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Permanent Suppression of Cortical Oscillations in Mice After Adolescent Exposure to Cannabinoids: Receptor Mechanisms

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Abstract

Marijuana use in adolescence, but not adulthood, may permanently impair cognitive functioning and increase the risk of developing schizophrenia. Cortical oscillations are patterns of neural network activity implicated in cognitive processing, and are abnormal in patients with schizophrenia. We have recently reported that cortical oscillations are suppressed in adult mice that were treated, in adolescence but not adulthood, with the cannabinoids WIN55,212-2 (WIN) or ⁹tetrahydrocannabinol (THC). WIN and THC are cannabinoid types 1 and 2 receptor (CB1R & CB2R) agonists, and also have activity at non-cannabinoid receptor targets. However, as acute WIN and THC administration can suppress oscillations through CB1Rs, we hypothesize that a similar mechanism underlies the permanent suppression of oscillations by repeated cannabinoid exposure in adolescence. Here we test the prediction that cannabinoid exposure in adolescence permanently suppresses cortical oscillations by acting through CB1Rs, and that these suppressive effects can be antagonized by a CB1R antagonist. We treated adolescent mice with various cannabinoid compounds, and pharmacologically-evoked oscillations *in vitro* in adult mice. We find that WIN exposure for six days in early adolescence suppresses oscillations preferentially in adult medial prefrontal cortex (mPFC) via CB1Rs, and that a similar CB1R mechanism accounts for the suppressive effects of long-term (20 day) adolescent THC in adult somatosensory cortex (SCx). Unexpectedly, we also find that CB2Rs may be involved in the suppression of oscillations in both mPFC and SCx by long-term adolescent cannabinoid exposure, and that non-cannabinoid receptors may also contribute to oscillation suppression in adult mPFC. These findings represent a novel attempt to antagonize the effects of adolescent cannabinoid exposure on neural network activity, and reveal the contribution of non-CB1R targets to the suppression of cortical oscillations.

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Keywords

development; marijuana; schizophrenia

1: Introduction

Marijuana is the most commonly used illicit drug in the United States. Of the nearly 20 million Americans ages 12 and older that report using marijuana within the past month, approximately 40% are characterized as “heavy” users, having used marijuana at least 20 of the last 30 days (Substance Abuse and Mental Health Administration (SAMHSA), 2013). Regular marijuana use is concentrated among adolescents and young adults (SAMHSA, 2013), and accumulating evidence indicates that heavy marijuana use before adulthood can permanently impair cognitive functioning (Pope *et al.*, 2003; Gruber *et al.*, 2012; Meier *et al.*, 2012) and greatly increase the risk, in some users, for developing severe psychiatric disorders, such as schizophrenia (Arseneault *et al.*, 2002; Zammit *et al.*, 2002). Significantly, these adverse cognitive and psychiatric consequences are not as apparent when marijuana use is initiated in adulthood (Moore *et al.*, 2007; Meier *et al.*, 2012). The vulnerable adolescent period coincides with the maturation of synchronized, neural network activity in the neocortex, termed cortical oscillations (Uhlhaas *et al.*, 2009), as well as with the anatomical and physiological development of the neural networks (Giedd *et al.*, 1999; Gogtay *et al.*, 2004; Uhlhaas *et al.*, 2010), the glutamatergic and GABAergic neurotransmitter systems (Bahn *et al.*, 1994; Cruz *et al.*, 2003;), and the endocannabinoid (eCB) system that shape oscillations (Ellgren *et al.*, 2008; Heng *et al.*, 2011; Long *et al.*, 2012). Cortical oscillations are implicated in cognitive and sensory processing (Buzsaki and Draguhn, 2004; Wang, 2010), and are abnormal in patients with schizophrenia, in which these functions are altered (Gonzalez-Burgos *et al.*, 2011; Uhlhaas and Singer, 2010). We have proposed a link between adolescent marijuana use and abnormal cortical network activity in adulthood, and recently demonstrated that chronic exposure to cannabinoids during adolescence — but not adulthood — permanently suppresses pharmacologically-evoked cortical oscillations both *in vitro* and *in vivo* in adult mice, and that these effects are more pronounced in more rostral cortical areas that are less developmentally mature at the time of cannabinoid exposure (Raver *et al.*, 2013).

Previous studies have demonstrated a similar attenuation of oscillatory power after *acute* administration of the cannabinoids WIN55,212-2 (WIN) and ⁹tetrahydrocannabinol (THC), the two CB1R/CB2R receptor ligands that we tested (Hajos *et al.*, 2000; Hajos *et al.*, 2008; Holderith *et al.*, 2011). These acute suppressive effects are mediated by the CB1R, as they can be antagonized by pre-treatment or co-administration of CB1R antagonists, such as AM251 or SR141716 (Robbe *et al.*, 2006; Hajos *et al.*, 2008; Holderith *et al.*, 2011; Sales-Carbonell *et al.*, 2013). Similarly, recent evidence indicates that 5 days of WIN exposure early in adolescence impairs the modulation of oscillatory local field potential (LFP) activity in mPFC evoked by ventral hippocampus stimulation, and that this impairment is mediated by the CB1R (Cass *et al.*, 2014). As we have discovered similar suppression of oscillations after chronic exposure to either WIN or THC (Raver *et al.*, 2013)— two structurally different cannabinoids that both act as CB1R agonists — we hypothesize that CB1Rs may

be responsible for these chronic effects as well. However, as WIN and THC have additional targets including CB2Rs (Showalter *et al.*, 1996), other G-protein coupled receptors (Breivogel *et al.*, 2001; Ryberg *et al.*, 2007), and non-cannabinoid receptor targets (Pertwee, 2008; Pertwee, 2010), it is possible that non-CB1Rs may underlie suppression of oscillations in adult mice.

Here we test the hypothesis that suppression of cortical oscillations in adult neocortex by adolescent cannabinoid exposure is mediated by CB1Rs. We used a well-established *in vitro* method to evoke robust oscillations in cortical networks (Buhl *et al.*, 1998; Oke *et al.*, 2010; Holderith *et al.*, 2011), as previously reported (Raver *et al.*, 2013). This method—kainic acid (KA) and carbachol (CCh) administration—provides a uniform stimulus to all tissue, and reliably evokes oscillations in the beta (13–29 Hz) and gamma (30–80 Hz) bandwidths, while also increasing oscillatory power at lower frequencies (theta: 4–7 Hz; alpha: 8–12 Hz). These pharmacologically-evoked oscillations strongly resemble network synchrony in the intact neocortex (Steriade *et al.*, 1996), and *in vitro* oscillations evoked with similar pharmacological methods are suppressed by acute (Hajos *et al.*, 2000; Holderith *et al.*, 2011) and chronic exposure to CB1R/CB2R agonists (Raver *et al.*, 2013). Our results indicate that the CB1R dependence of oscillation suppression in adult mice after repeated cannabinoid exposure during adolescence varies according to the duration of adolescent treatment, the identity of the cannabinoid administered, and the cortical region examined.

2: Materials and Methods

2.1. Animals

All experiments were performed according to the University of Maryland School of Medicine Institutional Animal Use and Care Committee protocols, and in compliance with the National Institute of Health guide for the care and use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used. Male CD-1 mice (Harlan Laboratories, Inc. Frederick, MD, USA) were obtained at postnatal day 30 (P30) and were treated with a variety of cannabinoid compounds, or a vehicle solution, via intraperitoneal (i.p.) injection. Treatment was administered for 20 days during adolescence (from P35–P55), or for 6 days during early (P35–P40) or late (P47–P52) adolescence (Figure 1A). After the last injection, animals were left undisturbed until adulthood (> P80). Animals assigned to groups in which the effects of antagonist + agonist administration were tested were given 2 separate injections, spaced 20–30 minutes apart, with the antagonist administered first. In antagonist alone groups, these injections were a) antagonist + vehicle; in agonist alone groups, they were b) vehicle + agonist; and in antagonist + agonist groups, animals received injections of c) antagonist + agonist. All injections were titrated such that total injection volume was 1 mL/kg.

2.2. Drugs

The CB1R/CB2R agonist WIN55,212–2 (1 or 2 mg/kg; Sigma Aldrich, St. Louis, MO, USA), the CB1R inverse agonist/antagonist AM251 (0.3, 0.5, 1, or 2 mg/kg), the CB1R/CB2R agonist ⁹tetrahydrocannabinol (THC; 5 mg/kg; National Institute on Drug Abuse Drug Supply Program, Bethesda, MD, USA), and the putative CB1R-inactive enantiomer

WIN55,212-3 were dissolved in 100% ethanol and administered in a 1:1:18 solution of ethanol : Emulphor (Alkamuls EL-620, Rhodia Chemicals, USA) : 0.9% saline at a final volume of 1 mL/kg. Control animals were injected with vehicle (1:1:18 ethanol: Emulphor: saline). The CB1R neutral antagonist AM4113 (1 mg/kg; a generous gift from Dr. Alexandros Makriyannis, Northeastern University, Boston, MA) and the CB2R inverse agonist/antagonist AM630 (1 mg/kg; Tocris Bioscience, Bristol, UK) were dissolved in 1:1 dimethyl sulfoxide (DMSO) : 100% ethanol with sonication, and were injected in a 0.5:0.5:1:18 solution of DMSO solution: 100% ethanol: Emulphor: 0.9% saline. A separate cohort of control animals were injected with the DMSO-containing vehicle (0.5:0.5:1:18 DMSO: ethanol: Emulphor: saline) and were compared to the 1:1:18 vehicle animals to ensure that DMSO had no effect on its own. Pharmacologically-evoked cortical oscillations recorded *in vitro* did not differ significantly between DMSO-containing vehicle-administered mice and those exposed to the non-DMSO containing vehicle (data not shown). Therefore, we used only 1:1:18 (ethanol: Emulphor: saline) administered animals for our vehicle control population.

Doses of antagonist were chosen from the literature (Guindon *et al.*, 2007; McMahon and Koek, 2007; Jarbe *et al.*, 2008) and from preliminary studies in our lab that indicate antagonism of behavioral effects of 5 mg/kg WIN — a dose 2.5 and 5 times higher than what we repeatedly administered to adolescent mice — by 1 mg/kg AM251. Throughout the Methods and Results sections, AM251 (a CB1R inverse agonist/antagonist), AM4113 (a neutral CB1R antagonist), and AM630 (a CB2R inverse agonist/antagonist) will be classified as “antagonists,” and their respective mechanisms of antagonism will be discussed later. Kainic acid (KA) was dissolved in normal artificial cerebrospinal fluid (ACSF; recipe described below) at a concentration of 1 mM. The dissolved KA solution was brought to pH 7.4 by adding 1M NaOH, 1 μ L at a time. Carbachol (CCh) was dissolved in MQ pure water at a concentration of 10 mM.

2.3. *In vitro* Slice Preparation and LFP Recordings

Experiments were performed by experimenters blind to the adolescent treatment condition of the animals. Adult male mice (> P80) were deeply anesthetized with ketamine (100 mg/kg) and rapidly decapitated. Two or three 400 μ m-thick coronal sections were cut from each cortical region: medial prefrontal cortex (mPFC; ~ 1.3 – 2.3 mm anterior to bregma) and primary somatosensory cortex (SCx; ~1.0 – 2.1 mm posterior to bregma) from either the left or the right hemisphere, using a microtome (Integraslice 7550MM, Campden Instruments, IN, USA). During brain extraction and cutting, tissue was immersed in ice-cold or 4°C thermoregulated ACSF, respectively, containing (in mM): 26 NaHCO₃, 5 BES, 15 glucose, 200 sucrose, 3 KCL, 1.5 MgSO₄, 2 CaCl₂ (sucrose ACSF). Slices were then incubated for 30 minutes at 36°C, followed by 30 minutes at 22°C in ACSF containing (in mM): 120 NaCl, 25 NaHCO₃, 5 BES, 15 glucose, 3 KCl, 1.3 MgSO₄, 2 CaCl₂ (normal ACSF). Throughout cutting, recovery, and recording, slices were continually saturated with 95% O₂/5% CO₂. Slices were maintained at 36°C in an interface-type recording chamber and were perfused at 0.7 mL/min with ACSF containing (in mM): 120 NaCl, 25 NaHCO₃, 5 BES, 15 glucose, 5 KCl, 1.3 MgSO₄, 2 CaCl₂ (High K⁺ ACSF). Kainic acid (KA; 400 nM) and carbachol (CCh; 20 μ M) were added to to reliably evoke robust beta (13–29 Hz) and

gamma (30–80 Hz) oscillations (Buhl *et al.*, 1998; Oke *et al.*, 2010; Raver *et al.*, 2013) that strongly resemble network activity in the intact neocortex (Steriade *et al.*, 1996). We employed this *in vitro* pharmacological approach to provide a uniform stimulus to all samples. Local field potential (LFP) recordings were made through a glass pipette filled with normal ACSF (impedance ~0.5–1 M Ω) with an ER-1 amplifier (Cygnus Technology, USA), sampled at 5 kHz, filtered between 0.1 and 1 KHz, and stored on an Apple computer using Igor Pro (Version 6.1, Wavemetrics, Portland, OR, USA). LFPs were recorded from cortical layers II/III of the prelimbic area (PL) of mPFC, and layers II/III in the barrel field of SCx after at least 45 minutes of KA + CCh perfusion. Example KA + CCh evoked LFP oscillations are presented in Figure 1. These LFP recordings from adult animals treated in adolescence with a vehicle solution show that, in baseline conditions (ACSF alone), there is little oscillatory activity in either mPFC or SCx (Figures 1B&C). Robust oscillatory activity is evoked in slices containing mPFC (Figure 1B) or SCx (Figure 1C) during perfusion of KA + CCh.

2.4. *In vitro* LFP Data Analysis

Data were analyzed by experimenters blind to the treatment condition of the animals. We analyzed *in vitro* LFP data with custom-written Igor Pro scripts. Discrete fast Fourier transforms (FFTs) were performed on 10 seconds of LFP data and oscillation power (area under the FFT curve) was integrated at different frequencies [theta (θ 4–7 Hz); alpha (α 8–12 Hz); beta (β ; 13–29 Hz); gamma (γ 30–80 Hz)]. Designation of frequency bandwidths is based on previous reports (Buhl *et al.*, 1998; Uhlhaas and Singer, 2010). Example FFTs of LFPs from the mPFC and SCx of adolescent vehicle-treated animals are presented in Figure 1B and C, respectively. KA + CCh evokes robust oscillations *in vitro* in both cortical areas, as indicated by higher power at all frequencies in the presence of KA + CCh relative to baseline conditions, and peaks of oscillation power in the beta and gamma bandwidth in mPFC (Figure 1B), and in the gamma frequency range in SCx (Figure 1C). Average power in each frequency bandwidth was calculated for vehicle-administered mice and was used to normalize all other oscillation power values in the different treatment conditions. Two or three slices of mPFC and SCx were collected for each animal, and oscillation power in each bandwidth was normalized to the average oscillation power in that frequency bandwidth for vehicle-treated mice. Four normalized values (theta, alpha, beta, gamma) were obtained from each LFP (2–3 LFPs per animal, one per slice) and were combined for each treatment condition. This allowed us to reassemble the entire oscillation frequency spectrum, to test whether antagonists altered normalized oscillation power compared to vehicle or WIN/THC. Statistical analyses were performed with STATA (Version 12, StataCorp, College Station, TX, USA). Data were tested for normality, normalized oscillation power values were plotted with cumulative probability distributions and were analyzed with non-parametric Kolmogorov-Smirnoff (KS) tests (significant $p < 0.05$) to examine the effect of adolescent cannabinoid exposure on cortical oscillations in adulthood. The KS test statistic represents the distance between distributions of normalized oscillation power in animals treated with different cannabinoid compounds, or a vehicle solution. A KS test statistic that fails to reach statistical significance ($p > 0.05$) indicates that the distributions of normalized oscillation power overlap, and, therefore, that the cannabinoid treatments do not significantly alter the power of pharmacologically-evoked cortical oscillations relative to each other, or relative to

oscillations in vehicle-treated animals. A KS test statistic that reaches statistical significance ($p < 0.05$) indicates that the distributions of normalized oscillation power from two different treatments are distinct. A significant leftward shift of one distribution relative to another (Figure 2B, for example) represents an attenuation of oscillation power, as a greater proportion of oscillations in the leftward-shifted treatment occur with lower power.

3: Results

3.1. WIN exposure during early, but not late adolescence permanently suppresses cortical oscillations through CB1Rs

We tested the hypothesis that adolescent cannabinoid exposure suppresses pharmacologically-evoked oscillations in the adult neocortex by acting through CB1Rs. We predicted that WIN exposure in early adolescence (P35–P40) would attenuate oscillations in adult mice, and that this suppression would be mediated by the CB1R, and therefore antagonized by co-administration of the CB1R antagonist AM251. We also predicted that these suppressive effects would be more pronounced after WIN exposure early in adolescence than after similar exposure late in adolescence (P47–P52) (Figure 1A), due to more active development of the cortical eCB system earlier in adolescence (Heng *et al.*, 2011; Cass *et al.*, 2014).

Male CD-1 mice were treated with vehicle, WIN (2 mg/kg), AM251 (2 mg/kg) or AM251 + WIN for six days, from P35–P40 (Figure 2A), and oscillations were pharmacologically evoked *in vitro* with KA + CCh in adulthood (Figure 1B&C). In support of our prediction, WIN exposure during early adolescence significantly suppressed oscillations in adult mPFC, relative to vehicle control levels (Figure 2B, Table 1A). This is apparent in the significant leftward shift of the normalized distribution of oscillation power by WIN from that of vehicle (Figure 2B). We found no significant effect of AM251 administration alone (Figure 2C, Table 1A). Oscillation power in adult mice exposed to AM251 + WIN in early adolescence was significantly higher than in those exposed to WIN alone, and equal to oscillation power in animals treated with vehicle (Figure 2D, Table 1A), indicating that AM251 prevented the effects of early adolescent WIN exposure in mPFC. These data suggest that early adolescent WIN exposure suppresses oscillations in mPFC by acting on CB1Rs. SCx is less sensitive to the effects of early adolescent cannabinoid exposure, as neither WIN nor AM251 altered pharmacologically-evoked cortical oscillations when administered from P35–P40 (Figures 2E&F, Table 1B).

We further tested the temporal window of cortical oscillation sensitivity to adolescent cannabinoid exposure by restricting WIN exposure to 6 days *late in adolescence*, from P47–P52 (Figure 1A). In mPFC, neither WIN nor AM251 exposure in the late adolescent period significantly altered the power of pharmacologically-evoked oscillations, relative to vehicle levels (Table 1A). Similarly, late adolescent WIN or AM251 exposure had no effect on oscillations in SCx (Table 1B). These data further support a gradient of adolescent sensitivity to exogenous cannabinoid exposure that parallels the development of the adolescent cortical eCB system (Eggan *et al.*, 2010; Heng *et al.*, 2011; Lee *et al.*, 2013), and complement a previous report from our laboratory indicating that cortical regions that are

less mature at the time of adolescent cannabinoid exposure display the greatest suppressive effects (Raver *et al.*, 2013).

3.2. Long-term adolescent THC exposure permanently suppresses pharmacologically-evoked cortical oscillations *in vitro* in SCx through CB1Rs

We have recently reported that a long-term, 20 day exposure to THC and WIN in adolescence significantly suppresses pharmacologically-evoked oscillations in adult mPFC and SCx (Raver *et al.*, 2013). We tested the hypothesis that these effects of long-duration cannabinoid administration are mediated by CB1R activation. We predicted that this suppression could be antagonized by long-term exposure to the CB1R antagonist AM251. Adolescent mice were exposed to THC (5 mg/kg), AM251 (0.3 or 0.5 mg/kg), AM251 + THC, or vehicle from P35–P55, and cortical oscillations were pharmacologically evoked in mPFC and SCx once animals reached adulthood (Figure 3A). Statistical summaries of results from mPFC are presented in Table 2, and summaries of results from SCx are presented in Table 3. Long-term adolescent THC exposure significantly suppressed the power of pharmacologically-evoked oscillations in both adult mPFC (Figure 3B, Table 2) and SCx (Figure 3D, Table 3). To test whether the CB1R antagonist AM251 can prevent these suppressive effects, we first analyzed oscillations in animals administered AM251 alone, as our data and that from other groups predict no effect on oscillations of either acute (Robbe *et al.*, 2006; Holderith *et al.*, 2011; Sales-Carbonell *et al.*, 2013) or short-term CB1R antagonist exposures (Figure 2C&F, Table 1) (Cass *et al.*, 2014). Surprisingly, in mPFC, long-term adolescent treatment with both 0.3 and 0.5 mg/kg AM251 significantly suppressed the power of oscillations relative to vehicle levels (Figure 3B, Table 2). We therefore reasoned that we cannot use these doses of AM251 to try to prevent the chronic effects of THC in mPFC, as AM251 produced the same phenotype as THC exposure.

Pharmacologically-evoked oscillations in SCx were insensitive to long-term exposure to either 0.3 or 0.5 mg/kg AM251 during adolescence (Figure 3E, Table 3), and this allowed us to test the prediction that these doses of AM251 could attenuate the suppression of oscillation power by THC. AM251 (0.3 mg/kg) paired with THC prevented oscillation suppression by adolescent THC exposure (Figure 3F, Table 3). Oscillation power in animals treated with 0.3 mg/kg AM251 + 5 mg/kg THC was significantly higher than in mice exposed to THC alone, and was equal to vehicle power (Figure 3F, Table 3), indicating a full attenuation of oscillation suppression by CB1R antagonism. AM251 (0.5 mg/kg) + THC (5 mg/kg) partially attenuated the effects of THC. Oscillation power in SCx of animals treated with 0.5 mg/kg AM251 + THC fell between that of animals exposed to THC or vehicle, and was therefore statistically indistinguishable from either THC or vehicle power (Figure 3G, Table 3). The full attenuation of THC's oscillation suppression by 0.3 mg/kg AM251, and the partial reversal by 0.5 mg/kg AM251, indicate that chronic adolescent THC suppresses oscillations in SCx by acting on CB1Rs.

3.3. Long-term adolescent CB1R antagonist (AM251) exposure permanently suppresses pharmacologically-evoked cortical oscillations *in vitro* in mPFC and SCx

We tested the prediction that long-term adolescent WIN exposure suppresses pharmacologically-evoked oscillations in adult neocortex by acting on CB1Rs. Adolescent

mice were exposed to WIN (5 mg/kg), AM251 (1 mg/kg), AM251 + WIN, or vehicle from P35–P55, and cortical oscillations were evoked in adult animals, as described above (Figure 4A). Long-term adolescent exposure to WIN (1 mg/kg) significantly suppressed the power of pharmacologically-evoked oscillations in both mPFC (Figure 4B, Table 2) and SCx (Figure 4D, Table 3). We analyzed oscillations in animals exposed to AM251 (1 mg/kg) (Figure 4A) to test the prediction that AM251 has no effect on its own in mPFC. Animals were exposed to a slightly higher dose of AM251 in this study than what was used in the THC study (1 mg/kg vs. 0.3 or 0.5 mg/kg) because WIN is a full CB1R agonist, whereas THC displays partial agonist activity at the CB1R (Pertwee, 2008; Pertwee, 2010). Therefore, we predicted that a higher dose of AM251 would be necessary to antagonize WIN's effects. Oscillations in mice chronically exposed to 1 mg/kg AM251 during adolescence were significantly suppressed in both mPFC (Figure 4B, Table 2) and SCx (Figure 4D, Table 3). We therefore reasoned that we could not use AM251 at this dose to try to antagonize the effects of chronic WIN exposure, as exposure to the antagonist alone mimicked the effects seen with WIN.

3.4. Long-term adolescent CB1R antagonist (AM4113) exposure permanently suppresses pharmacologically-evoked cortical oscillations *in vitro* in mPFC, but not SCx, and does not reverse WIN's effects in SCx

AM251 can antagonize CB1R mediated effects of cannabinoid agonist through its action as an inverse agonist. Acting as such, AM251 both blocks the activity of endogenous and exogenous CB1R agonists and decreases the constitutive activity of CB1Rs (Pertwee, 2005). Therefore, we tested the prediction that the neutral CB1R antagonist AM4113 would antagonize WIN's effects without altering oscillations on its own, since a neutral antagonist should not affect constitutive CB1R activity, but only block CB1R agonist action (Jarbe *et al.*, 2008).

However, as was the case with AM251, long-term adolescent administration of the neutral CB1R antagonist AM4113 alone (1 mg/kg) (Figure 5A) significantly suppressed oscillations in mPFC, relative to vehicle-treated animals (Figure 5B, Table 2). We again reasoned that we could not use AM4113 to try to antagonize chronic WIN's oscillation suppression in mPFC, as it produces the same phenotype as WIN alone. In SCx, AM4113 (1 mg/kg) did not alter oscillation power when administered alone (Figure 5D, Table 3), which allowed us to test the prediction that AM4113 could attenuate WIN's suppression. This was not the case. Chronic adolescent administration of AM4113 did not attenuate the significant oscillation suppression induced by WIN in SCx, but, rather, further suppressed power relative to both vehicle and WIN levels (Figure 5E, Table 3).

3.5. Long-term adolescent CB2R antagonist (AM630) exposure permanently suppresses pharmacologically-evoked cortical oscillations *in vitro* in mPFC and SCx

While WIN acts as a full agonist at the CB1R, it also has high affinity for CB2Rs (Showalter *et al.*, 1996; Pertwee, 2010). Although CB2R expression is thought to be confined to the peripheral tissues, mounting evidence indicates the presence of neuronal and glial CB2Rs in the CNS (Gong *et al.*, 2006; Onaivi *et al.*, 2006; Atwood and Mackie, 2010). Therefore, we

tested the prediction that the CB2R antagonist, AM630, would not alter the response to WIN, if WIN suppressed oscillations exclusively via CB1Rs (Figure 5A).

As above, the requisite experiment is predicated on AM630 alone not having lasting effects on cortical oscillations. However, long-term adolescent AM630 exposure alone significantly suppressed oscillations in adult mPFC (Figure 5C, Table 2) and SCx (Figure 5F, Table 3). We were therefore unable to test whether AM630 can antagonize WIN's effects, as AM630 produced similar results to those seen with WIN. We were also unable to exclude the possibility that CB2Rs may contribute to the effects of WIN, as a CB2R antagonist mimicked WIN's effects.

3.6. Long-term adolescent exposure to a compound with putative CB1R inactivity (WIN-3) persistently suppresses pharmacologically-evoked cortical oscillations *in vitro* in mPFC, but not SCx

Thus far, we have shown that the CB1R/CB2R agonists WIN and THC, a CB1R inverse agonist/antagonist (AM251), a CB1R neutral antagonist (AM4113), and a CB2R inverse agonist/antagonist (AM630) suppress oscillations in adult neocortex when chronically administered during adolescence (for 20 days), with milder effects in SCx than mPFC. To further test whether these suppressive effects are specific to compounds with CB1R or CB2R activity, we exposed adolescent animals to a compound that is presumed to be inactive at cannabinoid receptors (Figure 6A). The chemical enantiomer of WIN55,212–2 (WIN)—WIN55,212-3 (WIN-3)—has been described to be inactive at the CB1R at low concentrations (Pacheco *et al.*, 1991; Felder *et al.*, 1992). If the oscillation suppressing effects of chronic adolescent WIN are due to its action at cannabinoid receptors, we predict that exposure to WIN-3 would have no effect on cortical oscillations.

Unexpectedly, chronic exposure to WIN-3 during adolescence significantly suppressed the power of oscillations in adult mPFC, relative to those recorded in vehicle-treated adult mice (Figure 6B, Table 2). In agreement with our prediction, we found no effect of adolescent WIN-3 on oscillations recorded in adult SCx (Figure 6C, Table 3), indicating that WIN-3's lack of effect in SCx was due to its predicted mechanism as an inactive enantiomer. However, WIN-3 may suppress oscillations in mPFC via a non-CB1R mechanism. Therefore, while short-term (6 day) adolescent and acute WIN exposure suppress oscillations through CB1Rs, it appears that chronically administered WIN in adolescence may exert these suppressive effects in part through CB2Rs, or via non-cannabinoid receptor targets.

4. Discussion

4.1. Overall Summary

Here we test the hypothesis that repeated cannabinoid exposure during adolescence permanently suppresses oscillations in adult neocortex by acting at CB1Rs. A short period of exposure (6 days) to the potent, synthetic cannabinoid WIN during early adolescence suppresses oscillations in mPFC, but not SCx, and this effect is mediated by CB1Rs. The same administration paradigm late in adolescence does not alter oscillations in either cortical region. The early adolescent sensitivity of mPFC to CB1R activation that we describe

parallels recent work demonstrating a selective impairment of LFP modulation in the adult mPFC that is induced by short-term WIN exposure during early and mid, but not late, adolescence, and is attenuated by the CB1R antagonist AM251 (Cass *et al.*, 2014). Data from adult SCx also support the hypothesis of a CB1R-mediated effect, as oscillation suppression by long-term adolescent THC exposure (20 days) is restored to vehicle levels by low doses of AM251 that do not produce effects on their own. Multiple attempts to test the prediction that mPFC exhibits similar CB1R-mediated suppression of oscillations were inconclusive, as CB1R or CB2R antagonists, at all doses attempted, suppressed oscillations in mPFC when administered alone.

4.2. CB1R modulation of cortical oscillations

Kainic acid and carbachol can evoke robust beta (13–29 Hz) and gamma (30–80 Hz) oscillations *in vitro*, in isolated hippocampal or cortical preparations. These drugs act by enhancing excitatory drive (Buhl *et al.*, 1998; Cunningham *et al.*, 2003) and by activating cholinergic receptors, primarily on GABAergic interneurons (Fisahn *et al.*, 1998; Gulyas *et al.*, 2010). In certain hippocampal circuits CCh can generate theta (4–7 Hz) rhythms by acting solely on inhibitory interneurons, when excitatory transmission is blocked (Nagode *et al.*, 2011; Nagode *et al.*, 2014). Experiments performed in the CA3 region of the hippocampus suggest that *acute* cannabinoid exposure suppresses pharmacologically-evoked gamma oscillations *in vitro* by acting at CB1Rs expressed on glutamatergic terminals (Holderith *et al.*, 2011). This action may preferentially attenuate the frequency and firing precision of fast-spiking GABAergic interneurons that are crucial for sculpting oscillations in the gamma bandwidth (Cardin *et al.*, 2009), resulting in smaller and less synchronized field potential activity (Holderith *et al.*, 2011) that manifests as reduced oscillation power. A similar mechanism may underlie the CB1R-mediated suppression of oscillations that we find in SCx after long-term adolescent THC exposure, and in mPFC after early adolescent WIN exposure. However, the mechanism by which acute cannabinoid administration suppresses high-frequency oscillations in the neocortex is less clear. Genetic ablation of CB1Rs from glutamatergic neurons does not completely prevent the suppression of cortical oscillations faster than 12 Hz by acute cannabinoid exposure (Sales-Carbonell *et al.*, 2013), as would be expected if cannabinoid-induced suppression of neocortical oscillations is mediated by a similar excitatory-CB1R mechanism as in CA3 hippocampus (Holderith *et al.*, 2011). Furthermore, while activation of CB1Rs on inhibitory interneurons may not contribute to oscillation suppression by CB1R agonists in CA3 hippocampus *in vitro* (Holderith *et al.*, 2011), removal of CB1Rs from all GABAergic forebrain neurons *increases* cannabinoid agonist-mediated suppression of oscillation power in cortical oscillations *in vivo* (Sales-Carbonell *et al.*, 2013), suggesting a potential bi-directional regulation of oscillatory activity by CB1Rs at glutamatergic and GABAergic neurons. Interestingly, in the CA1 region of the hippocampus, CB1R-positive interneurons can generate theta oscillations (~4–7 Hz) when driven by CCh when excitatory neurotransmission is blocked, and these rhythms are completely abolished by exogenous or endogenous CB1R agonist activity (Nagode *et al.*, 2011; Nagode *et al.*, 2014), revealing a role for inhibitory-localized CB1Rs in pharmacologically-evoked oscillation suppression. Further tests of the contribution of CB1Rs expressed by excitatory or inhibitory neuronal populations to adolescent cannabinoid-induced suppression of cortical oscillations are necessary, and could be

achieved by using transgenic mouse strains lacking CB1Rs in specific neuronal populations. Such experiments may help to clarify whether cannabinoid activity attenuates cortical oscillations through similar mechanisms as in hippocampal networks, or whether there are alternative mechanisms at work in the neocortex.

4.3. Preferential sensitivity of rostral mPFC vs. caudal SCx

In agreement with our recent report (Raver *et al.*, 2013), we find that the adolescent mPFC is highly sensitive to manipulation of the eCB system. Long-term exposure to CB1R/CB2R agonists, CB1R antagonists, a CB2R antagonist, and a putative CB1R inactive enantiomer of WIN (WIN-3) all significantly attenuate pharmacologically-evoked oscillations *in vitro* in adult mPFC. We find that the SCx is less sensitive to adolescent cannabinoid exposure, in parallel with the caudal-to-rostral gradient of cortical and eCB system development (Gogtay *et al.*, 2004; Heng *et al.*, 2011). Oscillations in adult SCx, unlike in mPFC, are unaffected by either lower dose of the CB1R inverse agonist/antagonist AM251, or by the CB1R neutral antagonist AM4113. Furthermore, while a shorter period of cannabinoid exposure during early adolescence significantly suppresses oscillations in adult mPFC, oscillations in adult SCx are unaffected. Although this caudal-to-rostral gradient of cortical sensitivity probably reflects the prolonged adolescent and early adult maturation of prefrontal cortical circuitry (Gogtay *et al.*, 2004) and network oscillations (Uhlhaas and Singer, 2011), it may be somewhat specific to cannabinoid exposure. The density of CB1Rs declines more dramatically from juvenile to adult ages in rostral, associative cortical areas than in caudal, primary sensory cortical regions (Heng *et al.*, 2011), and expression of the CB1R is significantly higher in mPFC than in SCx from juvenile to adult ages (Heng *et al.*, 2011) (Dr. K.Y. Tseng, personal communication). This gradient of CB1R expression may underlie the sensitivity of the mPFC to chronic adolescent cannabinoid exposure that we report, as well as the preferential suppression of oscillations in mPFC by short WIN treatment in early adolescence.

4.4. CB1R antagonism and cortical oscillations

We are surprised to discover that long-term adolescent exposure to the CB1R antagonists AM251 or AM4113, an inverse agonist and a neutral antagonist, respectively, significantly attenuate the power of oscillations in adult mPFC. Although several previous studies have shown that oscillation suppression by *acute* CB1R agonist exposure is reversed with co-administration of a CB1R antagonist (Hajos *et al.*, 2000; Robbe *et al.*, 2006; Hajos *et al.*, 2008; Holderith *et al.*, 2011; Sales-Carbonell *et al.*, 2013), there have been few tests of whether these agents modulate oscillatory activity by themselves. Such studies often show that acute administration of CB1R inverse agonists do not affect oscillation power (Robbe *et al.*, 2006; Holderith *et al.*, 2011; Sales-Carbonell *et al.*, 2013), leading to the conclusion that tonic eCB activity does not contribute to the generation of oscillations, at least those generated pharmacologically (Gulyas *et al.*, 2010). However, there exists ample evidence to the contrary (Kim *et al.*, 2002; Fortin *et al.*, 2004; Nagode *et al.*, 2014), as eCBs have been shown to be released by exposure to CCh at doses similar to what we have used, and these eCBs potently suppress pharmacologically-evoked oscillations by acting at CB1Rs (Nagode *et al.*, 2014). Our data suggest a role for tonic eCB activity during adolescence, as long-term

blockade of CB1Rs in adolescence with AM251 or AM4113 leads to a persistent suppression of oscillation power in the adult neocortex.

While we find it surprising that exposure to cannabinoid receptor antagonists recapitulates the effects seen with agonist administration, these effects are not unprecedented (Manzanedo *et al.*, 2010). Indeed, chronic administration of CB1R antagonists can induce lasting changes in CB1R distribution and eCB levels (Castelli *et al.*, 2007; Guidali *et al.*, 2011), reminiscent of those seen after repeated CB1R agonist administration (Hunter and Burstein, 1997; Romero *et al.*, 1997; Sim-Selley and Martin, 2002; Rubino and Parolaro, 2008).

Through its action as a CB1R inverse agonist, AM251 modulates tonic CB1R activity by both reducing the receptor's constitutive activity in the absence of an agonist, and by occluding the action of eCBs (Pertwee, 2005). AM4113 also interferes with endogenous CB1R agonist activity, but it lacks the ability to modulate constitutive CB1R signaling (Chambers *et al.*, 2007; Sink *et al.*, 2008). Therefore, similar results produced by both AM251 and AM4113 in mPFC suggest that chronic interference with ongoing CB1R activity during adolescence—such as antagonism or displacement of eCBs at the CB1R—impairs the ability of the adult mPFC to generate pharmacologically-evoked oscillations. Further tests of this hypothesis could be performed by chronically administering indirect cannabinoid agonists that prevent the synthesis or breakdown of eCBs, and would therefore interfere with tonic eCB signaling in adolescence.

4.5. Non-CB1R contribution to oscillation suppression

In SCx, we find that long-term adolescent exposure to the neutral CB1R antagonist AM4113 alone does not alter oscillations in adults, but does not reverse long-term adolescent WIN-induced oscillation suppression. Surprisingly, AM4113 paired with WIN suppresses oscillation power further from that seen with WIN alone, suggesting that AM4113 and WIN may act synergistically and suppress oscillations, or act at different targets. Because WIN has potent CB2R agonist activity in addition to its action as CB1Rs (Showalter *et al.*, 1996; Atwood *et al.*, 2012), we posit that CB2Rs may contribute to oscillation suppression by WIN. CB2Rs are expressed in both mPFC and SCx (Gong *et al.*, 2006; Onaivi *et al.*, 2006), positioning them to contribute to the phenotype we observe. This hypothesis of a CB2R contribution is supported by our data showing a significant suppression of oscillations in adult mPFC and SCx by the CB2R antagonist AM630. Although centrally expressed CB2Rs are increasingly implicated in diverse functions, including cannabinoid-mediated memory impairments and drug reward and reinforcement (Atwood and Mackie, 2010; Xi *et al.*, 2011; Garcia-Gutierrez *et al.*, 2013), to date, little information exists regarding a possible role of the CB2R in network activity or oscillations. Interestingly, in entorhinal cortex, CB2R activation suppresses GABAergic inhibition that is not occluded by CB1R antagonism (Morgan *et al.*, 2009), revealing the functional expression of CB2Rs at central inhibitory synapses that may contribute to oscillation generation. Additional tests of a possible contribution of CB2Rs to oscillations are warranted.

Further evidence that non-CB1Rs may contribute to oscillation suppression in mPFC by long-term adolescent cannabinoid exposure comes from experiments with the putative inactive enantiomer of WIN, WIN-3. We find that WIN-3 unexpectedly and significantly

attenuates oscillations in mPFC, but has no effect in SCx. Although converging evidence of mPFC oscillation suppression by chronic adolescent exposure to CB1R/CB2R agonists, CB1R antagonists, and a CB2R antagonist suggest the involvement of CB1Rs and/or CB2Rs in this suppression, similar suppression by WIN-3 suggest that the effects of WIN may be, at least in part, cannabinoid receptor independent. Indeed, previous reports reveal that WIN-3 can produce identical effects as WIN that are not mediated by CB1Rs (Price *et al.*, 2004; Price *et al.*, 2007; Nemeth *et al.*, 2008). Our data present the possibility that long-term adolescent WIN exposure may affect the development of cortical oscillations through non-CB1R/CB2R mechanisms (Pertwee, 2010). These mechanisms may involve the non-CB1R/CB2R putative cannabinoid receptor GPR55 (Ryberg *et al.*, 2007), the TRPV-1 like receptors (Pertwee, 2006) that modulate glutamate release, as well as allosteric modulation of receptors for other neurotransmitters (Barann *et al.*, 2002), or direct modulation of cation permeable channels (Shen and Thayer, 1998).

5. Conclusions

Our experiments reveal that repeated exposure to cannabinoids in adolescence permanently suppresses pharmacologically-evoked oscillations in the adult neocortex, in part through CB1Rs, with evidence for the involvement of CB2Rs and non-cannabinoid receptors. The rostral medial prefrontal cortex (mPFC) is more sensitive to adolescent cannabinoid exposure, compared to the caudal, primary somatosensory cortex (SCx). A short period of exposure to the potent, synthetic CB1R/CB2R agonist WIN during the early adolescent period, compared to later in adolescence, suppresses oscillations selectively in mPFC that can be antagonized with a CB1R antagonist. Long-term adolescent exposure to THC, the primary psychoactive ingredient in marijuana, suppresses oscillations in the caudal SCx through a similar CB1R mediated mechanism. Multiple attempts to test the CB1R dependence of the oscillation suppressing effects of long-term WIN exposure reveal that *antagonism* of the CB1R or CB2R produces a similar suppression of oscillation power as seen after long-term exposure to CB1R/CB2R agonists. Similarly, long-term adolescent exposure to a compound presumed to be inactive at CBRs (WIN-3) significantly attenuates oscillations in adult mPFC. These data reveal a novel potential contribution of CB2Rs or non-cannabinoid receptors to the generation of pharmacologically-evoked cortical oscillations. Our findings are relevant to the current public discussion regarding the legalization and growing public acceptance of recreational marijuana use and its availability to teenagers, and the use of potent, synthetic CB1R-targeting cannabinoids such as “K2” and “spice” in adolescents (Atwood *et al.*, 2010; Hu *et al.*, 2011).

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Highlights

- Early-adolescent WIN exposure suppresses oscillations in adult mPFC.
- The suppression of cortical oscillations occurs through activation of CB1Rs.
- Long-term adolescent THC exposure suppresses oscillations in adult SCx through CB1Rs.
- Oscillation suppression by adolescent cannabinoids also involves CB2Rs and non-CBRs.

Figure 1. Kainic acid (KA) and carbachol (CCh) evoke robust oscillations *in vitro* in adult neocortex

(A) Experimental time course: comparisons of human and rodent development are adapted from (Andersen, 2003). Cannabinoids were administered to mice either for 6 days during early (P35–P40) or late adolescence (P47–P52), or for 20 days from P35–P55. LFPs were recorded *in vitro* from adult mice (>P80). (B) One-second example of LFPs recorded *in vitro* from mPFC of an adult mouse exposed to a vehicle solution from P35–P55. LFPs were recorded before (Baseline) and after perfusion of KA (400 nM) + CCh (20 μ M), which together evoke robust oscillations in LFPs. Oscillation power is quantified in the accompanying Fourier transform of 10-second LFP recordings. KA + CCh (black trace) increases power at all frequencies [theta (θ ; 4–7 Hz); alpha (α 8–12 Hz); beta (β ; 13–29 Hz); gamma (γ 30–80 Hz)] relative to baseline conditions (gray trace). (C) Similar to B, but example LFPs and Fourier transforms are from adult SCx. Note that KA + CCh evokes gamma oscillations in SCx that have approximately double the power as those recorded in mPFC.

Figure 2. Exposure to WIN in early adolescence suppresses oscillations *in vitro* in adult mPFC, but not SCx, via CB1Rs

(A) Experimental time course. Animals were injected with the CB1R/CB2R agonist WIN (2 mg/kg) or vehicle + the CB1R antagonist AM251 (2 mg/kg) in early adolescence (P35–P40) once daily for six days. LFPs were recorded *in vitro* in slices of adult mPFC (B–D) or SCx (E,F). (B,C) Cumulative probability distributions of normalized oscillation power plotted on a log scale from LFPs recorded in mPFC of adult mice treated with vehicle (solid line) or (B) WIN (dashed line) or (C) AM251 (dashed line) from P35–P40. Kolmogorov-Smirnoff tests were used to compare the effect of cannabinoid treatment on normalized oscillation

power. (D) Plots as in (B,C), except normalized power was compared between animals treated with WIN or AM251 + WIN, and between vehicle and AM251 + WIN with KS tests. (E,F) Plots as in (B,C) for LFPs recorded in SCx.

Figure 3. Long-term exposure to THC in adolescence suppresses oscillations *in vitro* in adult mPFC and via CB1Rs in SCx

(A) Experimental time course. Animals were injected with the CB1R/CB2R agonist THC (5 mg/kg) or vehicle + the CB1R antagonist AM251 (0.3 or 0.5 mg/kg) in adolescence (P35–P55) once daily for 20 days. LFPs were recorded *in vitro* in slices of adult mPFC (B,C) or SCx (D–G). (B, C) Cumulative probability distributions of normalized oscillation power plotted on a log scale from LFPs recorded in mPFC of adult mice treated with vehicle (solid line) or (B) THC (dashed line) or (C) AM251 (dashed line) from P35–P55. KS tests compared the effect of adolescent cannabinoid treatment on normalized oscillation power. (D,E) Plots as in (B,C) of LFPs from adult SCx. (F,G) Plots as in (D,E), except normalized power was compared between animals treated with THC or AM251 + THC [0.3 mg/kg AM251 in (F); 0.5 mg/kg AM251 in (G)], and between vehicle and AM251 + THC with KS tests.

Figure 4. Long-term exposure to WIN or AM251 in adolescence suppresses oscillations *in vitro* in adult mPFC and SCx

(A) Experimental time course. Animals were injected with the CB1R/CB2R agonist WIN (1 mg/kg) or vehicle + the CB1R antagonist AM251 (1 mg/kg) in adolescence (P35–P55) once daily for 20 days. LFPs were recorded *in vitro* in slices of adult mPFC (B,C) or SCx (D,E). (B,C) Cumulative probability distributions of normalized oscillation power plotted on a log scale from LFPs recorded in mPFC of adult mice treated with vehicle (solid line) or (B) WIN (dashed line) or (C) AM251 (dashed line) from P35–P55. KS tests compared the effect of adolescent cannabinoid treatment on normalized oscillation power. (D,E) Plots as in (B,C) of LFPs from adult SCx.

Figure 5. Long-term exposure to CB1R or CB2R antagonists during adolescence suppresses oscillations *in vitro* in adult mPFC, with milder effects in SCx

(A) Experimental time course. Animals were injected with the CB1R/CB2R agonist WIN (1 mg/kg) or vehicle + the CB1R antagonist AM4113 (1 mg/kg) or the CB2R antagonist AM630 (1 mg/kg) in adolescence (P35–P55) once daily for 20 days. LFPs were recorded *in vitro* in slices of adult mPFC (B,C) or SCx (D–F). (B,C) Cumulative probability distributions of normalized oscillation power plotted on a log scale from LFPs recorded in mPFC of adult mice treated with vehicle (solid line) or (B) AM4113 (dashed line) or (C) AM630 (dashed line) from P35–P55. KS tests compared the effect of adolescent cannabinoid treatment on normalized oscillation power. (D) Plot as in (B) of LFPs from adult SCx. (E) Plot as in (D), except normalized power was compared between animals treated with WIN or AM4113 + WIN, and between vehicle and AM4113 + WIN with KS tests. (F) Plot as in (C) of LFPs from adult SCx.

Figure 6. Long-term exposure to the putative inactive enantiomer WIN55,212-3 (WIN-3) in adolescence suppresses oscillations *in vitro* in adult mPFC, but not SCx
 (A) Experimental time course. Animals were injected with WIN-3 (1 mg/kg) or vehicle in adolescence (P35–P55) once daily for 20 days. LFPs were recorded *in vitro* in slices of adult mPFC (B) or SCx (C). Cumulative probability distributions of normalized oscillation power plotted on a log scale from LFPs recorded in adult mice treated with vehicle (solid line) or WIN-3 (dashed line) from P35–P55. KS tests compared the effect of adolescent WIN-3 treatment on normalized power.

Table 1
Summary of statistical analysis of early (P35–P40) or late adolescent (P47–P52)
cannabinoid effects on normalized oscillation power in adult neocortex

(A) In adult mPFC, effects of early or late-adolescent cannabinoid exposure were compared between treatment conditions with KS tests. Percentages reported are the median normalized LFP power in the treatment after “vs” relative to the median normalized LFP power in the treatment before “vs” in the statistical comparison.

(B) Same as in (A), but in adult SCx.

A	
Adolescent Cannabinoid Treatment	mPFC
Early Adolescent (P35–P40) WIN Treatment Vehicle (n = 11 slices/4 mice) vs. WIN (2 mg/kg) (n = 10 slices/4 mice)	p = 0.017, 52%
Early Adolescent (P35–P40) AM251 Treatment Vehicle (n = 11 slices/4 mice) vs. AM251 (2 mg/kg) (n = 13 slices/5 mice)	p = 0.707
Early Adolescent (P35–P40) AM251 + WIN Treatment WIN (2 mg/kg) (n = 10 slices/4 mice) vs. AM251 (2 mg/kg) + WIN (2 mg/kg) (n = 13 slices/5 mice) Vehicle (n = 11 slices/4 mice) vs. AM251 (2 mg/kg) + WIN (2 mg/kg) (n = 13 slices/5 mice)	p = 0.008, 194% p = 0.278
Late Adolescent (P47–P52) WIN Treatment Vehicle (n = 14 slices/5 mice) vs. WIN (2 mg/kg) (n = 13 slices/5 mice)	p = 0.057
Late Adolescent (P47–P52) AM251 Treatment Vehicle (n = 14 slices/5 mice) vs. AM251 (2 mg/kg) (n = 15 slices/5 mice)	p = 0.137

B	
Adolescent Cannabinoid Treatment	SCx
Early Adolescent (P35–P40) WIN Treatment Vehicle (n = 12 slices/4 mice) vs. WIN (2 mg/kg) (n = 11 slices/4 mice)	p = 0.104
Early Adolescent (P35–P40) AM251 Treatment Vehicle (n = 12 slices/4 mice) vs. AM251 (2 mg/kg) (n = 15 slices/5 mice)	p = 0.104
Late Adolescent (P47–P52) WIN Treatment Vehicle (n = 15 slices/5 mice) vs. WIN (2 mg/kg) (n = 15 slices/5 mice)	p = 0.064
Late Adolescent (P47–P52) AM251 Treatment Vehicle (n = 15 slices/5 mice) vs. AM251 (2 mg/kg) (n = 15 slices/5 mice)	p = 0.160

Table 2
Summary of statistical analysis of long-term adolescent (P35–P55) cannabinoid effects on normalized oscillation power in adult mPFC

Cannabinoid effects were compared between treatment conditions with KS tests. Percentages reported are the median normalized LFP power in the treatment after “vs” relative to the median normalized LFP power in the treatment before “vs” in the statistical comparison.

Long-Term Adolescent Cannabinoid Treatment	mPFC
Adolescent THC Treatment Vehicle (n = 60 slices/26 mice) vs. THC (5 mg/kg) (n = 21 slices/7 mice)	p < 0.001, 51%
Adolescent AM251 Treatment Vehicle (n = 60 slices/26 mice) vs. AM251 (0.3 mg/kg) (n = 12 slices/4 mice) Vehicle (n = 60 slices/26 mice) vs. AM251 (0.5 mg/kg) (n = 11 slices/4 mice) Vehicle (n = 60 slices/26 mice) vs. AM251 (1.0 mg/kg) (n = 7 slices/3 mice)	p < 0.001, 43% p < 0.001, 58% p = 0.010, 63%
Adolescent WIN Treatment Vehicle (n = 60 slices/26 mice) vs. WIN (1 mg/kg) (n = 50 slices/20 mice)	p < 0.001, 56%
Adolescent AM 4113 Treatment Vehicle (n = 60 slices/26 mice) vs. AM4113 (1 mg/kg) (n = 19 slices/8 mice)	p = 0.031, 78%
Adolescent AM630 Treatment Vehicle (n = 60 slices/26 mice) vs. AM630 (1 mg/kg) (n = 15 slices/6 mice)	p < 0.001, 53%
Adolescent WIN-3 Treatment Vehicle (n = 60 slices/26 mice) vs. WIN-3 (1 mg/kg) (n = 18 slices/7 mice)	p < 0.001, 49%

Table 3
Summary of statistical analysis of long-term adolescent (P35–P55) cannabinoid effects on normalized oscillation power in adult SCx

Cannabinoid effects were compared between treatment conditions with KS tests. Percentages reported are the median normalized LFP power in the treatment after “vs” relative to the median normalized LFP power in the treatment before “vs” in the statistical comparison.

Long-Term Adolescent Cannabinoid Treatment	SCx
Adolescent THC Treatment Vehicle (n = 61 slices/24 mice) vs. THC (5 mg/kg) (n = 24 slices/7 mice)	p = 0.006, 66%
Adolescent AM251 Treatment Vehicle (n = 61 slices/24 mice) vs. AM251 (0.3 mg/kg) (n = 10 slices/4 mice) Vehicle (n = 61 slices/24 mice) vs. AM251 (0.5 mg/kg) (n = 12 slices/4 mice) Vehicle (n = 61 slices/24 mice) vs. AM251 (1.0 mg/kg) (n = 8 slices/3 mice)	p = 0.068 p = 0.519 p < 0.010, 59%
Adolescent THC + AM251 Treatment THC (5 mg/kg) (n = 24 slices/7 mice) vs. + AM251 (0.3 mg/kg) + THC (5 mg/kg) (n = 12 slices/4 mice) Vehicle (n = 61 slices/24 mice) vs. + AM251 (0.3 mg/kg) + THC (5 mg/kg) (n = 12 slices/4 mice)	p = 0.004, 160% p = 0.519
Adolescent THC + AM251 Treatment THC (5 mg/kg) (n = 24 slices/7 mice) vs. + AM251 (0.5 mg/kg) + THC (5 mg/kg) (n = 12 slices/4 mice) Vehicle (n = 61 slices/24 mice) vs. + AM251 (0.5 mg/kg) + THC (5 mg/kg) (n = 12 slices/4 mice)	p = 0.973 p = 0.144
Adolescent WIN Treatment Vehicle (n = 61 slices/24 mice) vs. WIN (1 mg/kg) (n = 55 slices/21 mice)	p = 0.003, 74%
Adolescent AM4113 Treatment Vehicle (n = 61 slices/24 mice) vs. AM4113 (1 mg/kg) (n = 22 slices/8 mice)	p = 0.621
Adolescent AM4113 + WIN Treatment WIN (1 mg/kg) (n = 55 slices/21 mice) vs. AM4113 (1 mg/kg) + WIN (1 mg/kg) (n = 20 slices/7 mice) Vehicle (n = 61 slices/24 mice) vs. AM4113 (1 mg/kg) + WIN (1 mg/kg) (n = 20 slices/7 mice)	p = 0.021, 63% p < 0.001, 46%
Adolescent AM630 Treatment Vehicle (n = 61 slices/24 mice) vs. AM630 (1 mg/kg) (n = 19 slices/6 mice)	p = 0.009, 67%
Adolescent WIN-3 Treatment Vehicle (n = 61 slices/24 mice) vs. WIN-3 (1 mg/kg) (n = 21 slices/7 mice)	p = 0.312