Disruption of frontal theta coherence by Δ9-tetrahydrocannabinol is associated with positive psychotic symptoms.

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Running Title: THC and theta

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Abstract: 219 words

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Abstract

The main ingredient in cannabis, Δ^9-tetrahydrocannabinol (THC), can elicit acute psychotic reactions in healthy individuals and precipitate relapse in schizophrenic patients. However, the neural mechanism of this is unknown. We tested the hypothesis that THC-psychopatholgy is related to changes in EEG power or inter-regional coherence. In a within-subjects design, participants (n=16) were given intravenous (IV) THC (1.25mg) or placebo under double-blind conditions during EEG recordings. Using fast-Fourier transform, EEG data was analysed for power and coherence in the delta (1-3.5 Hz), theta (3.5-7 Hz), alpha (8-13 Hz), beta (14-25 Hz), low-gamma (30-40Hz) and high-gamma (60-70Hz) bands during engagement in the n-back test of working-memory (WM). Compared to placebo, THC evoked positive and negative psychotic symptoms, as measured by the PANSS scale ($p<0.001$) and slowed WM performance ($p<0.05$). Under THC, theta power was specifically reduced, ($p<0.001$) regardless of WM load, however, the reduction showed no relationship with psychotic symptoms or WM impairment. Coherence between bi-frontal electrodes in the theta band was also reduced by THC ($p<0.05$) and these reductions correlated with the change in positive psychotic symptoms (rho=0.79, $p<0.001$). Bi-frontal specificity was suggested by the absence of a relationship between psychotic symptoms and fronto-parietal coherence. The results reveal that the pro-psychotic effects of THC might be related to impaired network dynamics with impaired communication between the right and left frontal lobes.

Keywords

Δ^9-tetrahydrocannabinol; theta; psychosis; EEG

INTRODUCTION

There has been a renewed interest in the cannabis-model of acute psychosis, driven in part by epidemiological studies which have demonstrated that the use of cannabis is a risk factor for the development of schizophrenia (Arseneault *et al*, 2004; Moore *et al*, 2007). The major psychoactive effects of cannabis stem from the action of Δ^9-tetrahydrocannabinol (THC) at central CB_1 receptors (CB_1Rs) (Howlett *et al*, 2002; Mechoulam *et al*, 1970). Recent laboratory studies have confirmed earlier reports which used plant-derived preparations by showing that pure intravenous (IV) THC elicits acute schizophrenia-like symptoms and cognitive impairments in healthy volunteers (D'Souza *et al*, 2004; Morrison *et al*, 2009). Similarly, schizophrenic patients can experience a sudden and transient relapse of psychotic symptoms following IV THC despite having been previously well on dopamine-based anti-psychotic medication (D'Souza *et al*, 2005).

At present the neurophysiological mechanism responsible for the pro-psychotic properties of THC is unknown. The CB_1 receptor is found at high density in the prefrontal and association cortices, the anterior, mediodorsal, and intralaminar thalamic nuclei, the hippocampal complex, amygdala, entorhinal cortex, basal ganglia, substantia-nigra pars-reticulata, and cerebellum (Eggan and Lewis, 2007; Glass *et al*, 1997; Herkenham *et al*, 1991). Typically, CB_1 receptors are localised at the pre-synaptic terminals of glutamate and GABA-ergic neurons (Bodor *et al*, 2005; Eggan *et al*, 2010; Hill *et al*, 2007). Their endogenous transmitters, (endocannabinoids, eCBs) are released from dendritic spines and function as retrograde signalling molecules (meaning that neurochemical 'information' is passed from dendrite *to* axon terminal) (Piomelli, 2003). At the synaptic level, the eCBs inhibit GABA and glutamate release from nerve endings, over short and sustained durations, whereas at the psychological

level eCBs are vital for learning and memory (Chevaleyre *et al*, 2006; Freund *et al*, 2003). Exogenous cannabinoids such as THC probably do not capture the subtleties of eCB signalling (which involves tightly regulated local release and re-uptake) and hence are associated with disruption of learning and memory performance, and presumably their underlying neural correlates (Murray *et al*, 2007; Ranganathan and D'Souza, 2006).

In the last two decades it has become clear that neural oscillations are intrinsic to higher CNS functions (Buzsaki and Draguhn, 2004; Uhlhaas *et al*, 2009; Varela *et al*, 2001). Oscillations arise from the synchronised firing of large numbers of neurons. In turn, the precise timing of spikes from individual neurons is constrained by ongoing rhythms within the network. Oscillations are believed to be essential for rapid, dynamic integration within and between brain regions. Synchronised gamma (30-200Hz) rhythms "bind" local assemblies, whereas lower frequencies (theta, alpha and beta) sub-serve long-distance communication between brain areas (Kopell *et al*, 2000; Rodriguez *et al*, 1999; von Stein *et al*, 2000). Studies in animals have begun to unravel the effects of THC on such oscillations. Robbe and colleagues showed that THC decreased the power of theta, gamma and ripple oscillations in the rat hippocampus, effects which could be blocked by CB_1 antagonists (Robbe *et al*, 2006). Theta and gamma rhythms are believed to be essential for working memory (WM) and the encoding of episodic memories, whereas ripples are involved in the transfer of memory to cortical stores. In agreement, Hajos and co-workers found that CB_1 agonists disrupted theta and gamma oscillations within the septo-hippocampal system in rats, and disrupted auditory-gating; effects which were inhibited by CB_1 antagonists (Hajos *et al*, 2008). In humans there have been several recent reports of the effects of cannabis on neuronal oscillations, recorded using electroencephalography (EEG). A study by IIan and colleagues analysed EEG power in 10 subjects, during the performance of cognitive tasks (Ilan *et al*,

2004). Working and episodic memory performance was poorer following inhaled marijuana (3.5% THC) and the principal EEG findings were decreased global theta power and reduced alpha band reactivity. Similarly in a study of inhaled marijuana (0, 29, 49 & 69mg) in 16 participants, Bocker and co-workers observed a dose-dependent decrease of resting theta and beta power. Furthermore, they found the greater the decrease in theta power during a WM task, the greater the slowing of response time (Bocker *et al*, 2010).

Our primary interest in this study was to characterise EEG changes linked to the pro-psychotic properties of THC. Neither of the previous EEG studies of THC had investigated psychotic symptoms and one possibility was that psychotic symptoms would be related to the reductions in theta power demonstrated in these studies. However, more recent work has highlighted the importance of brain connectivity in the generation of psychotic symptoms. In particular, reductions in EEG coherence have been linked to specific positive symptoms (auditory hallucinations; Ford *et al*, 2002). EEG coherence is a measure of the correlation of activity in different brain regions. A high coherence indicates that EEG activity within a specific frequency band in one brain region predicts EEG activity in same frequency band in another and that the temporal relationship (phase) of the activity at the two sites is relatively constant. The implication of such amplitude and phase relationships is of cross-talk and shared function between the two regions. The link between positive symptoms and reductions in EEG coherence found in schizophrenia suggested a working hypothesis that the pro-psychotic properties of THC relates to a reduction in EEG coherence, specifically in the theta band given the animal and human data outlined above.

METHODS

The study was approved by the Joint Institute of Psychiatry and Maudsley Hospital Ethics Committee. All subjects provided written informed consent. Safety protocols have previously been described (Morrison *et al*, 2009).

Design

A randomised, double-blind placebo controlled crossover study in healthy volunteers of the effects of IV THC (1.25mg) on working-memory performance, psychopathology and concurrent EEG activity.

Participants

Twenty healthy participants were recruited via the King's College email lists. Inclusion criteria were: age between 21-50 years, previous cannabis use $\geq$ 1; a score of $<$ 15 on the General Health Questionnaire (GHQ-12, Goldberg *et al*, 1970). Exclusion criteria included: current pregnancy; a history of mental illness; drug or alcohol dependence (excluding nicotine); current or past severe medical disorders, or a history of major mental illness in a first-degree family member. Previous alcohol and drug use was recorded and a urine drug screen was carried out. Participants were asked to avoid alcohol and drugs for 24 h before, and to abstain from driving for 24 h after experiments. Sessions were performed at least two weeks apart and started between 0900 and 1400 h. Placebo and THC were administered under double-blind conditions, in a randomised counterbalanced order. Subjects received remuneration for their participation.

Pharmaceuticals

Synthetic Δ^9-tetrahydrocannabinol was supplied by THC Pharm GmbH (Frankfurt am Main, Germany) and prepared as 1mg/ml vials for intravenous injection, by Bichsel Laboratories (Interlaken, Switzerland) as previously described (Naef *et al*, 2004). After dilution in normal saline, preparations for injection contained 1.25 % (v/v) ethanol absolute. Sterile cannulae were inserted into veins in the antecubital fossa of both arms: one for administration of pharmaceuticals and one for plasma sampling. THC was administered in 1ml/min pulses over a period of 5 min (total dose = 1.25mg). Blood samples were taken at baseline and at 1, 5, 15, 60, and 120 min after dosing, for analysis of [THC] as previously described (Morrison 2009). The plasma concentration of THC following IV delivery follows a similar time-course to that observed following inhalation (Naef *et al*, 2004). The dose, (1.25mg) was selected on the basis of previous studies, and *roughly* corresponds to one standard 'joint' (D'Souza *et al*, 2004; Morrison *et al*, 2009; Bhattacharyya *et al*, 2010).

Psychopathological and cognitive measures

Psychotic symptoms were rated using the positive and negative syndrome scale (PANSS, (Kay *et al*, 1987). A completely independent PANSS-trained senior psychiatrist rated 3 x 10-minute periods - at baseline, at 30-minutes post-pharmaceutical and finally at 90-minutes post-pharmaceutical. Apart from these 3 periods, the psychiatrist and participant were kept separate to minimise potential bias. The PANSS consists of a positive sub-scale (*7 items; delusions, conceptual disorganisation, hallucinations, hyperactivity, grandiosity, suspiciousness/persecution and hostility*), a negative sub-scale (*7 items*;

blunted affect, emotional withdrawal, poor rapport, passive/apathetic social withdrawal, difficulty in abstract thinking, lack of spontaneity and flow of conversation, stereotyped thinking) and a general psychopathological sub-scale (*14 items*). Items are rated from 1-7 (absent-severe), thus the range of scores on the positive subscale is 7-49. It is recognised that there is wide inter-individual variation in PANSS scores following IV THC (D'Souza *et al*, 2004; Morrison *et al*, 2009). Previously we found that investigator-rated (PANSS) and participant-rated (CAPE, Community Assessment of Psychic Experiences) measures of THC-elicited positive symptoms were in agreement (rho=0.62, $p<0.001$), and that both measures of positive symptoms were distinct from anxiety (Morrison *et al*, 2009). Considered as a group however, positive symptoms following THC are modest and short-lived. Overall, in earlier studies approximately 35-50% of healthy participants showed changes of >= 3-4 points on the PANSS positive subscale under THC conditions (D'Souza *et al*, 2004; Morrison *et al*, 2009).

Immediately following the psychiatric assessment at 30-mins post-pharmaceutical, participants were administered a standard computerised version of the n-back task. The n-back procedure has been used extensively to measure human working memory performance (Owen *et al*, 2005). Participants were required to monitor a series of letters and report when the current letter matched the letter n integers back, where n=1 (1-back) or n=2 (2-back), the latter being more difficult. The task requires continuous updating of information stores. In contrast, in the 0-back condition (which does not require manipulation of material in WM), participants responded to the appearance of a pre-specified letter. Overall, the task consisted of alternating 30-second blocks of 0-back, 1-back and 2-back conditions, and lasted 6 minutes in total. Within blocks, letters were displayed every 2 seconds for 1 second. Written instructions were read out and participants were given a practice run to demonstrate their understanding of the rules. Subjects were seated ~66cm from a CRT monitor and instructed to report

correct answers as rapidly as possible by pressing a joy-pad button with their R-index finger. Accuracy of responses and reaction-times were measured and stored digitally.

Electroencephalography

All data recording and signal processing was performed in Neuroscan 4.3. Electroencephalographic (EEG) activity was recorded from 63 electrode sites using a Quik-Cap system (Compumedics inc.), with a linked mastoid reference and ground at AFz. All impedances were maintained below 10 kΩ. Additional electrodes were placed at the outer canthi to measure horizontal electrooculographic activity (EOG; monopolar with linked mastoid reference). Vertical EOG was measured using a bipolar recording with electrodes above and below the left eye. The EEG was sampled at 2000 Hz and corrected for eye-blinks using a regression approach. The corrected EEG was epoched, using a 10% Hanning window, into 2048ms segments (-24 to 2024ms with respect to each n-back letter stimulus). Epochs were baseline corrected. For each of the three n-back conditions, average power within the frequency bands delta (1-3.5 Hz), theta (3.5-7 Hz), alpha (8-13 Hz), beta (14-25 Hz), low-gamma (30-40Hz), and high-gamma (60-70Hz) bands was calculated using Fast Fourier Transform (FFT).

For the power analysis, individual electrodes were grouped as left-frontal, LF (F1, F3, F5, F7, AF3); right-frontal, RF (F2, F4, F6, F8, AF4); left-central, LC (C1, C3, FC1, FC3); right-central, RC (C2, C4, FC2, FC4); left-temporal, LT (FT7, T7, TP7, CP5, P7); right-temporal, RT (FT8, T8, TP8, CP6, P8) left occipito-parietal, LOP (O1, PO5, PO3, P3, P1); and right occipito-parietal, ROP (O2, PO6, PO4, P4, P2). The mean value from each group of electrodes was used for statistical analysis. The midline electrodes FZ, CZ and PZ were analysed individually.

For the coherence analysis the data was transformed to bipolar derivations. These derivations consist of pairs of neighbouring electrodes at different scalp locations to eliminate the contribution of activity from a common reference to the coherence estimate. Bipolar channels were derived for left and right frontal and parietal regions (F3/F5; PO3/PO5; F4/F6; PO4/PO6). The measure of coherence used is equivalent to a Pearson correlation performed with complex numbers. It measures the correlation (a value between 0 and 1) of EEG activity in a specific frequency band between two scalp locations. For each of the three n-back conditions, coherence measures were calculated between three pre-specified inter-regions, left frontal-left parietal F3/F5-PO3/PO5; right frontal-right parietal F4/F6-PO4/PO6; and left frontal-right frontal F3/F5-F4/F6. Laplacian derivations were also derived from frontal sites over the left hemisphere (LpF3, LpF5 and LpFT7) and right hemisphere (LpF4, LpF6 LpFT8). In Laplacian derivations, the influence of deep brain sources is minimised by referencing each site to the average of its 4 surrounding neighbouring electrodes (see results).

Statistical analyses

All analyses were conducted in SPSS 15.0. Distributions were checked for normality using Kolmogorov-Smrnov statistics. Non-parametric tests were used to analyse PANSS scores, because of floor effects under placebo conditions; Thus differences between THC v placebo sessions were assessed using Friedman's test and relationships between PANSS scores and EEG measures were analysed using Spearman's rho. Accuracy and speed of performance in the n-back were analysed by repeated measures ANOVA, with Task-Difficulty (0-back, 1-back, 2-back) and THC-Treatment (placebo, THC) as within-subjects factors. Relationships between accuracy/speed of processing in the

most challenging (2-back) condition of the n-back and EEG measures were analysed by Pearson's correlation coefficient.

A repeated measures ANOVA was used to analyse EEG power in each frequency band (delta, theta, alpha, beta, gamma-low, gamma-high), Within subject factors were: Region (LF, RF, LC, RC, LT, RT, LOP, ROP, Fz, Cz and Pz), Task-Difficulty (0-back, 1-back, 2-back) and THC-Treatment (placebo, THC). A repeated measures ANOVA was used to analyse EEG coherence. Factors (4) were; Frequency (delta, theta, alpha, beta, gamma-low, gamma-high), inter-Region (left frontal-left parietal, right frontal-right parietal and left frontal-right frontal), Task-Difficulty (0-back, 1-back, 2-back) and THC-Treatment (placebo, THC). Separate ANOVAs were also conducted for each frequency band (delta *to* gamma-high) in which factors were inter-Region, Task-Difficulty and THC-Treatment. Where sphericity assumptions were violated, Huynh-Feldt corrected statistics were used. Post-hoc t-tests were carried out where appropriate.

Correlations between psychological outcomes and EEG measures were Bonferroni corrected to adjust for multiple comparisons. Otherwise significance was accepted at $p<0.05$. All analyses were 2-tailed.

RESULTS

Overall, 16 of 20 participants (7 male, 9 female) completed both sessions of the study. Two participants were lost to follow up. One subject discontinued her involvement and one subject experienced short-lived panic and the session was stopped prematurely. Mean age was 26 ± 6 years. Prior to experimental sessions, all urine drug screens were negative. Previous use of cannabis ranged from 2 to approximately 1000 occasions (median = 40). With regard to other drugs, 11 (of 16) had previously taken stimulants (cocaine/amphetamines), 6 had taken ecstasy, 6 had taken psychedelics (psilocybin/LSD) and there was a single case each of previous ketamine and gamma-hydroxybutyric acid (GHB). Plasma concentrations of THC over the course over the experiment are summarised in Figure 1.

Psychopathology and cognitive performance

Compared to placebo, THC increased positive (Friedman's χ^2=63.7, p<0.001) negative (Friedman's χ^2=56.0, p<0.001) and general PANSS scores (Friedman's χ^2=36.1, p<0.001). Increases were most pronounced at the 30-minute assessment point and tended back towards baseline by 90-minutes (Figure 2a, b). Overall, 40% of participants showed increases in PANSS positive symptom scores of >4 points at 30-minutes post-injection.

There was a trend towards reduced accuracy in the n-back task following treatment with THC (F=2.95, p=0.11). Accuracy was robustly affected by task-difficulty (F=5.38, p<0.005), with poorer performance in the 2-back condition compared to both the 1-back (p<0.05) and 0-back (p<0.01) conditions (Figure

3a). In terms of accuracy, there was no THC-treatment x task-difficulty interaction.

Response times in the n-back task were slower under THC versus placebo conditions ($F=6.8$, $p<0.05$), and as task-difficulty increased ($F=32.3$, $p<0.001$). There was an interaction between THC-treatment x task-difficulty, in that the effect of THC was significantly greater as the n-back became more challenging ($F=7.74$, $p<0.005$) (Figure 3b).

Electroencephalography

Power

The effect of THC at each frequency band is shown in Figure 4a. THC decreased theta power ($F=23.5$, $p<0.001$), regardless of Task-Difficulty or Region (Figure 4b, c). There was also a trend towards decreased alpha power under THC ($F=3.74$, $p=0.07$), with no THC-Treatment x Task-Difficulty or THC-Treatment x Region interactions. Power in the delta and beta bands also reduced under THC while that in the gamma-low and gamma-high bands increased, although none of these effects were significant: beta ($p=0.45$), delta ($p=0.34$), low-gamma ($p=0.21$) and high-gamma ($p=0.21$).

EEG coherence

There were overall effects of region ($F=9.8$, $p<0.01$), frequency ($F=16.6$, $p<0.001$), task- difficulty ($F=24.3$, $p<0.001$), and THC ($F=6.1$, $p<0.05$). EEG coherence was greater between bi-frontal electrodes, compared to L-fronto-parietal ($p<0.05$) and R-fronto-parietal ($p<0.05$) electrode pairs.

Compared to the 0-back condition, overall coherence increased under the 1-back ($p<0.001$) and 2-back conditions ($p<0.005$).

Interactive effects were region x frequency ($F=4.0$, $p=0.01$) and frequency x THC ($F=3.1$, $p<0.05$). There was a trend towards a 3-way interaction between region, frequency and THC-treatment ($F=2.0$, $p=0.09$).

Under placebo, bi-frontal coherence was largest in the theta band and theta coherence was greater between bi-frontal region compared to both the left ($p=0.01$) and right ($p<0.005$) fronto-parietal regions. THC selectively decreased bi-frontal coherence in the theta ($p<0.05$) and alpha ($p<0.05$) bands (Figure 5).

The observed decrease in theta coherence between bi-frontal electrodes under THC could reflect a decrease in connectivity between frontal regions in both hemispheres. However, another interpretation might be that it reflects a decrease in activity of a deep prefrontal source detected by both frontal regions. To explore this issue further we examined theta coherence in Laplacian derivations over the left and right hemispheres to minimise the contribution from deep sources. Coherence under placebo conditions (0.18 ± 0.02; mean±SEM averaged over 3 frontal Laplacian sites) was reduced by THC (0.11 ± 0.02) and differences emerged at the level of a strong trend ($F=4.0$, $p=0.08$). This suggests the coherence changes do not relate to activity in a deep source but reflect true changes in bi-frontal connectivity.

Correlations

EEG Power and psychopathology

Overall there was no correlation between *change-in* theta power, either globally or specifically at electrode Fz, and (1) change in reaction-time in the most challenging (2-back) level of the n-back task, (2) *change in* positive PANSS scores (THC – placebo at 30 minutes) (3) *change in* negative PANSS scores (THC – placebo at 30 minutes).

EEG coherence and psychopathology

We investigated possible relationships between *change in* theta and alpha coherence and (1) change in reaction-time in the most challenging (2-back) level of the n-back task, (2) *change in* positive PANSS scores (THC – placebo at 30 minutes) (3) *change in* negative PANSS scores (THC – placebo at 30 minutes). Since there were six comparisons in total, Bonferroni-corrected statistical significance was set at $p < 0.008$.

The *change-in* theta coherence (averaged over bi-frontal, and left and right fronto-parietal) under THC conditions was strongly associated with the *change-in* positive PANSS scores (rho=0.75, p=0.001), but neither *change in* negative symptoms nor reaction-time. The relationship between theta coherence and positive symptoms was specific for the bi-frontal region (rho=0.79, p<0.001). Neither change in left or right fronto-parietal theta coherence was related to positive symptoms. The larger the reduction in bi-frontal theta coherence, the larger the increase in positive symptoms on the PANSS. Reduced bi-

frontal theta coherence occurred at all levels of the n-back and the correlation between the decrease in theta and increase in positive symptoms survived the removal of 2 potential outliers (rho=0.69, p=0.006) (Figure 6).

There was a weaker relationship between the *change-in* alpha coherence and the *change-in* negative symptoms (Averaged rho=0.57, p=0.02) which was insufficiently robust to survive correction for multiple testing, and no relationship with positive symptoms nor reaction-time in the n-back.

DISCUSSION

The major finding of the present study is that THC decreased theta coherence between bi-frontal brain regions compared to the placebo day and that the reduction in coherence was strongly associated with positive psychotic symptoms. Additional effects of THC – transient psychosis as reflected in the PANSS score, slower working-memory performance and global suppression of power in the theta band - are consistent with previous reports.

Methodological issues - coherence

Our measure of coherence is influenced by both the amplitude and phase of EEG activity. A high theta coherence value indicates a linear relationship between theta amplitude in left frontal and right frontal cortices across sequential WM epochs and a consistent phase relationship. The reduction in coherence under THC indicates either that the theta amplitude relationship between the two frontal sites is weaker of that theta phase varies from trial to trial. Our coherence measure does not disambiguate these two

possibilities; however, a reduction in either amplitude or phase relationship points to a weakening of shared function or cross-talk between the frontal sites. The coherence seems to be driven by activity in the lateral frontal cortex and not from a deep source (for example on the medial surface of the frontal lobes) as the reduction in coherence under THC is preserved in the Laplacian data which is sensitive to local sources but not deeper ones. The THC effects in the Laplacian data are less significant than those in the bipolar montage analysis suggesting either that the Laplacian electrodes used were not directly above the cortical regions involved in the cross–talk or that large regions of frontal cortex are involved as this reduces the sensitivity of Laplacian data.

THC, theta coherence and psychosis

Previously there has been speculation that the pro-psychotic effects of THC stem from disruption of synchronised neural rhythms (Hajos *et al*, 2008; Sewell *et al*, 2009). The major finding here provides the first experimental support for this idea. It may be that THC elicits a 'lesion', at the molecular level which can 'push' an otherwise healthy nervous system towards acute psychosis, hastens the onset of psychotic-breakdown in those destined to develop schizophrenia and provokes acute relapse in established cases (Kuepper *et al*, 2010). The precise nature (and location) of the molecular lesion, downstream of CB_1 receptors is unknown. But the most likely scenario is that THC disrupts the intricacies of fast amino-acid based neurotransmission; and in doing so, disrupts network oscillations which depend, in-part, upon reciprocal glutamate and GABA-ergic connections (Buzsaki, 2006; Ford and Mathalon, 2008; Hajos *et al*, 2008; Robbe *et al*, 2006; Uhlhaas *et al*, 2008).

The findings point to the pre-frontal cortex and implicate the theta band in the generation of psychotic

symptoms. Reduced bi-frontal (but not fronto-parietal) coherence from placebo day levels was strongly associated with positive psychotic symptoms. This was not the case for theta power. The simplest interpretation is that acute THC-psychosis is associated with disruption in long-distance synchrony (reflected in coherence) but not with disruption of local theta rhythms (reflected in power). There is a consensus that theta oscillations are important for long-distance 'cross-talk' between brain regions (Uhlhaas *et al*, 2010; Varela *et al*, 2001). The findings here are broadly in keeping with the disconnection hypothesis of schizophrenia, in which impaired functional connectivity between brain regions underlies psychotic symptoms (Stephan *et al*, 2009). Some caution is required in attributing the pro-psychotic effects of THC to a direct action within the frontal cortices. It is likely that, in the present study, theta oscillations within limbic regions were also disrupted by THC (Hajos *et al*, 2008; Robbe *et al*, 2006). Thus an apparent cortical lesion might *only* be a marker for a cortico-limbic lesion which is 'closer' to the pathophysiology of THC. For example Df(16)$A^{+/-}$ mice, (which mimic one of the largest genetic risk-factors for schizophrenia, a microdeletion on human chromosome 22q11.2), show reduced phase-locking of pre-frontal cells to hippocampal theta rhythms and reduced coherence of pre-frontal and hippocampal local field potentials. (Sigurdsson *et al*, 2010).

It is unclear whether the link between positive psychotic symptoms and decreased bi-frontal theta coherence reported here is specific to THC and WM tasks or constitutes a more generally applicable biomarker for positive psychotic symptoms whatever their context. We did not find a robust association between EEG coherence and negative symptoms suggesting that these symptoms may be related to a different mechanism or disconnection between different areas

The link between positive symptoms and coherence was specific to the theta band. It is possible that the

gamma band would also have been linked to psychotic symptoms. It is known that gamma oscillations 'nest' within slower oscillations; thus the power of gamma rhythms rises and falls on a slower theta oscillation (Canolty *et al*, 2006). However, clear separation of neural and muscle-derived gamma in the human scalp-recorded EEG can be difficult, although algorithms have been developed to permit identification of neural gamma in humans (Nottage, 2010). It is possible that changes in the gamma band in addition to theta will be detected using these novel methods. Studies in schizophrenic patients have reported decreased power of frontal theta oscillations during performance of WM tasks (Haenschel *et al*, 2009; Schmiedt *et al*, 2005). Ford and colleagues compared the EEG of speech production versus listening, in patients and controls. Speech production was associated with increased coherence across classical left hemisphere language regions within the theta band, in healthy controls but not in schizophrenic patients (Ford *et al*, 2002). The authors concluded that reduced fronto-temporal functional connectivity in schizophrenia could lead to the attribution of self-generated speech to an external source; auditory hallucinations. Our subjects did not experience auditory hallucinations and our findings suggest that other classes of psychotic symptom by be linked to reductions in bi-frontal coherence.

THC, theta oscillations and cognition

Under THC, there were decreases in theta power and theta coherence regardless of the level of difficulty in the n-back task. The reduction in theta power is unlikely to relate to a non-specific decrease in arousal as drowsiness and the transition to sleep has the opposite effect, increasing theta activity. All three n-back levels differed from the 'at-rest' state in placing explicit demands on attention. Generally, the impact of THC on n-back performance was relatively subtle. As demands on working

memory increased, performance was significantly slower, but accuracy was maintained. Indeed the majority of participants continued to 'achieve' 100% success, even on the most challenging level. In agreement with previous findings, THC-elicited deficits in WM showed no relationship with acute psychosis (Morrison *et al*, 2009). Additionally, in the present data set, we found no relationship between WM deficits and the decreases in EEG power. The latter finding is in contrast with the recent report of Bocker et al, who found the greater the reduction in theta power under THC the greater the slowing of responses (but not reduction in accuracy) in a WM task (Bocker *et al*, 2010). It also has been reported that fronto-parietal coherence (specifically within the theta band) increases during WM performance (Sarnthein *et al*, 1998). This effect was not observed in the present study. One possibility is that the n-back task employed here was insufficiently challenging.

The neurophysiology of THC

Recently, cannabinoid agonists have emerged as a 'tool' for the study of brain network dynamics and for the study of psychotic mental states (Buzsaki, 2006; Sewell *et al*, 2009). In the former case, CB_1 agonists are unique in that they can disrupt network synchrony whilst preserving the firing-rates of individual pyramidal and GABA-ergic interneurons (Buzsaki, 2006; Robbe *et al*, 2006). Comparing different drug-models of psychosis; CB_1 agonists can elicit a psychotic reaction in otherwise healthy controls after a single exposure, in contrast to stimulants (amphetamine/cocaine) where repeated use is a pre-requisite. And repeated use of cannabis (especially forms high in THC-content) is a risk factor for the genesis of schizophrenia, in contrast to ketamine where no association has as yet been found.

The endocannabinoids are key components in plasticity and a rapidly developing field is the overlap between neural oscillations and plasticity. The role of theta oscillations in the generation of hippocampal long-term potentiation (LTP) has long been appreciated (Huerta and Lisman, 1993). But more recently it has become apparent that gamma rhythms and spike-timing dependent plasticity (STDP) occur within the same, critical time-frame (tens of milliseconds) suggestive of mechanistic links between the two phenomena (Buzsaki, 2006; Uhlhaas *et al*, 2010). Spike-timing dependent long-term depression (LTD) in the cortex is implemented by retrograde endocannabinoid signalling (Chevaleyre *et al*, 2006). And animal work has shown that THC disrupts network oscillations and network plasticity simultaneously (Robbe *et al*, 2006). Potentially this could offer insight into how THC can precipitate an acute, reversible psychosis (disruption of oscillations) and a chronic psychotic illness (*additional* disruption of plasticity).

Strengths and limitations

A weakness of the current study is that only one dose of THC was used. The sample size is also small for teasing out interactions, but typical for studies of this type. There are two main strengths. Experimentally-induced psychoses permit the testing of healthy participants before, during and after administration of a pro-psychotic drug, and intra-individual comparisons. We also took advantage of pure synthetic preparations of THC. Plant derived material contains further cannabinoid molecules such as cannabidiol (CBD) which counteracts the pro-psychotic effects of THC (Bhattacharyya *et al*, 2010), and tetrahydrocannabivarin (THCV), an antagonist at CB_1 receptors (Pertwee *et al*, 2008).

Conclusion

Global theta power was reduced by THC – without any manifest psychopathological consequences. In contrast, there was a strong and specific association between THC-induced positive psychotic symptoms and reduced bi-frontal theta coherence. Impaired functional 'cross-talk' between the frontal lobes in the theta band might account for the pro-psychotic effects of THC/cannabis. Whether this is also true of psychotic symptoms more generally is a question for further study.

DISCLOSURE/CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose

ACKNOWLEDGEMENTS

Supported by the MRC (UK) and the Beckley Foundation.

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Titles & Legends to figures

Figure 1. Plasma concentrations of THC (mean±SEM) following the intravenous injection of THC (1.25mg).

Figure 2. (a) Positive and (b) negative psychotic symptoms (mean±SEM) as measured by the PANSS scale were increased from baseline (time 0 min) following the administration of intravenous THC (1.25mg).

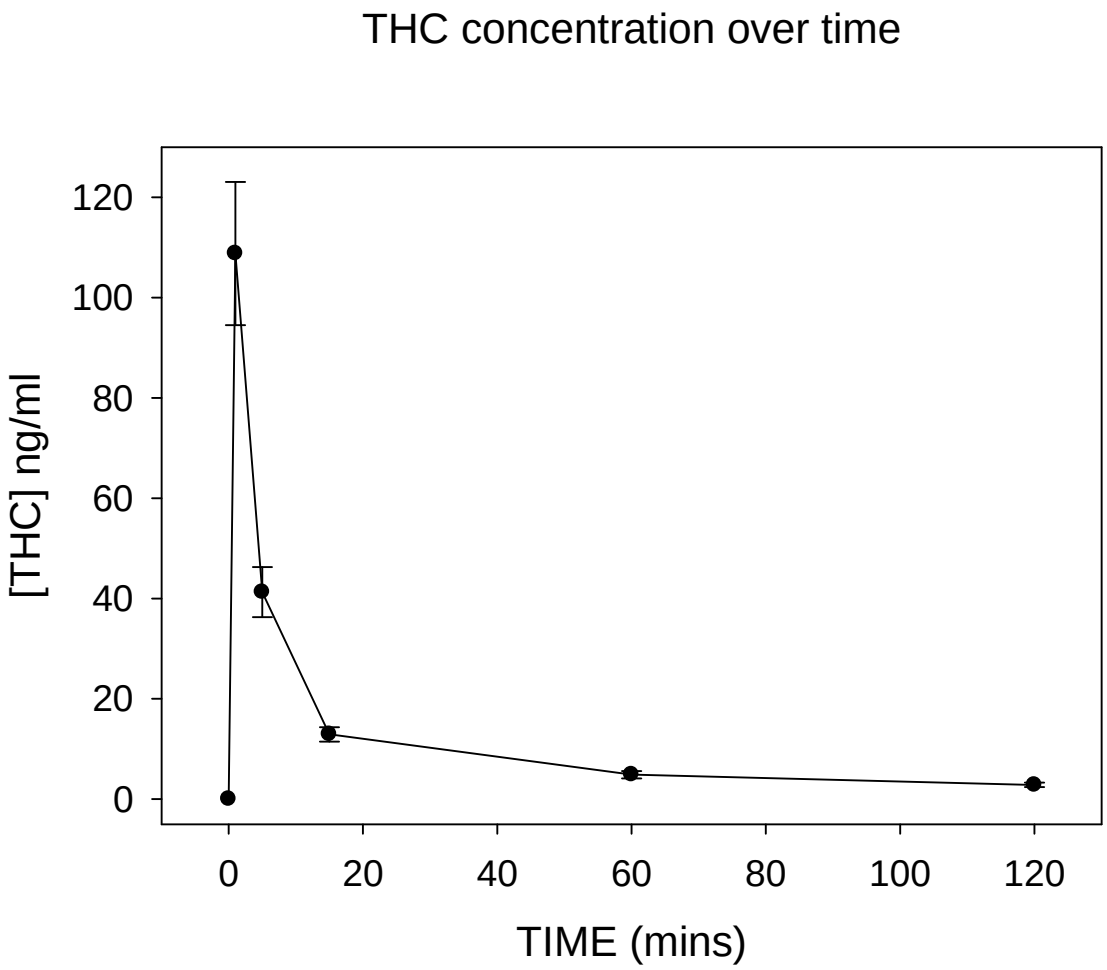
Figure 3. (a) Accuracy and (b) reaction-time in the n-back task (mean±SEM) under THC versus placebo conditions. (a) THC had no effect on accuracy. (b) Performance was slower under THC conditions ($p<0.05$) and showed an interaction with task-difficulty ($p<0.005$).

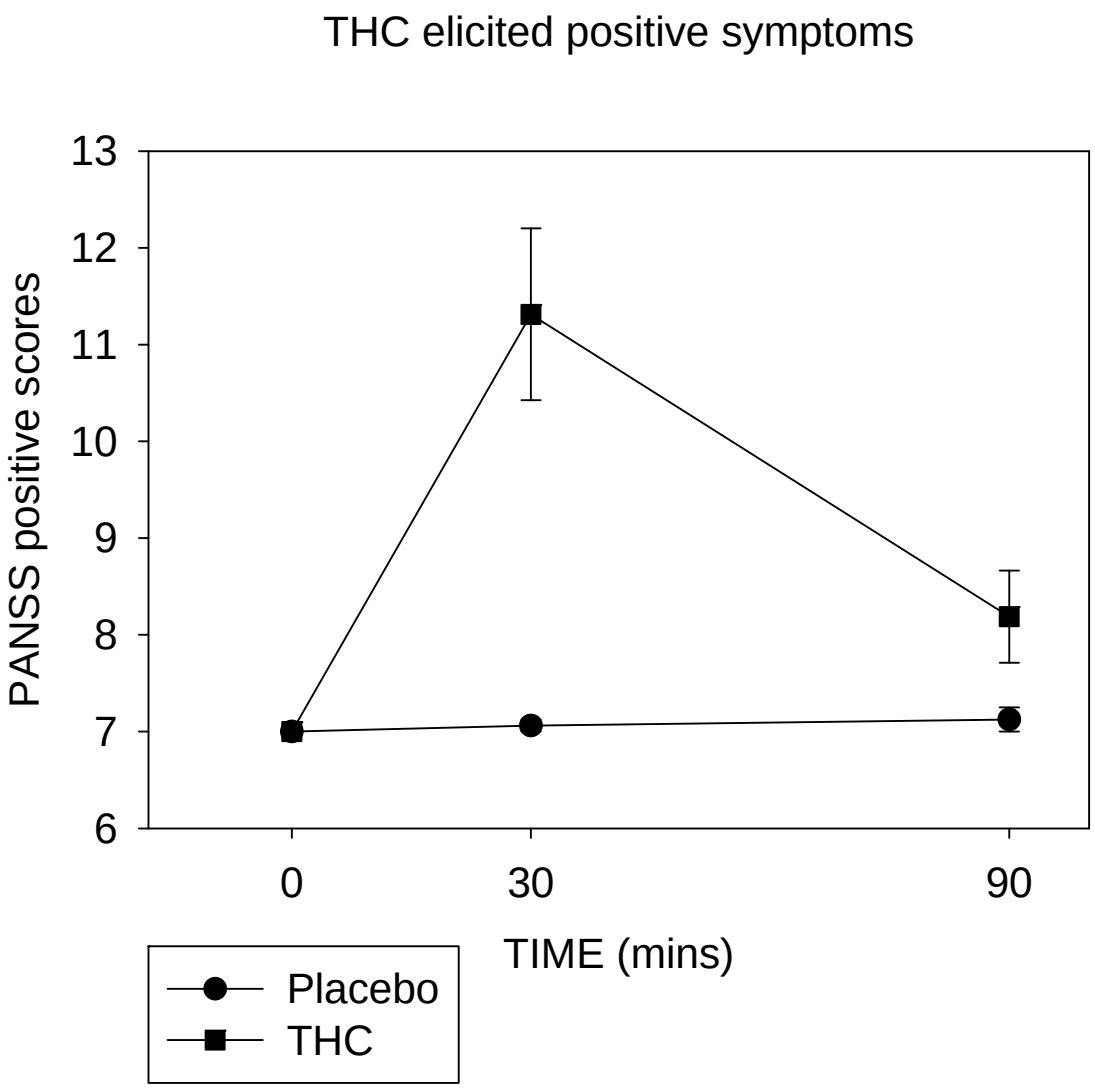
Figure 4. (a) The effect of THC at each frequency band. Compared to placebo, theta power (mean±SEM) was decreased by THC ($p<0.001$). Decreases or increases under THC in other frequency bands are not significant. Theta power was reduced regardless of task-difficulty (b) or recording site (c). Left frontal (LF), right-frontal (RF), left central (LC), right central (RC), left temporal (LT), right temporal (RT), left occipitoparietal (LOP), right occipitoparietal (ROP), midline frontal (FZ), midline parietal (PZ) and midline central (CZ).

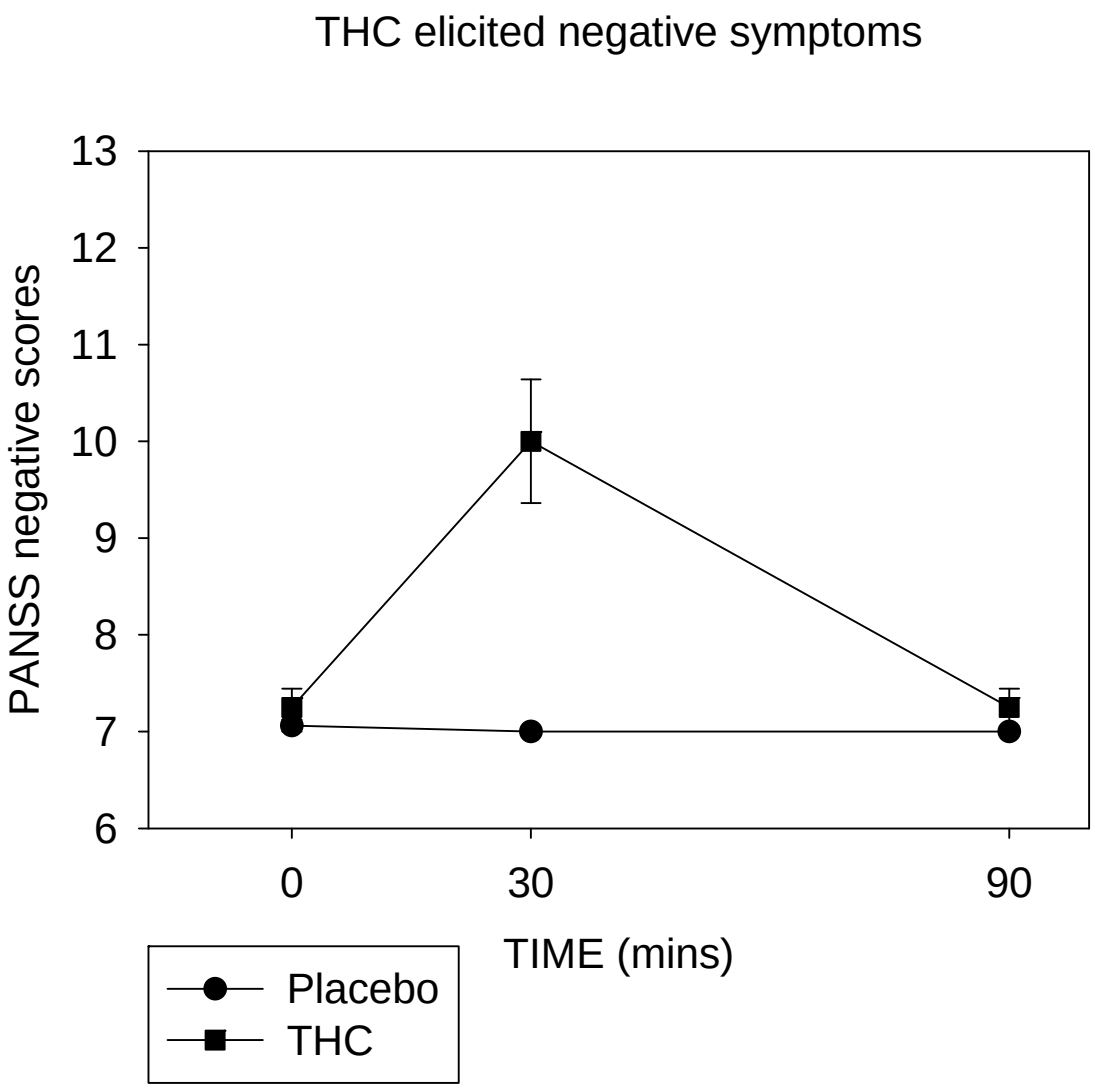
Figure 5. Coherence between left and right prefrontal regions, under THC versus placebo, in the control (0-back) and most challenging (2-back) levels of the n-back task.
Bars show coherence (mean±SEM) by frequency. (*Diagonal stripes* – delta. *Black* – theta. *Grey* – alpha. *White* – beta.) THC treatment selectively decreased coherence in the theta ($p<0.05$) and alpha

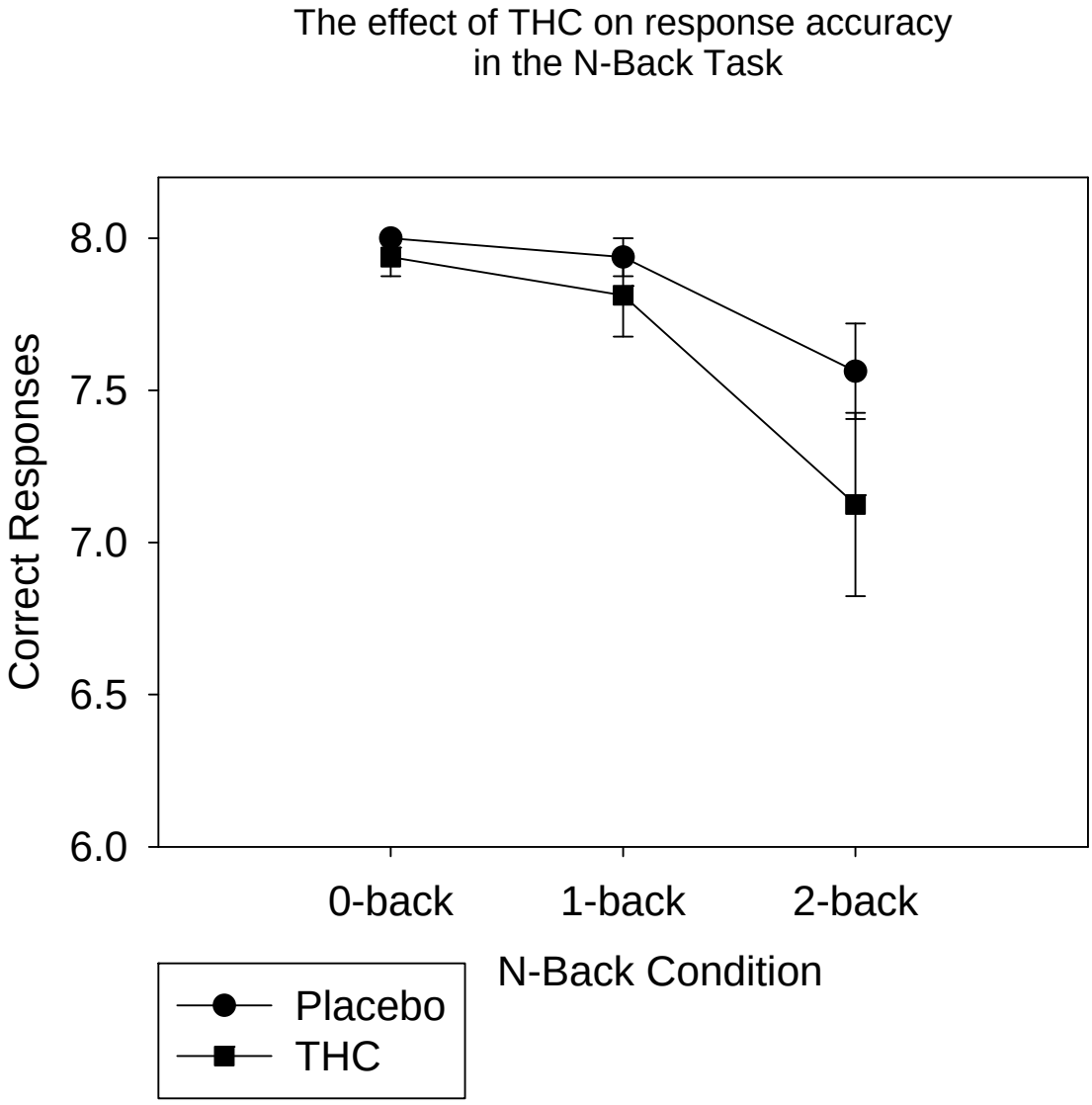
($p<0.05$) bands.

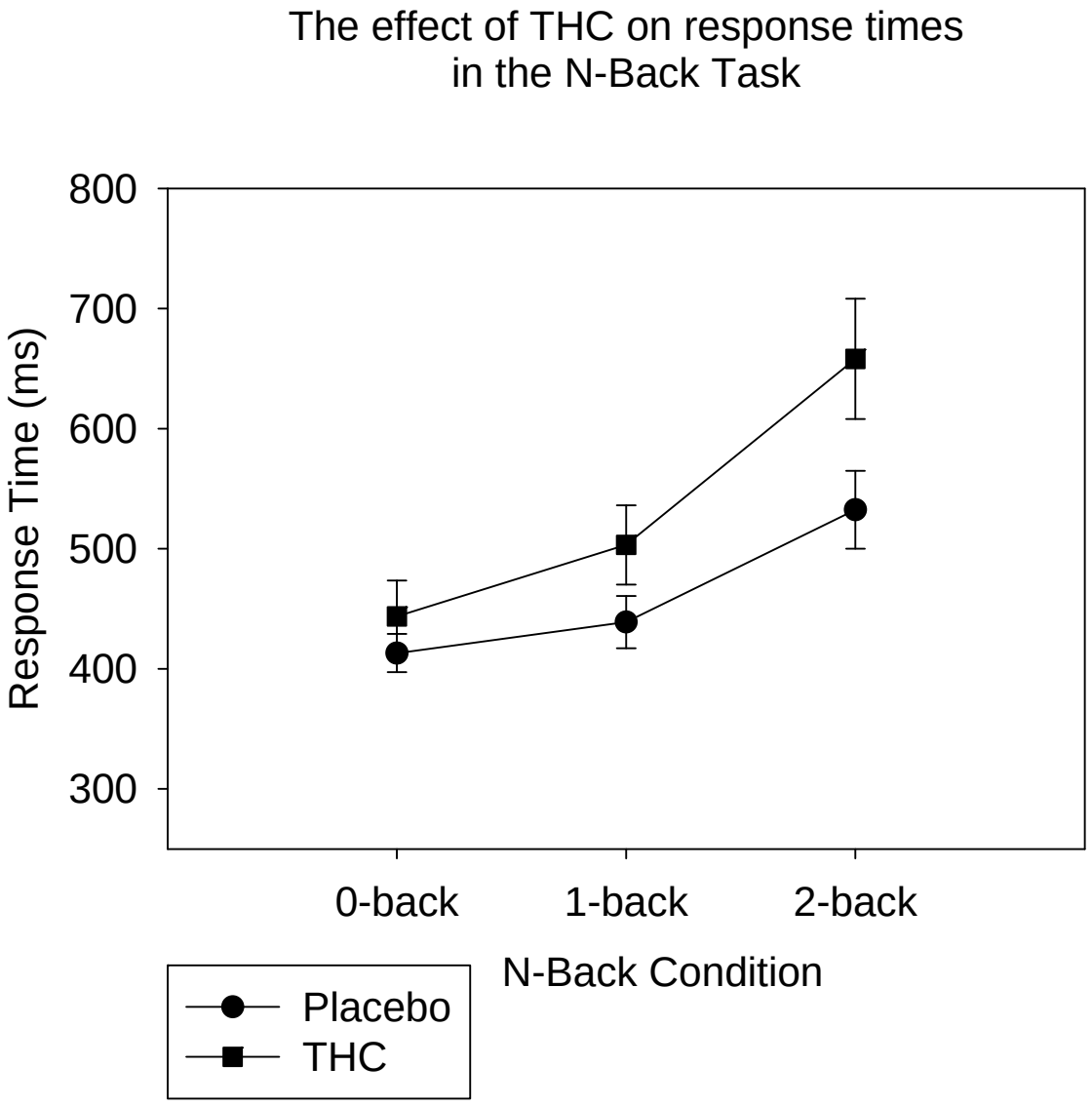
Figure 6. Reductions in theta coherence between left and right prefrontal regions under THC was correlated with positive psychotic symptoms (rho=0.79, $p<0.001$), and survived the removal of 2 potential outliers (rho=0.69, $p=0.006$).

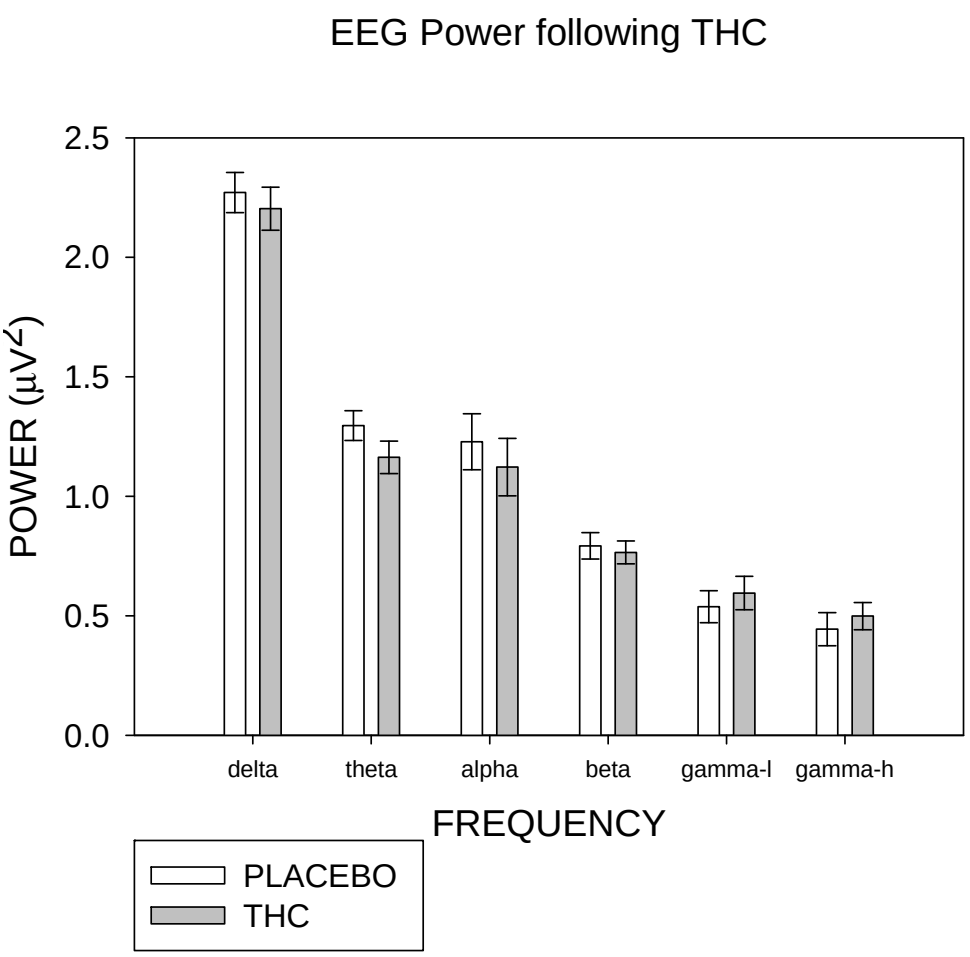

THC concentration over time
[THC] ng/ml
120
100
80
60
40
20
0
0
20
40
60
80
100
120
TIME (mins)


THC elicited positive symptoms
PANSS positive scores
13
12
11
10
9
8
7
6
0
30
90
TIME (mins)
Placebo
THC

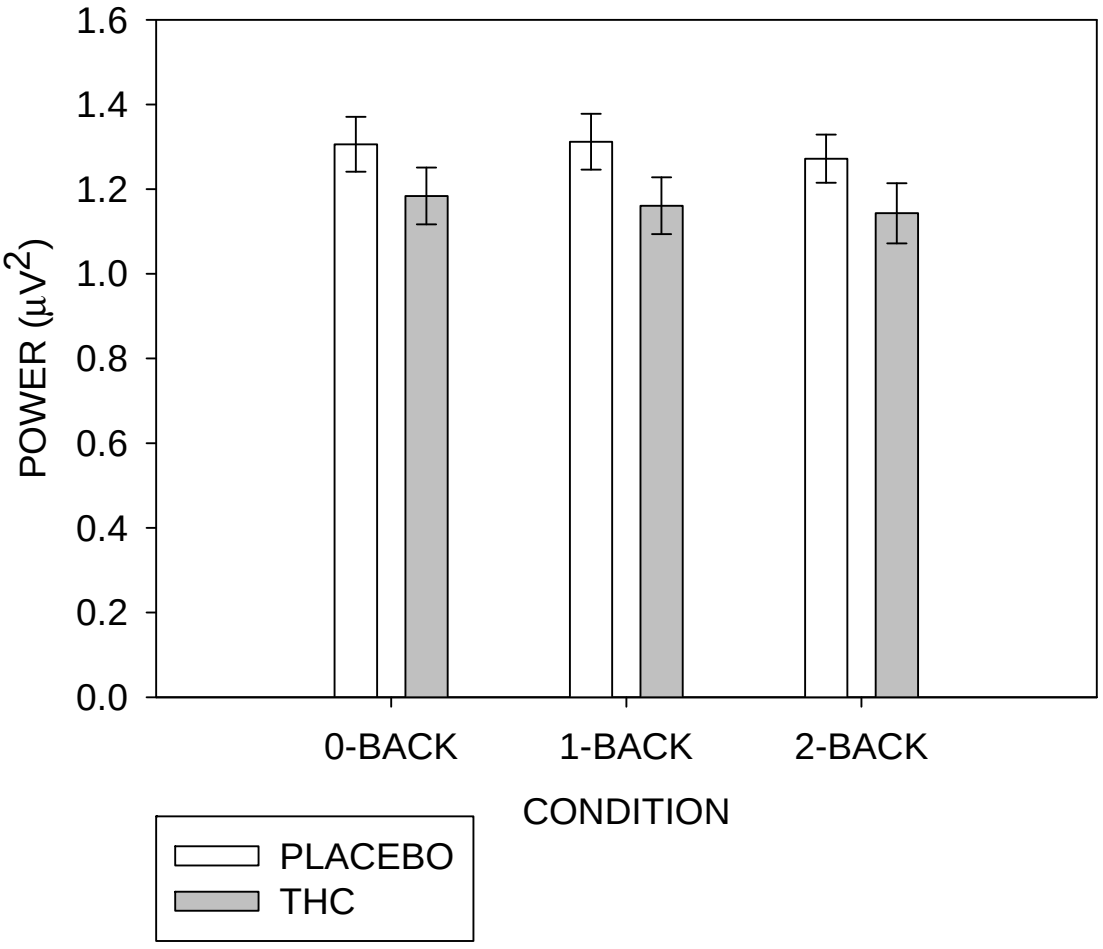

THC elicited negative symptoms
PANSS negative scores
13
12
11
10
9
8
7
6
0
30
90
TIME (mins)
Placebo
THC


The effect of THC on response accuracy
in the N-Back Task
Correct Responses
8.0
7.5
7.0
6.5
6.0
0-back
1-back
2-back
N-Back Condition
Placebo
THC

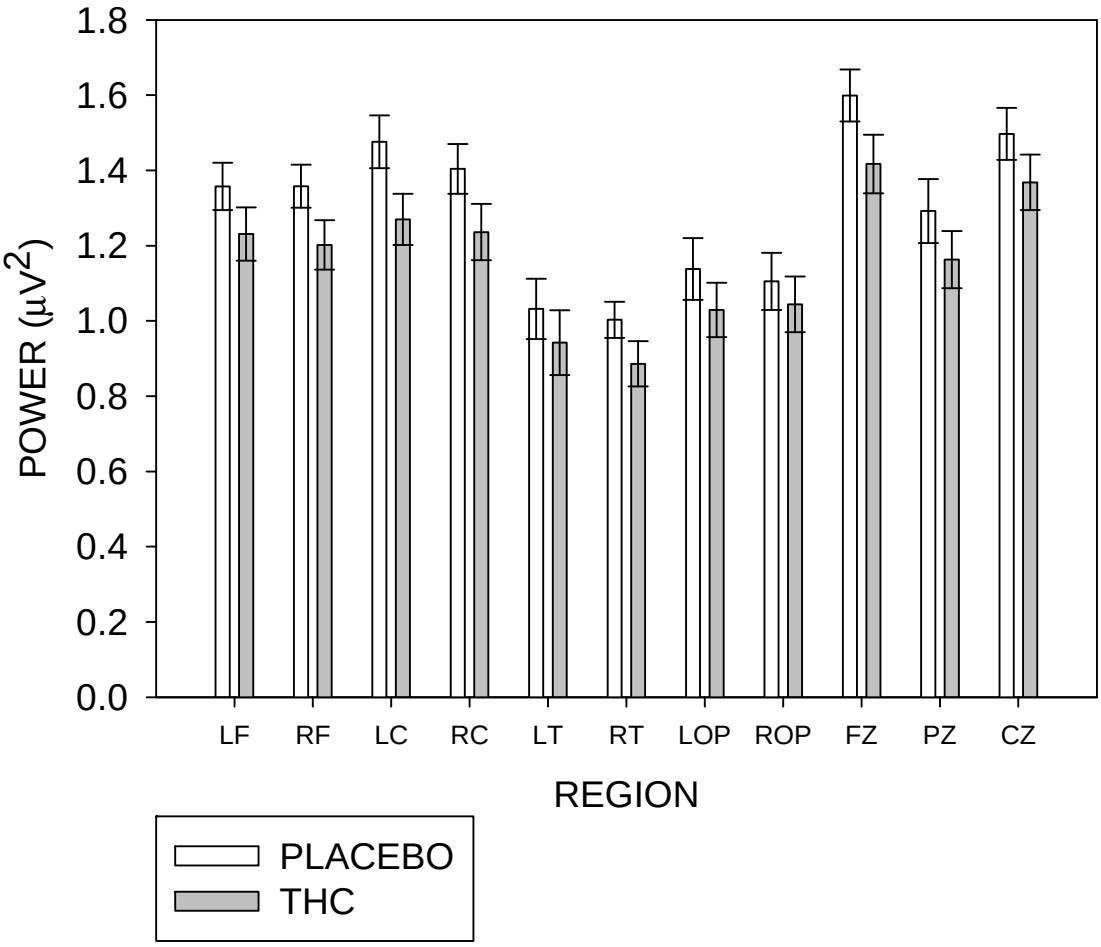

The effect of THC on response times
in the N-Back Task
800
700
600
500
400
300
Response Time (ms)
0-back
1-back
2-back
N-Back Condition
Placebo
THC


EEG Power following THC
2.5
2.0
1.5
1.0
0.5
0.0
POWER (μV²)
delta
theta
alpha
beta
gamma-l
gamma-h
FREQUENCY
PLACEBO
THC

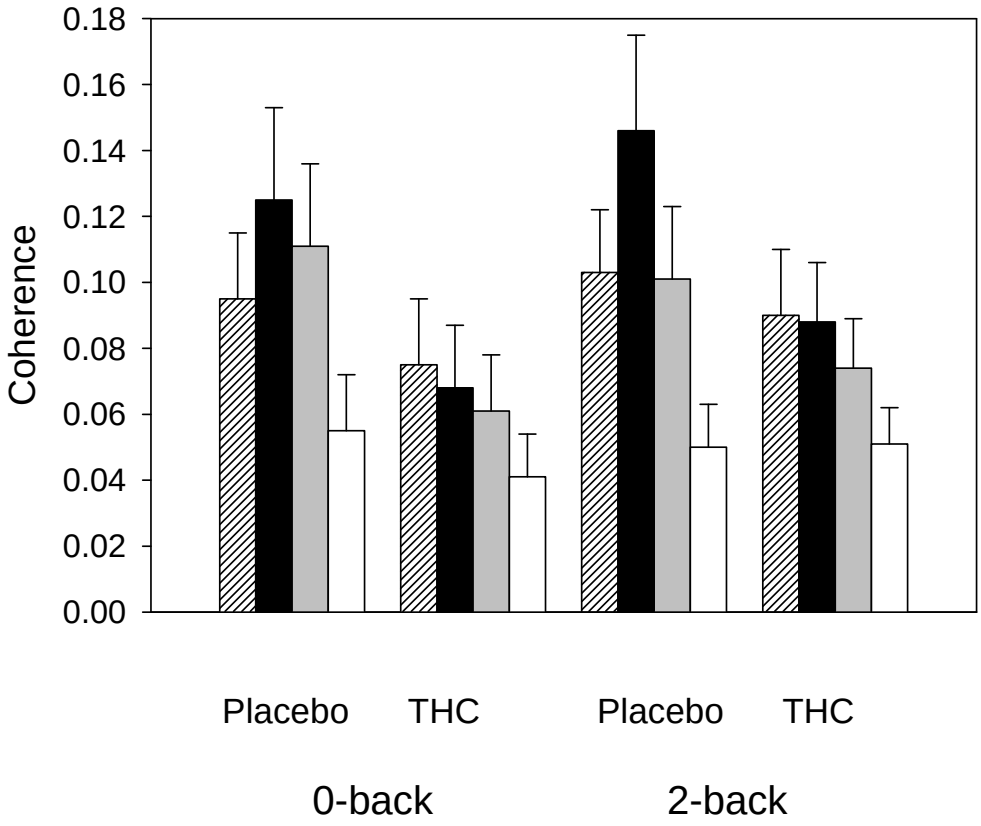
THETA power decreases under THC regardeless of memory load



THETA power under THC v placebo according to region



Bi-frontal coherence in the n-back task under placebo v THC conditions



The Relationship between positive psychotic symptoms
& bi-frontal theta coherence