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Adolescent Cannabinoid Exposure Permanently Suppresses Cortical Oscillations in Adult Mice

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Regular marijuana use during adolescence, but not adulthood, may permanently impair cognition and increase the risk for psychiatric diseases, such as schizophrenia. Cortical oscillations are integral for cognitive processes and are abnormal in patients with schizophrenia. We test the hypothesis that adolescence is a sensitive period because of the active development of cortical oscillations and neuromodulatory systems that underlie them. The endocannabinoid system upon which marijuana acts is one such system. Here we test the prediction that adolescent cannabinoid exposure alters cortical oscillations in adults. Using *in vitro* local field potential, *in vivo* electrocorticogram recordings and cognitive behavioral testing in adult mice, we demonstrate that chronic adolescent, but not adult, cannabinoid exposure suppresses pharmacologically evoked cortical oscillations and impairs working memory performance in adults. The later-maturing prefrontal cortex is more sensitive to adolescent exposure than the earlier-maturing, primary somatosensory cortex. These data establish a link between chronic adolescent cannabinoid exposure and alterations in adult cortical network activity that underlie cognitive processes.

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INTRODUCTION

Marijuana is the most commonly used illicit drug among adolescents and young adults in the United States (Substance Abuse and Mental Health Services Administration (SAMHSA), 2011). Persistent marijuana use before adulthood may permanently impair cognitive functioning (Solowij *et al*, 2002; Meier *et al*, 2012) and confer a higher risk of developing psychiatric diseases, such as schizophrenia, in susceptible individuals (Arseneault *et al*, 2004). Chronic adolescent, but not adult, cannabinoid exposure produces lasting working memory impairments and recapitulates other schizophrenia endophenotypes in rodents, including impaired sensorimotor gating, social avoidance, and anhedonia/avolition (Schneider and Koch, 2003; O'Shea *et al*, 2004; Quinn *et al*, 2008).

Adolescents are especially sensitive to repeated marijuana use, presumably because marijuana interferes with ongoing anatomical and physiological maturation of the brain, particularly the cerebral cortex (Andersen, 2003). The cortex matures along a caudal-to-rostral gradient, with primary sensory cortical areas maturing earlier, and prefrontal regions developing into early adulthood (Gogtay *et al*, 2004). The cortical endocannabinoid (eCB) system, in which marijuana acts, develops along the same gradient, with changes in cannabinoid-1 receptor (CB1R) density and distribution (Heng *et al*, 2011) and eCB metabolism (Long *et al*, 2012).

Coincident with eCB development is the maturation of cortical oscillations (Uhlhaas *et al*, 2009). Oscillations reflect the synchronous activity of neural networks (Buzsaki and Draguhn, 2004) and are integral to sensory processing, working memory, and nearly all cognitive functions, as they may provide a mechanism by which the brain 'binds' the activity of distributed neural ensembles into a coherent representation of cognitive or sensory content (Singer and Gray, 1995; Fries, 2005; Buzsaki, 2006). Cortical oscillations are abnormal in diseases in which these functions are impaired, such as schizophrenia (Gonzalez-Burgos and Lewis, 2008; Uhlhaas and Singer, 2010). Furthermore, oscillations underlie efficient cortical network processing (Buzsaki and Draguhn, 2004) and are dependent on the anatomical and physiological processes that mature throughout adolescence (Uhlhaas *et al*, 2009, 2010).

Acute cannabinoid administration attenuates the power of oscillations (Hajós *et al*., 2000, 2008; Robbe *et al*, 2006; Kucewicz *et al*, 2011) by acting at CB1Rs to suppress glutamate release from pyramidal neurons (Holderith *et al*, 2011; Sales-Carbonell *et al*, 2013). Furthermore, oscillations are suppressed in chronic marijuana users, particularly in those with an earlier age of onset of use (Skosnik *et al*, 2012). The adolescent maturation of cortical oscillations

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and the ongoing development of the eCB system that modulates this network activity, suggest that oscillations may be vulnerable to adolescent marijuana use. Here we test the hypothesis that chronic adolescent, but not adult, cannabinoid exposure alters cortical oscillations, particularly in less developed rostral cortical areas, and impairs cognitive behavioral performance in adults.

MATERIALS AND METHODS

Animals

All experiments were performed according to University of Maryland School of Medicine Institutional Animal Use and Care Committee protocols. Data were collected and analyzed by experimenters 'blind' to animals' treatment condition. Male CD-1 mice (Harlan Laboratories, Frederick, MD) were obtained at P30 (adolescent treatment) or P65 (adult treatment) and injected once daily i.p. from P35 to P55 or P70 to P90. Mice were group housed with cage mates exposed to both vehicle and WIN/Δ-^{9} tetrahydrocannabinol (THC). After the last injection, adolescent-treated mice were left undisturbed until adulthood (Figure 1a); adult-treated mice were left undisturbed for at least 10 days after the final injection to allow WIN to wash out of tissue. For electrocorticogram (ECoG) experiments, mice were injected once per day i.p. for 3 days with saline, 10 or 20 mg/kg ketamine; the order of injections was alternated between animals.

Drugs

WIN55-212,2 (0.25 or 1 mg/kg; Sigma Aldrich, St Louis, MO) and THC (5 mg/kg; National Institute on Drug Abuse Drug Supply Program, Bethesda, MD) were dissolved in 100% ethanol and injected in a 1 : 1 : 18 solution of ethanol: castor oil (Alkamuls EL-620, Rhodia Chemicals): 0.9% saline (1 ml/kg). Control animals were injected vehicle (1 : 1 : 18 ethanol:oil:saline). Ketamine (10 or 20 mg/kg; Bioniche Pharma, IL) was dissolved 1 : 5 in 0.9% saline and injected i.p. (0.1 ml).

In Vitro Slice Preparation and Local Field Potential (LFP) Recordings

Adult mice (>P100) were anesthetized with ketamine (100 mg/kg) and decapitated. Two or three 400 μm coronal sections were cut from each cortical region (mPFC or SCx) from one hemisphere using a microtome (Integraslice 7550 MM, Campden Instruments, IN). During cutting, tissue was immersed in ice-cold ACSF containing (in mM): 26 $NaHCO_3$, 5 BES, 15 glucose, 200 sucrose, 3 KCl, 1.5 $MgSO_4$,

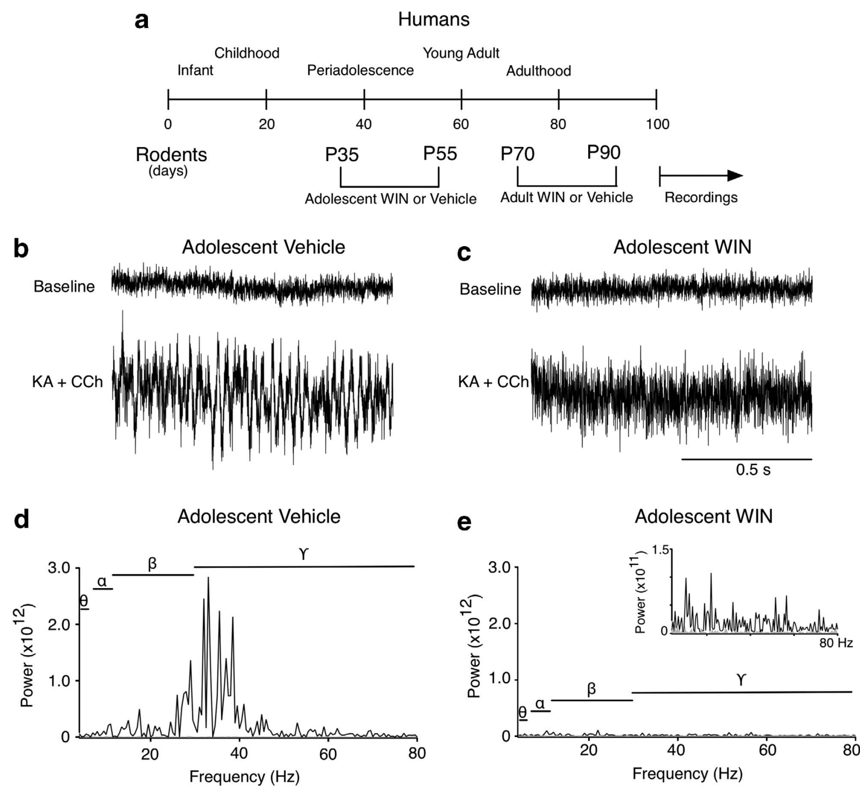

Figure 1 Robust oscillations are pharmacologically induced *in vitro* in adult mouse neocortex in adolescent vehicle but not WIN-treated mice. (a) Experimental time course: comparisons of human and rodent development are modified from (Andersen, 2003). The CB1R agonist WIN55,212-2 (WIN; 0.25 or 1 mg/kg) or vehicle were administered to adolescent (P35–P55) or adult mice (P70–P90) once daily for 20 days. LFPs were recorded in brain slices from adult mice (>P100). (b, c) 1-s epoch of *in vitro* LFP from mPFC slice from an adult mouse administered vehicle (b) or 1 mg/kg WIN (c) during adolescence. LFPs were recorded before (baseline) and during kainic acid (KA; 400 nM) and carbachol (CCh; 20 μM) perfusion (KA + CCh). (d, e) Fourier transform of 10-s LFP recordings in (b) and (c), respectively. KA + CCh (black trace) markedly increases power at all frequencies compared with baseline conditions (grey trace). Frequency ranges: $\theta = 4$–7 Hz; $\alpha = 8$–12 Hz; $\beta = 13$–29 Hz; $\gamma = 30$–80 Hz. Inset in (e) shows a magnified view of the FFT in (e).

and 1 $CaCl_2$. Next, slices were incubated for 30 min at 36 °C and 30 min at 22 °C in ACSF containing (in mM): 120 NaCl, 25 $NaHCO_3$, 5 BES, 15 glucose, 3 KCl, 1.3 $MgSO_4$, and 2 $CaCl_2$ (normal ACSF). Slices were continually saturated with 95% O_2–5% CO_2. Slices were maintained at 36 °C in an interface-type recoding chamber and were perfused at 0.7 ml/min with ACSF containing (in mM): 120 NaCl, 25 $NaHCO_3$, 5 BES, 15 glucose, 5 KCl, 1.3 $MgSO_4$, 2 $CaCl_2$ (high K^+ ACSF), kainic acid (KA; 400 nM) and carbachol (CCh; 20 μM). LFP recordings were made through a glass pipette filled with normal ACSF (resistance ~ 0.5–1 MΩ) with an ER-1 amplifier (Cygnus Technology), sampled at 5 kHz, filtered between 0.1 Hz and 1 KHz, and stored on a Mac computer using Igor Pro (Version 6.1, Wavemetrics, Portland, OR).

In Vitro Data Analysis

We analyzed *in vitro* LFP data with custom-written Igor Pro scripts and the Time-Frequency Toolkit (http://www.igorexchange.com/project/TFPlot). Discrete fast Fourier transforms (FFTs) were performed on 10 s of LFP data and oscillation power (area under the curve) was integrated at different frequencies ($\theta = 4$–7 Hz; $\alpha = 8$–12 Hz; $\beta = 13$–29 Hz; $\gamma = 30$–80 Hz). Frequency bandwidth boundaries were based on Buhl *et al*, 1998 and Uhlhaas and Singer, 2010. Statistical analyses were performed with STATA (Version 12, StataCorp, College Station, TX). Data were tested for normality and extreme outliers were identified using the fourth-spread outlier detection test and removed (Hoaglin *et al*, 1986). Kruskal–Wallis (KW) or Mann–Whitney *U* (MWU) tests were used to determine a significant main effect of treatment (significant $P < 0.05$). Pairwise comparisons were performed with MWU tests (significant $P < 0.05$).

In Vivo Surgical Procedures and ECoG Recordings

Adult mice (> P100) were anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg). A F20-EET radiotelemetric transmitter (Data Sciences International, Minneapolis, MN) was implanted subcutaneously and its leads implanted over the dura above the frontal cortex (1.7 mm anterior to bregma) and the cerebellum (6.4 mm posterior to bregma). Animals were individually housed and recovered from surgery for 48 h before recording.

Mice were acclimated to the behavior testing room for 1 h before ECoG recordings. ECoGs were recorded with the Dataquest A.R.T. acquisition system (Data Sciences International) with frontal ECoG recordings referenced to the cerebellum. Baseline ECoG (10 min) recordings were followed by an i.p. injection of saline, 10 or 20 mg/kg ketamine, and 40 min of post-injection recordings.

In Vivo Data Analysis

We analyzed *in vivo* ECoGs with custom-written MATLAB scripts (Version 2012a, Mathworks, MA) and the mtspecgramc routine in the Chronux Toolbox (http://chronux.org; Mitra and Bokil, 2008). Oscillation power in each bandwidth ($\delta = 1$–3 Hz; $\theta = 4$–7 Hz; $\alpha = 8$–12 Hz; $\beta = 13$–29 Hz; $\gamma = 30$–80 Hz) was computed in 10 s bins from spectrograms for each animal, and averaged into 10 m bins. Post-injection power was compared with the baseline period with MWU tests (significant $P < 0.05$) using STATA.

Novel Object Recognition Behavior

Adult mice (> P100) were habituated to a black, plastic behavioral arena (12" × 9" × 11") for 1 h in low light conditions. On the next day, mice were introduced to two identical objects (two shot glasses, 1.5" wide × 3" tall, or two white glass vases, 3" wide × 4" tall) for 10 min in the arena, returned to their home-cage for 60 min, and then exposed to one object from the first session (vase or shot glass) and one novel object (shot glass or vase) for 10 min. The objects and the arena were cleaned thoroughly with 70% ethanol between sessions to eliminate olfactory cues. Object identity (shot glass or vase) and the location of novel/familiar objects (left or right) were counterbalanced between animals. Behavior was videotaped during these sessions and the time spent interacting with each object during the first 3 min of the test session was manually scored offline by an experimenter blind to animals' treatment condition and to the novel/familiar nature of the objects. Object interaction was said to occur when the animal's snout was oriented within 2 cm of the object. Object interaction data were tested for normality and analyzed with two-tailed Student's *t*-tests (significant $P < 0.05$).

RESULTS

Adolescent, but Not Adult, WIN Administration Suppresses *In Vitro* Cortical Oscillations Preferentially in Rostral Cortical Areas

In adolescent vehicle or WIN-treated mice (0.25 or 1 mg/kg), we assessed the ability of the adult neocortex to produce synchronous activity by pharmacologically evoking oscillations. The method we used—KA (400 nM) and CCh (20 μM)—reliably produces β and γ oscillations in cortical slices (Buhl *et al*, 1998; Oke *et al*, 2010) that strongly resemble network synchrony in the intact neocortex (Steriade *et al*, 1996). KA and CCh promote robust LFP oscillations *in vitro* by enhancing excitatory drive (Buhl *et al*, 1998; Cunningham *et al*, 2003) and activating cholinergic receptors on GABAergic interneurons (Fisahn *et al*, 1998; Gulyás *et al.*, 2010).

Coronal brain slices containing medial prefrontal cortex (mPFC) or primary somatosensory cortex (SCx) were prepared from adult mice administered WIN or vehicle during adolescence (Figure 1a). An example LFP recorded *in vitro* from adult mPFC of an adolescent vehicle-treated mouse before and during KA + CCh perfusion (Figure 1b) shows robust β and γ oscillations and increased power in θ and α bandwidths ($\theta = 4$–7 Hz; $\alpha = 8$–12 Hz; $\beta = 13$–29 Hz; $\gamma = 30$–80 Hz) evoked by KA + CCh. This is quantified in the accompanying FFTs of the LFPs (Figure 1d) and in the spectrogram and power spectral density (PSD) in Figure 2a.

In mPFC slices from adolescent WIN-treated mice, pharmacologically evoked oscillations were profoundly suppressed. Figure 1c shows an example *in vitro* LFP from mPFC of an adolescent 1 mg/kg WIN-treated adult before and during KA + CCh perfusion. Note the markedly

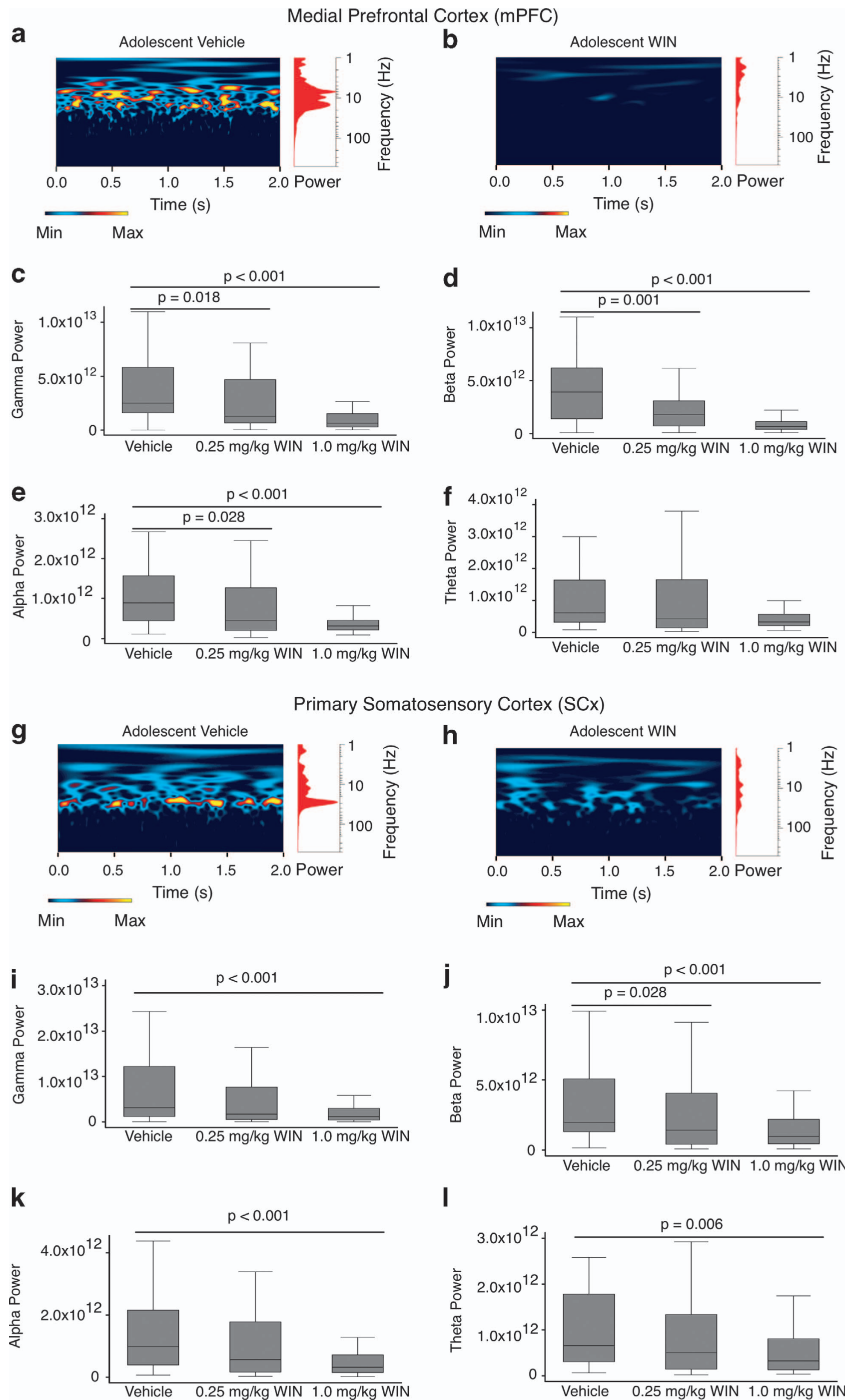

Figure 2 Chronic adolescent WIN administration attenuates pharmacologically evoked oscillations *in vitro* in mPFC and SCx. (a, b) Spectrogram (left) and power spectral density (right) of a 1-s representative KA + CCh LFP recorded in mPFC of an adult mouse administered vehicle (a) or 1 mg/kg WIN (b) during adolescence. Minimum–maximum scales are the same in a and b. (c–f) Box and whisker plots (box: 25th percentile, median, 75th percentile; whiskers: adjacent value to 25% or 75% values) of power from FFTs of LFPs in mPFC of adult mice with KA + CCh perfusion. Mice were administered WIN (0.25 or 1 mg/kg) or vehicle during adolescence (Figure 1a). KW tests determined significant differences between the three treatment conditions and pairwise comparisons were performed using MWU tests (significant $P < 0.05$). (g, h) Spectrograms and power spectral densities of 1-s example KA + CCh LFP recorded in SCx of adult mouse administered vehicle (g) or 1 mg/kg WIN (h) during adolescence. Minimum–maximum scales are the same in g and h. (i–l) Box and whisker plots of power from FFTs of LFPs in SCx of adult mice in the presence of KA + CCh. Mice were treated and statistics were performed as described for (c–f).

attenuated power of these oscillations as quantified in the FFT (Figure 1e) and spectrogram and PSD (Figure 2b) compared with oscillation power in an adolescent vehicle-treated mouse (Figures 1b and d and Figure 2a).

In slices of adult mPFC, power in γ, β, and α bandwidths was significantly and dose-dependently attenuated by adolescent WIN exposure (Figures 2c–e, Table 1a). Although it approached statistical significance, there

Table 1 Summary of Cannabinoid Effects *In Vitro*

	Gamma (30–80 Hz)	Beta (13–29 Hz)	Alpha (8–12 Hz)	Theta (4–7 Hz)
(*a*) *Medial Prefrontal Cortex (mPFC)*				
Chronic adolescent WIN treatment	$P<0.001$	$P<0.001$	$P<0.001$	$P=0.071$
Vehicle ($n=12$ slices per 8 mice) *vs* WIN (0.25 mg/kg) ($n=16$ slices per 10 mice)	$P=0.018$, 52%	$P=0.001$, 46%	$P=0.028$, 51%	
Vehicle *vs* WIN (1 mg/kg) ($n=14$ slices/10 mice)	$P<0.001$, 26%	$P<0.001$, 17%	$P<0.001$, 35%	
Chronic adult WIN treatment				
Vehicle ($n=8$ slices per 3 mice) *vs* WIN (1 mg/kg) ($n=12$ slices per 5 mice)	$P=0.543$	$P=0.918$	$P=0.662$	$P=0.264$
Chronic adolescent THC treatment				
Vehicle ($n=12$ slices per 8 mice) *vs* THC (5 mg/kg) ($n=21$ slices per 8 mice)	$P<0.001$, 35%	$P<0.001$, 14%	$P<0.001$, 18%	$P<0.001$, 24%
(*b*) *Primary Somatosensory Cortex (SCx)*				
Chronic adolescent WIN treatment	$P=0.002$	$P<0.001$	$P<0.001$	$P=0.006$
Vehicle ($n=21$ slices per 12 mice) *vs* WIN (0.25 mg/kg) ($n=26$ slices per 12 mice)	$P=0.051$	$P=0.028$, 72%	$P=0.066$	$P=0.103$
Vehicle *vs* WIN (1 mg/kg) ($n=24$ slices per 12 mice)	$P<0.001$, 37%	$P<0.001$, 49%	$P<0.001$, 33%	$P<0.001$, 49%
Chronic adult WIN treatment				
Vehicle ($n=8$ slices per 3 mice) *vs* WIN (1 mg/kg) ($n=13$ slices per 5 mice)	$P=0.523$	$P=0.305$	$P=0.294$	$P=0.225$
Chronic adolescent THC treatment				
Vehicle ($n=21$ slices per 12 mice) *vs* THC (5 mg/kg) ($n=24$ slices per 12 mice)	$P=0.131$	$P=0.901$	$P=0.129$	$P=0.025$, 75%

(a) Summary of statistical analyses of cannabinoid effects on the power of pharmacologically evoked LFP oscillations recorded *in vitro* in mPFC. Kruskal–Wallis (KW) tests determined significant differences among three treatment conditions, and pairwise comparisons were tested with Mann–Whitney *U*-tests (MWU; significant $P<0.05$). No pairwise comparisons were performed if KW $P>0.05$. MWU tests compared two treatment conditions. Percentages reported are relative to median LFP power recorded in vehicle-treated mice. (b) Summary of statistical analysis of LFP oscillations recorded *in vitro* in SCx. Analysis was performed as in (a)

was no significant main effect on θ power (Figure 2f, Table 1a).

We next tested the prediction that the caudally located SCx would be less sensitive to adolescent cannabinoid exposure than the rostral mPFC. In SCx of a vehicle-treated mouse, KA + CCh evoked robust γ oscillations (Figure 2g), in contrast to oscillations recorded in an adolescent 1 mg/kg WIN-administered mouse (Figure 2h). Adolescent WIN administration significantly suppressed γ, β, α, and θ oscillations in adult SCx (Figures 2 i–l, Table 1b). However, SCx was less sensitive than mPFC to adolescent WIN exposure as evidenced by the relatively weak effects of adolescent 0.25 mg/kg WIN and the markedly smaller effects of 1 mg/kg (Table 1b).

Long-term cognitive impairments and elevated risk of psychiatric disorders in regular marijuana users are less pronounced when use is initiated in adulthood, instead of in adolescence (Arsenault *et al*, 2004; Meier *et al*, 2012). Our hypothesis predicts that the long-term effects of WIN on cortical oscillations would be restricted to adolescent exposure. Adult mice were administered WIN (1 mg/kg) or vehicle for 20 days (P70–P90; Figure 1a) and oscillations were evoked *in vitro* with KA + CCh. We found that adult treatment had no significant effect on oscillation power in any frequency examined in mPFC (Table 1a) or SCx (Table 1b). The persistent suppression of pharmacologically evoked oscillations recorded in mPFC and SCx of adult mice after WIN exposure during adolescence, but not adulthood, supports our hypothesis that cortical oscillations are markedly sensitive to repeated adolescent cannabinoid exposure.

Adolescent THC Administration Suppresses *In Vitro* Cortical Oscillations Preferentially in Rostral Cortical Areas

Although WIN is used experimentally to induce cannabinoid-mediated effects, treating mice with the primary active ingredient in marijuana, THC, allowed us to directly test our hypothesis that repeated adolescent marijuana use persistently disrupts cortical oscillations. We administered THC (5 mg/kg) to adolescent mice and recorded oscillations *in vitro* as above (Figure 3a).

In adult mPFC, LFP power in γ, β, α, and θ bandwidths was significantly attenuated by adolescent THC administration (Figures 3b–e, Table 1a). SCx was less sensitive than mPFC to adolescent THC exposure (Figures 3f–i, Table 1b) as evidenced in a lack of significant effect on γ, β, or α oscillations and the mild attenuation of θ power by adolescent THC.

Adolescent WIN Administration Attenuates *In Vivo* Cortical Oscillations

To test whether adolescent cannabinoid administration impairs cortical oscillations in the intact adult brain, we recorded ECoGs *in vivo* from freely moving adult mice administered WIN (1 mg/kg) or vehicle during adolescence (Figure 1a). Robust oscillations were pharmacologically evoked with the non-competitive NMDA receptor antagonist ketamine as sub-anesthetic doses evoke robust cortical oscillations in rodents and humans (Plourde *et al*, 1997;

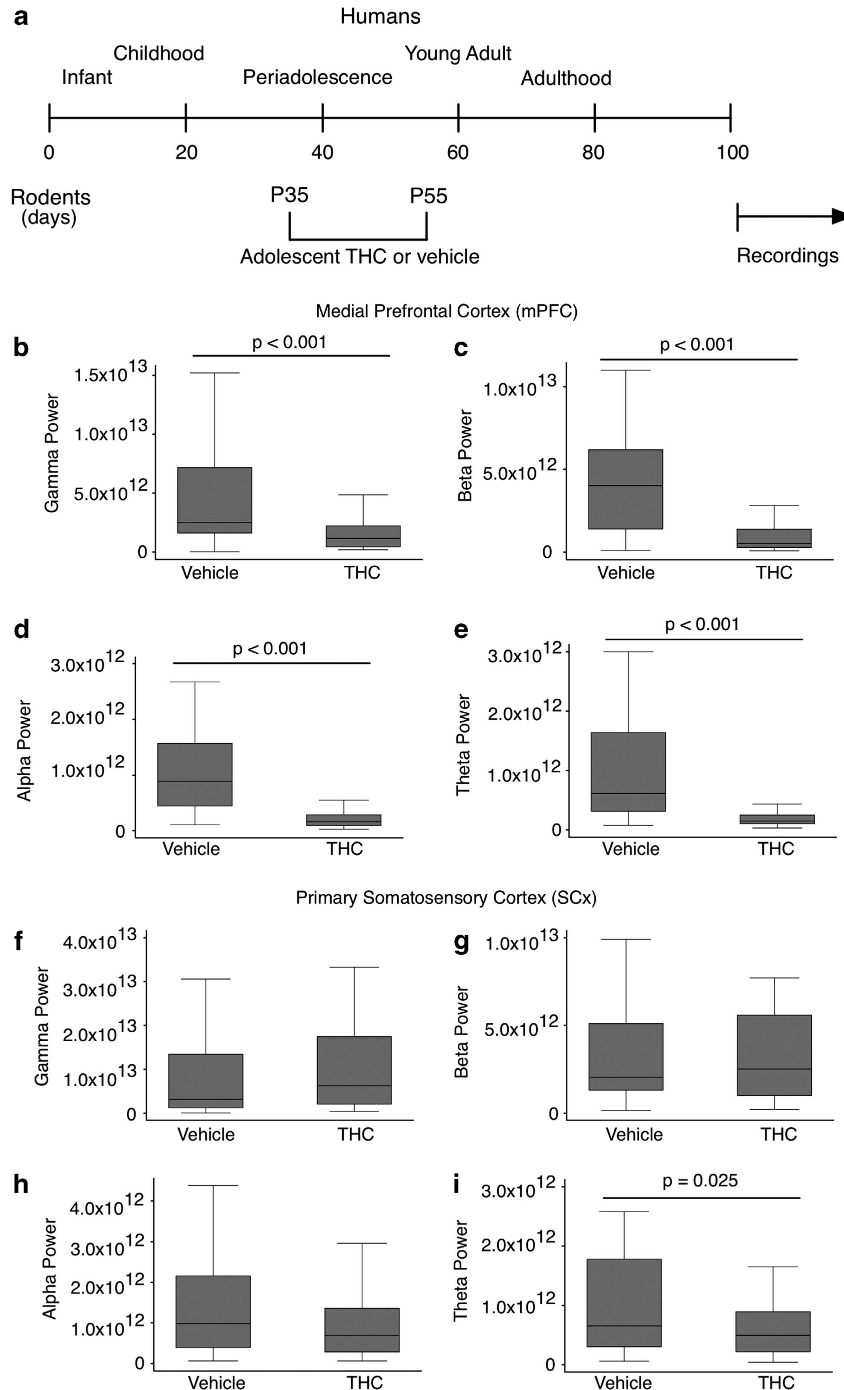

Figure 3 Chronic adolescent THC administration suppresses oscillations *in vitro* in mouse mPFC but not SCx. (a) THC (5 mg/kg) or vehicle was administered to adolescent mice (P35–P55) and LFPs were recorded in slices from adult mice (>P100). (b–e) Box and whisker plots of power from FFTs of LFPs in mPFC of adult mice with KA + CCh perfusion. Power from adolescent THC or vehicle-treated mice were compared using MWU tests (significant $P<0.05$). (f–i) Box and whisker plots of power extracted from FFTs of LFPs in SCx of adult mice in the presence of KA + CCh. Mice were treated and statistics were performed as in (b–e).

Hakami *et al*, 2009) that are independent of animals' behavioral states (Hakami *et al*, 2009).

Ten-minute baseline ECoG recordings were followed by an i.p. injection of saline, 10 or 20 mg/kg ketamine, and 40 min of post-injection recordings (Figure 4a). Immediately after ketamine injection, all mice displayed characteristic movement abnormalities (tottering, falling over, and turning in circles). In vehicle-treated mice, ketamine (10 or 20 mg/kg) markedly increased γ and α power (Figures 4a and c) but did not increase power at other frequencies (data not shown). Ketamine-evoked γ (Figures 4a and b) and α power (Figures 4c and d) were normalized to power before and after saline injection, which did not produce changes in power at either frequency (data not shown).

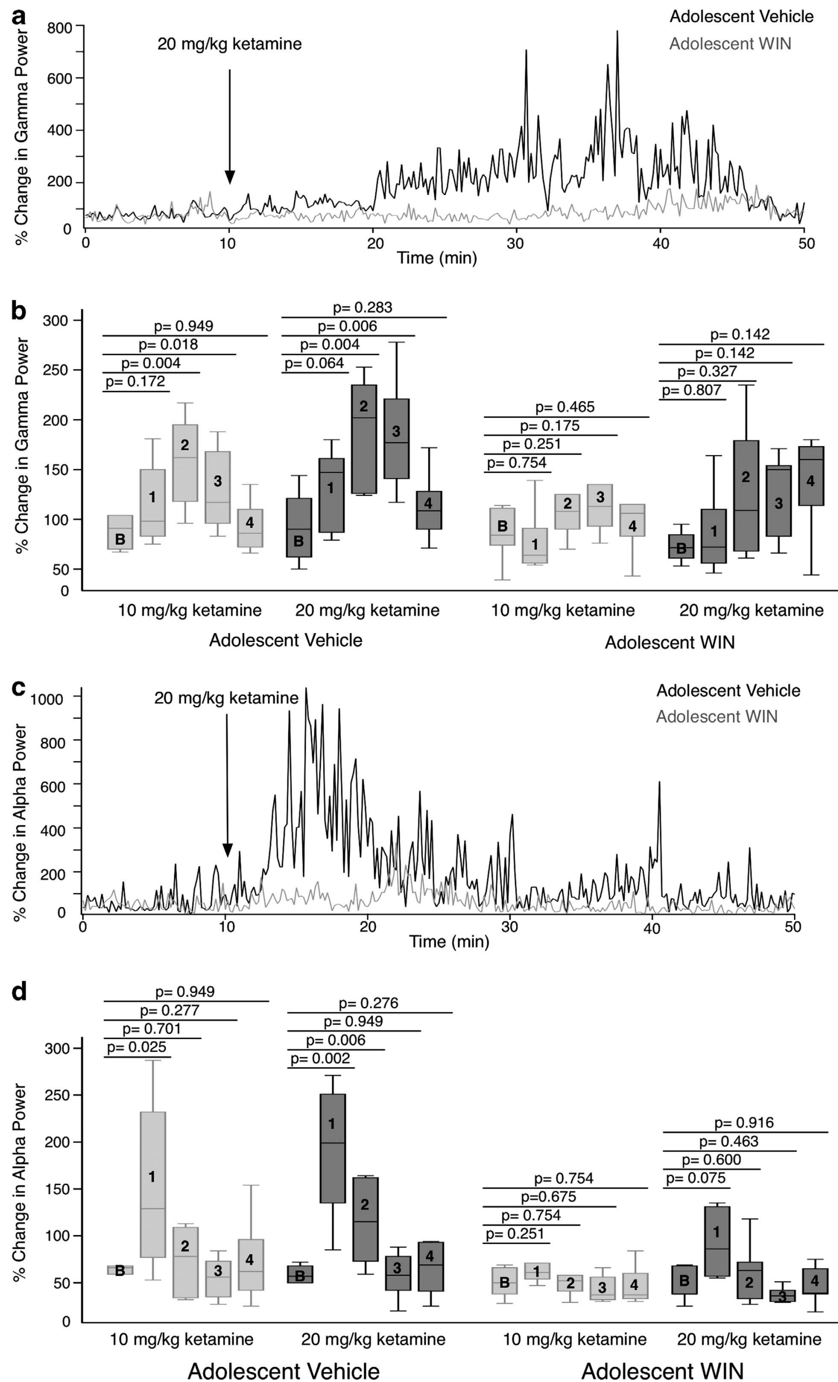

Figure 4 Chronic adolescent administration of WIN attenuates cortical oscillations *in vivo*. (a) Representative time course of γ power in frontal ECoG before and after injection of 20 mg/kg ketamine. Mice were treated with 1 mg/kg WIN (grey trace) or vehicle (black trace) from P35 to P55 and ECoGs were recorded from adults (>P100). γ Power after ketamine injection was normalized to γ power after saline injection. (b) Box and whisker plots of ECoG γ power in adolescent vehicle ($n=5$) or WIN-treated ($n=7$) mice after injection of 10 mg/kg (light grey) or 20 mg/kg (dark grey) ketamine, normalized as above. B = minutes 0–10 of recording; 1 = minutes 10–20 of recording; 2 = minutes 20–30 of recording; 3 = minutes 30–40 of recording; 4 = minutes 40–50 of recording. Post-injection power was compared with baseline power with MWU tests (significant $P<0.05$). (c) Representative time course of α power in frontal ECoG before and after injection of 20 mg/kg in an adolescent vehicle (black trace) or WIN-treated (grey trace) adult mouse. Data are presented as in (a). (d) Box and whisker plots of ECoG α power in adolescent vehicle ($n=5$) or WIN-treated ($n=7$) mice after injection of 10 mg/kg (light grey) or 20 mg/kg (dark grey) ketamine, normalized to power before and after saline injection. Time segments are indicated and statistics were performed as in (b).

γ Oscillations. An example time course from a vehicle-treated mouse demonstrates that γ power increased approximately 10 min after 20 mg/kg ketamine and peaked at nearly 800% of saline levels from 20 to 30 min before it returned to baseline (Figure 4a). Group data analysis (Figure 4b) revealed that 10 or 20 mg/kg ketamine significantly increased γ power above baseline from 10 to 30 min after injection in adolescent vehicle-treated mice. In

contrast, neither 10 or 20 mg/kg ketamine had a significant effect on γ power in ECoGs of adolescent WIN-treated mice, as shown in the example time course of γ power (Figure 4a) and in group data (Figure 4b).

α Oscillations. Acute administration of 10 or 20 mg/kg ketamine to adolescent vehicle-treated mice also dose-dependently increased α power, as shown in the example time course (Figure 4c) and in group data (Figure 4d). In adolescent WIN-treated mice, ketamine failed to elevate α power above baseline levels (Figures 4c and d). Therefore, while both 10 and 20 mg/kg ketamine significantly increased γ and α power in ECoGs of freely moving adolescent vehicle-treated mice, adolescent WIN treatment prevented this ketamine-induced increase in oscillatory power.

Adolescent WIN Administration Impairs Cognitive Behavioral Performance

To test whether chronic adolescent cannabinoid exposure produces lasting cognitive impairments, we tested adult mice that had been treated in adolescence with WIN (1 mg/kg) or vehicle in the novel object recognition test of working memory. Although vehicle-treated mice spend significantly more time exploring a novel object, WIN-treated animals spend equal time exploring familiar and novel objects ((vehicle ($n = 7$ mice): mean % time with novel object = 70.4 ± 5.1%; mean % time with familiar object = 29.6 ± 5.1%; $P < 0.001$, two-tailed t-test) and (WIN ($n = 8$ mice): mean % time with novel object = 51.2 ± 4.5%; mean % time with familiar object = 48.8 ± 4.54%; $P = 0.710$, two-tailed t-test)). Total object interaction time did not differ significantly between vehicle and WIN-treated mice (vehicle: mean = 15.4 ± 3.9 s; WIN: mean = 23.5 ± 4.4 s; $P = 0.198$, two-tailed t-test), suggesting that the above results are not biased by differences in locomotion or object engagement during the task.

DISCUSSION

We present evidence that 20-day adolescent, but not adult, cannabinoid exposure attenuates adult cortical oscillations predominantly in cortical areas that are less developed in adolescence. These findings are consistent with reports that regular marijuana users have suppressed evoked γ oscillations with those who initiated use as adolescents showing the greatest subsequent effects (Skosnik *et al*, 2012). However, as these studies were conducted in adults who still regularly used marijuana, a link between marijuana use specifically during adolescence and suppressed oscillations in adulthood could not be determined. Our findings are also consistent with reports of impaired ketamine-evoked γ synchrony in animal models of psychiatric illnesses (Phillips *et al*, 2012) that are characterized by abnormal network activity (Uhlhaas and Singer, 2010) and more likely in those that regularly used marijuana as adolescents (Arseneault *et al*, 2004).

We also report impaired novel object recognition behavior in adolescent WIN-treated mice, which is consistent with previous reports of cognitive impairments after persistent adolescent cannabis exposure in both rodents (Schneider and Koch, 2003; O'Shea *et al*, 2004; Quinn *et al*, 2008) and humans (Solowij *et al*, 2002; Meier *et al* 2012). Impairments in working memory, processing speed, perceptual reasoning, and other executive functions have been documented in long-term adolescent cannabis users (Solowij *et al*, 2002; Meier *et al* 2012). Poor cognitive performance in persistent marijuana users may be related to the cortical oscillation attenuation that we report, as synchronous cortical neural activity plays a role in selective attention (Fries *et al*, 2001), integration of sensory information (Singer and Gray, 1995), working memory (Roux *et al*, 2012), and other cognitive functions (Buzsaki, 2006; Wang, 2010). Chronic adolescent, but not adult, cannabinoid-induced oscillation suppression suggests that attenuated network synchrony is not merely a consequence of repeated cannabinoid exposure, but reflects a unique sensitivity of the adolescent brain to modification by cannabinoids. Our data are consistent with findings that adolescent, but not adult, cannabinoid exposure produces lasting cognitive impairments in rodents (Schneider and Koch, 2003; O'Shea *et al*, 2004; Quinn *et al*, 2008) and humans (Solowij *et al*, 2002; Meier *et al*, 2012).

The heightened sensitivity of the adolescent cortex is probably attributable to anatomical and neurochemical development that occurs during this period (Andersen, 2003). Postnatal maturation of cortical grey and white matter progresses along a caudal-to-rostral gradient (Giedd *et al*, 1999; Gogtay *et al*, 2004). As more caudal, sensory cortical areas develop earlier than rostral prefrontal regions (Luna, 2009), prefrontal cortical circuitry is particularly vulnerable to adolescent drug use (Andersen, 2003). Our data confirm this vulnerability, as the magnitude of oscillation suppression seen after adolescent cannabinoid exposure is greater in the rostral mPFC than the caudal SCx.

Cortical glutamatergic and GABAergic networks that synchronize the firing of pyramidal neurons and sculpt the temporal profile of cortical oscillations (Whittington *et al*, 2000; Cardin *et al*, 2009) also undergo substantial adolescent maturation (Cao *et al*, 2000; Hashimoto *et al*, 2009). Coincident adolescent development of the cortical eCB system includes reduced CB1R expression (Deshmukh *et al*, 2007; Heng *et al*, 2011) and changes in eCB metabolism (Long *et al*, 2012). The highly dynamic nature of glutamatergic, GABAergic, and eCB system development parallels the maturation of cortical oscillations that are suppressed by cannabinoids, and may underlie the attenuation of cortical oscillations that we observe after adolescent cannabinoid exposure.

Acute cannabinoid exposure attenuates the power of neural oscillations recorded *in vitro* (Hajós *et al*., 2000, 2008) and *in vivo,* in freely moving animals (Robbe *et al*, 2006; Hajós *et al*., 2008; Kucewicz *et al*, 2011) and during working memory tasks (Kucewicz *et al*, 2011) by CB1R-mediated suppression of glutamate release from pyramidal neurons (Holderith *et al*, 2011; Sales-Carbonell *et al*, 2013). This CB1R-mediated attenuation of excitatory transmission preferentially suppresses the frequency and firing precision of fast-spiking GABAergic interneurons, resulting in smaller and less synchronized field potentials (Holderith *et al*, 2011). As we have found similar oscillation suppression after chronic exposure to either WIN or THC—two structurally different cannabinoids that both act as CB1R agonists—we suggest that CB1Rs may be responsible for the chronic effects that we observe. However, as WIN and THC

have additional targets including CB2Rs (Showalter *et al*, 1996) and other GPCRs (Breivogel *et al*, 2001; Ryberg *et al*, 2007), non-CB1R receptors may underlie oscillation suppression by chronic adolescent WIN or THC administration. Elucidating the receptor mechanism responsible for the effects of adolescent WIN and THC exposure is beyond the scope of this report and related studies are currently underway in our laboratory.

To our knowledge, ours is the first study to demonstrate a direct link between cannabinoid exposure specifically during adolescence and abnormal electrophysiological activity in the adult neocortex, as well as to report a differential vulnerability of cortical regions that parallels their maturational state at the time of drug exposure.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)