

UCSF

UC San Francisco Previously Published Works

Title

Δ^9 -THC Disrupts Gamma (γ)-Band Neural Oscillations in Humans

Permalink

<https://escholarship.org/uc/item/6ms753qw>

Journal

Neuropsychopharmacology, 40(9)

ISSN

0893-133X

Authors

Cortes-Briones, J
Skosnik, PD
Mathalon, D
et al.

Publication Date

2015-08-16

DOI

10.1038/npp.2015.53

Peer reviewed



Δ^9 -THC Disrupts Gamma (γ)–Band Neural Oscillations in Humans

Jose Cortes-Briones, Patrick D Skosnik, Daniel Mathalon, John Cahill, Brian Pittman, Ashley Williams, R Andrew Sewell, Mohini Ranganathan, Brian Roach, Judith Ford, Deepak Cyril D'Souza

Cite this article as: Jose Cortes-Briones, Patrick D Skosnik, Daniel Mathalon, John Cahill, Brian Pittman, Ashley Williams, R Andrew Sewell, Mohini Ranganathan, Brian Roach, Judith Ford, Deepak Cyril D'Souza, Δ^9 -THC Disrupts Gamma (γ)–Band Neural Oscillations in Humans, *Neuropsychopharmacology* accepted article preview 24 February 2015; doi: [10.1038/npp.2015.53](https://doi.org/10.1038/npp.2015.53).

This is a PDF file of an unedited peer-reviewed manuscript that has been accepted for publication. NPG are providing this early version of the manuscript as a service to our customers. The manuscript will undergo copyediting, typesetting and a proof review before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers apply.

Received 5 November 2014; revised 17 January 2015; accepted 26 January 2015;
Accepted article preview online 24 February 2015

Δ^9 -THC Disrupts Gamma (γ)–Band Neural Oscillations in Humans

Jose Cortes-Briones, Ph.D.,^{1,3} Patrick D. Skosnik, Ph.D.,^{1,2,3} Daniel Mathalon, Ph.D.,
M.D.,^{3,4,5} John Cahill, M.D.,^{1,2,3} Brian Pittman, M.S.,^{2,3} Ashley Williams B.A.,^{1,2} R.
Andrew Sewell, M.D.,^{1,2,3}, Mohini Ranganathan, M.D.,^{1,2,3} ⁵Brian Roach, B.A.,⁴, Judith
Ford, Ph.D.,^{3,4,5} Deepak Cyril D'Souza, M.D.,^{1,2,3},

¹ Psychiatry Service, VA Connecticut Healthcare System, West Haven, CT, USA

² Abraham Ribicoff Research Facilities, Connecticut Mental Health Center, New Haven,
CT, USA

³ Department of Psychiatry, Yale University School of Medicine, 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

Corresponding Author: Deepak Cyril D'Souza

Psychiatry Service 116A, VA Connecticut Healthcare System, 950 Campbell Avenue,
West Haven, CT 06516

Phone: (203) 932-5711 x 2594 Fax: (203) 937-4860

E-mail: deepak.dsouza@yale.edu

Keywords: Δ^9 -THC, cannabinoids, cannabis, ASSR, cognition, perception, psychosis, schizophrenia

Total number of words: Abstract = 246, Article Body = 3949

Total number of: Figures = 2, Tables = 2

Supplementary Tables: 2

Supplementary Figures: 1

Accepted Manuscript

Abstract

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 delta-9-tetrahydrocannabinol (Δ^9 -THC) on the auditory steady-state response (ASSR) were studied in humans ($n=20$) who completed three test days during which they received intravenous Δ^9 -THC (placebo, 0.015 and 0.03mg/kg) in a double-blind, randomized, cross-over, and counterbalanced design. Electroencephalography (EEG) was recorded while subjects listened to auditory click trains presented at 20, 30 and 40Hz. Psychosis-relevant effects were measured with the Positive and Negative Syndrome scale (PANSS).

Δ^9 -THC (0.03mg/kg) reduced inter-trial coherence (ITC) in the 40Hz condition compared to 0.015mg/kg and placebo. No significant effects were detected for 30 and 20Hz stimulation. Furthermore, there was a negative correlation between 40Hz ITC and PANSS subscales and total scores under the influence of Δ^9 -THC. Δ^9 -THC (0.03mg/kg) reduced evoked power during 40Hz stimulation at a trend level. Recent users of cannabis showed blunted Δ^9 -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.

Accepted manuscript

INTRODUCTION

With the legalization of “medical” marijuana (cannabis) (Kleber and DuPont, 2012; Procon.org, 2014) and recreational cannabis use in some states of the U.S (Bly, 2012), 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 (e.g., 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 delta-9-tetrahydrocannabinol (Δ^9 -THC), the primary psychoactive constituent in cannabis.

Δ^9 -THC, via activation of brain cannabinoid 1 receptors (CB1R), 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 (30-80Hz) 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 Δ^9 -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*, 2008; Hajos *et al*, 2000; 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, while 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 electroencephalogram (EEG) by measuring the brain's auditory steady-state response (ASSR) to sensory stimulation, i.e., the entrainment of EEG activity to regular auditory stimuli (e.g. 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 (~40Hz) responses in the ASSR

paradigm (Krishnan *et al*, 2009; Kwon *et al*, 1999; Light *et al*, 2006; Spencer *et al*, 2008a), which is associated with some of the characteristic abnormalities of the disorder (e.g., 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 the acute Δ^9 -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 Δ^9 -THC on several electrophysiological indices of information processing relevant to psychosis (e.g., P300 (D'Souza *et al*, 2012)) and to determine the relationship between the electrophysiological and behavioral effects of Δ^9 -THC. In this study we examined the acute, dose-related effects of intravenous (IV) Δ^9 -THC on the ASSR in a number of frequency bands, and the relationship between these effects and the psychosis-relevant effects induced by Δ^9 -THC. We hypothesized that Δ^9 -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 Δ^9 -THC.

MATERIALS AND METHODS

This randomized, double-blind, placebo-controlled, counterbalanced, cross-over 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 Δ^9 -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 about 4 weeks of the first test day. Cannabis-naïve 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 blunted 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 (8am-3pm) and their consumption did not exceed 10 cigarettes/day. Recent users of cannabis were instructed to abstain from smoking for at least 24 hours prior to test day. Test days were rescheduled if subjects reported using cannabis within 24 hours of the test day.

Subjects who were not categorized as recent users of cannabis were reminded not to use cannabis for two 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 Δ^9 -THC (placebo [vehicle (ethanol)], 0.015mg/kg, or 0.03mg/kg) over 10 minutes by IV 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 Δ^9 -THC solutions are reported elsewhere (D'Souza *et al*, 2004). For the control placebo condition, an equivalent volume of ethanol (vehicle) was used, which was previously shown to be undetectable in multiple post-injection blood samples (D'Souza *et al*, 2004). The IV route of administration was chosen to standardize the delivery of Δ^9 -THC as discussed previously (D'Souza *et al*, 2004).

General EEG Acquisition and Processing

Multichannel electroencephalography (EEG) was recorded from the scalp using a 22-lead electrode cap (Physiometrix Inc., Billerica, MA, USA) based on the International 10-20 System (see supplementary text 5). Electrode impedances were maintained <5kohms. Recordings were made using a linked ear reference. The data were recorded using Neuroscan Synamps amplifiers (Neuroscan SynAmps, Compumedics Neuroscan, Charlotte, NC, USA). Codes indicating stimulus onset and type were incorporated into the data stream for off-line averaging and analysis using Neuroscan Acquire software for data acquisition and software for stimulus presentation (Presentation, Neurobehavioral Systems, Berkeley, CA, USA). 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, Inc., Elk Grove Village, IL, USA). Stimuli consisted of standard, unattended (non-target) auditory click trains from a three-stimulus oddball task as reported previously (D'Souza *et al*, 2012). The auditory click trains were presented at 3 different frequencies (20, 30 and 40Hz; 80 dB SPL). Each block contained 150 trials of a single frequency presented for 500ms each (ISI 750ms). Each trial lasted 1250ms, and each block lasted 4 minutes. 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 (0.5-100Hz), 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 1200ms epochs time-locked to stimulus onset, with a 300ms pre-stimulus baseline. A ± 95 μ V 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, USA) using either custom-made scripts (for Inter-Trial Coherence, evoked power) or the EEGLAB toolbox' scripts and plugins (for signal preprocessing) (Delorme and Makeig, 2004; Gómez-Herrero *et al*, 2006).

Inter-Trial Coherence (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, 40Hz) and electrode (Fz, Cz, Pz), the signal epochs (trials) were band-pass filtered around the stimulation frequency (stimulation frequency ± 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 50-550ms after the onset of the auditory stimulation, and the average ITC of the baseline period (-300 to -50ms) 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 ± 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 50-550ms after the onset of auditory stimulation, and the average power of the baseline period (-300 to -50ms) 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, which are known to be sensitive to the effects of Δ^9 -THC (Radhakrishnan *et al*).

General Procedure and Test Days (see supplementary table 1)

Test days were separated by 3 days to minimize carryover effects. 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 post-study 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, standard deviations and graphs. For each drug condition (placebo, 0.015mg/kg, and 0.03mg/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, which, compared to 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, since there were no significant differences in pre-drug ASSR across test days (for further details, see supplementary text 3), for parsimony, these data were dropped.~~ However, since there were no significant differences in pre-drug measures of ASSR between the 3 test days (for further details, see supplementary text 3), pre-drug data were not included in the analysis to detect drug effects.

The effects of Δ^9 -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. *Post-hoc* pairwise comparisons (0.03mg/kg vs placebo, 0.015mg/kg vs placebo, and 0.03mg/kg vs 0.015mg/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 x 3 drug condition pairs).

To characterize the relationship (correlation) between the effects of Δ^9 -THC on both EEG (ITC and evoked power) and PANSS scores, longitudinal regressions were conducted on the data of both Δ^9 -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 Δ^9 -THC-active conditions (maximum number of regressions =8: 2 EEG measures x 4 behavioral measures). Furthermore, in order to approximate the regression coefficients to partial correlation coefficients, standardized regression coefficient (β s) 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 Δ^9 -

THC-active conditions, composite z scores were calculated using the mean and standard deviation of the data of both Δ^9 -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, USA) and custom-made scripts in Matlab (MathWorks, Natick, MA, USA).

RESULTS

Demographic and cannabis use data are listed in table 1. Of 56 subjects who were 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 three test days (21% dropout rate) of which 5 had to be dropped due to 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 due to artifactual contamination (for details, see supplementary text 1), and 3 were categorized as outliers and dropped during the statistical inspection of the EEG 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 1 outlier for the PANSS general and total scores during the 0.015mg/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 (ASSR)

Inter-Trial Coherence (ITC): there was a HB-corrected significant main effect of dose on ITC with 40Hz stimulation (Wald $\chi^2(2)=12.677, p_{Adj}=0.006$). No significant main effect of dose was observed with the 20 and 30Hz stimulation frequencies either before or after HB-correction ($p_{S_{Adj}}>0.1$). *Post-hoc* analyses of the 40Hz data revealed HB-corrected (3 comparisons) significantly lower ITC in the 0.03mg/kg condition compared to the 0.015mg/kg ($p_{Adj}=0.005$) and the placebo ($p_{Adj}=0.002$) conditions (figure 1). No significant differences were found between the 0.015mg/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 40Hz stimulation (Wald $\chi^2(2)=5.023, p=0.081$), while no effects were detected with 20 and 30Hz stimulation. Inspection of the means (table 2) showed that in the 40Hz condition, evoked power had a dose-related reduction as observed in ITC.

Behavioral and Subjective effects

As reported elsewhere (D'Souza *et al*, 2012), Δ^9 -THC significantly increased PANSS positive, negative, general and total scores (all $p_s<0.001$). All doses were significantly different from each other at 10 minutes drug post-infusion for all these measures (all $p_s<0.01$).

Relationship between electrophysiological measures and psychosis-related measures:

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

Inter-trial coherence (ITC): For the active Δ^9 -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 (figures 2).

Exploratory Analyses on the Effects of Recent Cannabis Exposure:

Exploratory analyses were conducted to determine the association between cannabis use in the 30 days prior to 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 (“non-users”, $n=11$) 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. *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 x 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 towards significance but the group x 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 Δ^9 -THC, there were significant ($p_{Adj}<0.001$) increases in plasma levels of Δ^9 -THC and its metabolite THC-COOH (see supplementary figure 1 and text 4 for more information).

Safety:

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

DISCUSSION

To our knowledge, this is the first report of exogenous cannabinoids (IV Δ^9 -THC) disrupting evoked γ -band neural oscillations in humans. For the 40Hz ASSR, there was a dose dependent reduction of ITC, with the higher dose (0.03mg/kg) showing reduced ITC compared to both the lower dose (0.015mg/kg) and placebo. No significant effects of Δ^9 -THC were detected on the 20 or 30Hz ASSRs, suggesting that cannabinoids selectively affected time-locked γ -band activity. While 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 40Hz) and preserved response to 20Hz stimulation in first episode and chronic schizophrenia patients compared to healthy controls (Krishnan *et al*, 2009; Kwon *et al*, 1999; Light *et al*, 2006; Roach *et al*, 2012; Spencer *et al*, 2008a; Spencer *et al*, 2008b).

Inter-Trial 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 intra-individual variability of the brain's response to identical stimuli/events. This intra-individual variability may reflect greater randomness in the neural processes underlying the ASSR. As observed in schizophrenia, Δ^9 -THC-induced increased intra-individual variability was more pronounced at 40Hz than 20Hz 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.

Gamma 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*; 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 involved in mediating the effects of Δ^9 -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). Further, 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; Eggen and Lewis, 2007; Eggen *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). Further, CCK cells themselves have collaterals that provide input onto PV cells (Karson *et al*, 2009). Thus, while 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 which 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 *et al*, 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, thus their random activity will remain inhibited (i.e., 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 Δ^9 -THC produces long lasting and less localized effects. This nonphysiological activation of CB1Rs on CCK-positive interneurons by Δ^9 -THC will therefore interfere with the fine-tuning of network oscillations. While 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, while 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 day) history of cannabis exposure had blunted responses to the disruptive effects of Δ^9 -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 Δ^9 -THC across behavioral, subjective, cognitive (memory), neuroendocrine (cortisol) and neurochemical (brain derived neurotrophic factor [BDNF]) outcomes (D'Souza *et al*, 2008a; D'Souza *et al*, 2009; D'Souza *et al*, 2008b; 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 Δ^9 -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 Δ^9 -THC effects.

Strengths, limitations and conclusions

This study has several strengths and some limitations. The IV route of administration and the weight-adjusted dose addressing the inter- and intra-individual variability associated with oral or smoked Δ^9 -THC is a strength. Further, the use of

multiple doses allowed for establishing a dose-response relationship. The exclusion of heavy cannabis users who are likely tolerant to Δ^9 -THC effects (D'Souza *et al*, 2008b) also maximized the chance to capture acute Δ^9 -THC effects on neural oscillations. Finally, the use of novel preprocessing techniques (described in the supplement) 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, due to 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 Δ^9 -THC effects on evoked power. Therefore, in order to characterize the acute effect of cannabinoid agonists on the ASSR in humans, further research is needed to replicate our findings. 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 which 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.

Accepted manuscript

ACKNOWLEDGMENTS

The authors wish to acknowledge support from the (1) Department of Veterans Affairs, (2) National Institute of Mental Health, (3) National Institute of Drug Abuse, (4) National Institute of Alcoholism and Alcohol Abuse (NIAAA) and (5) the Yale Center for Clinical Investigation (YCCI). This research project was funded in part by grants from NIDA (R21 DA020750 to DCD) and a NARSAD Young Investigator Award to PDS. The authors also thank Angelina Genovese, R.N.C., M.B.A.; Michelle San Pedro, R.N.; Elizabeth O'Donnell, R.N.; Brenda Breault, R.N., B.S.N.; Sonah Yoo, R.Ph.; Rachel Galvan, R.Ph.; and Willie Ford of the Neurobiological Studies Unit at the VA Connecticut Healthcare System, West Haven Campus for their central contributions to the success of this project. Finally, this manuscript is dedicated to the memory of our dear friend and colleague, the late Dr. R. Andrew Sewell.

DISCLOSURE/CONFLICT OF INTEREST

Jose Cortes-Briones, Patrick Skosnik, John Cahill, Ashley Williams, Brian Roach, Mohini Ranganathan and R. Andrew Sewell (deceased) report no financial relationships with commercial interests. Deepak Cyril D'Souza has in the past 3 years or currently receives research grant support administered through Yale University School of Medicine from AbbVie and Pfizer Inc., and is a consultant for Bristol Meyers Squibb and Johnson and Johnson. Dr. Mathalon is a consultant for Bristol Myers Squibb, Roche, and Amgen.

Accepted manuscript

REFERENCES

- Ali AB, Todorova M (2010). Asynchronous release of GABA via tonic cannabinoid receptor activation at identified interneuron synapses in rat CA1. *Eur J Neurosci* **31**(7): 1196-1207.
- Bacci A, Huguenard JR, Prince DA (2004). Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids. *Nature* **431**(7006): 312-316.
- Bartos M, Elgueta C (2012). Functional characteristics of parvalbumin-and cholecystinin-expressing basket cells. *The Journal of Physiology* **590**(4): 669-681.
- Bly LS (2012). Colorado, Washington OK recreational marijuana use. *USA Today*.
- Bodor AL, Katona I, Nyiri G, Mackie K, Ledent C, Hajos N, *et al* (2005). Endocannabinoid signaling in rat somatosensory cortex: laminar differences and involvement of specific interneuron types. *J Neurosci* **25**(29): 6845-6856.
- Buzsaki G, Wang XJ (2012). Mechanisms of gamma oscillations. *Annu Rev Neurosci* **35**: 203-225.
- Cannon J, McCarthy MM, Lee S, Lee J, Borgers C, Whittington MA, *et al* (2014). Neurosystems: brain rhythms and cognitive processing. *Eur J Neurosci* **39**(5): 705-719.
- Carbuto M, Sewell RA, Williams A, Forselius-Bielen K, Braley G, Elander J, *et al* (2011). The safety of studies with intravenous Delta(9)-tetrahydrocannabinol in humans, with case histories. *Psychopharmacology (Berl)*.
- Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P (1995). Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* **378**(6552): 75-78.
- Csicsvari J, Jamieson B, Wise KD, Buzsáki G (2003). Mechanisms of gamma oscillations in the hippocampus of the behaving rat. *Neuron* **37**(2): 311-322.
- Curley AA, Lewis DA (2012). Cortical basket cell dysfunction in schizophrenia. *J Physiol* **590**(Pt 4): 715-724.
- D'Souza DC, Braley G, Blaise R, Vendetti M, Oliver S, Pittman B, *et al* (2008a). Effects of haloperidol on the behavioral, subjective, cognitive, motor, and neuroendocrine effects of Delta-9-tetrahydrocannabinol in humans. *Psychopharmacology (Berl)* **198**(4): 587-603.

D'Souza DC, Fridberg DJ, Skosnik PD, Williams A, Roach B, Singh N, *et al* (2012). Dose-Related Modulation of Event-Related Potentials to Novel and Target Stimuli by Intravenous Δ 9-THC in Humans. *Neuropsychopharmacology* **37**(7): 1632-1646.

D'Souza DC, Perry E, MacDougall L, Ammerman Y, Cooper T, Wu YT, *et al* (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology* **29**(8): 1558-1572.

D'Souza DC, Pittman B, Perry E, Simen A (2009). Preliminary evidence of cannabinoid effects on brain-derived neurotrophic factor (BDNF) levels in humans. *Psychopharmacology (Berl)* **202**(4): 569-578.

D'Souza DC, Ranganathan M, Braley G, Gueorguieva R, Zimolo Z, Cooper T, *et al* (2008b). Blunted Psychotomimetic and Amnestic Effects of Delta-9-Tetrahydrocannabinol in Frequent Users of Cannabis. *Neuropsychopharmacology*.

Davies L, Gather U (1993). The identification of multiple outliers. *Journal of the American Statistical Association* **88**(423): 782-792.

De Clercq W, Vergult A, Vanrumste B, Van Paesschen W, Van Huffel S (2006). Canonical correlation analysis applied to remove muscle artifacts from the electroencephalogram. *Biomedical Engineering, IEEE Transactions on* **53**(12): 2583-2587.

Delorme A, Makeig S (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of neuroscience methods* **134**(1): 9-21.

Eggan SM, Lewis DA (2007). Immunocytochemical distribution of the cannabinoid CB1 receptor in the primate neocortex: a regional and laminar analysis. *Cereb Cortex* **17**(1): 175-191.

Eggan SM, Melchitzky DS, Sesack SR, Fish KN, Lewis DA (2010). Relationship of cannabinoid CB1 receptor and cholecystokinin immunoreactivity in monkey dorsolateral prefrontal cortex. *Neuroscience* **169**(4): 1651-1661.

First MB, Spitzer RL, Gibbon M, Williams JBW (2002). *Structured Clinical Interview for DSM-IV-TR Axis I Disorders- Non-patient Edition* American Psychiatric Association: Washington,DC.

Foldy C, Aradi I, Howard A, Soltesz I (2004). Diversity beyond variance: modulation of firing rates and network coherence by GABAergic subpopulations. *Eur J Neurosci* **19**(1): 119-130.

Foldy C, Neu A, Jones MV, Soltesz I (2006). Presynaptic, activity-dependent modulation of cannabinoid type 1 receptor-mediated inhibition of GABA release. *J Neurosci* **26**(5): 1465-1469.

Freund TF (2003). Interneuron Diversity series: Rhythm and mood in perisomatic inhibition. *Trends Neurosci* **26**(9): 489-495.

Gandal MJ, Edgar JC, Klook K, Siegel SJ (2012). Gamma synchrony: towards a translational biomarker for the treatment-resistant symptoms of schizophrenia. *Neuropharmacology* **62**(3): 1504-1518.

Gómez-Herrero G, De Clercq W, Anwar H, Kara O, Egiazarian K, Van Huffel S, *et al* (eds) (2006). Automatic removal of ocular artifacts in the EEG without an EOG reference channel. *Signal Processing Symposium, 2006. NORSIG 2006. Proceedings of the 7th Nordic*. IEEE.

Goncharova I, McFarland DJ, Vaughan TM, Wolpaw JR (2003). EMG contamination of EEG: spectral and topographical characteristics. *Clinical Neurophysiology* **114**(9): 1580-1593.

Gonzalez-Burgos G, Lewis DA (2008). GABA neurons and the mechanisms of network oscillations: implications for understanding cortical dysfunction in schizophrenia. *Schizophr Bull* **34**(5): 944-961.

Gonzalez S, Cebeira M, Fernandez-Ruiz J (2005). Cannabinoid tolerance and dependence: a review of studies in laboratory animals. *Pharmacol Biochem Behav* **81**(2): 300-318.

Haig AR, Gordon E, De Pascalis V, Meares RA, Bahramali H, Harris A (2000). Gamma activity in schizophrenia: evidence of impaired network binding? *Clin Neurophysiol* **111**(8): 1461-1468.

Hajos M, Hoffmann WE, Kocsis B (2008). Activation of cannabinoid-1 receptors disrupts sensory gating and neuronal oscillation: relevance to schizophrenia. *Biological psychiatry* **63**(11): 1075-1083.

Hajos N, Katona I, Naiem SS, MacKie K, Ledent C, Mody I, *et al* (2000). Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. *Eur J Neurosci* **12**(9): 3239-3249.

Hampel FR (1974). The influence curve and its role in robust estimation. *Journal of the American Statistical Association* **69**(346): 383-393.

Hill EL, Gallopin T, Ferezou I, Cauli B, Rossier J, Schweitzer P, *et al* (2007). Functional CB1 receptors are broadly expressed in neocortical GABAergic and glutamatergic neurons. *J Neurophysiol* **97**(4): 2580-2589.

Hirvonen J, Goodwin RS, Li CT, Terry GE, Zoghbi SS, Morse C, *et al* (2012). Reversible and regionally selective downregulation of brain cannabinoid CB1 receptors in chronic daily cannabis smokers. *Mol Psychiatry* **17**(6): 642-649.

Holderith N, Nemeth B, Papp OI, Veres JM, Nagy GA, Hajos N (2011). Cannabinoids attenuate hippocampal gamma oscillations by suppressing excitatory synaptic input onto CA3 pyramidal neurons and fast spiking basket cells. *J Physiol* **589**(Pt 20): 4921-4934.

Johnson LA, Johnson RL, Alfonzo C (2011). Spice: a legal marijuana equivalent. *Mil Med* **176**(6): 718-720.

Johnston LD, O'Malley PM, Bachman HG, Schulenberg JE (2012). Monitoring the Future national results on adolescent drug use: Overview of key findings, 2011. . Ann Arbor: Institute for Social Research, The University of Michigan.

Jones RT, Benowitz NL, Hering RI (1981). Clinical relevance of cannabis tolerance and dependence. *Journal of Clinical Pharmacology* **21**(8-9 Suppl): 143S-152S.

Karson MA, Tang AH, Milner TA, Alger BE (2009). Synaptic cross talk between perisomatic-targeting interneuron classes expressing cholecystokinin and parvalbumin in hippocampus. *J Neurosci* **29**(13): 4140-4154.

Katona I, Sperlagh B, Magloczky Z, Santha E, Kofalvi A, Czirjak S, *et al* (2000). GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. *Neuroscience* **100**(4): 797-804.

Katona I, Urban GM, Wallace M, Ledent C, Jung KM, Piomelli D, *et al* (2006). Molecular composition of the endocannabinoid system at glutamatergic synapses. *J Neurosci* **26**(21): 5628-5637.

Kawamura Y, Fukaya M, Maejima T, Yoshida T, Miura E, Watanabe M, *et al* (2006). The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. *J Neurosci* **26**(11): 2991-3001.

Kay SR, Opler LA, Lindenmayer JP (1989). The Positive and Negative Syndrome Scale (PANSS): rationale and standardisation. *British Journal of Psychiatry - Supplementum*(7): 59-67.

Keimpema E, Straiker A, Mackie K, Harkany T, Hjerling-Leffler J (2012). Sticking out of the crowd: the molecular identity and development of cholecystokinin-containing basket cells. *J Physiol* **590**(Pt 4): 703-714.

Kleber HD, DuPont RL (2012). Physicians and medical marijuana. *Am J Psychiatry* **169**(6): 564-568.

- Krishnan GP, Hetrick WP, Brenner CA, Shekhar A, Steffen AN, O'Donnell BF (2009). Steady state and induced auditory gamma deficits in schizophrenia. *NeuroImage* **47**(4): 1711-1719.
- Kwon JS, O'Donnell BF, Wallenstein GV, Greene RW, Hirayasu Y, Nestor PG, *et al* (1999). Gamma frequency-range abnormalities to auditory stimulation in schizophrenia. *Arch Gen Psychiatry* **56**(11): 1001-1005.
- Liang K-Y, Zeger SL (1986). Longitudinal data analysis using generalized linear models. *Biometrika* **73**(1): 13-22.
- Light GA, Hsu JL, Hsieh MH, Meyer-Gomes K, Sprock J, Swerdlow NR, *et al* (2006). Gamma band oscillations reveal neural network cortical coherence dysfunction in schizophrenia patients. *Biological psychiatry* **60**(11): 1231-1240.
- Mehmedic Z, Chandra S, Slade D, Denham H, Foster S, Patel AS, *et al* (2010). Potency trends of Delta9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. *J Forensic Sci* **55**(5): 1209-1217.
- Morgan NH, Stanford IM, Woodhall GL (2008). Modulation of network oscillatory activity and GABAergic synaptic transmission by CB1 cannabinoid receptors in the rat medial entorhinal cortex. *Neural plasticity* **2008**: 808564.
- Morrison PD, Zois V, McKeown DA, Lee TD, Holt DW, Powell JF, *et al* (2009). The acute effects of synthetic intravenous Delta9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. *Psychological medicine* **39**(10): 1607-1616.
- ONDCP OoNDP (2008). Marijuana: The Greatest Cause of Illegal Drug Abuse. *The Marijuana Factbook*. Executive Office of the President Washington, DC 20503.
- Partha M, Hemant B (2007). *Observed brain dynamics* Oxford University Press: New York.
- Procon.org (2014). Medical marijuana. <http://medicalmarijuana.procon.org/>. Vol 2014.
- Puthusserypady S, Ratnarajah T (2005). H[∞] adaptive filters for eye blink artifact minimization from electroencephalogram. *Signal Processing Letters, IEEE* **12**(12): 816-819.
- Radhakrishnan R, Wilkinson ST, D'Souza DC (2014). Gone to Pot—A Review of the Association between Cannabis and Psychosis. *Frontiers in Psychiatry* **5**.
- Ranganathan M, Braley G, Pittman B, Cooper T, Perry E, Krystal J, *et al* (2009). The effects of cannabinoids on serum cortisol and prolactin in humans. *Psychopharmacology (Berl)* **203**(4): 737-744.

- Roach B, Ford J, Hoffman R, Mathalon D (2012). Converging evidence for gamma synchrony deficits in schizophrenia. *Supplements to Clinical neurophysiology* **62**: 163-180.
- Robbe D, Montgomery SM, Thome A, Rueda-Orozco PE, McNaughton BL, Buzsaki G (2006). Cannabinoids reveal importance of spike timing coordination in hippocampal function. *Nat Neurosci* **9**(12): 1526-1533.
- Sales-Carbonell C, Rueda-Orozco PE, Soria-Gomez E, Buzsaki G, Marsicano G, Robbe D (2013). Striatal GABAergic and cortical glutamatergic neurons mediate contrasting effects of cannabinoids on cortical network synchrony. *Proc Natl Acad Sci U S A* **110**(2): 719-724.
- SAMHSA (2011). Results from the 2010 National Survey on Drug Use and Health: Summary of National Findings, . Substance Abuse and Mental Health Services Administration: Rockville, MD.
- SAMHSA (2012). Drug Abuse Warning Network, 2010: National Estimates of Drug-Related Emergency Department Visits. Center for Behavioral Health Statistics and Quality (formerly the Office of Applied Studies), Substance Abuse and Mental Health Services Administration (SAMHSA): Rockville, MD.
- Skosnik P, Krishnan G, Aydt E, Kuhlensmidt H, O'Donnell B (2006). Psychophysiological evidence of altered neural synchronization in cannabis use: relationship to schizotypy. *American Journal of Psychiatry* **163**(10): 1798-1805.
- Skosnik PD, D'Souza DC, Steinmetz AB, Edwards CR, Vollmer JM, Hetrick WP, *et al* (2012). The Effect of Chronic Cannabinoids on Broadband EEG Neural Oscillations in Humans. *Neuropsychopharmacology* **37**(10): 2184-2193.
- Sohal VS (2012). Insights into cortical oscillations arising from optogenetic studies. *Biol Psychiatry* **71**(12): 1039-1045.
- Sohal VS, Zhang F, Yizhar O, Deisseroth K (2009). Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature* **459**(7247): 698-702.
- Spencer KM, Salisbury DF, Shenton ME, McCarley RW (2008a). Gamma-band auditory steady-state responses are impaired in first episode psychosis. *Biological psychiatry* **64**(5): 369-375.
- Spencer KM, Salisbury DF, Shenton ME, McCarley RW (2008b). γ -Band Auditory Steady-State Responses Are Impaired in First Episode Psychosis. *Biological psychiatry* **64**(5): 369-375.

Tallon-Baudry C, Bertrand O, Delpuech C, Pernier J (1996). Stimulus specificity of phase-locked and non-phase-locked 40 Hz visual responses in human. *The Journal of Neuroscience* **16**(13): 4240-4249.

Tukker JJ, Fuentealba P, Hartwich K, Somogyi P, Klausberger T (2007). Cell type-specific tuning of hippocampal interneuron firing during gamma oscillations in vivo. *The journal of neuroscience* **27**(31): 8184-8189.

Uhlhaas PJ, Haenschel C, Nikolić D, Singer W (2008). The role of oscillations and synchrony in cortical networks and their putative relevance for the pathophysiology of schizophrenia. *Schizophrenia bulletin* **34**(5): 927-943.

Uhlhaas PJ, Singer W (2010). Abnormal neural oscillations and synchrony in schizophrenia. *Nat Rev Neurosci* **11**(2): 100-113.

Uhlhaas PJ, Singer W (2013). High-frequency oscillations and the neurobiology of schizophrenia. *Dialogues Clin Neurosci* **15**(3): 301-313.

Van Drongelen W (2006). *Signal processing for neuroscientists: an introduction to the analysis of physiological signals* Academic press.

Vardakou I, Pistos C, Spiliopoulou C (2010). Spice drugs as a new trend: mode of action, identification and legislation. *Toxicol Lett* **197**(3): 157-162.

Wang XJ (2010). Neurophysiological and computational principles of cortical rhythms in cognition. *Physiol Rev* **90**(3): 1195-1268.

Wilson RI, Nicoll RA (2002). Endocannabinoid signaling in the brain. *Science* **296**(5568): 678-682.

Zeger SL, Liang K-Y (1986). Longitudinal data analysis for discrete and continuous outcomes. *Biometrics*: 121-130.

LEGENDS TO FIGURES

Figure 1: 40Hz inter-trial 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, 0.015mg/kg, and 0.03mg/kg conditions during 40Hz 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, while the green and red lines represent the ITC of the 0.015mg/kg and 0.03mg/kg doses, respectively.

c) ITC (mean \pm S.E.M) by drug condition at electrode Cz during the time interval 50-550ms after stimulation onset. Significant differences ($p_{Adj}<0.01$) between drug conditions are marked with **.

Figure 2: Relationship between 40Hz inter-trial coherence (ITC) and psychosis-relevant measures under the effects of Δ^9 -THC.

The scatter plots show the z-normalized values of the 40Hz ITC (x axes) versus the PANSS **(a)** positive, **(b)** negative, **(c)** general, and **(d)** total scores (y axes) under the effects of Δ^9 -THC. The relationship between the variables is represented by the regression lines and the standardized regression coefficients (β). **Data from recent users (last 30 days) and non-users of cannabis are depicted as circles and triangles, respectively.**

Table 1. Sample demographics

General Characteristics		
No. 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 (# of subjects)	Recent Users	Nonusers
	9	11
Frequency of Cannabis Use Within Past 30 Days (# 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 (# 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 (# 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*

Daily Cigarette Smokers (# of subjects)	1
Social Cigarette Smoker (# of subjects)	1
Average No. of Alcoholic Drinks Per Week [Mean (SD)]	6.01 (6.06)

 (# of subjects)

No exposure to other drugs (# of subjects)	6																
Exposure to drugs other than Cannabis (# of subjects)	<table> <tr> <td>Psilocybin</td> <td>11</td> </tr> <tr> <td>LSD</td> <td>2</td> </tr> <tr> <td>Salvinorin</td> <td>3</td> </tr> <tr> <td>Nitrous Oxide</td> <td>2</td> </tr> <tr> <td>Cocaine</td> <td>5</td> </tr> <tr> <td>Amphetamines</td> <td>3</td> </tr> <tr> <td>MDMA</td> <td>2</td> </tr> <tr> <td>Opioids</td> <td>2</td> </tr> </table>	Psilocybin	11	LSD	2	Salvinorin	3	Nitrous Oxide	2	Cocaine	5	Amphetamines	3	MDMA	2	Opioids	2
Psilocybin	11																
LSD	2																
Salvinorin	3																
Nitrous Oxide	2																
Cocaine	5																
Amphetamines	3																
MDMA	2																
Opioids	2																

**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.*

Table 2: Δ^9 -THC effects on inter-trial coherence (ITC) and evoked power

<i>Measure</i>	<i>Placebo</i> M (SD)	<i>0.015 mg/kg</i> M (SD)	<i>0.03 mg/kg</i> M (SD)
Inter-trial 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)

Figure 1

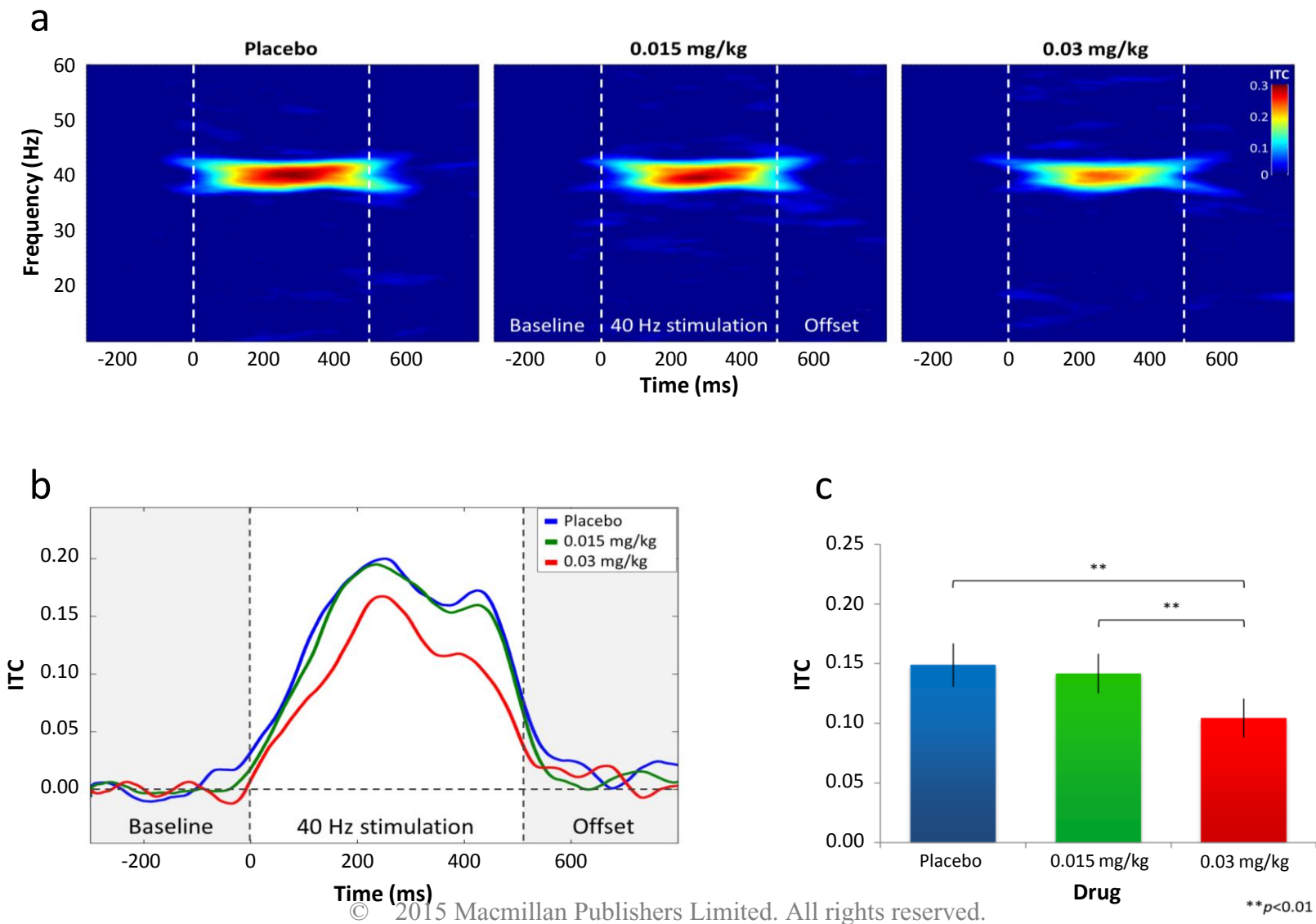


Figure 2

