Δ⁹-THC Disrupts Gamma (γ)-Band Neural Oscillations in Humans

Jose Cortes-Briones[1,2], Patrick D Skosnik[1,2,3], Daniel Mathalon[2,4,5], John Cahill[1,2,3], Brian Pittman[2,3], Ashley Williams[1,3], R Andrew Sewell[1,2,3], Mohini Ranganathan[1,2,3], Brian Roach[4,5], Judith Ford[2,4,5] and Deepak Cyril D'Souza*,[1,2,3]

[1]Psychiatry Service, VA Connecticut Healthcare System, West Haven, CT, USA; [2]Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA; [3]Abraham Ribicoff Research Facilities, Connecticut Mental Health Center, New Haven, CT, USA; [4]Department of Psychiatry, University of California, San Francisco, San Francisco, CA, USA; [5]Mental Health Service Line, San Francisco VA Medical Center, San Francisco, CA, USA

Gamma (γ)-band oscillations play a key role in perception, associative learning, and conscious awareness and have been shown to be disrupted by cannabinoids in animal studies. The goal of this study was to determine whether cannabinoids disrupt γ-oscillations in humans and whether these effects relate to their psychosis-relevant behavioral effects. The acute, dose-related effects of Δ-9-tetrahydrocannabinol (Δ⁹-THC) on the auditory steady-state response (ASSR) were studied in humans ($n = 20$) who completed 3 test days during which they received intravenous Δ⁹-THC (placebo, 0.015, and 0.03 mg/kg) in a double-blind, randomized, crossover, and counterbalanced design. Electroencephalography (EEG) was recorded while subjects listened to auditory click trains presented at 20, 30, and 40 Hz. Psychosis-relevant effects were measured with the Positive and Negative Syndrome scale (PANSS). Δ⁹-THC (0.03 mg/kg) reduced intertrial coherence (ITC) in the 40 Hz condition compared with 0.015 mg/kg and placebo. No significant effects were detected for 30 and 20 Hz stimulation. Furthermore, there was a negative correlation between 40 Hz ITC and PANSS subscales and total scores under the influence of Δ⁹-THC. Δ⁹-THC (0.03 mg/kg) reduced evoked power during 40 Hz stimulation at a trend level. Recent users of cannabis showed blunted Δ⁹-THC effects on ITC and evoked power. We show for the first time in humans that cannabinoids disrupt γ-band neural oscillations. Furthermore, there is a relationship between disruption of γ-band neural oscillations and psychosis-relevant phenomena induced by cannabinoids. These findings add to a growing literature suggesting some overlap between the acute effects of cannabinoids and the behavioral and psychophysiological alterations observed in psychotic disorders.

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INTRODUCTION

With the legalization of 'medical' marijuana (cannabis) (Kleber and DuPont, 2012; Procon.org, 2014) and recreational cannabis use in some states of the United States (Wilkinson and D'Souza, 2014), the high rates (ONDCP, 2008; SAMHSA, 2011) and earlier onset of cannabis use (Johnston *et al*, 2012), the increasing potency of cannabis (Mehmedic *et al*, 2010), the recreational use of highly potent synthetic cannabinoids (eg, Spice and K-2) (Johnson *et al*, 2011; Vardakou *et al*, 2010), and the high rates of emergency department visits related to cannabis (SAMHSA, 2012), there is a need to understand the basic mechanisms underlying the behavioral effects of cannabinoids such as Δ-9-tetrahydrocanabinol (Δ⁹-THC), the primary psychoactive constituent in cannabis.

*Correspondence: Professor DC D'Souza Psychiatry Service 116A, VA Connecticut Healthcare System, 950 Campbell Avenue, West Haven, CT 06516, USA, Tel: +1 203 932 5711 (2594), Fax: +1 203 937 4860, E-mail: deepak.dsouza@yale.edu

Δ⁹-THC, via activation of brain cannabinoid 1 receptors (CB1Rs), induces a range of acute alterations in perceptual, emotional, and cognitive functions that are relevant to psychotic states and psychotic disorders such as schizophrenia (reviewed in Radhakrishnan *et al*, 2014). Neural oscillations in the gamma (γ)-band (30–80 Hz) are thought to play a key role in the operation of these functions by participating in sensory registration and integration, associative learning, and conscious awareness among other processes (Uhlhaas *et al*, 2008). Therefore, it is expected that alterations in these processes will be associated with abnormalities in γ-band oscillations. Consistent with this view, a number of studies in schizophrenia patients have confirmed the existence of an association between functional and γ-band abnormalities as measured by electroencephalography (EEG) (see Uhlhaas *et al*, 2008 for review). This raises the intriguing possibility that some of the acute psychosis-relevant functional abnormalities induced by Δ⁹-THC may be associated with γ-band alterations (Skosnik *et al*, 2006).

Consistent with this possibility, both *in vitro* and *in vivo* studies in animals have shown that CB1R agonists modulate

γ-oscillations in the cerebral cortex and hippocampus (Hajos et al, 2000, 2008; Morgan et al, 2008; Robbe et al, 2006). For example, systemic administration of the CB1R agonist CP-55940 has been shown to decrease the amplitude of γ-band oscillations in mice as measured via neocortical electrocorticograms (Sales-Carbonell et al, 2013). Similar findings have been observed via local field potential (LFP) recordings in rats (Hajos et al, 2008).

However, although γ-band abnormalities have been reported in schizophrenia, cannabinoids have been shown to acutely induce psychosis-relevant phenomena in healthy humans, and cannabinoids have been shown to disrupt γ-band oscillations in animals, we are unaware of any studies in humans examining the acute effects of CB1R agonists on either γ-band oscillations or the relationship between γ-band oscillations and the acute psychosis-relevant phenomena induced by CB1R agonists.

In humans, macroscopic synchronized neural oscillations can be evaluated noninvasively with the EEG by measuring the brain's auditory steady-state response (ASSR) to sensory stimulation, that is, the entrainment of EEG activity to regular auditory stimuli (eg, auditory click trains) of varying frequencies. Neural circuits act as oscillators that tune their activity to the frequency and phase of the presented stimuli, providing an indicator of the brain's ability to engage in transient states of synchronized oscillatory activity at the frequency used for stimulation. Furthermore, a number of studies have shown that the ASSR is sensitive to the abnormalities present in schizophrenia. For example, first-episode and chronic schizophrenia patients show attenuated γ-band (~40 Hz) responses in the ASSR paradigm (Krishnan et al, 2009; Kwon et al, 1999; Light et al, 2006; Spencer et al, 2008a) that is associated with some of the characteristic abnormalities of the disorder (eg, positive symptoms and working memory deficits) (Light et al, 2006; Spencer et al, 2008b). In view of this, we selected the ASSR paradigm to study the relationship between acute Δ⁹-THC-induced alterations in γ-band oscillations and psychosis-relevant effects.

This study was part of a larger project that aimed to assess the dose-related effects of Δ⁹-THC on several electrophysiological indices of information processing relevant to psychosis (eg, P300; D'Souza et al, 2012) and to determine the relationship between the electrophysiological and behavioral effects of Δ⁹-THC. In this study we examined the acute, dose-related effects of intravenous (i.v.) Δ⁹-THC on the ASSR in a number of frequency bands, and the relationship between these effects and the psychosis-relevant effects induced by Δ⁹-THC. We hypothesized that Δ⁹-THC would specifically reduce the γ-band (40 Hz) ASSR, and that γ-band ASSRs measures would be inversely correlated with the psychosis-relevant effects of Δ⁹-THC.

MATERIALS AND METHODS

This randomized, double-blind, placebo-controlled, counterbalanced, crossover study was conducted at the Neurobiological Studies Unit (VA Connecticut Healthcare System (VACHS), West Haven, CT). Subjects were recruited by advertisements and by word of mouth, and were paid for their participation. The study was approved by the institutional review boards of the VACHS and Yale University

School of Medicine and was carried out in accordance with the Helsinki Declaration of 1975. Subjects were informed about the potential for adverse effects of Δ⁹-THC including psychosis, anxiety, panic, and abuse liability.

Subjects

After obtaining written informed consent, subjects ($n = 30$; 18–35 years) underwent a Structured Clinical Interview for DSM-IV (SCID; First et al, 2002) and were carefully screened for any DSM Axis I lifetime psychiatric or substance use disorder (excluding tobacco and cannabis use) and family history of major Axis I disorder. Subjects were screened at a separate session within ~4 weeks of the first test day. Cannabis-naive individuals were excluded to minimize any risk of promoting future cannabis use/abuse. Subjects with DSM-IV cannabis dependence based on the SCID were excluded because cannabis dependence has been associated with a bunted response to THC (tolerance). Subjects were categorized into those who had not used cannabis in the past 30 days (recent users) and those who had not (nonusers) for exploratory analyses to determine the effects of cannabis exposure on study outcomes. The history provided by subjects was confirmed by a telephone interview conducted with a spouse or family member identified by the subject. A general physical and neurological examination, electrocardiogram, and laboratory tests (serum electrolytes, liver function tests, complete blood count with differential, urine toxicology, and urine pregnancy in women) were also conducted. After screening, subjects were instructed to refrain from alcohol, caffeinated beverages, illicit drugs (other than cannabis), or prescription drugs not approved by the research team for 2 weeks before the study and throughout study participation. Cigarette smokers were permitted to participate only if their pattern of cigarette consumption would allow them to abstain for each test day (0800–1500 h) and their consumption did not exceed 10 cigarettes/day. Recent users of cannabis were instructed to abstain from smoking for at least 24 h before test day. Test days were rescheduled if subjects reported using cannabis within 24 h of the test day. Subjects who were not categorized as recent users of cannabis were reminded not to use cannabis for 2 weeks before testing and throughout study participation. For this group, abstinence was confirmed by urine drug testing on the morning of each test day.

Subjects completed 3 test days during which they received Δ⁹-THC (placebo (vehicle (ethanol)), 0.015 mg/kg, or 0.03 mg/kg) over 10 min by i.v. route in a randomized, counterbalanced order under double-blind conditions. Both staff and subjects received identical information without reference to any hypothesized group differences.

Drugs

The preparation, formulation, and storage of the Δ⁹-THC solutions are reported elsewhere (D'Souza et al, 2004). For the control placebo condition, an equivalent volume of ethanol (vehicle) was used that was previously shown to be undetectable in multiple postinjection blood samples (D'Souza et al, 2004). The i.v. route of administration was chosen to standardize the delivery of Δ⁹-THC as discussed previously (D'Souza et al, 2004).

General EEG Acquisition and Processing

Multichannel EEG was recorded from the scalp using a 22-lead electrode cap (Physiometrix, Billerica, MA) based on the International 10–20 System (see Supplementary Text 5). Electrode impedances were maintained < 5 kohms. Recordings were made using a linked ear reference. The data were recorded using Neuroscan Synamps amplifiers (Neuroscan SynAmps, Compumedics Neuroscan, Charlotte, NC). Codes indicating stimulus onset and type were incorporated into the data stream for offline averaging and analysis using Neuroscan Acquire software for data acquisition and software for stimulus presentation (Presentation, Neurobehavioral Systems, Berkeley, CA). EEG was continuously monitored on a screen outside the recording chamber to detect drowsiness.

ASSR Paradigm

For assessment of ASSRs, subjects sat in an acoustically shielded booth in front of a computer monitor with eyes open, while passively listening to click trains presented through Etymotic insert ER-1 earphones (Etymotic Research, Elk Grove Village, IL). Stimuli consisted of standard, unattended (nontarget) auditory click trains from a three-stimulus oddball task as reported previously (D'Souza *et al*, 2012). The auditory click trains were presented at three different frequencies (20, 30, and 40 Hz; 80 dB SPL). Each block contained 150 trials of a single frequency presented for 500 ms each (ISI 750 ms). Each trial lasted 1250 ms, and each block lasted 4 min. Across subjects, the order of blocks was counterbalanced and the order of conditions was randomized.

EEG Signal Analysis

A detailed account of EEG methods is provided in the Supplementary Text 1. Continuous EEG data were band-pass filtered (0.5–100 Hz), line noise was removed using a multi-tapering technique (Partha and Hemant, 2007), muscle artifacts were removed using a blind source separation algorithm (De Clercq *et al*, 2006), and eye movement and blink artifacts were removed with an adaptive filter algorithm (Puthusserypady and Ratnarajah, 2005). Data were segmented in 1200 ms epochs time-locked to stimulus onset, with a 300-ms prestimulus baseline. A $\pm$ 95 µV voltage criterion was used to reject bad epochs. EEG data from three midline electrodes (Fz, Cz, and Pz) were used for ASSR analyses, given that the ASSR is typically maximal at the midline electrodes (Skosnik *et al*, 2012). All analyses were done in Matlab (MathWorks, Natick, MA) using either custom-made scripts (for inter-trial coherence (ITC) and evoked power) or the EEGLAB toolbox' scripts and plugins (for signal pre-processing) (Delorme and Makeig, 2004; Gómez-Herrero *et al*, 2006).

ITC reflects the consistency across trials of the phases of the brain's electrophysiological response to equivalent stimuli/events (Tallon-Baudry *et al*, 1996). ITC values range from 0 to 1, being maximal at a given time point (relative to the onset of the stimulation/event) when the phases of the EEG signals of all the trials are the same at the time point, and minimal when the phases at the time point are randomly distributed across the trials. For each auditory stimulation frequency (20, 30, and 40 Hz) and electrode (Fz, Cz, ad Pz), the signal epochs (trials) were band-pass filtered around the stimulation frequency (stimulation frequency $\pm$ 5 Hz) and then Hilbert-transformed to obtain the analytic signal. Instantaneous ITC was obtained as the norm of the point-by-point average across trials of the normalized analytic signals. ITC values were averaged in the time window 50–550 ms after the onset of the auditory stimulation, and the average ITC of the baseline period (-300 to -50 ms) was subtracted from it.

Evoked power reflects both the magnitude of the brain's voltage response to a stimulus/event and the consistency across trials of the time course of this time-locked response. For each auditory stimulation frequency and electrode, signal epochs (trials) were band-pass filtered (stimulation frequency $\pm$ 5 Hz), averaged across trials in a point-by-point manner, and then Hilbert-transformed to obtain the analytic signal. Instantaneous evoked power was obtained by squaring the norm of the analytic signal. Evoked power was then averaged in the interval 50–550 ms after the onset of auditory stimulation, and the average power of the baseline period (-300 to -50 ms) was subtracted from it.

Behavioral Psychosis-Relevant Measures

Psychosis-relevant effects were captured with the Positive and Negative Syndrome Scale (PANSS) (Kay *et al*, 1989) positive, negative, general, and total scores that are known to be sensitive to the effects of Δ⁹-THC (Radhakrishnan *et al*, 2014).

General Procedure and Test Days

Test days were separated by 3 days to minimize carryover effects (see Supplementary Table 1). On the morning of each test day, subjects underwent drug and pregnancy testing. EEG was recorded during an ASSR task, administered before and when peak drug effects were expected to occur on each test day. In-study safety procedures and poststudy prospective safety assessments were performed; their results were reported in further detail elsewhere (Carbuto *et al*, 2011; D'Souza *et al*, 2004).

Statistical Analysis

Initially, data were examined descriptively using means, SDs, and graphs. For each drug condition (placebo, 0.015 mg/kg, and 0.03 mg/kg), EEG (evoked power and ITC) and behavioral (PANSS positive, negative, general, and total scores) measures were assessed for normality with the Kolmogorov–Smirnov test. Outliers were detected using a median absolute deviation (MAD)-based (Hampel, 1974) criterion that, compared with the common 'standard deviations above the mean' criterion, is highly robust against the presence of multiple outliers (Davies and Gather, 1993) (for further details, see Supplementary Text 2). Using this approach, no greater than 12% of the data for any outcome variable were excluded from the analysis.

Given that the auditory steady-state stimulation response was maximal at the electrode Cz, only this electrode was used for statistical analyses. EEG measures were obtained both before drug administration (baseline) and around predicted

occurrence of peak drug effects. However, as there were no significant differences in predrug measures of ASSR between the 3 test days (for further details, see Supplementary Text 3), predrug data were not included in the analysis to detect drug effects.

The effects of Δ9-THC on ITC and evoked power were assessed using generalized estimating equations (GEE) with unstructured working correlation matrices (Liang and Zeger, 1986; Zeger and Liang, 1986). The effect of drug condition on each EEG measure (two measures: evoked power and ITC) was modeled by fitting three separate GEEs to the data, one for each stimulation frequency. For each EEG measure, the *p*-values of the GEE models were adjusted for three comparisons (one for each stimulation frequency) with the Holm–Bonferroni (HB) sequential procedure. The *post hoc* pairwise comparisons (0.03 mg/kg *vs* placebo, 0.015 mg/kg *vs* placebo, and 0.03 mg/kg *vs* 0.015 mg/kg) were performed for the GEE models with a significant main effect of drug condition in order to localize the source of the effect. For each EEG measure, *p*-values of the pairwise comparisons were HB adjusted in accordance with the total number of comparisons performed for the EEG measure (maximum possible number of comparisons for each EEG measure = 9: 3 frequencies × 3 drug condition pairs).

To characterize the relationship (correlation) between the effects of Δ9-THC on both EEG (ITC and evoked power) and PANSS scores, longitudinal regressions were conducted on the data of both Δ9-THC-active conditions. The behavioral measures were regressed on the EEG measure/s presenting a significant main effect of drug by fitting GEE models with unstructured working correlation matrices to the data of both Δ9-THC-active conditions (maximum number of regressions = 8: 2 EEG measures × 4 behavioral measures). Furthermore, in order to approximate the regression coefficients to partial correlation coefficients, standardized regression coefficient (βs) were obtained by transforming the data into composite *z*-scores before fitting the GEE models. To preserve the relative differences in the values of each variable between the two Δ9-THC-active conditions, composite *z*-scores were calculated using the mean and SD of the data of both Δ9-THC-active conditions pooled together. The *p*-values of the coefficients were HB adjusted for the total number of regression performed (maximum number = 8). Analyses were done using SPSS 21 (IBM Corporation, Armonk, NY) and custom-made scripts in Matlab (Math-Works, Natick, MA).

RESULTS

The demographic and cannabis use data are listed in Table 1. Of the 56 subjects who initially consented for the study, 10 failed the screening process and 8 chose not to initiate the study. Of the remaining 38 subjects, 30 completed all 3 test days (21% dropout rate), of which 5 had to be dropped because of technical difficulties during EEG acquisition. There were no obvious differences between dropouts and completers (see Supplementary Table 2). Of the remaining 25 subjects, 2 were dropped during the EEG preprocessing because of artifactual contamination (for details, see Supplementary Text 1), and 3 were categorized as outliers and dropped during the statistical inspection of the EEG

Table 1 Sample Demographics

General characteristics		
No. of male (female)		14 (6)
Age (mean (SD))		25.700 (7.623)
Handedness		1 Left handed
Years of education (mean (SD))		15.200 (2.142)
Estimated IQ (mean (SD))		116.850 (3.883)
Body mass index (mean (SD))		24.25 (2.99)
Cannabis exposure		
Age of first cannabis use		16.150 (4.522)
Days since last use (mean (SD) range)		402.725 (854.634) 1–3650
Subgrouping (no. of subjects)	Recent users	9
	Nonusers	11
Frequency of cannabis use within past 30 days (no. of subjects)	0 Days	11
	1–3 Days	4
	4–8 Days	1
	9–15 Days	2
	16–29 Days	2
Estimated lifetime exposure to cannabis (no. of subjects)	Only once	0
	2–5 Days	1
	5–10 Days	2
	11–20 Days	4
	21–30 Days	1
	31–50 Days	1
	51–100 Days	1
	101–200 Days	1
	201–300 Days	0
	301–500 Days	4
	501–1000 Days	2
	> 1000 Days	3
Estimated cannabis exposure during heaviest use (no. of subjects)	< 1 Per year	1
	1 Per year	2
	1 Per 6 months	4
	1 Per 3 months	3
	1–3 Per month	2
	1–2 Per week	1
	3–6 Per week	3
	7 Per week	4
Other drug exposure[a]		
Daily cigarette smokers (no. of subjects)		1
Social cigarette smoker (no. of subjects)		1
Average no. of alcoholic drinks per week (mean (SD))		6.01 (6.06)
No exposure to other drugs (no. of subjects)		6
Exposure to drugs other than cannabis (no. of subjects)	Psilocybin	11
	LSD	2
	Salvinorin	3
	Nitrous oxide	2
	Cocaine	5
	Amphetamines	3
	MDMA	2
	Opioids	2

[a]None of the subjects met criteria for abuse or dependence of tobacco, alcohol, and other drugs of abuse. Exposure to the drugs listed above was limited and remote.
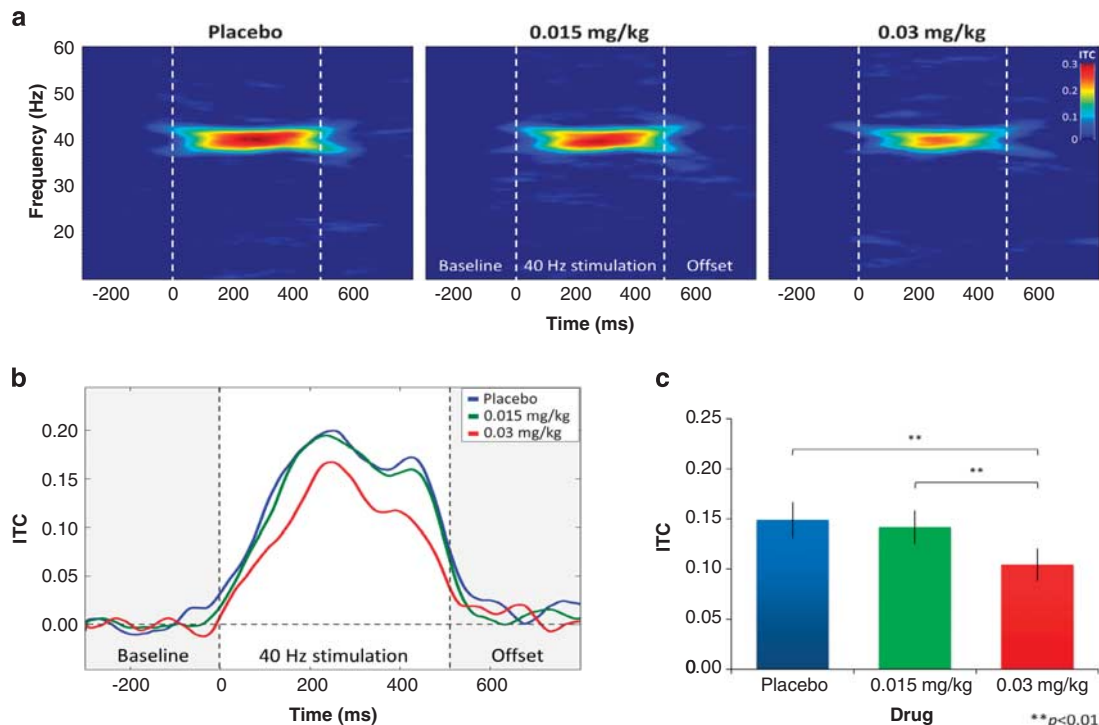
Figure 1 The 40 Hz intertial coherence (ITC) per drug condition at electrode Cz. (a) Hilbert transform-based time–frequency plots for ITC (for details, see Supplementary Text 1). From left to right, the plots show the group-averaged time–frequency plots of the placebo, 0.015 mg/kg, and 0.03 mg/kg conditions during 40 Hz stimulation at electrode Cz. (b) Time course of the group-averaged ITC per drug condition at electrode Cz. The blue line represents the ITC of the placebo condition, whereas the green and red lines represent the ITC of the 0.015 and 0.03 mg/kg doses, respectively. (c) ITC (mean ± SEM) by drug condition at electrode Cz during the time interval 50–550 ms after stimulation onset. **: Significant differences ($p_{Adj} < 0.01$) between drug conditions.

measures. Thus, EEG data from 20 subjects who completed all test days were used for statistical analyses. The inspection of the behavioral measures of these 20 subjects revealed one outlier for the PANSS general and total scores during the 0.015 mg/kg condition. This data point was excluded from the regression analyses (for details about the rejections of outliers, see Supplementary Text 2).

Auditory Steady-State Response

Intertrial coherence. There was a HB-corrected significant main effect of dose on ITC with 40 Hz stimulation (Wald QUOTE (2) = 12.677, $p_{Adj} = 0.006$). No significant main effect of dose was observed with the 20 and 30 Hz stimulation frequencies either before or after HB correction ($ps_{Adj} > 0.1$). The *post hoc* analyses of the 40 Hz data revealed HB-corrected (3 comparisons) significantly lower ITC in the 0.03 mg/kg condition compared with the 0.015 mg/kg ($p_{Adj} = 0.005$) and the placebo ($p_{Adj} = 0.002$) conditions (Figure 1). No significant differences were found between the 0.015 mg/kg and the placebo conditions ($p_{Adj} > 0.1$). Means and SDs of the EEG measures are reported in Table 2.

Evoked power. No significant main effect of dose on evoked power was observed with any stimulation frequency after HB correcting for multiple comparisons ($p_{Adj} > 0.1$). Before corrections a trend to significance was observed with 40 Hz stimulation (Wald QUOTE (2) = 5.023, $p = 0.081$), whereas no effects were detected with 20 and 30 Hz

stimulation. Inspection of the means (Table 2) showed that in the 40 Hz condition, evoked power had a dose-related reduction as observed in ITC.

Behavioral and Subjective Effects

As reported elsewhere (D'Souza *et al*, 2012), Δ⁹-THC significantly increased PANSS positive, negative, general, and total scores (all $ps < 0.001$). All doses were significantly different from each other at 10 min drug postinfusion for all these measures (all $ps < 0.01$).

Relationship Between Electrophysiological Measures and Psychosis-Related Measures

There was a significant main effect of dose only for the 40 Hz stimulation ITC. Thus, only the 40 Hz ITC was used in the regressions of psychosis-relevant symptoms (PANSS scores) on EEG measures. Results were HB adjusted for four comparisons (ITC for 1 frequency × 4 psychosis-relevant measures).

Intertrial coherence. For the active Δ⁹-THC conditions, significant HB-adjusted inverse relationships were found between ITC and the (1) PANSS positive ($\beta = -0.385$, Wald $\chi^2(1) = 4.847$, $p_{Adj} = 0.028$), (2) negative ($\beta = -0.358$, Wald $\chi^2(1) = 7.247$, $p_{Adj} = 0.021$), (3) general ($\beta = -0.383$, Wald $\chi^2(1) = 5.618$, $p_{Adj} = 0.036$), and (4) total ($\beta = -0.453$, Wald $\chi^2(1) = 11.800$, $p_{Adj} = 0.002$) scores (Figure 2).

Table 2 Δ^9-THC Effects on Intertrial Coherence (ITC) and Evoked Power

Measure	Placebo, M (SD)	0.015 mg/kg, M (SD)	0.03 mg/kg, M (SD)
Intertrial coherence			
40 Hz	0.149 (0.082)	0.142 (0.073)	0.104 (0.073)
30 Hz	0.027 (0.042)	0.042 (0.044)	0.043 (0.043)
20 Hz	0.038 (0.040)	0.030 (0.040)	0.039 (0.041)
Evoked power (μV^2)			
40 Hz	0.288 (0.209)	0.269 (0.167)	0.196 (0.167)
30 Hz	0.075 (0.122)	0.114 (0.137)	0.107 (0.100)
20 Hz	0.252 (0.393)	0.134 (0.178)	0.184 (0.194)

Exploratory Analyses on the Effects of Recent Cannabis Exposure

Exploratory analyses were conducted to determine the association between cannabis use in 30 days before study participation and the effects of acute Δ^9-THC on ITC and evoked power. The sample was divided into those who reported using cannabis in the past 30 days ('recent users', $n = 9$) and those who did not ('nonusers', $n = 11$) as per (D'Souza *et al*, 2012). The effects of cannabis exposure (recent user *vs* nonuser) and the interaction between dose and cannabis exposure on each EEG measure (evoked power and ITC) were examined by fitting a GEE model with an unstructured working correlation matrix to the data. The *post hoc* analyses were performed whenever significant main effects or interaction effects were observed.

These exploratory analyses revealed that recent users of cannabis had lower ITC than nonusers at a trend level (group effect: $p = 0.07$). As reported earlier, the dose effect was significant, but the group × dose interaction was not significant (Wald $\chi^2(2) = 2.328$, $p = 0.312$). Furthermore, recent users of cannabis had significantly lower evoked power than nonusers (Wald $\chi^2(1) = 5.188$, $p = 0.023$). As reported earlier the dose effect showed a trend toward significance but the group × dose interaction was not significant (Wald $\chi^2(2) = 2.372$, $p = 0.306$).

Plasma THC and THC-COOH Levels

For both active doses of $\Delta9$-THC, there were significant ($p_{Adj} < 0.001$) increases in plasma levels of $\Delta9$-THC and its metabolite THC-COOH (see Supplementary Figure 1 and Supplementary Text 4 for more information).

Safety

As reported elsewhere (D'Souza *et al*, 2012), there were five nonserious adverse events and no serious adverse events that occurred on test days.

DISCUSSION

To our knowledge, this is the first report of exogenous cannabinoids (i.v. Δ^9-THC) disrupting evoked γ-band neural oscillations in humans. For the 40 Hz ASSR, there was a dose-dependent reduction of ITC, with the higher dose (0.03 mg/kg) showing reduced ITC compared with both the lower dose (0.015 mg/kg) and placebo. No significant effects of Δ^9-THC were detected on the 20 or 30 Hz ASSRs, suggesting that cannabinoids selectively affected time-locked γ-band activity. Although not statistically significant, Δ^9-THC also reduced evoked power.

These findings are similar to a large body of evidence from ASSR studies showing reduced ITC during γ-band stimulation (maximal effect at 40 Hz) and preserved response to 20 Hz stimulation in first-episode and chronic schizophrenia patients compared with healthy controls (Krishnan *et al*, 2009; Kwon *et al*, 1999; Light *et al*, 2006; Roach *et al*, 2012; Spencer *et al*, 2008a, b).

Intertrial Coherence

ITC is a measure of the consistency in the phases of the brain's oscillatory responses to a stimulus/event across different trials. Thus, as reflected by reductions in ITC, it seems that both schizophrenia and relevant phenomena acutely induced by Δ^9-THC are associated with an increase in the intraindividual variability of the brain's response to identical stimuli/events. This intraindividual variability may reflect greater randomness in the neural processes underlying the ASSR. As observed in schizophrenia, Δ^9-THC-induced increased intraindividual variability was more pronounced at 40 than 20 Hz oscillations.

Relationship to Psychotomimetic Effects

Consistent with other studies, Δ^9-THC induced psychotomimetic effects in a dose-dependent manner (D'Souza *et al*, 2004; Morrison *et al*, 2009). Thus, at doses that induce psychotomimetic effects, Δ^9-THC concurrently disrupted evoked γ-band neural oscillations. Of note, there were robust ($\beta = -0.35$ to -0.45) inverse relationships between ITC and several subscales of the PANSS that assess symptoms relevant to schizophrenia.

The γ-band neural oscillations are essential for processes such as sensory registration, integration and binding of perceptual features, associative learning, and conscious awareness (for a review, see Cannon *et al*, 2014; Gandal *et al*, 2012; Uhlhaas *et al*, 2008; Uhlhaas and Singer, 2013). If disrupted, this could contribute to the Δ^9-THC-induced psychotomimetic effects (Skosnik *et al*, 2006) captured by the PANSS. These observations are consistent with other reports of inverse relationships between clinical variables and measures of γ-band oscillations in schizophrenia. For example, in a study by Haig *et al* (2000), it was demonstrated that the magnitude of γ deficits in schizophrenia patients correlated with PANSS scores (one of the primary dependent measures in the current study) (Haig *et al*, 2000).

Neural Mechanisms Underlying Δ^9-THC Effects on γ-Band Oscillations

Given the extensive crosstalk between the CB1R system and the GABAergic and glutamatergic systems, below herewith we speculate on how the latter two might be mechanistically
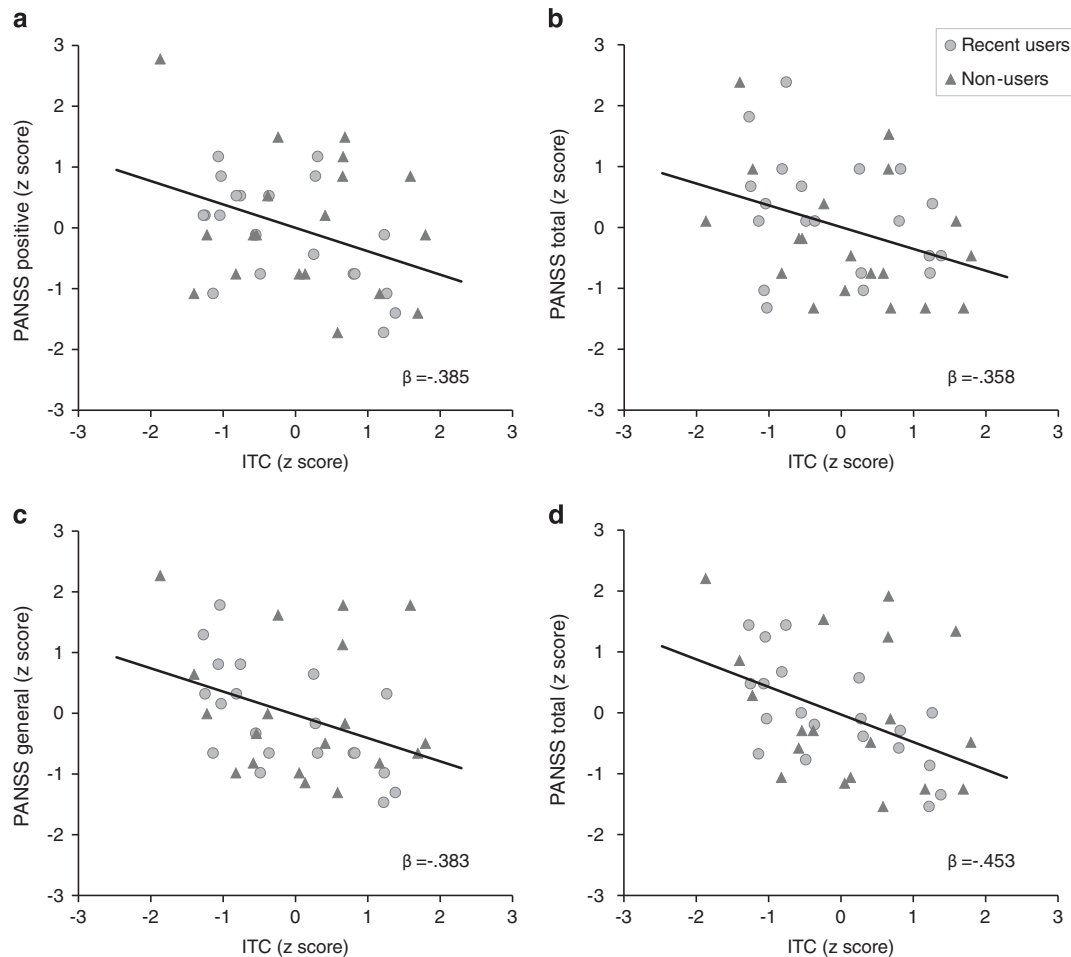
Figure 2 Relationship between 40 Hz intertrial coherence (ITC) and psychosis-relevant measures under the effects of Δ⁹-THC. The scatter plots show the z-normalized values of the 40 Hz ITC (x axes) *versus* the PANSS (a) positive, (b) negative, (c) general, and (d) total scores (y axes) under the effects of Δ⁹-THC. The relationship between the variables is represented by the regression lines and the standardized regression coefficients (β). Data from recent users (past 30 days) and nonusers of cannabis are depicted as circles and triangles, respectively.

involved in mediating the effects of Δ⁹-THC on γ-band oscillations.

The potential role of GABA. A large body of research has shown that GABAergic interneurons are the generators of neural oscillations in the γ-range (Buzsaki and Wang, 2012; Foldy *et al*, 2004; Gonzalez-Burgos and Lewis, 2008; Sohal, 2012; Uhlhaas and Singer, 2010; Wang, 2010). Furthermore, the sizeable branching of outputs from GABAergic interneurons are ideally suited to synchronize large numbers of pyramidal cells (Cobb *et al*, 1995). However, there are diverse types of GABAergic interneurons based upon their electrophysiological characteristics, their expression of calcium-binding proteins, and/or whether or not they are 'fast' or 'non-fast' spiking neurons. The fast-spiking parvalbumin (PV)-expressing interneurons appear to be the primary generator of neural oscillations in the brain (Curley and Lewis, 2012; Sohal, 2012; Sohal *et al*, 2009). PV cells typically target the pyramidal cell axon initial segment and cell body, thus positioning their terminals in an optimal location to synchronize numerous pyramidal cells. The non-fast spiking

CCK-positive interneurons are in a prime location to 'fine-tune' the network oscillations generated by PV cells (Freund, 2003). Germane to the current study, CCK-positive interneurons cells appear to be the only cortical and hippocampal interneuron type to express CB1Rs (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). CCK-positive interneurons also target pyramidal cell bodies, putting them in an ideal location to modulate PV to pyramidal cell oscillations (Keimpema *et al*, 2012). Furthermore, CCK cells themselves have collaterals that provide input onto PV cells (Karson *et al*, 2009). Thus, whereas PV interneurons are involved in the *generation* of γ-rhythms (like a 'metronome'), CCK interneurons enhance the signal-to-noise ratio of γ–activity through a CB1R-mediated mechanism (Bartos and Elgueta, 2012; Csicsvari *et al*, 2003; Tukker *et al*, 2007).

Under physiological conditions, the release of neurotransmitters such as GABA or glutamate results in the on-demand synthesis of endocannabinoids that travel retrogradely to activate presynaptic CB1R. The activation of these presynaptic CB1Rs results in the inhibition of further neurotransmitter

release. In the case of CCK interneurons this would lead to the disinhibition of PCs. Only PCs that are *strongly* recruited by a stimulus/task (signal) can mount an adequate endocannabinoid release to overcome CCK-BC-mediated inhibition (Bartos and Elgueta, 2012; Wilson and Nicoll, 2002). Conversely, PCs that are *weakly* recruited by a stimulus/task (noise) will not be able to overcome the CCK-BC-mediated inhibition, and thus their random activity will remain inhibited (ie, their contribution is 'filtered' out).

In contrast to endocannabinoids that are synthesized on demand, released locally, and quickly removed after release, the administration of exogenous cannabinoids such as Δ⁹-THC produces long-lasting and less localized effects. This nonphysiological activation of CB1Rs on CCK-positive interneurons by Δ⁹-THC will therefore interfere with the fine-tuning of network oscillations. Although admittedly speculative, interference with the fine-tuning of network oscillations would be expected to disrupt sensory, perceptual, and cognitive functions, leading to psychosis-like phenomena. Confirming this postulate, the current study found that 40 Hz ITC during high-dose THC inversely correlated with PANSS scores. Hence, although this finding of an association between disruptions in neural oscillations and psychosis does not prove a causal relationship, these data suggest a potential relationship between THC-induced disruption in γ-oscillations and measures of psychosis.

The potential role of glutamate. In addition to the suppression of GABA release from CCK-positive interneurons, cannabinoids can disrupt stimulus-locked γ-band responses by suppressing glutamate release from PCs through the activation of CB1Rs located on their synaptic terminals (Holderith et al, 2011; Katona et al, 2006; Kawamura et al, 2006). *In vitro* evidence shows that the CB1R-mediated reduction of glutamate release onto PCs and fast-spiking PV-BCs decreases γ-band local field potentials (spectral power) by reducing the firing rate and by increasing the temporal variability (randomness) of spiking (with respect to the phase of local field potentials) of both types of postsynaptic neurons (Holderith et al, 2011).

Impact of Cannabis Exposure

The limited power to examine the influence of cannabis exposure in this study notwithstanding, the results of the current study suggest that individuals with a recent (past 30 days) history of cannabis exposure had blunted responses to the disruptive effects of Δ⁹-THC on ITC and evoked power. These findings are consistent with the observation that relatively limited exposure to cannabis is associated with a blunted response to the effects of Δ⁹-THC across behavioral, subjective, cognitive (memory), neuroendocrine (cortisol), and neurochemical (brain-derived neurotrophic factor (BDNF)) outcomes (D'Souza et al, 2008a, b, 2009; Ranganathan et al, 2009). This is likely related to the known tolerance and CB1R downregulation that occurs after repeated cannabis exposure (Gonzalez et al, 2005; Hirvonen et al, 2012; Jones et al, 1981). Interestingly, recent cannabis use did not influence the disruptive effects of Δ⁹-THC on P300a/b amplitude and P300b latency (D'Souza et al, 2012) suggesting that measures of ITC and evoked power might be more sensitive to Δ⁹-THC effects.

Strengths, Limitations, and Conclusions

This study has several strengths and some limitations. The i.v. route of administration and the weight-adjusted dose addressing the inter- and intraindividual variability associated with oral or smoked Δ⁹-THC is a strength. Furthermore, the use of multiple doses allowed for establishing a dose–response relationship. The exclusion of heavy cannabis users who are likely tolerant to Δ⁹-THC effects (D'Souza et al, 2008b) also maximized the chance to capture acute Δ⁹-THC effects on neural oscillations. Finally, the use of novel preprocessing techniques (described in the Supplementary Text 1) to clean the muscular artifacts that frequently contaminate EEG data allowed more data to be retained, resulting in increased signal-to-noise ratio of evoked measures (Van Drongelen, 2006) and reduced the likelihood of spurious findings (Goncharova et al, 2003). There are some limitations regarding the use of EEG that should be taken into account. Despite the fact that muscular and ocular artifactual activity was minimized by preprocessing, it is not possible to rule out the possibility that some drug-induced effects may have been either obscured or erroneously boosted by residual artifactual activity. Furthermore, because of dropouts and the process of removing artifacts, not all the data that could have been used were available for analysis. This should be considered when interpreting the lack of statistically significant effects on some outcomes including power. The study may have had limited power to detect Δ⁹-THC effects on evoked power. Therefore, in order to characterize the acute effect of cannabinoid agonists on the ASSR in humans, future studies with a larger sample, the inclusion of a sufficient sample of subjects with a wide range of cannabis exposure, and the inclusion of cognitive measures that are influenced by γ-band oscillations are warranted.

In summary, we show for the first time in humans that exogenous cannabinoids acutely disrupt evoked γ-band neural oscillations that are essential for processes such as sensory registration, integration and binding of perceptual features, associative learning, and conscious awareness. Furthermore, we show a relationship between the disruption of neural oscillations and the presence of abnormalities in these cognitive processes similar to the ones observed in psychosis.

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