Chapter 76

Effects of Δ⁹-Tetrahydrocannabinol, Synthetic Cannabinoids, and Fatty Acid Amide Hydrolase Inhibitors on Mood and Serotonin Neurotransmission

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Abbreviations

5-HT 5-Hydroxytryptamine, serotonin
CB1R Cannabinoid type 1 receptor
DR Dorsal raphe
FAAH Fatty acid amide hydrolase
FST Forced swim test
GABA γ-Aminobutyric acid
ip Intraperitoneal
iv Intravenous
mPFCv Ventromedial prefrontal cortex
PFC Prefrontal cortex
SSRI Selective serotonin reuptake inhibitor
Δ⁹-THC (−)*trans*-Δ⁹-tetrahydrocannabinol

INTRODUCTION

For many centuries, the *Cannabis sativa* plant has been known to possess psychotropic effects linked to the regulation of mood. It was early in the seventeenth century when Robert Burton considered cannabis as a possible treatment for depression in his book: *The Anatomy of Melancholy*. The idea that the mood-elevating properties of cannabis could treat depression was soon set aside because of multiple adverse side effects and inconsistent efficacy, leaving a controversy over its mechanisms of action.

Certainly, one of the most salient effects of cannabinoids is related to elevation of mood: subjective effects include a sensation of "high," anxiety relief, and the feeling of being "relaxed" (Earleywine, 2005; Iversen, 2003). This subjective experience is highly variable depending on the dose of drug, the environment, and the experience and expectations of the drug user. The high produced by cannabis is a complex experience, characterized by a quickening of mental associations and a sharpened sense of humor, sometimes described as a state of "fatuous euphoria."

A breakthrough discovery in the twentieth century was the acknowledgment of a multifaceted endogenous cannabinoid system (i.e., endocannabinoid system) that plays a vital role in the etiology and pathophysiology of brain dysfunctions. Despite this knowledge, very little is known about the mechanism(s) that underlies the mood-elevating effects of cannabis, but it is very likely mediated by the central cannabinoid receptor CB1 (CB1R) since the CB1R antagonist rimonabant blocks this effect (Huestis et al., 2001).

When our laboratory started this line of research in 2002, only a few articles were published on the link between cannabis and serotonin (5-HT) function. At that time, it was known that CB1R was present at the level of the nucleus of the dorsal raphe (DR) (Matsuda, Bonner, & Lolait, 1993; Moldrich & Wenger, 2000; Tsou et al., 1998), the major source of 5-HT neurons in the brain, and that fatty acid amide hydrolase (FAAH), the enzyme responsible for the degradation of the endocannabinoid anandamide, was present in its oligodendrocytes (Egertova, Cravatt, & Elphick, 2003). These anatomical data were consistent with an electrophysiological study reporting that postsynaptic orexin receptors can modulate glutamatergic synaptic transmission to DR 5-HT neurons through retrograde endocannabinoid signaling (Haj-Dahmane & Shen, 2005). It was known that 5-HT1B, 5-HT2A (Devlin & Christopoulos, 2002), and 5-HT3 receptor subtypes were coexpressed with CB1R (Hermann, Marsicano, & Lutz, 2002) in γ-aminobutyric acid (GABA)-ergic interneurons (Morales, Wang, Diaz-Ruiz, & Jho, 2004), as was the inhibitory functional interaction between CB1R and 5-HT2A receptors (Darmani, 2001; Kimura, Ohta, Watanabe, Yoshimura, & Yamamoto, 1998) and between CB1R and 5-HT3 receptors (Barann et al., 2002; Fan, 1995). Moreover, it was reported that somatodendritic 5-HT1A receptors are involved in the hypothermic effects of Δ⁹-tetrahydrocannabinol (Δ⁹-THC) administration (Malone & Taylor, 2001), suggesting an interaction between these two systems at the hypothalamic level. At this time,

the Gorzalka lab at the University of British Columbia showed that chronic treatment with the CB1R agonist HU-210 alters pharmacological responses to 5-HT2A and 5-HT1A receptor agonists (Hill, Sun, Tse, & Gorzalka, 2006).

However, the extent to which activation of the endocannabinoid system could directly modulate 5-HT firing properties was unknown. For these reasons, our laboratory started to better dissect the possible involvement of these compounds in modulating 5-HT firing activity. The activity of a putative antidepressant drug on 5-HT firing is essential for predicting its potential therapeutic effect on mood regulation.

HOW TO ASSESS IF A PUTATIVE DRUG MODULATES MOOD USING ANIMAL MODELS

Major depression is a heterogeneous disorder, but despite its complexity, several animal models able to mimic symptoms of human depression have been developed. Importantly, these tests, such as the forced swim test (FST), tail suspension test (TST), learned helplessness model, and chronic unpredictable stress paradigm, have been repeatedly validated and are currently the most popular models for detecting antidepressant-like activity because of their ease of use, reliability, and high predictive validity (see our review, Bambico & Gobbi, 2008).

It is well known that people suffering from mood disorders show impaired function of the 5-HT and/or norepinephrine system and that different classes of currently available antidepressants mostly act by modulating, directly or indirectly, these two neurotransmitter systems. However, compelling evidence indicates that another monoamine, dopamine, plays a role in the pathophysiology and treatment of depression. Using in vivo electrophysiology, it is possible to examine the modulation of these monoamines by clinically prescribed antidepressants. All conventional antidepressants increase/decrease monoaminergic electrical activity as final pathways, albeit through different mechanisms (for more details, see Gobbi & Blier, 2005; Bambico & Gobbi, 2008). Consequently, testing whether a novel compound modulates monoaminergic neurotransmission using in vivo electrophysiology represents a valid framework to assess its potential for antidepressant action.

Δ^9-TETRAHYDROCANNABINOL AND SEROTONIN FIRING ACTIVITY

The primary pharmacologically active component of cannabis, Δ^9-THC, probably mediates most of its psychoactive and mood-related effects. Although heavy or high-dose cannabis use has been associated with an elevated risk for developing mood disorders, anxiety, psychosis, and cognitive impairment, especially among teenagers, cannabis has been routinely used for self-medicating depressive symptoms, suggesting that it could have therapeutic benefits in primary and secondary depression (for review see Bambico & Gobbi, 2008). Nabilone (Cesamet), a synthetic Δ^9-THC derivative commercialized in Canada, has been reported to increase mood in 38% of people and to induce euphoria in another 14% (from *Compendium of Pharmaceuticals and Specialties*, Canada, 2012).

Similarly in rodents, antidepressant-like activity following Δ^9-THC administration has been reported in preclinical animal models such as the FST (Bambico, Hattan, Garant, & Gobbi, 2012; El-Alfy et al., 2010; Moreira, Grieb, & Lutz, 2004), the olfactory bulbectomy model (Elbatsh, Spicer, Marsden, Fone, & Kendall, 2009; Rodriguez-Gaztelumendi, Rojo, Pazos, & Diaz, 2009), and the TST (El-Alfy et al., 2010). On the other hand, Egashira et al. (2008) reported that Δ^9-THC administration increased, rather than decreased, immobility in the FST, which is indicative of behavioral despair. These conflicting results suggest that Δ^9-THC may also exert depressogenic effects under certain conditions, similar to cannabis use in humans.

Given the dual effects of cannabis and cannabis constituents on mood and antidepressant-like behavior, respectively, we examined whether Δ^9-THC modulates single-unit 5-HT firing in vivo. The intravenous administration of various doses of Δ^9-THC (0–1.6 mg/kg) yielded a complex response profile. In fact, we identified three different groups of 5-HT neurons on the basis of their response to Δ^9-THC within this dose range: 25.58% ($n=22$) were excited (≥10% of baseline), 32.56% ($n=28$) were inhibited (<10% of baseline), and 41.86% ($n=36$) were nonresponding ($\chi^2=3.5$, $p=0.17$). These neurons, regardless of their response, exhibited identical electrophysiological characteristics and were all recorded from the rostrocaudal midline extent of the DR nucleus. The excitatory responses were mainly produced by doses >0.45 mg/kg and were maximal at 1.0 mg/kg. The inhibitory responses were mainly produced by doses <0.45 mg/kg and were maximal at 0.4 mg/kg. Inert responses were equally distributed between low and high dose ranges. In the brain, Δ^9-THC binds mostly to CB1R, but at higher doses may also bind to the transient receptor potential vanilloid type 1 (TRPV1) channel, also known as the capsaicin receptor or vanilloid receptor 1. In an attempt to determine which receptor subsystems were responsible for the contrasting excitatory and inhibitory responses, the CB1R antagonist rimonabant (1.0 mg/kg) or the TRPV1 channel antagonist capsazepine (0.01 mg/kg) was intravenously administered following a Δ^9-THC-induced increase or decrease in DR 5-HT firing. We found that the excitatory response was attenuated by rimonabant, but not by capsazepine, suggesting that Δ^9-THC-induced increases in 5-HT firing are mediated by CB1R activation. On the other hand, the inhibitory response was only partially reversed by rimonabant in one of three neurons and was not at all sensitive to capsazepine, indicating a non-CB1R- and a non-TRPV1-mediated mechanism (Bambico et al., 2012).

Acute intraperitoneal (ip) administration of the dose of Δ^9-THC shown earlier to evoke the maximal excitatory response from 5-HT neurons (1.0 mg/kg) produced a modest but nonsignificant enhancement of spontaneous 5-HT firing. Increasing the dose to 2 and 4 mg/kg similarly yielded nonsignificant elevations in the mean 5-HT firing rate. However, repeated administration (once daily for 5 days) of Δ^9-THC (1.0 mg/kg, ip) produced a significant increase in the mean spontaneous DR 5-HT firing rate ($p<0.05$), which was blocked by the coapplication of rimonabant (1.0 mg/kg, ip), which is suggestive of a CB1R-mediated effect (Figure 1). The reason for this discrepancy between acute vs chronic Δ^9-THC treatment is unknown, although it may be due to the pharmacodynamic/pharmacokinetic properties of Δ^9-THC or possibly a

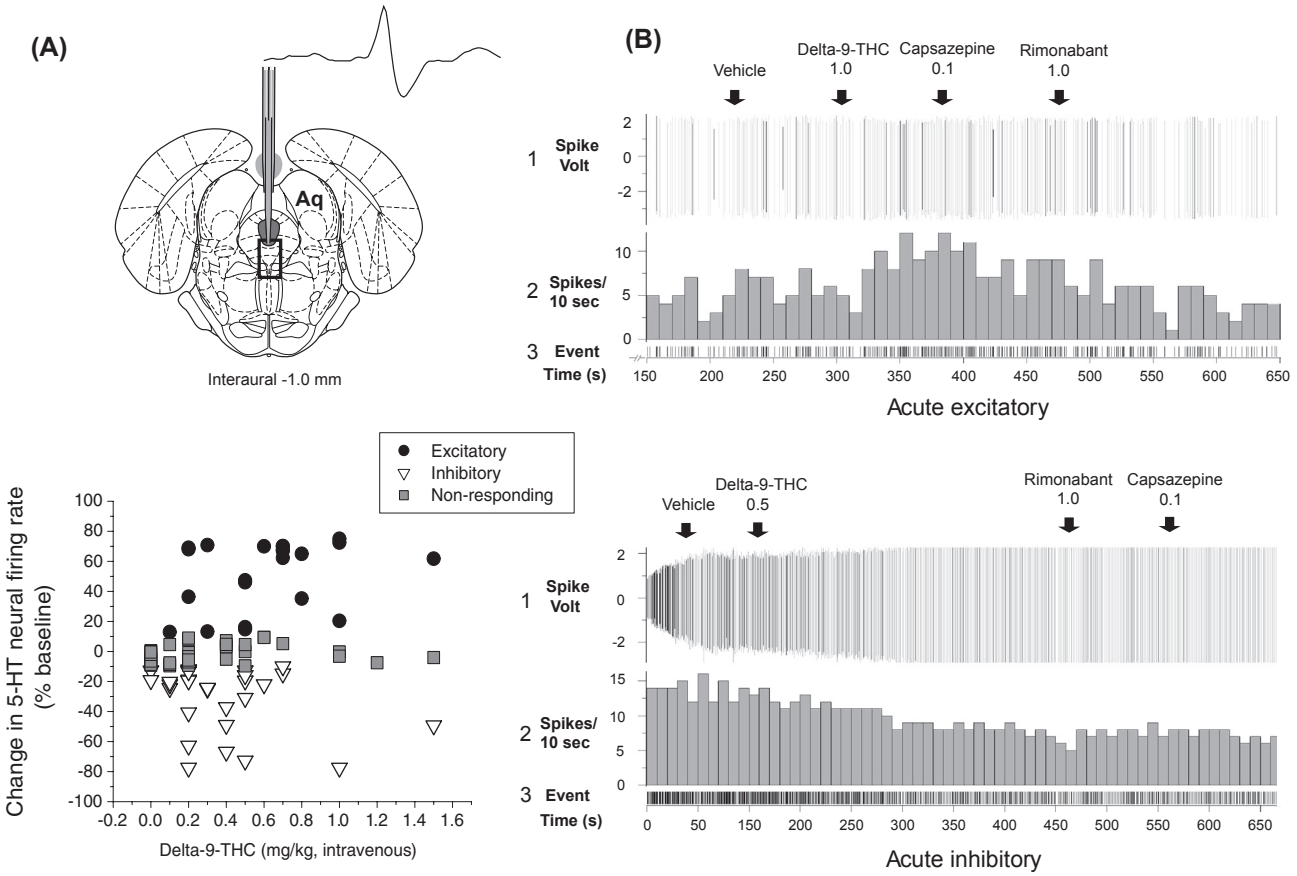

FIGURE 1 (A) Acute intravenous (iv) administrations of variable doses of Δ9-THC elicited a complex response profile from DR 5-HT neurons, with neurons exhibiting either excitatory or inhibitory responses, as well as inert ones. A plate from Paxinos and Watson (2007) illustrating a brain coronal section through the DR nucleus is shown. The boxed area shows where putative 5-HT neurons were recorded. An exemplary spike waveform of a putative 5-HT neuron is shown above. (B, top panel) A representative 5-HT neural firing rate histogram showing that a Δ9-THC-evoked excitatory response was reversed by the CB1R antagonist rimonabant but not by the vanilloid (TRPV1) antagonist capsazepine. (B, bottom panel) A representative 5-HT neural firing rate histogram showing that a Δ9-THC-evoked inhibitory response was reversed neither by rimonabant nor capsazepine. Numbers above arrows indicate the iv dose administered. *From Bambico et al. (2012), Progress in Neuropsychopharmacology and Biological Psychiatry, with permission.*

long-term neuroplastic or synaptic modification produced by prolonged CB1R activation (Bambico et al., 2012).

Similar data were found in the FST, a popular preclinical paradigm used to assess the putative antidepressant-like properties of a drug (Castagne, Moser, Roux, & Porsolt, 2011). Following FST exposure, we found that acute Δ9-THC administration (1.0 mg/kg, ip) was not sufficient to elicit differences in either the total duration or the frequency of active vs passive coping behaviors compared to control-treated rats. Conversely, a repeated administration schedule (5 days) elicited an antidepressant-like response, characterized by an increase in swimming duration and a decrease in immobility, similar to the conventional selective 5-HT reuptake inhibitor (SSRI) citalopram (Figure 1, Bambico et al., 2012). Moreover, chronic Δ9-THC, but not citalopram, significantly increased climbing duration ($p = 0.007$), suggesting that the antidepressant-like effects of Δ9-THC administration are mediated by the noradrenergic system.

Last, we showed that repeated (5 days), but not acute, administration of Δ9-THC (1.0 mg/kg, ip) increased the excitatory response of hippocampal CA3 pyramidal neurons to the 5-HT1A receptor antagonist WAY 100635 in a manner similar to 5-day treatment with citalopram (Bambico et al., 2012). These findings suggest that Δ9-THC, similar to other classes of antidepressants, increases tonic 5-HT1A postsynaptic receptor activity, but only following a long-term treatment regimen (Besson, Haddjeri, Blier, & de Montigny, 2000; Haddjeri, Blier, & de Montigny, 1998). In summary, these electrophysiological experiments revealed that administration of various doses of Δ9-THC produces a complex multimodal response, with excitation, inhibition, and nonresponse among different populations of 5-HT neurons. Additional research examining the pharmacological effects of Δ9-THC on monoaminergic transmission and emotional processes is warranted. Evidence suggests that Δ9-THC could act both as a partial and as a full CB1R agonist, depending on whether the receptor is localized to GABAergic or glutamatergic synapses (Laaris, Good, & Lupica, 2010).

Repeated Δ9-THC treatment (1.0 mg/kg) yielded a significant elevation in the mean discharge rate of spontaneously active DR 5-HT neurons, an effect that was reversed by coadministration with the CB1R antagonist rimonabant. Similar results have also been obtained with the synthetic CB1R agonist WIN 55,212-2

ex vivo (Mendiguren & Pineda, 2009). Moreover, these researchers showed that both rimonabant and the CB1R inverse agonist AM251 decreased DR 5-HT neural activity in approximately 50% of neurons recorded (Mendiguren & Pineda, 2009).

Thus, there appears to be a general consensus that the endocannabinoid system modulates 5-HT neurotransmission, with CB1R activation resulting in enhanced DR 5-HT firing and increased postsynaptic 5-HT1A receptor activation. However, the response profile of CB1R agonists and antagonists is far from uniform, which could be due to the ubiquitous expression profile of CB1Rs and their ability to modulate both excitatory and inhibitory transmission in an on-demand fashion.

THE EFFECT OF WIN 55,212-2 ON 5-HT FIRING AND ANTIDEPRESSANT-LIKE ACTIVITY

In our laboratory, we have assessed the spontaneous single-unit firing activity of DR 5-HT neurons after cumulative intravenous (iv) administration of the CB1R agonist WIN 55,212-2. Increasing doses of WIN 55,212-2 (0.05–0.2 mg/kg) evoked a dose-dependent increase in 5-HT unit firing activity, which was half-maximal (ED_{50}) at a dose of 0.1 mg/kg and was not blocked by capsazepine (20 µg/kg, iv), but was blocked by rimonabant (1 mg/kg, iv) in 100% of neurons tested (Figure 2), (Bambico, Katz, Debonnel, & Gobbi, 2007).

WIN 55,212-2 treatment also increased 5-HT burst activity, a pattern that is associated with antidepressant-like activity (Gobbi et al., 2005). The maximal increase in burst frequency and in the mean number of spikes in a burst from baseline was recorded following the 0.2 mg/kg dose (Figure 2(E); Bambico et al., 2007). Among all neurons recorded, 66.67% of 5-HT neurons responded to increasing dose injections of WIN 55,212-2, while 33.33% of neurons were nonresponding. All responding and nonresponding neurons showed the same electrophysiological characteristics, were inhibited by the $GABA_B$ agonist baclofen, and were localized to the DR, thus indicating that not all DR neurons are activated by CB1R stimulation.

Importantly, cumulative doses higher than 0.2 mg/kg of WIN 55,212-2 injected iv produced a general decline in neuronal excitation that was significant at both 0.3 and 0.4 mg/kg and achieved a maximal level 45% below baseline (i.e., vehicle) following 0.4 mg/kg WIN 55,212-2. A waning of stimulatory effects was also observed with different parameters of burst activity: burst frequency, mean number of spikes in a burst, and mean burst length. In two of three neurons tested, capsazepine reversed the decrease induced by high doses of WIN 55,212-2, suggesting that TRPV1 receptors, but not CB1Rs, are involved in the inhibitory effects of high doses of WIN 55,212-2 on DR 5-HT firing. Interestingly, neither rimonabant (1 mg/kg, iv) nor capsazepine (20 µg/kg, iv) alone had a significant effect on 5-HT single-unit firing activity, meaning that at low doses these two drugs may act as inert antagonists or that such doses are unable to unmask a putative tonic cannabinoid- or vanilloid-mediated regulation of 5-HT firing.

We found that in the FST, WIN 55,212-2 at the dose of 0.1–0.2 mg/kg administered 23, 5, and 0.75 h before the test produced antidepressant-like effects characterized by decreased immobility and increased swimming activity (Bambico et al., 2007). However, the antidepressant-like activity disappeared when higher doses were injected (Bambico et al., 2007).

Incremental doses of WIN 55,212-2 and the mean spontaneous firing rate of 5-HT neurons showed a biphasic relationship. One-way analysis of variance revealed a dose-dependent increase with lower doses of WIN 55,212-2, while coadministration of rimonabant prevented this increase. A dose of 0.2 mg/kg WIN 55,212-2 yielded a maximal 126.32% increase in neuronal activity. On the other hand, a much higher dose of WIN 55,212-2 (2.0 mg/kg) yielded a significant 64% decrease in 5-HT firing compared to vehicle. We also calculated the mean number of neurons per electrode descent, which serves as an indirect measure of spontaneously active neurons (Gobbi et al., 2007). In comparison with vehicle injections, there were 28% more spontaneously active 5-HT neurons encountered after treatment with 0.1 mg/kg WIN 55,212-2 and 33.33% more active neurons with 0.2 mg/kg WIN 55,212-2 ($p<0.01$), while a high dose of WIN 55,212-2 (2.0 mg/kg) had 48.8% fewer active neurons than the control ($p<0.01$). The number of spontaneously active neurons in rats treated with a low dose of WIN 55,212-2 (0.2 mg/kg) coapplied with rimonabant (1.0 mg/kg) did not significantly differ from that of those treated with vehicle (Bambico et al., 2007).

We also asked if the WIN 55,212-2-induced stimulation of DR 5-HT neurons is mediated by CB1Rs located in neurons of the prefrontal cortex (PFC) that project to the DR. Indeed, DR 5-HT neurons receive important excitatory inputs from pyramidal (glutamatergic) cells of the PFC (Jankowski & Sesack, 2004). We performed systematic transections of the PFC–DR pathway prior to conducting electrophysiological recordings. Three types of transections were performed: a total bilateral PFC transection (tPFC), a selective transection of PFC focusing on the medial PFC (mPFC; areas transected included the dorsal peduncular, infralimbic, prelimbic Cg3, and cingulate Cg1 cortices), and a transection of the lateral aspect of the PFC (latPFC): mainly the lateral prefrontal/agranular insular, but also the frontal Fr2, Fr1, and Fr3; the ventrolateral and lateral orbital cortices; and some parts of parietal area 1 (modified after Hajós, Hajós-Korcsok, & Sharp, 1999). 5-HT single-unit recordings were conducted 1.5–2 h after each transection.

Following tPFC transection, WIN 55,212-2 failed to increase 5-HT single-unit firing activity at otherwise stimulatory doses in intact brains. To pinpoint the specific subregion of the PFC that is critical in mediating the modulation of 5-HT single-unit activity, we compared transection of the mPFC with that of the latPFC. The response of 5-HT single units to the latPFC transection did not significantly differ from the control, but on the other hand, mPFC transection produced an effect similar to tPFC transection and was significantly different from the control, thus indicating that the medial, but not lateral, subregions of the PFC contribute to the enhanced 5-HT firing activity following CB1R activation (Bambico et al., 2007). To further corroborate this hypothesis we also injected WIN 55,212-2 directly into the mPFC and then performed the FST; and we observed that the injection of the CB1 agonist into the mPFC was sufficient to induce an antidepressant-like effect and increase 5-HT firing activity of DR neurons (Bambico et al., 2007).

Altogether these results indicate that the ventromedial PFC (mPFCv) plays an instrumental role in mediating DR 5-HT firing and that local CB1R activation within this region exerts antidepressant-like effects via enhanced mPFCv-driven stimulation of DR 5-HT neuronal firing.

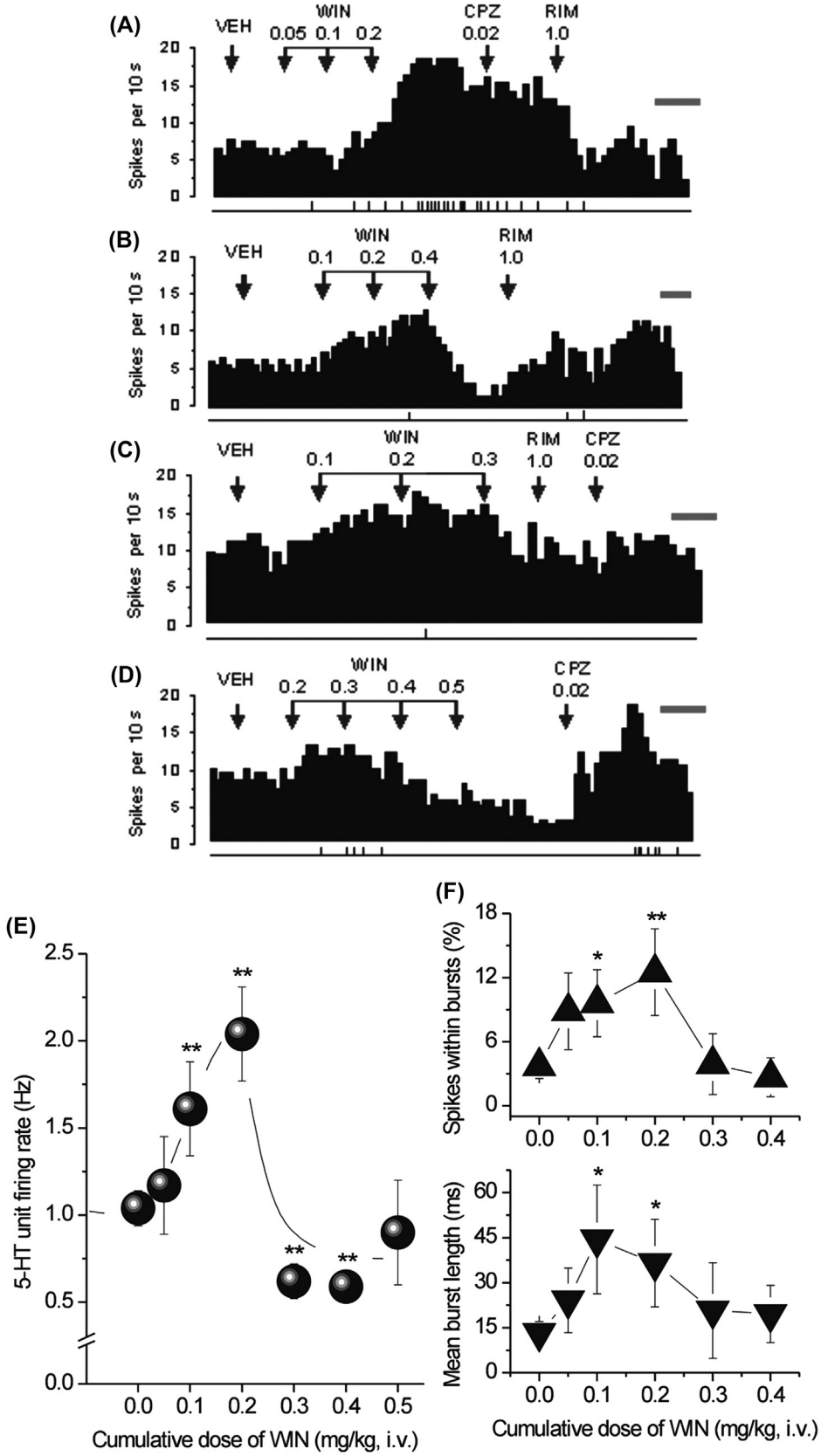

FIGURE 2 Effect of iv administration of cumulative doses of WIN 55,212-2 on DR 5-HT neurons. (A–D) Integrated firing rate histograms of 5-HT neurons illustrating that low doses of WIN (0.1–0.2 mg/kg, iv) rapidly increased single-unit firing activity. (A) This effect was reversed by rimonabant (RIM) (1.0 mg/kg, iv; $n=4$) but not by capsazepine (CPZ) (0.02 mg/kg, iv; $n=4$). (B–D) High dose of WIN (0.30–0.50 mg/kg, iv) rapidly decreased 5-HT single-unit firing activity. This effect was reversed by CPZ (0.02 mg/kg, iv) in two of three neurons (D) and partially reversed (B) or not reversed (C) by RIM (1 mg/kg, iv) in one and three neurons, respectively. 5-HT neuronal firing rate in each histogram is plotted as spikes per 10 s. Calibration bar on the right side of each histogram, 1 min. The vertical lines depicted below each histogram represent the frequency of neuronal burst activity such that each tick corresponds to a burst discharge event. (E) WIN (0.05–0.5 mg/kg, iv) produced a biphasic response profile in 5-HT single-unit activity. (F) Line graphs showing that cumulative doses of WIN modulated 5-HT neuronal burst activity measured as percentage of spikes within bursts and mean burst length (bottom). *$p<0.05$ or **$p<0.01$ vs baseline (vehicle). *From Bambico et al. (2007), Journal of Neuroscience, with permission.*

FATTY ACID AMIDE HYDROLASE INHIBITION, 5-HT FIRING, AND ANTIDEPRESSANT-LIKE ACTIVITY

The selective inhibitor of the enzyme FAAH, which catalyzes the intracellular hydrolysis of the endocannabinoid anandamide, has been proposed to be a useful alternative to direct CB1R agonists because of their capacity to increase endogenous CB1R signaling without inducing the typical cannabis-related side effects such as dependence and sedation (see Bambico & Gobbi, 2008 for review).

One of the first experiments carried out in our laboratory was to test whether the FAAH inhibitor URB 597 influences DR 5-HT transmission. We first measured spontaneous activity of 5-HT neurons in the DR of anesthetized rats. Single injections of URB 597 (0.03–0.3 mg/kg, iv) evoked a slow increase in 5-HT neuron firing activity, which was half-maximal at a dose of ≈0.06 mg/kg and was blocked by pretreatment with rimonabant (1 mg/kg, iv; Figure 3). Interestingly, the increase in firing was not as immediate as observed with the CB1R direct agonist, occurring only after 15–20 min; this delay is compatible with the pharmacodynamics of URB 597, which after passing the blood–brain barrier, inhibits FAAH, leading to a gradual accumulation in anandamide content, which subsequently activates CB1Rs (Gobbi et al., 2005).

This response was recapitulated following subchronic URB 597 treatment. Indeed, 4-day treatment with URB 597 (0.1 mg/kg, ip, once daily for 4 days) evoked an even stronger response, which was also reversed by rimonabant (1 mg/kg, ip) (Gobbi et al., 2005). This sustained 5-HT increase after a subchronic treatment regimen was also associated with an increase in 5-HT bursting activity and sustained 5-HT release in the hippocampus, but not in the PFC, as assessed by in vivo microdialysis in awake rats (Gobbi et al., 2005). A single injection of URB 597 had no such effect.

Finally, 4-day treatment with URB 597 did not affect the responsiveness of 5-HT neurons to local iontophoretic administration of the 5-HT1A receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT), suggesting that URB 597, unlike classical antidepressants (Artigas, Romero, de Montigny, & Blier, 1996; Gobbi & Blier, 2005), did not produce

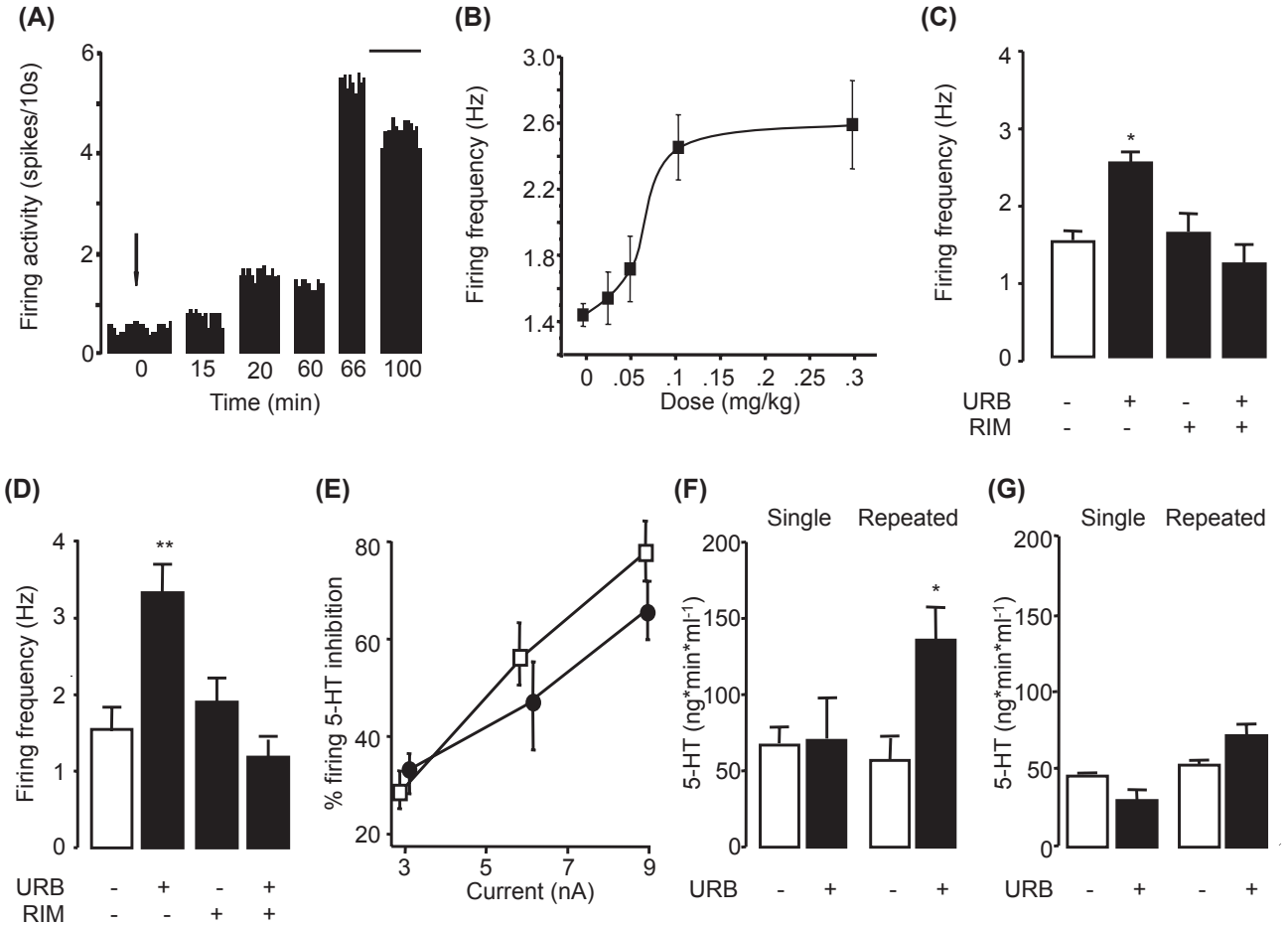

FIGURE 3 Effects of URB 597 on 5-HT neuron firing in the rat DR. (A) Integrated firing rate histogram of DR neurons, illustrating the time-dependent effects of URB 597; arrow indicates time of URB 597 injection (0.1 mg/kg, iv; calibration bar, 1 min). (B) Dose-dependent effects of URB 597 on spontaneous firing rate. (C and D) Single administration of rimonabant (RIM) (1 mg/kg, iv) prevents the effects of single (0.1 mg/kg) (C) and repeated (D) URB 597 injections (0.1 mg/kg, ip, once daily for 4 days) on 5-HT neuron firing. (E) Repeated URB 597 administration does not affect the response of 5-HT neurons to 8-hydroxy-2-(di-*n*-propylamino)tetralin, expressed as percentage inhibition of 5-HT-neuron firing rate. Open symbols represent vehicle. (F and G) Effects of single or repeated URB 597 injections on 5-HT outflow over 3 h in hippocampus (F) and PFC (G) of awake rats. *$p<0.05$ vs vehicle; **$p<0.01$ vs vehicle. *From Gobbi et al. (2005), Proceedings of the National Academy of Sciences, with permission.*

desensitization of 5-HT1A autoreceptors. Importantly, at the same doses (0.1 and 0.3 mg/kg) and duration (once a day for 4 days), URB 597 induced antidepressant-like effects in the mouse TST and the rat FST. This effect was more robust after repeated injections (4 days) and was reversed by the preadministration of the CB1R antagonist rimonabant (Figure 3; Gobbi et al., 2005).

Since we have previously shown that intra-mPFCv administration of WIN 55,212-2 elicits antidepressant-like responses as well as increases in DR 5-HT firing, we next examined whether *endogenous* medial prefrontocortical cannabinoid signaling modulates coping behaviors in the FST using a combination of behavioral, pharmacological, biochemical, and electrophysiological approaches. We first examined how FST exposure affects endocannabinoid ligand content in the mPFC using mass spectrometry and revealed that anandamide content experiences a rapid and robust decline immediately following the first FST exposure session. Anandamide content was partially (but not fully) restored when examined 24 h later, but was subject to an even greater decline following a second FST exposure session, and this was accompanied by behavioral despair (i.e., increased immobility) (McLaughlin et al., 2012). Thus, fluctuations in anandamide signaling in the mPFC were suspected to mediate the transition between active and passive coping strategies. To support this claim, we next demonstrated that local inhibition of FAAH in the mPFCv with URB 597 reduced the expression of passive, despair-like coping responses in the FST and consequently augmented the expression of a subset of active coping responses (i.e., swimming) that are known to be 5-HT mediated (McLaughlin et al., 2012). The enhancement of swimming cannot be attributed to a general increase in locomotion, since previous reports have demonstrated that URB 597 does not significantly affect basal locomotor activity (Adamczyk, Mccreary, & Filip, 2008). This effect in the FST was blocked by coadministration of the CB1R inverse agonist AM251, as well as by global pharmacological depletion of 5-HT precursors, suggesting that the ability of FAAH inhibition within the mPFCv to promote active coping strategies in the FST is both CB1R dependent and 5-HT mediated. Finally, using in vivo single-unit extracellular recordings, we demonstrated that local inhibition of FAAH within the mPFCv enhanced the firing rate of DR 5-HT neurons on a time course that mirrors the behavioral effects in the FST (McLaughlin et al., 2012). Together, these studies argue that anandamide/CB1R activity in the mPFCv mediates the expression of active coping responses in the FST via an enhancement of DR 5-HT neuronal firing, which is in line with previous findings from our group following intra-mPFCv administration of exogenous CB1R agonists (Bambico et al., 2007).

We were also able to recapitulate these electrophysiological and behavioral findings in FAAH-knockout mice ($FAAH^{-/-}$), in which we observed a marked increase (+34.68%) in DR 5-HT neural firing compared to their littermates, which was reversed by rimonabant (Bambico et al., 2010). This effect was particularly significant in a subset of neurons exhibiting high firing rates (33.15% mean decrease). $FAAH^{-/-}$ mice also showed a resilience profile characterized by reduced immobility in the FST and TST, predictive of antidepressant activity, which again was attenuated by rimonabant administration.

The delay in therapeutic onset of antidepressants has been attributed to gradual neuroplastic adaptations at the presynaptic and postsynaptic levels that result from the progressive augmentation of 5-HT activity. These modifications include desensitization of autoinhibitory 5-HT1A receptors and sensitization or increased tonic activation of postsynaptic 5-HT1A receptors (Besson et al., 2000; Haddjeri et al., 1998; Szabo & Blier, 2001). The hippocampal pyramidal response to the 5-HT1A receptor antagonist WAY 100635 indicates enhanced tonus on the hippocampal 5-HT1A heteroreceptors, a hallmark of antidepressant-like action. $FAAH^{-/-}$ mice, compared to their wild-type littermates, showed increased tonic activity of 5-HT1A receptors, as tested with ip administration of WAY 100635 (0.5 mg/kg), which potently disinhibited hippocampal pyramidal neural activity (Bambico et al., 2010). Together, these results suggest that genetic deletion or pharmacological inhibition of FAAH (systemically or intra-mPFCv) elicits antidepressant-like effects, paralleled by increased 5-HT transmission and augmented postsynaptic 5-HT1A and 5-HT2A/2C receptor function.

CHRONIC ADMINISTRATION OF CB1R AGONISTS IN ADOLESCENCE AND ADULTHOOD: IMPLICATIONS FOR 5-HT FIRING ACTIVITY

Cannabis remains the most abused illicit substance worldwide. During the period of 2010–2012, 11.69% of Americans age 12 and older had abused marijuana at least once in the year prior to being surveyed (Substance Abuse and Mental Health Services Administration, 2014). It is important to mention that there have been studies that suggest that heavy and/or prolonged use of cannabis early in life increases the risk for neuropsychiatric disorders such as anxiety, depression, and schizophrenia (Bambico, Duranti, Tontini, Tarzia, & Gobbi, 2009; Bambico & Gobbi, 2008; Howlett et al., 2004) independent of whether the individual uses other illicit drugs (Hayatbakhsh et al., 2007). The limited neurobiological data scrutinizing this association are somewhat inconsistent, with reports of both increased (O'Shea, McGregor, & Mallet, 2006; O'Shea, Singh, McGregor, & Mallet, 2004) and decreased (Biscaia et al., 2003; Rubino et al., 2008) emotional reactivity following adolescent cannabis use. The impact of adolescent vs adult cannabis exposure, as well as the neural mechanisms underlying the increased predisposition for developing mood disorders, deserves more attention, especially given the recent changes in laws governing recreational cannabis use in some US states.

The neurobiological impact of drug use is especially compounded during the critical adolescence period when brain development is punctuated by constant neuroplastic shaping, synaptic reorganization, and extensive neurochemical changes, along with reckless or impulsive behaviors, novelty and sensation seeking, risk taking, and partial anhedonia (Spear, 2000). During this stage, corticolimbic CB1R density is at its peak, undergoing gradual pruning thereafter (Belue, Howlett, Westlake, & Hutchings, 1995), which may well relate to the crucial role of endocannabinoids in brain developmental processes including neurogenic control, neural progenitor proliferation, lineage segregation, and the migration and phenotypic specification of immature neurons (Harkany, Keimpema, Barabas, & Mulder, 2008). Several lines of evidence support the notion that prolonged aberrations in CB1R signaling may dramatically alter CB1R density in ways that potentially disrupt the development of the monoaminergic systems, thereby influencing mood and anxiety control (Ellgren et al., 2008).

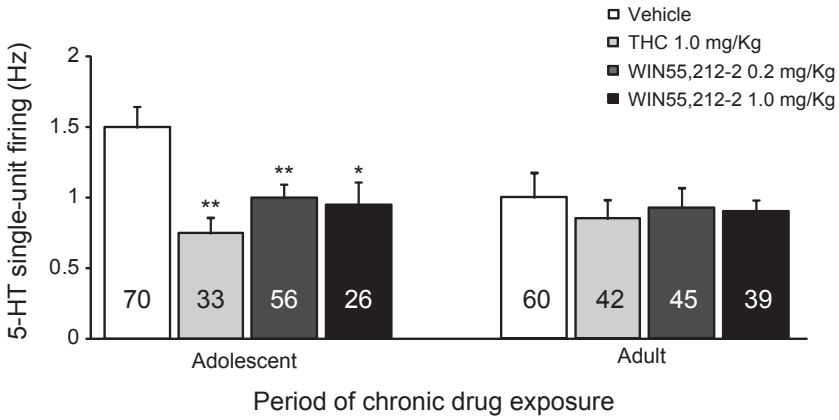

FIGURE 4 Alterations in 5-HT neurotransmission following chronic adolescent cannabinoid exposure. Chronic daily treatment with WIN 55,212-2 (0.2 or 1.0 mg/kg, ip) or THC (1.0 mg/kg, ip) when administered during adolescence but not when administered during adulthood resulted in a significant decrease in DR 5-HT spontaneous single-unit firing. Values within each bar denote the number of neurons recorded. $*p<0.05$, $**p<0.01$. *Modified after Bambico et al. (2010), with permission.*

In light of this evidence, we studied the impact of exogenous CB1R agonists on 5-HT firing activity after long-term exposure during adolescence or adulthood, hypothesizing that the adolescent brain would be subject to more detrimental, insidious effects of chronic CB1R agonism compared to that of mature subjects. Adolescent rats were treated with WIN 55,212-2 from postnatal day (PND) 30 to 50 (0.1 or 1 mg/kg, once a day) or with Δ^9-THC (1 mg/kg, once a day) or vehicle. At PND 70, they were tested using behavioral assays for emotional behavior or in vivo electrophysiology for assessing changes in 5-HT neurotransmission. Four distinct groups of adult rats were similarly treated for 20 days (from PND 70 to 90) and tested 20 days later (PND 110).

In the adolescent-exposed groups, all drug treatments significantly attenuated spontaneous 5-HT single-spike activity in adulthood (Bambico et al., 2010, Figure 4). Further analyses of neural activity revealed a modest nonsignificant decrease in burst firing activity (decreased number of spikes per burst and burst length, increased burst interspike interval, and decreased ratio (%) of spikes within bursts to the total number of spikes) following exposure to the low dose of WIN 55,212-2 (Bambico et al., 2010). Opposite effects (nonsignificant *increase* in burst activity) were observed after exposure to the high dose of WIN 55,212-2 as well as Δ^9-THC, which corresponded to positively skewed interspike interval distributions, further indicating irregular and burst-like neural firing (Bambico et al., 2010). Drug exposure during adulthood did not yield significant changes in 5-HT single-spike and burst firing activity.

From a behavioral point of view, chronic adolescent exposure, but not adult exposure, to WIN 55,212-2 (low 0.2 mg/kg and high 1.0 mg/kg) and Δ^9-THC (1 mg/kg) led to depressive-like behavior in the FST and sucrose preference test, while the high dose also induced anxiety-like responses in the novelty-suppressed feeding test.

Together these data suggest that the 5-HT system in adolescents is particularly sensitive to chronic consumption of CB1R agonists leading to a net decrease in 5-HT electrical activity and behavioral consequences such as vulnerability to depression and anxiety. Adult chronic cannabinoid exposure failed to elicit profound or persistent effects on emotional behavior and 5-HT neurotransmission. These findings are particularly relevant from a translational perspective in human populations, although more studies are needed to understand the reasons for these plastic changes induced by exogenous CB1R agonists. Moreover, greater efforts are needed in the prevention and treatment of mental health consequences induced by heavy and/or prolonged cannabis use during adolescence.

CONCLUSION

Major depression is a complex multifaceted disease whose etiology and pathophysiology is poorly understood. It is estimated to afflict 10–15% of the general population, resulting in profound alterations in emotional, cognitive, and neuroendocrine functioning (Lépine & Briley, 2011). The World Heath Organization predicts that major depression will become the most prevalent cause of illness-induced disability by the year 2030, leading to premature fatalities and disabilities. It is one of the most prevalent psychiatric disorders. The STAR-D (largest prospective randomized antidepressant trial) showed that 16% of study dropouts were due to intolerance of medication. Also 38.6% developed moderate/severe impairments due to an adverse event. It was also noted that the remission rate of first-line treatment with the SSRI Citalopram over a 12-week period was relatively modest (36.8%) (Rush et al. 2006). Therefore, the discovery and validation of new targets for the treatment of depression are consequently of extreme importance.

From our studies, we have demonstrated that direct activation of CB1R via Δ^9-THC or synthetic cannabinoid agonists and indirect activation of CB1R via inhibition of FAAH both exert putative antidepressant-like effects at low doses by modulating 5-HT neurotransmission, thereby producing effects on mood and behavior via a mechanism similar to that of conventional antidepressants. However, with respect to Δ^9-THC and other synthetic cannabinoid agonists, the opposite effect is observed at higher doses and the side effects induced by these cannabinoids prevent them from being considered as viable antidepressant compounds.

Another major concern of cannabinoid use for therapeutic purposes is the potentially harmful effects on brain plasticity and

development following adolescent consumption. We revealed that 5-HT neurotransmission during adolescence is particularly sensitive to chronic consumption of CB1R agonists, leading to a net decrease in 5-HT activity, with adverse behavioral consequences such as increased vulnerability to developing depression and anxiety. Even if data from our laboratory and others suggest that the association between cannabis and depression during adulthood is restricted to early onset (i.e., adolescent) of cannabis use, it cannot be ruled out that vulnerable adult populations may also be sensitive to the adverse consequences of heavy and/or prolonged cannabis consumption.

The development of FAAH inhibitors might instead represent a valid alternative to cannabis and cannabinoid-based compounds, since they increase endogenous cannabinoids in a natural fashion, thereby avoiding many of the side effects of exogenous cannabinoids (Gobbi et al., 2005). However, more studies are clearly needed to better understand the neurobiological underpinnings of cannabis use and depression and to test novel FAAH inhibitors or other cannabis-derived medicines for the treatment of mood disorders in clinical settings.

APPLICATIONS TO OTHER ADDICTIONS AND SUBSTANCE MISUSE

1. The cannabinoid receptors can be stimulated by either endogenous or exogenous cannabinoids orchestrating a well-defined signaling response with multiple points of interaction with the mesocorticolimbic reward/stress circuit.
2. The mesocorticolimbic reward/stress circuit is also implicated in cocaine dependence, amphetamine dependence, opioid dependence, and alcohol dependence.
3. Cannabis abuse is associated with the abuse of other substances, such as nicotine and alcohol, and possibly harder drugs such as heroin, cocaine, and ecstasy.
4. Activation of CB1R affects mood regulation through indiscriminate modulation of brain structures that are crucial for stress responsivity and reward seeking (e.g., DR, hippocampus, amygdala, hypothalamus, ventral striatum, mPFC, ventral tegmental area). As such, dysfunctional CB1R activity may stimulate drug seeking and enhance vulnerability for developing affective disorders.
5. Our studies showed that chronic use of cannabis during adolescence, similar to chronic use of amphetamine or nandrolone, can have serious and irreversible effects on 5-HT and norepinephrine neurotransmission.

DEFINITION OF TERMS

Depression This is a complex heterogeneous disease clinically characterized by a constellation of symptoms impairing an individual's ability to function and cope with daily life activities. Its main alterations are in the cognitive, affective, somatic, and vegetative domains. The symptoms must be present mainly for 2 weeks, representing a change in previous functioning. Core symptoms are anhedonia and depressed mood.

Serotonin (5-hydroxytryptamine) This is the principal monoamine neurotransmitter that it is implicated in mood, sleep, and appetite. It has been also implicated in learning processes as well as in memory functions. Most conventional antidepressants act by modulating the amount of this neurotransmitter.

Δ^9-Tetrahydrocannabinol This is the main psychoactive constituent of cannabis, with multiple functions and effects including changes in emotionality, behavior, and cognitive functions. Moreover, its analgesic, antinausea, hypnotic, and hyperphagic properties have been exploited for medicinal purposes for centuries. However, its potential for abuse and dependence and its association with many neuropsychiatric disorders make it necessary to better understand its complex neurobiological mechanism of action.

Forced swim test This is a behavioral test to examine antidepressant-like activity of putative antidepressant drugs.

Dorsal raphe This is the main source of 5-HT neurons in the brain. It has two subdivisions, the rostral and caudal regions, with many projections, mainly to the amygdala. The rostral subdivision has been associated with depression, as individuals suffering from depression display morphological abnormalities (i.e., smaller size) within this brain area.

Agonist This refers to a molecule that activates a receptor to produce a maximal biological response.

Antagonist This refers to a molecule that blocks a biological response by binding to a receptor.

Partial agonist This is a molecule that binds to a receptor, producing a biological response that is less than that of a full agonist. In the presence of a high concentration of endogenous agonists, the partial agonist acts like an antagonist.

KEY FACTS

Key Facts about Cannabis

- Cannabis remains one of the most abused illicit drugs for recreational purposes.
- Its main psychoactive component, Δ^9-THC, elicits a complex subjective experience that depends on the potency of Δ^9-THC, the user, and the environment in which the drug is consumed.
- There has been a steady rise in the use of cannabis in recent years. We are still trying to unravel its complex physiological and behavioral effects.
- Cannabis use has been shown to produce alterations in emotional and cognitive processes and has been linked to the onset of many neuropsychiatric disorders, most notably schizophrenia, in individuals with a genetic predisposition, as well as depression, possibly in nonpredisposed individuals.
- There is an ongoing debate regarding the therapeutic effects and detrimental consequences of cannabis consumption; accordingly, more studies are warranted.

Key Facts about Depression

- Depression is a relatively common debilitating mental disease, affecting almost 350 million people according to the World Health Organization.
- Epidemiological studies have reported that up to 9.5% of adults in the United States suffer from varying degrees of depression, while in Canada the prevalence of depression is estimated to be between 3.2% and 6%.

- Depression is characterized by persistent symptoms of depressed mood and anhedonia, feelings of worthlessness, alterations in somatic and vegetative functions, cognitive deficits, sleep disturbances, change in appetite, fatigue, anxiety, and psychomotor agitation.
- Numerous studies have revealed a greater likelihood for substance abusers to develop mood disorders, especially depression.
- Only one-third of people with depression respond to antidepressants, one-third have a partial response, and one-third have no response at all. Mental health researchers are still searching for novel treatments for drug-resistant depression and working toward a better comprehension of its pathophysiological underpinnings.

SUMMARY POINTS

- Psychoactive constituents of cannabis, synthetic cannabinoids, and endocannabinoid enhancers (FAAH inhibitors) regulate mood and affective states by modulating 5-HT neurons located in the DR nuclei.
- Δ^9-THC modulates 5-HT firing activity, as repeated administration of low doses (1 mg/kg) of Δ^9-THC elicits an increase in DR 5-HT single-unit activity and enhances tonic activity of 5-HT1A receptors in the dorsal hippocampus, resulting in an antidepressant-like effect.
- Δ^9-THC produces a mixed response on 5-HT firing activity, with 26% of neurons showing an increase, 33% showing a decrease, and 42% showing no response. However, after 4 days of ip injections, Δ^9-THC (1 mg/kg) produces a significant elevation of 5-HT firing.
- The increase in firing following WIN 55,212 and Δ^9-THC administration is prevented by the CB1R antagonist rimonabant, providing empirical support for a CB1R-mediated mechanism of action.
- The mPFCv plays an instrumental role in mediating the increase in 5-HT firing and the antidepressant-like effects of CB1R agonists.
- Chronic administration of CB1R agonists during adolescence leads to a net decrease in 5-HT electrical activity and impairments in emotional behavior during adulthood.
- Genetic and pharmacological inhibition of FAAH produces anxiolytic and antidepressant-like effects.
- CB1R activation strongly interacts with the 5-HT system to regulate mood; however, the use of such compounds during adolescence could lead to later impairments in 5-HT neurotransmission and emotional behavior.
- More studies are warranted to understand and untangle the neuroplastic modifications provoked by cannabis consumption, and future research is needed to design treatments targeting the endocannabinoid system that may prevent the development of major depression in vulnerable populations.

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