic function, a number of laboratories have evaluated the physiological consequences of repeated THC exposure. Many of these studies were based on the observation that long-term THC treatment (usually 5–10 d) causes adaptations of CB1 receptors and/or their downstream cellular effectors (Breivogel et al. 1999; Rubino et al. 2000; Tonini et al. 2006).

NUCLEUS ACCUMBENS

The NAc, also known as the ventral striatum, is involved in motivational behavior, plays a pivotal role in mediating the effects of abused drugs, and undergoes significant molecular, structural, and physiological remodeling during passive and volitional drug administration (Lupica et al. 2004; Chen et al. 2010; Russo et al. 2010; Luscher and Malenka 2011). Both the NAc and the dorsal striatum (discussed in a later section) receive extensive cortical glutamatergic input onto the primary GABAergic output neurons, known as medium spiny neurons

(MSNs) that also receive input from ventral midbrain DA neurons. Because of its central role in the brain's motivation and reward circuitry, the actions of abused drugs on synaptic process in the NAc have received considerable attention. Early studies in our laboratory and others showed that, similar to the hippocampus, CB1 receptors are located on both glutamatergic and GABAergic axon terminals in the NAc, and that activation of these receptors could inhibit neurotransmitter release (Hoffman and Lupica 2001; Robbe et al. 2001). Subsequent studies found that repeated exposure to THC (10 mg/kg, i.p. for 7 d) resulted in tolerance to the inhibitory effects of WIN55,212-2 at both glutamate and GABA synapses in the NAc (Hoffman et al. 2003). Furthermore, because LTD of excitatory synaptic transmission in the NAc is dependent on the release of the endocannabinoid 2-arachidonoylglycerol, and its activation of CB1 receptors on glutamate axon terminals (Robbe et al. 2002), an early focus was to determine the effect of chronic THC on this form of synaptic plasticity. We found that the marked tolerance of CB1 receptors in the NAc following chronic THC exposure was associated with a loss of endocannabinoid-mediated LTD (Hoffman et al. 2003). Although the behavioral roles of endocannabinoid-dependent LTD in the NAc are largely unknown, it has been speculated that synaptic remodeling of striatal circuits by long-term plasticity may represent a mechanism through which drug use may progress from casual to impulsive (Gerdeman et al. 2003; Kauer and Malenka 2007). Therefore, from these studies we can conclude that either short-term (Mato et al. 2004) or long-term (Hoffman et al. 2003) exposure to THC can limit the degree to which NAc glutamate synapses undergo LTD, and this appears to result from a down-regulation of CB1 receptor function and the ability of endocannabinoids to initiate this form of synaptic plasticity. We propose that this effect of THC may alter motivational processes mediated by the NAc and may play a role in modulating the reinforcing properties of other abused drugs acting either directly within the NAc or indirectly by altering DA function in this brain structure.

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CEREBELLUM

Cannabinoid CB1 receptors are found at very high concentrations in the cerebellum, where they are expressed on excitatory parallel fiber (PF)–Purkinje cell (PC) synapses (Levenes et al. 1998; Safo and Regehr 2005). The firing patterns of PCs are thought to be involved in setting motor activity and gait, and cannabinoids are known to induce ataxia, catalepsy, and hypolocomotion, at least partly through interactions with cerebellar CB1 receptors (Patel and Hillard 2001). In addition, tolerance to these acute motoric effects of THC occurs with prolonged administration, and withdrawal from THC is associated with alterations in cerebellar cyclic AMP/PKA pathway function (Tzavara et al. 2000). For these reasons, Tonini and colleagues examined the effects of repeated THC exposure (10 mg/kg, 2 × daily, 4.5 d) in mice on PF synapses on cerebellar PCs (Tonini et al. 2006). This THC treatment produced tolerance to the inhibitory effects of the full CB1 receptor agonist, CP55,940, at PF glutamatergic synapses. The investigators also reported that chronic THC enhanced glutamatergic transmission at this synapse, and that the increase in glutamate release activated extrasynaptic metabotropic glutamate type-1 receptors (mGluR1) located on the cerebellar PCs. Endocannabinoid-dependent LTD and LTP are known to occur at glutamatergic PF-PC synapses, and these forms of plasticity are thought to underlie cerebellar-dependent motor learning (Edwards and Skosnik 2007). Therefore, the consequences of chronic THC exposure on cerebellar synaptic plasticity are of critical importance to our understanding of cannabis effects on this brain structure. Although LTD appeared to be unaffected by chronic THC, stimuli that would normally generate LTP at the PF–PC synapse produced LTD instead (Tonini et al. 2006). Furthermore, the investigators found that LTP could be rescued following blockade of adenosine A1 receptors, suggesting that THC treatment enhanced endogenous adenosine release that limited LTP, via presynaptic inhibition of glutamate release. These investigators also found that the effects of repeated THC on PF-

PC synapses could be prevented by a reduction in extracellular signal regulated kinase (ERK) signaling, through either pharmacological or genetic approaches (Tonini et al. 2006). As suggested by the investigators, this “pathological” shift of cerebellar synaptic plasticity from LTP to LTD following chronic THC would likely have profound consequences for cerebellar information processing (Tonini et al. 2006). This study shows the breadth of changes in downstream cellular signaling pathways that can be affected by chronic THC exposure (Rubino et al. 2004) and serves to highlight the critical link between these changes and altered synaptic processes.

HIPPOCAMPUS

In addition to the disruptive acute effect of THC on hippocampal synaptic function discussed above, there have been many studies detailing the deleterious effects of long-term cannabis use on cognitive executive function in humans, and the completeness of the reversibility of these impairments is currently a subject of debate (Bolla et al. 2002; Pope et al. 2002; Crean et al. 2011). For these reasons the effects of long-term THC treatment on synaptic function and long-term synaptic plasticity in the hippocampus in vitro have been examined. We compared LTP at Schaffer collateral glutamatergic synapses onto CA1 pyramidal neurons in brain slices obtained from adolescent rats treated with THC (10 mg/kg, for 1, 3, or 7 d) or vehicle (Hoffman et al. 2007). In contrast to the results of Mato et al. (2004), we did not observe an impairment of LTP 24 h following a single THC injection. However, a reduction in hippocampal LTP was observed 24 h following three consecutive days of THC administration, and LTP was completely absent following 7 d of THC treatment (Hoffman et al. 2007). The disruptive effects of 7 d THC on LTP were prevented when each THC injection was preceded by an injection with AM251, strongly suggesting that chronic THC interfered with hippocampal LTP via activation of CB1 receptors (Hoffman et al. 2007). Additional experiments showed that at the time of LTP induction THC was no longer detectable in

the brains of the chronically treated rats using liquid chromatography-mass spectrometry (LC-MS), suggesting that the LTP impairment was not simply the result of ongoing activation of CB1 receptors by residual THC, but rather that chronic exposure to the drug caused enduring neurobiological changes that interfered with synaptic plasticity.

When the time course for reversal of the LTP impairment was examined, it was found that LTP partially recovered beginning 3 d following THC treatment cessation, although complete recovery was not observed at time points up to 14 d post THC (Hoffman et al. 2007). This may have implications for interpreting the long-lasting effects of cannabis on human memory (Crean et al. 2011).

These data suggest that long-term THC exposure can disrupt hippocampal LTP by causing cellular or synaptic changes that endure following cessation of the drug. Therefore, in an effort to determine the mechanism of the chronic THC impairment of hippocampal LTP we examined glutamatergic and GABAergic synaptic function following this same 7-d THC treatment. Similar to results described in cerebellum (Tonini et al. 2006), we observed an increase in the probability of glutamate release following chronic THC, as well as alterations in AMPA receptor function (Hoffman et al. 2007), that might explain the LTP impairment. These results have recently been confirmed and expanded by another study, which also showed that chronic THC blocks LTP at glutamatergic dentate granule cell perforant path synapses onto CA3 pyramidal neurons (Fan et al. 2010). Additionally, these investigators describe increased synaptic glutamate release and a reduction in postsynaptic AMPA and NMDA receptor expression in hippocampal tissue from animals chronically treated with THC. The investigators suggest that the alterations in glutamatergic synapses likely result from CB1R-dependent reductions in phosphorylation of cAMP response element binding protein (CREB), as a consequence of the inhibition of adenylyl cyclase by these receptors (Fan et al. 2010). Although baseline GABAergic transmission was not altered by 7 d THC treatment, we observed a marked tol-

erance of GABAergic synapses to WIN55,212-2. This is consistent with the full agonist actions of THC at GABAergic synapses described previously (Laaris et al. 2010), as well as the critically defined role for these synapses in mediating the behavioral effects of THC (Puighermanal et al. 2009). Together these data suggest that long-term use of cannabis and exposure to THC results in specific alterations in both glutamatergic and GABAergic synaptic function that can prevent a form of synaptic plasticity that is likely central to the formation of hippocampal-dependent memories (Whitlock et al. 2006). Furthermore, it is likely that these changes are not restricted to the hippocampus, but likely occur throughout the CNS in brain regions where CB1 receptors are highly expressed, and this may explain the extensive effects of long-term cannabis use on executive cognitive function. The extent to which these cellular changes fully reverse following termination of cannabis use and whether there is an interaction between the use of the drug, the age of the individual, and the reversibility of these phenomena is currently under investigation.

DORSAL STRIATUM

The dorsal striatum plays an integral role in motor behavior, and the ability of THC to induce hypolocomotion and ataxia is thought to result from activation of CB1 receptors in this and other brain areas (Patel and Hillard 2001; Gerdeman et al. 2003). Furthermore, tolerance to the motoric effects of THC is readily observable (Rubino et al. 2005). Because the dorsal striatum processes information received from cortical, thalamic, and midbrain DA inputs it is also central to many forms of instrumental learning and habit formation, which is of importance to the process of addiction (Gerdeman et al. 2003; Everitt et al. 2008). Recent evidence suggests that the dorsomedial and dorsolateral striatum may be involved in instrumental, reward-based processing and habit-driven behavior that persists in the absence of overt reward, respectively (Yin et al. 2006, 2008, 2009). Also, similar to the NAc (ventral striatum), LTD of cortical glutamatergic inputs to the dorsal

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striatum is dependent on endocannabinoid activation of presynaptic CB1 receptors (Gerde et al. 2002; Ronesi et al. 2004), which may be involved in the establishment of striatal-dependent learning and habit formation (Kano et al. 2009). Because of the importance of the endogenous cannabinoid system in mediating motor behavior, instrumental learning, and habit formation and its potential involvement in compulsive drug seeking, the role of THC in regulating endogenous cannabinoid function has been studied (Nazzaro et al. 2012). This study found that, similar to previous work in other brain areas, treatment of mice with THC (10 mg/kg, i.p., 5 d) eliminated endogenous cannabinoid-dependent LTD of cortical inputs to MSNs in the dorsolateral striatum that was associated with behavioral tolerance to THC (Nazzaro et al. 2012). Striatal MSNs send their GABAergic output to downstream targets via two pathways known as indirect and direct, which are defined by various postsynaptically expressed receptors and neuropeptides (Kreitzer 2009). When electrophysiologically recorded dorsolateral striatal MSNs were segregated according to indirect and direct pathways, using post-hoc identification with single-cell reverse transcriptase polymerase chain reaction (RT-PCR) or immunohistochemistry, it was found that only the DA D2 receptor expressing MSNs associated with the indirect pathway showed endogenous cannabinoid-dependent LTD (Nazzaro et al. 2012). Furthermore, chronic THC treatment blocked LTD only at synapses onto these indirect pathway MSNs. The investigators also showed that endocannabinoid-independent LTD in the dorsomedial striatum was not affected by chronic THC (Nazzaro et al. 2012).

Depotential is another form of synaptic plasticity in which previously established LTP is reversed by appropriate subsequent low-frequency stimuli (Wagner and Alger 1996). Long-term potentiation can be induced at corticostriatal synapses onto MSNs in the dorsolateral striatum using high-frequency electrical stimulation when extracellular magnesium is reduced (Centonze et al. 1999). Nazzaro et al. (2012) found that depotential of cortico-

striatal synapses on indirect pathway MSNs could be blocked by the CB1 receptor antagonist AM-251, demonstrating a role for endocannabinoids in the weakening of these previously strengthened synapses. When the effects of chronic THC were examined on depotential, it was found that, like LTD, depotential was prevented. Thus, the investigators showed that both LTD and depotential of LTP in the dorsolateral striatum were mediated by endogenous cannabinoids and were both blocked by chronic THC exposure. Because habit formation and habitual responding is thought to be mediated by the dorsolateral striatum (Yin et al. 2008) and is impaired by cannabinoid receptor blockade (Hilario et al. 2007), it is important to evaluate the behavioral consequence of the aforementioned loss of endocannabinoid-dependent synaptic plasticity in the dorsolateral striatum on these behaviors. Therefore, Nazzaro (2012) evaluated the effect of chronic THC on habitual behavior in mice. They found that, in contrast to mice treated with vehicle, mice tolerant to THC continued to perform operant tasks in which the strength of a food reinforcer was weakened by a devaluation procedure. Based on these results, and those of the synaptic plasticity experiments, the investigators conclude that long-term treatment with THC results in a switch from goal directed to habitual behavior, in which mice continue to emit responses despite having information to suggest that the reinforcer associated with that response is no longer as “valuable” (Nazzaro 2012).

The investigators also make a strong causal case for endocannabinoid-dependent LTD and depotential in this behavioral process because both the behavioral change and the loss of synaptic plasticity could be reversed with the small conductance calcium-sensitive potassium channel (SK) channel blocker, apamin. Because SK channels are involved in limiting the duration of action potentials (Louise Faber 2009) blockade of this channel with apamin can increase action potential duration and thereby increase the calcium-dependent release of endogenous cannabinoids from neurons (Piomelli 2003; Riegel and Lupica 2004).

SUMMARY AND CONCLUSIONS

The studies reviewed above show that both acute and repeated exposure to THC have definable physiological consequences for synaptic function and several forms of synaptic plasticity in the CNS. Pharmacological and genetic approaches indicate that these effects of THC can largely be ascribed to activation of CB1 receptors, and although non-CB1 targets for THC have been described, the contribution of these effects to synaptic function appears to be small. Based on these findings and the ubiquitous expression of CB1 receptors in the CNS, it is clear that synaptic alterations caused by chronic THC can affect a wide variety of brain processes and behaviors. Future studies will likely focus more closely on causally linking the synaptic changes caused by acute or repeated THC exposure on the behaviors mediated by brain regions in which endocannabinoid-dependent plasticity is observed and CB1 receptors are expressed.

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Synaptic Targets of Δ^9 -Tetrahydrocannabinol in the Central Nervous System

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