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The Effect of Chronic Cannabinoids on Broadband EEG Neural Oscillations in Humans

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Animal and cellular work has shown that central cannabinoid-1 receptors modulate neural oscillations in the gamma range (40 Hz), which may be important for normal perceptual and cognitive processes. In order to assess the effect of cannabinoids on broadband-frequency neural oscillations in humans, the current study examined the effect of chronic cannabis use on auditory steady-state responses (ASSRs) utilizing electroencephalography (EEG). Passive ASSRs were assessed using varying rates of binaural stimulation (auditory click-trains; 10–50 Hz in increments of 5 Hz; 80 dB SPL) in carefully screened cannabis users and controls. Chronic cannabis users ($n = 22$; 12 h abstinence before study; positive 11-nor-9-carboxy-delta-9-tetrahydrocannabinol urine levels) and cannabis naïve controls ($n = 24$) were evaluated. Time X frequency analyses on EEG data were performed using Fourier-based mean trial power (MTP) and phase-locking (inter-trial coherence; ITC). Transient ERPs to stimulus onset (auditory N100 components) were also evaluated. As predicted, a decrease in spectral power (MTP) at 40 Hz was observed in the cannabis group ($p < 0.018$). No effects on phase-locking (ITC) or the N100 were observed. Further, within the cannabis group, lower 40 Hz power correlated with an earlier age of onset of cannabis use ($p < 0.04$). These data suggest that chronic exposure to exogenous cannabinoids can alter the ability to generate neural oscillations, particularly in the gamma range. This is consistent with preclinical animal and cellular data, which may have implications for understanding the short- and long-term psychopharmacological effects of cannabis.

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INTRODUCTION

Cannabis remains one of the most widely used psychoactive substances in the world (United Nations Office on Drugs and Crime, 2009). The principal psychoactive constituent in cannabis, Δ^9-tetrahydrocannabinol (THC; Gaoni and Mechoulam, 1971), affects' the brain via the action of central cannabinoid-1 receptors (CB1R; Devane *et al*, 1988; Pertwee *et al*, 2010). The CB1R is one of the most abundant G-protein-coupled receptors in the central nervous system, with high densities in areas such as the cerebral cortex, basal ganglia, hippocampus, and cerebellum (Egertova and Elphick, 2000; Eggan and Lewis, 2007; Glass *et al*, 1997; Herkenham *et al*, 1990; Pertwee, 1997, 1999; Tsou *et al*, 1998). CB1Rs are primarily located presynaptically, and their activation (by either endogenous or exogenous cannabinoids) inhibits the release of other neurotransmitters such as gamma-amino butyric acid (GABA) and glutamate by decreasing Ca^{2+} influx via the inhibition of adenylate cyclase and N-type Ca^{2+} channels (Freund *et al*, 2003). In the cerebral cortex and hippocampus, this neuromodulation principally occurs in networks of cholecystokinin-containing GABAergic interneurons (Ali and Todorova, 2010; Bacci *et al*, 2004; Bodor *et al*, 2005; Eggan and Lewis, 2007; Eggan *et al*, 2010; Foldy *et al*, 2006; Hill *et al*, 2007; Katona *et al*, 2000). Thus, it appears that CB1Rs may function as a molecular 'brake,' regulating the timing and release of GABA and other neurotransmitters (Farkas *et al*, 2010).

Concerning the precise role that cannabinoids have in GABA function, it has been hypothesized that 'cannabis' neurobehavioral effects may involve alterations in neural synchronization' (Skosnik *et al*, 2006a). Indeed, *in vitro* and *in vivo* animal studies have shown that CB1Rs modulate gamma- (30–80 Hz) and theta-band (4–7 Hz) synchronized oscillations in networks of GABAergic interneurons in the cerebral cortex and hippocampus (Hajos *et al*, 2000, 2008; Katona *et al*, 1999; Morgan *et al*, 2008; Reich *et al*, 2005; Robbe *et al*, 2006). This may be particularly germane to the psychopharmacological effects of cannabis, as neural

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oscillations are thought to be involved in several domains of cognitive and perceptual function. For example, cellular studies and computational models have suggested that gamma-band rhythmic synchronization across neuronal assembles has an important role in the integration and binding of perceptual features, associative learning, and conscious awareness (Melloni *et al*, 2007; Singer, 1999; Uhlhaas *et al*, 2009; Whittington *et al*, 2000).

Although several experiments have examined the effect of cannabinoid manipulations on synchronized oscillations using cellular and animal preparations (Hajos *et al*, 2000, 2008; Katona *et al*, 1999; Morgan *et al*, 2008; Reich *et al*, 2005; Robbe *et al*, 2006), there has been a paucity of studies assessing CB1R effects on frequency-specific neural oscillations in humans. One non-invasive technique that can be used to study neural oscillations in humans is electroencephalography (EEG). EEG is one of the few available neuroimaging methodologies that can directly measure neural events (postsynaptic potentials) with high temporal precision in humans (Luck *et al*, 2011). Neural network oscillations can be probed and assessed by entrainment of the EEG to rhythmic sensory stimulation (eg, auditory click trains at specific frequencies). Because the EEG waveform entrains to the frequency and phase of the presented stimulus, it serves as an indicator of the functional state of the neural circuits supporting synchronized oscillations. In the auditory modality, the output of such stimulation is termed the auditory steady-state response (ASSR). Evidence suggests that the ASSR has a preferred gamma-band resonance (Galambos *et al*, 1981; Picton *et al*, 2003), is generated by the brainstem, thalamus, cerebellum, and auditory cortex (Hari *et al*, 1989a, 1989b; Makela and Hari, 1987; Pantev *et al*, 1996; Pastor *et al*, 2002, 2006; Steinmann and Gutschalk, 2011), and is mediated by the GABAergic system (Vohs *et al*, 2010). As aptly described by Spencer *et al* (2008), 'Although it is not thought that the ASSR itself reflects any process related to the formation of cell assemblies, its 40-Hz resonance suggests that the underlying neural circuits preferentially oscillate at this frequency and thus might rely on some of the same circuit and intrinsic neuron properties as non-driven (sensory evoked and cognitive-related) oscillations (Spencer *et al*, 2008).' Thus, the auditory steady-state paradigm may represent a valid probe with which to test the ability of neural networks to oscillate at frequencies important for normal perceptual and cognitive processes. Indeed, the ASSR has been used successfully to demonstrate neural oscillation deficits in psychotic illnesses such as schizophrenia and bipolar disorder (Krishnan *et al*, 2009; Kwon *et al*, 1999; Light *et al*, 2006; O'Donnell *et al*, 2004; Rass *et al*, 2010; Spencer *et al*, 2008).

In the only previous study of the effects of cannabis use on neural oscillations using the ASSR paradigm, it was demonstrated that 20 and 40 Hz harmonic EEG spectral power were decreased during beta-band auditory stimulation (Skosnik *et al*, 2006a). However, this initial study only assessed a sample of self-reported cannabis users, with no objective confirmation of recent cannabis use (eg, detection of urinary THC metabolites). Further, only three frequencies of stimulation were utilized (20, 30, and 40 Hz), and the fast Fourier transform measure of power did not examine the temporal dynamics of the ASSR, or differentiate effects on power *vs* phase locking. Therefore, the current study examined the effect of chronic cannabinoids on broadband-frequency neural oscillations in confirmed cannabis users utilizing the ASSR paradigm. On the basis of previous animal and cellular work, it was hypothesized that the cannabis group would exhibit ASSR deficits (decreases in mean trial power (MTP) and inter-trial coherence (ITC)), specifically in the gamma band.

MATERIALS AND METHODS

Subjects

This study was approved by the Indiana University Bloomington Human Subjects Committee. Current cannabis users ($n = 22$) and healthy drug-naive controls ($n = 24$) were recruited from the local university community, paid for their participation, and written informed consent was obtained from each.

The inclusion criteria were as follows: (1) For the cannabis group: current cannabis consumption (smoked joints) at the rate of at least once per week during the last month, a positive urine toxicology screen for THC metabolites (THC-COOH), no other illicit substance use during the past 3 months (including a negative urine toxicology screen for other illicit drugs), and no DSM-IV diagnosis of Axis I or II disorders, including no current or past history of illicit substance abuse or dependence (other than cannabis); (2) For the control group: no history of illicit substance use, a negative urine toxicology screen for all drugs tested, and no history of psychiatric illness (Axis I or II); (3) For all participants: ages 18–35, completion of high-school education, no family history of schizophrenia or bipolar disorder, no history of cardiovascular disease, hearing problems, neurological disease, learning disability, or head injury resulting in loss of consciousness. In addition, participants were excluded if they reported consumption of more than two alcoholic drinks per day (one per day for females). The cannabis group drug-use inclusion criteria (cannabis use at least once per week; 12 h abstinence) were chosen to minimize acute cannabis effects. Human studies indicate that 80–90% of the total amount of THC is excreted within 5 days, so a minimum use of once per week enabled detection of THC metabolites (Hunt and Jones, 1980).

Clinical Interviews, Questionnaires, and Drug Use Assessment

The structured clinical interview for DSM-IV axis I and II disorders (SCID I and SCID II) were administered to assess current and past history of psychopathology. The SCID I module E and a locally developed drug-use questionnaire were used to ascertain current and past diagnoses for alcohol and substance abuse and dependence. Levels of cannabis consumption (estimated number of joints) were determined via the interview and questionnaire for lifetime, the past 6 months, 3 months, 1 month, and then for the week before the test session as has been described previously (Fridberg *et al*, 2010; Skosnik *et al*, 2006a, 2008). Participants were instructed to consider each day of the week and indicate, for an average week, how much they

Table 1 Demographic and Substance Use Data (mean ± SD)

	Controls ($n = 24$)	Users ($n = 22$)	*P*
Age (years)	21.6 (3.0)	21.1 (2.6)	0.59
Education (years)	15.0 (1.6)	14.4 (1.4)	0.20
Gender (no. of females)[a]	12	7	0.21
WAIS (piccom) Scores	12.9 (2.92)	12.3 (2.42)	0.43
WAIS (digit) scores	11.7 (2.53)	11.4 (2.40)	0.68
WAIS (sim) scores	12.4 (2.57)	12.5 (2.41)	0.91
WAIS (dspan) scores	10.8 (2.44)	11.7 (3.03)	0.28
Average number of alcoholic drinks/week	0.96 (2.5)	5.3 (4.5)	0.001[a]
Age of first cannabis use	—	15.3 (1.4)	—
Frequency of cannabis use (joints in last month)	—	54.0 (36.6)	—
Hours since last cannabis use (h)	—	41.4 (34.0)	—

Note: WAIS version was the WAIS-III. Subscales included were picture completion (piccom); digit symbol (digit); similarities (sim); digit span (dspan).
[a]Results of χ^2 test.

consumed per drug-use occasion for each length of time assessed. Age of first use, number of joints smoked in the last month, and time since last use are reported in Table 1.

Urine screens (Q10-1, Proxam Diagnostics, Sunnyvale, CA, USA) were administered immediately preceding EEG testing in order to corroborate self-reports from the drug questionnaire and clinical interviews. The Q10-1 kit screens for cannabis (THC-COOH; 50 ng/ml sensitivity), opiates, amphetamines, cocaine, MDMA (ecstasy), tricyclic antidepressants, phencyclidine, benzodiazepines, methamphetamines, and barbiturates.

In addition to assessment of psychopathology and substance use, subscales of the Wechsler Adult Intelligence Scale III (WAIS-III; picture completion, digit symbol, similarities, and digit span) were used to assess possible deficits in general neuropsychological function.

Auditory Steady-state Responses

During the assessment of ASSRs, participants were seated comfortably in a sound-attenuated room with eyes open while passively listening to click trains presented through Etymotic insert earphones (Etymotic Research, Elk Grove Village, IL, USA). Auditory stimuli consisted of click trains (square waves) presented at nine different frequencies in each of the ten blocks (10, 15, 20, 25, 30, 35, 40, 45, and 50 Hz; 80 dB SPL). Each block contained 100 trials of the frequency of interest, which were presented for 500 ms each (interstimulus interval of 1000 ms). The order of frequency blocks was randomized across subjects.

EEG Recording

The EEG was recorded continuously (band pass 0.1–100 Hz; sampling rate 1000 Hz) from the scalp using a 32 channel electrode cap with a nose reference, along with additional electrodes to record the vertical electrooculogram (VEOG; Neuroscan SynAmps, Compumedics Neuroscan, Charlotte, NC, USA). Electrode impedances were maintained below 10 kΩ. The recorded EEG was segmented into epochs consisting of the 500 ms during stimulus presentation, along with a 500 ms baseline and a 500 ms offset period. Any trial with a voltage $> \pm 100\,\mu V$ was excluded from analysis. Ocular movement correction was applied using Gratton's algorithm (Gratton *et al*, 1983).

EEG Signal Analysis

MTP and ITC were determined using a time X frequency spectrogram with the Signal Processing and EEGLab toolbox in MATLAB (Delorme and Makeig, 2004; Rass *et al*, 2010; Skosnik *et al*, 2006b). For MTP, a baseline-normalized event-related spectral perturbation (ERSP) was obtained by applying a fast Fourier Transform using a time-sliding window on single-trial data. This results in a time X frequency transform consisting of a complex number for every timepoint, frequency, and trial. The 500 ms interval before stimulus onset was used as the baseline for computing the ERSP, and the sliding window had a duration of 128 ms. After sufficient padding a frequency resolution of 0.98 Hz was obtained and the time resolution was 3.8 ms. A Hanning window (100%) was applied on the data before the fast Fourier transform. No other taper functions were used. Thus, MTP represents the average of spectral power from individual trials after subtracting the mean from the baseline period (500 ms before stimulus onset).

For ITC (which represents phase synchronization of EEG activity across trials at particular temporal intervals and frequencies), the complex output of the ERSP was divided by its complex norm (absolute value), which was then averaged across trials. The complex norm of this averaged value results in the ITC value for different time and frequency points. ITC values range from 0 (absence of synchronization) to 1 (perfect synchronization, or phase reproducibility across trials at a given frequency).

MTP and ITC values (frequency bins ± 5 Hz from the frequency of stimulation in each condition) were averaged using sequential 100 ms windows between onset and offset of the stimuli (500 ms) as has been reported previously (Rass *et al*, 2010; Skosnik *et al*, 2006b). This resulted in 5 MTP and ITC values for every subject and every channel.

For analysis of the transient auditory-evoked response (N100), epochs were low-pass filtered at 15 Hz (24 dB/octave) before averaging, and were baseline-corrected (300 ms prestimulus baseline) after averaging. Peak amplitude and latency values after stimulus onset were used as the dependent measures, and were obtained for each electrode within the time window of interest using an automated algorithm (Vision Analyzer, Brain Products GmbH, Gilching, Germany). The N100 was defined as the most negative voltage between 90 and 150 ms after stimulus onset as reported previously (Skosnik *et al*, 2008). The signal amplitude showed local maxima in the frontal region (FCz), and all subsequent statistical analyses were conducted on data from this site. All N100 EEG processing was performed using commercially available software (Vision Analyzer, Brain Products GmbH).

Statistical Analysis

All analyses were conducted in the software package PASW Statistics 18.0. For the primary EEG outcome measures (MTP and ITC), a repeated measures ANOVA was utilized to examine the between-subjects factor of group (2) and the within-subjects factor of time (5) at electrode FCz (where MTP and ITC were maximal). Separate ANOVAs were performed for each frequency condition. Greenhouse–Geisser corrections for non-sphericity were used, where appropriate. The addition of gender, age, and level of alcohol use as covariates did not alter the results for the MTP or ITC analyses. For the transient N100 ERP, amplitude and latency were assessed within each frequency condition using a one-way ANOVA (at electrode FCz). In order to examine possible relationships between the ASSR and cannabis use variables (age of first use, number of joints in the last month, and time since last use), Pearson correlation coefficients were utilized. For the correlational analyses, a single value was calculated for MTP and ITC at the frequency of interest for the entire interval after stimulus onset (average MTP and ITC between 0–500 ms). All EEG data were normally distributed, as assessed with Shapiro–Wilk tests for normality. A criterion of $p < 0.05$ was used throughout to determine statistical significance, and all tests were two-tailed.

RESULTS

Table 1 provides basic demographic information as well as cannabis/alcohol use rates. A one-way ANOVA revealed that there were no significant differences between the groups in age, years of education, or WAIS-III subscale scores. A χ^2 test showed that the gender distribution within each group was not significantly different (Table 1). Because of the stringent exclusion criteria (see below), alcohol use rates for both groups were extremely low. However, there was a significant difference in the average number of alcoholic drinks consumed per week between the two groups (Table 1).

In the ASSR EEG protocol, subjects in both groups entrained to all nine stimulus frequencies, which can be seen in the grand averaged time X frequency plots of MTP and ITC across all frequencies taken from electrode FCz (Figure 1a and b). Note the preferred resonance of the ASSR at 40 Hz, particularly in MTP (Figure 1a), which has been described previously (Galambos *et al*, 1981; Picton *et al*, 2003). For MTP, a repeated measures ANOVA revealed a main effect of time for the 35, 40, and 50 Hz frequency conditions, an indication of change in spectral power across the stimulation time window, with the strongest response at 40 Hz (Table 2, top). Moreover, a main effect of group was observed in the 40 Hz ($F(1,44) = 6.01$, $p < 0.018$) and 15 Hz ($F(1,44) = 4.19$, $p < 0.047$) frequency conditions, indicating that the cannabis group had significantly decreased spectral power in the gamma and low beta bands. No group X time interactions were observed for MTP. Time X frequency plots of MTP comparing cannabis users *vs* controls in the 40 Hz condition can be seen in Figure 2.

For ITC, a main effect of time was observed for all frequency conditions (Table 2, bottom). No group or group X time interactions were observed for ITC.

For transient ERPs to stimulus onset, no group differences were observed in the N100 component in any frequency condition, indicating that the group differences in MTP were not due to altered early stimulus processing and registration. For illustrative purposes, ERPs to stimulus onset in the 40 Hz condition can be seen in Figure 3.

On the basis of *a priori* hypotheses, and the fact that the cannabis group exhibited decreased MTP in the gamma band (40 Hz), correlational analyses were carried out to examine the relationship between cannabis use variables and 40 Hz MTP. Cannabis use variables analyzed were age of first cannabis use, number of joints in the last month, and time since last use. A significant correlation was observed between 40 Hz spectral power and age of first cannabis use ($r = 0.45$, $p < 0.041$; Figure 4). In other words, individuals with the earliest age of cannabis use onset exhibited the lowest gamma-band (40 Hz) MTP.

DISCUSSION

The present study evaluated neural oscillatory activity by way of EEG in chronic cannabis users. The primary finding was a decrease in spectral power (MTP) during gamma- (40 Hz) and low beta-band (15 Hz) auditory steady-state stimulation in the cannabis group. No group differences were observed in ITC. Across all frequency conditions, no differences were observed between the groups in the transient N100 ERP component, indicating intact early auditory processing and sensory registration. Lastly, within the cannabis group, earlier onset of cannabis use was associated with lower levels of 40 Hz oscillatory power.

The fact that cannabis users did not exhibit differences in the transient N100 ERP is not surprising, as previous work on this component has been mixed. The N100 is thought to be related to basic perceptual processing, and in the auditory domain, is likely generated by auditory and frontal cortices (Naatanen and Picton, 1987). Skosnik *et al* (2008) demonstrated that heavy cannabis users had decreased N100 amplitudes for discrete 1000 Hz tones during an associative learning task (Skosnik *et al*, 2008). However, a subsequent study utilizing the same auditory stimuli with a different sample of cannabis users failed to replicate this finding (Edwards *et al*, 2008). Whether heavy cannabis use can disrupt the transient N100 ERP therefore remains equivocal.

The current study showed that exogenous cannabinoid exposure decreased MTP, particularly in the gamma band. Although power is thought to reflect the amplitude of neural oscillations, ITC represents the variance of phase across single trials, and is mathematically independent of power and amplitude (Roach and Mathalon, 2008; Spencer *et al*, 2008). These results therefore suggest that for 40 Hz stimuli, cannabis preferentially affected amplitude, while the variance of phase across single trials was not disrupted.

The observed association between cannabis use and altered gamma band activity is in agreement with previous cellular work examining the effects of exogenous cannabinoids on neural oscillations. Hajos *et al* (2000) showed that the administration of the highly potent cannabinoid agonist

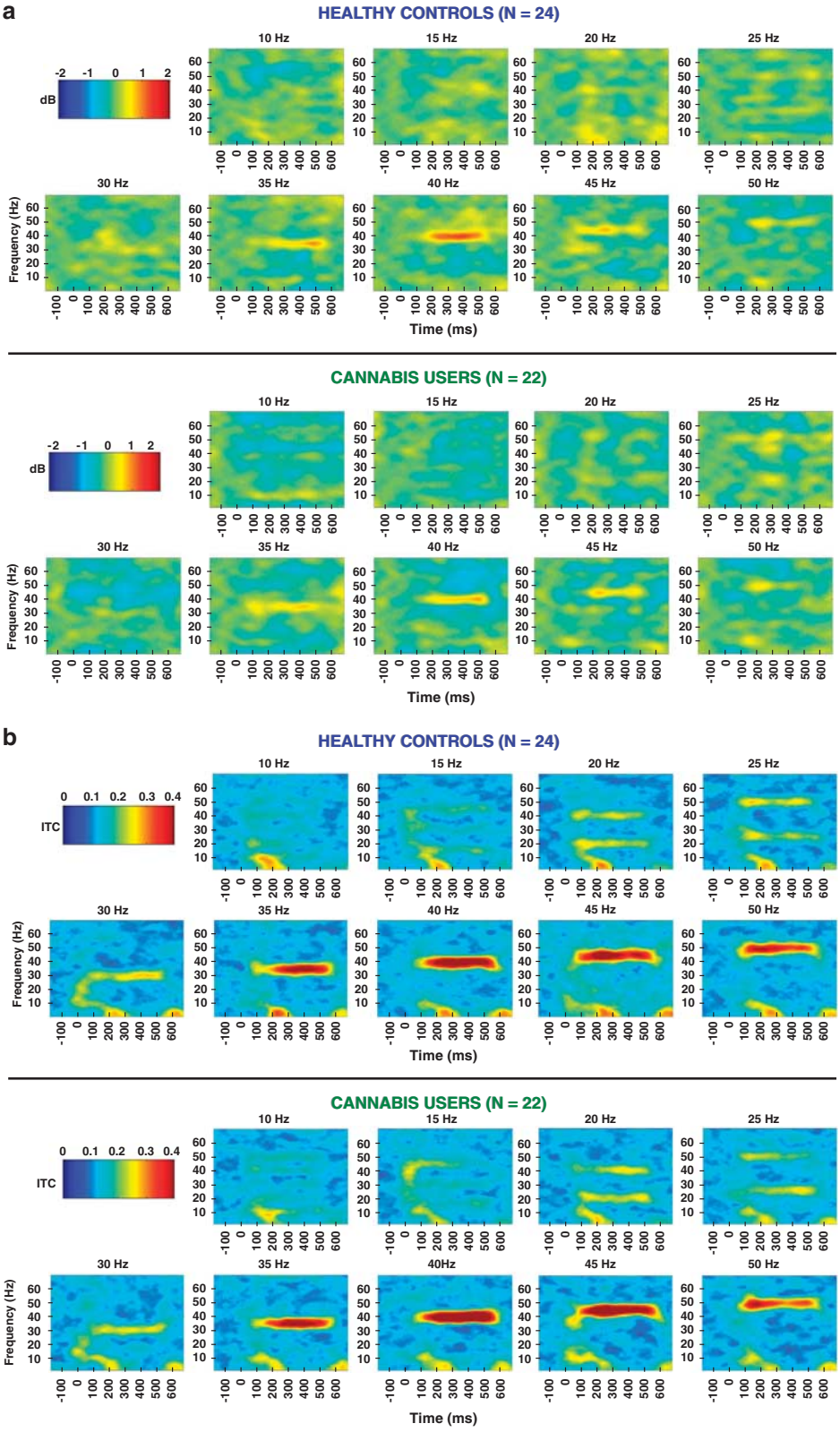

Figure 1 (a) Grand-averaged time X frequency plots demonstrating spectral power across all stimulation frequencies (FCz). Note the preferred resonance of mean trial power (MTP) at 40 Hz stimulation. (b) Grand-averaged time X frequency plots for inter-trial coherence (ITC) across all stimulation frequencies (FCz).

CP 55,940 robustly reduced the power of 40-Hz oscillations elicited in hippocampal slices by kainate *in vitro* (Hajos *et al*, 2000). In an *in vivo* study using rat entorhinal cortical neurons, it was found that while the CB1R agonist arachidonylcyclopropylamide had no effect on neural oscillations, the CB1R antagonist LY320135 increased

Table 2 ANOVA Table Showing Results for All Time X Frequency Analyses (MTP and ITC) Across All Frequency Conditions

ASSR condition (Hz)	Group	Time	Group X time
Spectral power (MTP)			
10	$F(1,44) = 0.645$, $p = 0.426$	$F(2.99,131.56) = 1.46$, $p = 0.23$	$F(2.99,131.56) = 0.37$, $p = 0.78$
15	**$F(1,44) = 4.19$, $p < 0.047$***	$F(2.57,112.85) = 0.224$, $p = 0.851$	$F(2.57,112.85) = 1.33$, $p = 0.27$
20	$F(1,44) = 0.771$, $p = 0.385$	$F(2.04,89.73) = 2.13$, $p = 0.12$	$F(2.04,89.73) = 1.01$, $p = 0.37$
25	$F(1,44) = 0.034$, $p = 0.854$	$F(3.46,152.1) = 0.6$, $p = 0.64$	$F(3.46,152.1) = 0.1$, $p = 0.72$
30	$F(1,44) = 0.96$, $p = 0.332$	$F(2.0,87.6) = 0.53$, $p = 0.59$	$F(2.0,87.6) = 1.0$, $p = 0.39$
35	$F(1,44) = 0.210$, $p = 0.649$	**$F(3.44,151.3) = 3.92$, $p = 0.007$***	$F(3.44,151.3) = 0.5$, $p = 0.71$
40	**$F(1,44) = 6.01$, $p < 0.018$***	**$F(3.15,138.6) = 7.8$, $p < 0.000$***	$F(3.15,138.6) = 1.47$, $p = 0.22$
45	$F(1,44) = 1.43$, $p = 0.24$	$F(2.53,111.46) = 2.58$, $p < 0.07$	$F(2.53,111.46) = 0.64$, $p = 0.56$
50	$F(1,44) = 0.14$, $p = 0.91$	**$F(3.32,146.2) = 4.0$, $p < 0.007$***	$F(3.32,146.2) = 0.48$, $p = 0.49$
Intertrial coherence (ITC)			
10	$F(1,44) = 0.04$, $p = 0.85$	**$F(2.49,109.32) = 22.08$, $p = 0.000$***	$F(2.49,109.32) = 0.75$, $p = 0.50$
15	$F(1,44) = 0.85$, $p = 0.36$	**$F(3.13,137.78) = 17.38$, $p < 0.000$***	$F(3.13,137.78) = 2.3$, $p = 0.08$
20	$F(1,44) = 0.21$, $p = 0.65$	**$F(3.23,142.17) = 20.1$, $p < 0.000$***	$F(3.23,142.17) = 1.69$, $p = 0.17$
25	$F(1,44) = 0.045$, $p = 0.83$	**$F(3.20, 140.61) = 5.22$, $p < 0.002$***	$F(3.20, 140.61) = 1.54$, $p = 0.21$
30	$F(1,44) = 0.02$, $p = 0.89$	**$F(2.58,113.38) = 12.6$, $p < 0.000$***	$F(2.58,113.38) = 0.34$, $p = 0.76$
35	$F(1,44) = 0.36$, $p = 0.55$	**$F(2.60,114.24) = 84.57$, $p < 0.000$***	$F(2.60,114.24) = 0.76$, $p = 0.50$
40	$F(1,44) = 1.71$, $p = 0.20$	**$F(2.17, 95.43) = 102.61$, $p < 0.000$***	$F(2.17, 95.43) = 1.34$, $p = 0.27$
45	$F(1,44) = 1.58$, $p = 0.22$	**$F(2.40,105.46) = 83.84$, $p < 0.000$***	$F(2.40,105.46) = 2.20$, $p = 0.11$
50	$F(1,44) = 0.62$, $p = 0.44$	**$F(2.39,105.36) = 57.23$, $p < 0.000$***	$F(2.39,105.36) = 1.52$, $p = 0.22$

Significant effects are denoted with an asterisk* and bold font.

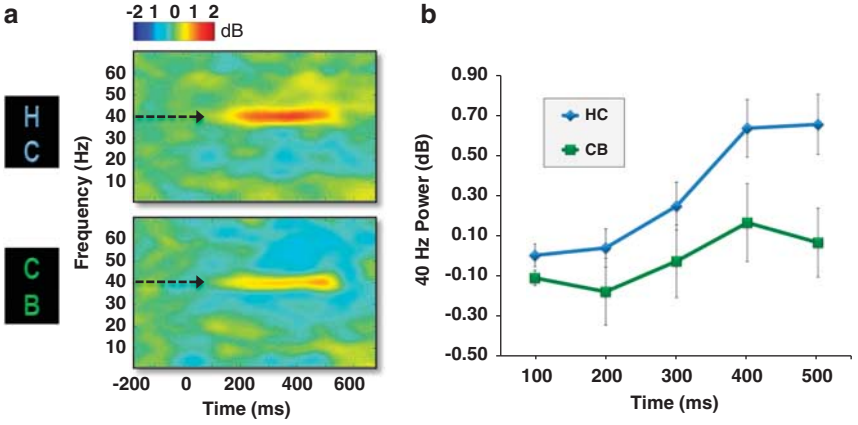

Figure 2 (a) Grand-averaged time X frequency plots of MTP during gamma-band (40 Hz) auditory stimulation at electrode FCz for healthy controls (HC; top; $n = 24$) and cannabis users (CB; bottom; $n = 22$). Greater 40 Hz power was seen in control subjects compared with cannabis users. (b) Average MTP values in 100 ms intervals during the 500 ms window after onset of the 40 Hz click trains for control subjects (blue line) and cannabis users (green line) at FCz (error bars indicate ± SE).

gamma-band power in the deep medial entorhinal cortex (Morgan *et al*, 2008). Interestingly, LY320135 suppressed gamma power in more superficial layers of the entorhinal cortex, illustrating the complex role of CB1Rs in cortical oscillations.

Although the results gleaned from slice preparations are highly informative, they are somewhat distal from the type of EEG data described in the current study. More analogous to the surface-based EEG data described here, several groups have examined the effects of cannabinoids on neural oscillations *in vivo* using animal-based local field potentials (LFPs). For example, it has been shown that both THC and CP 55,940 disrupt hippocampal theta and gamma oscillations in head-restrained and freely moving rats, effects that were blocked by the CB1R antagonist SR141716A (Robbe *et al*, 2006). Importantly, the alterations were shown to be functionally relevant, as the degree of theta power disruption was correlated with performance on a hippocampal-

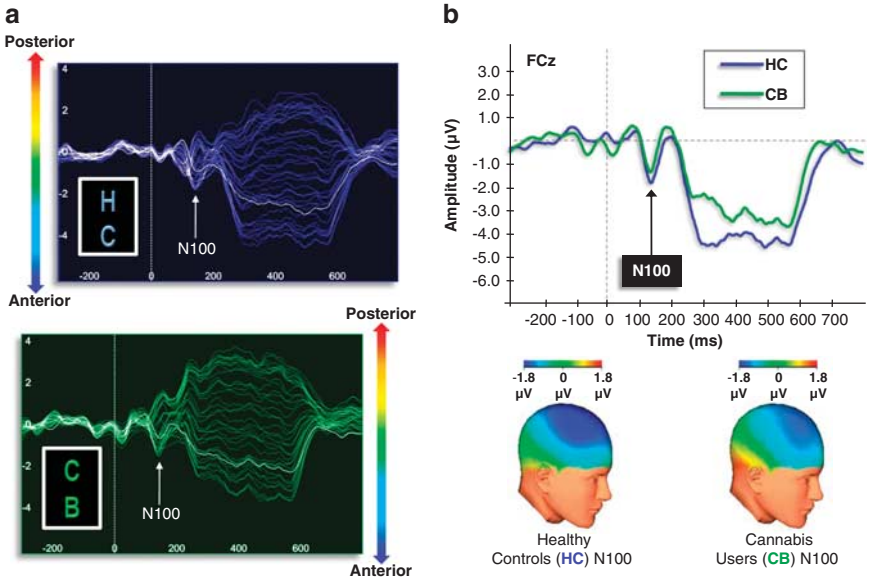

Figure 3 (a) Butterfly plots illustrating the grand-averaged ERPs across all electrodes during 40 Hz stimulation for healthy controls (HC) and cannabis users (CB). Waveforms from each source electrode are oriented generally from posterior (top) to anterior (bottom). (b) Grand-averaged ERPs (FCz) and topographic maps (at 100 ms) for healthy controls and cannabis users demonstrating the N100 to 40 Hz stimulation (top). No group differences were observed in the N100 component in any frequency condition, indicating normal early stimulus processing and registration.

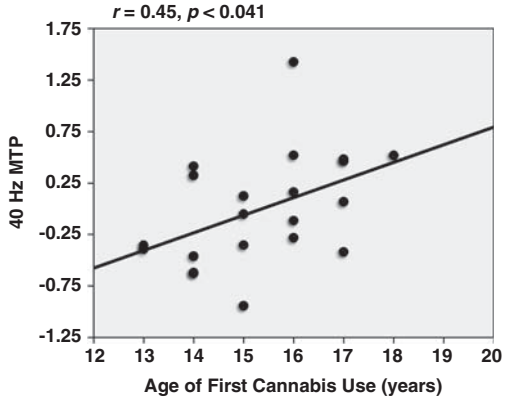

Figure 4 Correlation between 40 Hz spectral power (average MTP between 0–500 ms during 40 Hz stimulation) and age of first cannabis use within the cannabis group ($n = 22$).

dependent memory task. In a study exploring hippocampal and cortical LFPs, Hajos *et al* (2008) demonstrated that rats engaged in a sensory gating paradigm exhibited decreases in gamma- and theta-band spectral power after the administration of CP 55,940. CP 55,940 similarly affected prefrontal cortical recordings during free movement (attenuation of gamma and theta-band power). Importantly, these results were both CB1R-specific, as the disruption in neural oscillations was reversed by the CB1R antagonist AM-251. Taken together, these results suggest that theta and gamma oscillations in networks of GABAergic interneurons are regulated by the endocannabinoid system, which can be disturbed by the exogenous application of CB1R agonists.

In terms of human studies, the present findings are consistent with the results of a number of experiments examining the effects of both chronic and acute cannabinoids on neural oscillations. For example, two studies have shown that *chronic* cannabis users exhibit disrupted neural oscillatory activity using different EEG paradigms. Edwards *et al* (2009) implemented a human analogue of the sensory gating paradigm described above by Hajos *et al* (2008), and found decreased gamma-band power during the auditory click stimuli, which was negatively correlated with levels of cannabis use (ie, those with the lowest gamma power had the greatest levels of cannabis exposure) (Edwards *et al*, 2009). Further, Skosnik *et al* (2006a) found evidence of decreased EEG spectral power using several frequencies of stimulation in the ASSR paradigm (Skosnik *et al*, 2006a). Regarding the acute effects of cannabinoids in humans, Morrison *et al* (2011) recently demonstrated that intravenous THC administration decreased theta power and inter-electrode coherence during performance on an *n*-back task of working memory (Morrison *et al*, 2011). Two previous studies showed similar results with inhaled THC, including decreased resting state theta power and disruptions in working memory performance (Bocker *et al*, 2010; Ilan *et al*, 2004, 2005). To date, no human studies have shown altered gamma-band activity in the context of acute cannabinoid administration.

The current finding that 40 Hz power was associated with a younger age of onset of cannabis use suggests that long-term exposure to cannabis (and not recency of use or residual cannabinoids) contributed to the observed findings. This is noteworthy, given the known role of cannabinoids in neurodevelopment. Both cellular and animal studies have shown that the endogenous cannabinoid system has a key role in neurogenesis, neural specification, neural maturation, neuronal migration, axonal elongation, and glia formation (Harkany *et al*, 2007, 2008a, 2008b). Hence, earlier cannabis exposure during adolescence may alter neurodevelopmental trajectories,

which could permanently disrupt the ability of neural circuits to generate synchronized oscillations. Interestingly, cannabis use is now thought to represent a risk factor and component cause in the development of schizophrenia (Moore *et al*, 2007; Sewell *et al*, 2010). As schizophrenia patients and their first-degree relatives also demonstrate decreased 40 Hz ASSRs (Shin *et al*, 2011), it is reasonable to speculate that these alterations are mediated in part by disruptions in cannabinoid-GABA interactions. Future research is needed to explicitly test this postulate.

There are several limitations to the current experiment. First, the cross-sectional design of the study precludes the ability to ascertain precise cause and effect relationships. Hence, it remains unclear whether the observed results were due to the residual effects of THC, cannabis withdrawal, long-term cannabis exposure (eg, CB1R down-regulation), or premorbid neurodevelopmental and/or personality differences predisposing individuals to use cannabis. Second, the cannabis plant contains nearly 70 phytocannabinoids, so it is unclear which specific constituent has a role in neural oscillations. Each of these limitations could be resolved in future studies by examining the ASSR in the context of acute THC administration in humans. A third limitation is that while cannabis use status was confirmed by urinary THC-COOH, no quantitative assays of THC or THC-COOH were undertaken, which would have been a more valid means to determine the magnitude of recent cannabis exposure. Fourth, as previous studies have shown that CB1Rs are also involved in theta-band oscillations, future work should examine the effect of cannabis on ASSRs in the 4–7 Hz range. Finally, the functional significance of the ASSR remains unclear, as it is unknown whether the neural oscillations evoked during auditory steady-state stimulation are related to spectral power and phase locking measured during cognitive tasks. These limitations notwithstanding, the current data suggest that chronic exposure to cannabis may alter the ability to generate neural oscillations in the gamma range, which may have implications for understanding the short- and long-term psychopharmacological effects of exogenous cannabinoids.

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