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DOI:
10.1017/S0033291718000387

Document Version
Peer reviewed version

Link to publication record in King's Research Portal

Citation for published version (APA):
Bhattacharyya, S., Sainsbury, T., Allen, P., Nosarti, C., Atakan, Z., Giampietro, V., Brammer, M., & McGuire, P. K. (2018). Increased hippocampal engagement during learning as a marker of sensitivity to psychotomimetic effects of δ-9-THC. *Psychological Medicine*, 1-9. Advance online publication. https://doi.org/10.1017/S0033291718000387

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Psychological Medicine

Increased hippocampal engagement during learning as a marker of sensitivity to psychotomimetic effects of delta-9-THC

--Manuscript Draft--

Manuscript Number:	PSM-D-17-00440R3
Full Title:	Increased hippocampal engagement during learning as a marker of sensitivity to psychotomimetic effects of delta-9-THC
Article Type:	Original Article
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Manuscript Region of Origin:	UNITED KINGDOM
Abstract:	Background: Cannabis and its main psychoactive ingredient delta-9-tetrahydrocannibidiol (THC), can induce transient psychotic symptoms in healthy individuals and exacerbate them in those with established psychosis. However, not everyone experiences these effects, suggesting that certain individuals are particularly susceptible. The neural basis of this sensitivity to the psychotomimetic effects of THC is unclear. Methods: We investigated whether individuals who are sensitive to the psychotomimetic effects of THC (TP) under experimental conditions would show differential hippocampal activation compared to those who are not (NP). We studied 36 healthy males under identical conditions under the influence of placebo or THC (10 mg) given orally, on two separate occasions, in a pseudo-randomised, double-blind, repeated measures, within-subject, cross-over design, using psychopathological assessments and functional MRI while they performed a verbal learning task. They were classified into those who experienced transient psychotic symptoms (TP; n=14) following THC administration and those who did not (NP; n=22). Results: Under placebo conditions, there was significantly greater engagement of the left hippocampus ($p<0.001$) in the TP group compared to the NP group during verbal encoding, which survived leave-one-out analysis. The level of hippocampal activation was directly correlated (spearman's rho= 0.44, $p=0.008$) with the severity of transient psychotic symptoms induced by THC. This difference was not present when we compared two subgroups from the same sample that were defined by sensitivity to anxiogenic effects of THC.

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	Conclusions: These results suggest that altered hippocampal activation during verbal encoding may serve as a marker of sensitivity to the acute psychotomimetic effects of THC.

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Title Page *Words: 5031*

Figures: 3; Tables: 1

Increased hippocampal engagement during learning as a marker of sensitivity to psychotomimetic effects of delta-9-THC

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Number of figures: 3; Number of tables: 1

Supplementary Material: Supplementary Methods-1; Supplementary Tables- 5; Supplementary Figures- 5.

Word Count: Abstract: 250 words; Text: 5031 words

Key words: fMRI; cannabis; delta-9-tetrahydrocannabinol; verbal learning; psychosis; hippocampus

FUNDING & DISCLOSURES:

Conflict of Interest Disclosures: The authors do not report any financial relationships with commercial interests.

Grant Funding/Support: This work was supported by a Joint Medical Research Council/Priory Clinical research training fellowship from the Medical Research Council, United Kingdom, to Sagnik Bhattacharyya (SB). SB has been supported by a NIHR Clinician Scientist Award (NIHR CS-11-001).

Role of the Funder/ Sponsor: The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. The funding source had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and the decision to submit the manuscript for publication.

ABSTRACT

Background: Cannabis and its main psychoactive ingredient delta-9-tetrahydrocannibidiol (THC), can induce transient psychotic symptoms in healthy individuals and exacerbate them in those with established psychosis. However, not everyone experiences these effects, suggesting that certain individuals are particularly susceptible. The neural basis of this sensitivity to the psychotomimetic effects of THC is unclear.

Methods: We investigated whether individuals who are sensitive to the psychotomimetic effects of THC (TP) under experimental conditions would show differential hippocampal activation compared to those who are not (NP). We studied 36 healthy males under identical conditions under the influence of placebo or THC (10 mg) given orally, on two separate occasions, in a pseudo-randomised, double-blind, repeated measures, within-subject, cross-over design, using psychopathological assessments and functional MRI while they performed a verbal learning task. They were classified into those who experienced transient psychotic symptoms (TP; n=14) following THC administration and those who did not (NP; n=22). Results: Under placebo conditions, there was significantly greater engagement of the left hippocampus (p<0.001) in the TP group compared to the NP group during verbal encoding, which survived leave-one-out analysis. The level of hippocampal activation was directly correlated (spearman's rho= 0.44, p=0.008) with the severity of transient psychotic symptoms induced by THC. This difference was not present when we compared two subgroups from the same sample that were defined by sensitivity to anxiogenic effects of THC.

Conclusions: These results suggest that altered hippocampal activation during verbal encoding may serve as a marker of sensitivity to the acute psychotomimetic effects of THC.

Introduction

Regular cannabis use is associated with a dose-dependent increase in the risk of onset (Moore *et al.*, 2007, Zammit *et al.*, 2002) and exacerbation (Patel *et al.*, 2016, Schoeler *et al.*, 2016b, Schoeler *et al.*, 2016c, Schoeler *et al.*, 2016d) of psychotic disorders such as schizophrenia. Consistent with this, delta-9-tetrahydrocannabinol (THC), the main psychoactive ingredient in cannabis has been shown in experimental studies to induce transient psychotic symptoms in healthy subjects (Bhattacharyya *et al.*, 2015a, Bhattacharyya *et al.*, 2009, D'Souza *et al.*, 2004, Morrison *et al.*, 2011) and exacerbate them in schizophrenia patients (D'Souza *et al.*, 2005, Henquet *et al.*, 2006).

However, there is marked variation in the psychotomimetic and cognitive effects of cannabis based on genetic (Bhattacharyya *et al.*, 2012a, Bhattacharyya *et al.*, 2014) and personality and familial factors (Di Forti *et al.*, 2012, Henquet *et al.*, 2005, McGuire *et al.*, 1995, Stirling *et al.*, 2008, van Winkel *et al.*, 2011) as well as the composition of cannabis (Bhattacharyya *et al.*, 2010). Experimental studies in healthy individuals suggest that even when given substantial doses of pure THC, not all will experience a state of transient psychosis (Atakan *et al.*, 2013, Bhattacharyya *et al.*, 2010). While this may point towards fundamental neurobiological differences between those who are susceptible to the psychotomimetic effects of THC and those who are not, it is unclear if this is the case.

Evidence from animal models has led to the hypothesis that the development of psychosis may be associated with increased hippocampal activity (Lodge and Grace, 2007), in turn driving striatal dopaminergic overactivity (Lodge and Grace, 2011).

This is consistent with evidence of increased resting hippocampal regional cerebral blood flow (Allen *et al.*, 2016) and increased hippocampal activation during memory tasks (Valli *et al.*, 2011) in individuals at high risk of developing psychosis (Allen *et al.*, 2016). The hippocampus has a central role in memory formation and relational memory binding (Hannula and Ranganath, 2008). Patients with schizophrenia have a deficit in verbal learning and memory (Hannula and Ranganath, 2008, Lepage *et al.*, 2015, Schaefer *et al.*, 2013) and altered hippocampal function during memory processing (Hannula and Ranganath, 2008, Ragland *et al.*, 2015), leading to the suggestion that memory deficits and associated altered brain function may be potential neurocognitive markers of schizophrenia (Lepage *et al.*, 2015). Impairment in learning and memory, specifically dose-related (Curran *et al.*, 2002) impairment in verbal learning (Curran *et al.*, 2002, Henquet *et al.*, 2006, Ranganathan and D'Souza, 2006) that cannot be accounted for by effects on attention (Curran *et al.*, 2002, Ranganathan and D'Souza, 2006), is also one of the most prominent acute cognitive effects of THC in man that persists in chronic users (Schoeler and Bhattacharyya, 2013, Schoeler *et al.*, 2016a, Solowij *et al.*, 2002). Patients with schizophrenia are more vulnerable to dose-related verbal learning impairments under the influence of THC compared to healthy individuals (D'Souza *et al.*, 2005). The hippocampus has a high density of the type 1 central cannabinoid receptors (Eggan and Lewis, 2007, Elphick and Egertova, 2001), which are the main central target of THC and animal studies show that the effect of THC on learning correlate with its effect on hippocampal neuronal firing (Heyser *et al.*, 1993, Robbe *et al.*, 2006). Hence, one may predict that increased hippocampal activity may also underlie sensitivity to the psychotomimetic effects of THC. This hypothesis has not been tested before. The only previous study that investigated neurophysiological differences between those

who develop transient psychotic symptoms (TP group) and those that do not (NP group) in response to experimental THC challenge employed a cognitive (psychomotor control) task that does not normally engage the hippocampal region (Atakan *et al.*, 2013).

On the other hand, neuroimaging studies in healthy individuals have demonstrated that THC disrupts brain activity in regions associated with memory such as the medial temporal and prefrontal cortices as well functional connectivity between them during memory processing (Bhattacharyya *et al.*, 2012a, Bhattacharyya *et al.*, 2015b, Bhattacharyya *et al.*, 2009) and other cognitive tasks (Bhattacharyya *et al.*, 2012b, Bhattacharyya *et al.*, 2015b, Bhattacharyya *et al.*, 2010). In the present study, we therefore re-analysed previously reported data acquired employing a verbal memory paradigm that engages the hippocampus (Bhattacharyya *et al.*, 2012a) to examine whether differences in brain activation during task performance would differentiate those healthy individuals who experience transient psychotic (TP) symptoms from those who do not (NP), following an acute challenge with THC. Brain activation was indexed using blood oxygen level dependent (BOLD) haemodynamic response measured using fMRI while participants performed a verbal paired associates learning task. We predicted that when performing the task under placebo conditions, individuals who were sensitive to the psychotomimetic effects of THC (TP PLB) would show greater hippocampal activation than those who were not (NP PLB). We then tested the hypothesis that this difference in brain activation under the placebo condition between TP and NP groups would be directly associated with the magnitude of THC-induced psychotomimetic effects in the same individuals. Finally, we

hypothesized that THC administration would modulate brain activation differently in the TP and NP groups, reflecting the different symptomatic effects.

Furthermore, we carried out exploratory analysis to investigate whether the difference in neurophysiological response between TP and NP under placebo condition was a specific biomarker for THC-induced psychotomimetic effects as opposed to acute effects of THC on other symptoms such as anxiety(Bhattacharyya *et al.*, 2017), by testing whether brain activity differences between individuals who experienced acute anxiety (TA PLB) under THC versus those who did not experience anxiety (NA PLB) were in different brain regions compared to differences between 'TP PLB' and 'NP PLB' groups.

Methods and Materials

Using an established protocol (Bhattacharyya *et al.*, 2012a, Bhattacharyya *et al.*, 2012b, Bhattacharyya *et al.*, 2009), 36 healthy, occasional cannabis user male participants attended two sessions at least one month apart when they were given an identical capsule to be taken orally, containing either 10mg of THC or placebo using a pseudo-randomised, double-blind, repeated-measures, within-subject crossover design and a counterbalanced order of drug administration. Following administration, the subjects were required to complete a verbal paired associate learning task (Bhattacharyya *et al.*, 2009) while their brain activity was measured using fMRI. Methods and study participants are described in detail in Supplementary Methods. While we have previously reported the effects of THC and its genetic moderation at a group level (Bhattacharyya *et al.*, 2012a, Bhattacharyya *et al.*, 2009), the present study focuses on brain function

differences between those individuals who were sensitive to the psychotomimetic effects of THC compared to those who were not (see details below).

Participants were right-handed, English-speaking males, without a personal or family history of mental illness in first-degree relatives, mean age of 25.97±5.58 and mean National adult reading test (NART) score of 97.7±6. Alcohol, cannabis and other illicit drug use was assessed using the Addiction Severity index (McLellan *et al.*, 1980). They had used cannabis upto 25 times in their lifetime, drank less than 21 units/week of alcohol and had minimal exposure to other illicit drugs (see Supplementary Table 1). Participants were asked to abstain from all recreational drugs for the duration of the study and one month prior to it. Each participant passed a negative urine drug screen on the morning of each session for opiates, cocaine, amphetamines, benzodiazepines and THC to ensure that no traces of these drugs were in their systems. Psychological assessments (to assess mental state) were conducted and blood samples (to assess drug levels) were taken prior to and 1, 2, and 3 hours after drug administration. MRI scans were performed 1 hour after ingestion of the drug.

Psychotomimetic effects of THC were measured by an experienced clinical researcher using the positive and negative syndrome scale (PANSS) (Kay *et al.*, 1987). State-trait anxiety inventory- state (STAI) (Spielberger, 1983) and the analogue intoxication scale (AIS) (Mathew *et al.*, 1992) were used to measure anxiety and level of intoxication respectively. Psychological effects peaked 2 hours after THC administration, and hence ratings at this time point were used to compare the TP and NP groups.

Classification of participants on the basis of sensitivity to THC

For the purpose of this investigation, we established *a priori* criteria (also see Supplementary Methods) to define transient psychosis induced by THC, which were used to classify the participants into those who experienced transient psychotomimetic effects (TP) and those who did not (NP). Participants were identified as having experienced transient psychotic symptoms and allocated to the TP group if they scored at least 3 or more on any of the PANSS positive subscale items that measured psychotic symptoms (Delusions, Hallucinations, Suspiciousness/ Persecution) during any of the time-points when ratings were obtained following THC administration (Atakan *et al.*, 2013). Each item of PANSS is scored on a 7-point Likert scale, with a score of 1 denoting that the item being measured is "absent", a score of 2 denoting that it is "minimal" (indicating "questionable or subtle or suspect pathology" and a score of 3 denoting "mild" (indicating "a symptom whose presence is clearly established but not pronounced"). Higher scores on each of these items indicate greater severity. A score of 3 was used as the cut-off as it 3 denotes "mild" severity, indicating "a symptom whose presence is clearly established but not pronounced", and is the threshold used in the clinical setting to indicate clear, unambiguous presence of a psychotic symptom (Kay *et al.*, 1987). Psychotic symptoms scored in these participants were otherwise comparable to that observed in a clinical situation except for the transient nature of psychotic symptoms observed under the experimental THC challenge condition. For our exploratory analyses, participants were classified into those who experienced transient anxiety (TA) and those who did not (NA) under the influence of THC on the basis of a greater (TA) or lesser (NA) than 4-point change in their STAI (when baseline STAI score was deducted from their peak post-THC STAI score) in response to

THC administration. This cut-off was determined using the Reliable Change Index (Jacobson and Truax, 1991).

Image acquisition

fMRI scans were acquired using a 1.5 tesla scanner (see supplementary methods). During fMRI acquisition, subjects completed a verbal paired associate learning task (Bhattacharyya *et al.*, 2009), which comprised encoding, recall and baseline conditions. For the encoding condition, subjects were required to indicate whether visually presented word pairs were related in terms of their meaning, while during a subsequent recall condition, they were presented with one word from each pair presented before and were required to recall the missing word that had been previously associated with the word. During the baseline condition, subjects were presented with word-pairs with identical or different fonts and they were asked to indicate if the fonts were identical. For each of the conditions, 8 stimuli pairs were presented sequentially across 4 blocks. Recall score was used as a measure of task performance during the memory task. Analysis of performance data suggested a ceiling effect by the 3rd block (Supplementary Figure 1) and hence only data from the first 3 blocks were analysed.

Data analysis

Psychological ratings and memory task performance were analyzed using SPSS version 22. Socio-demographic characteristics (such as age, NART score and number of years in education) and task performance (recall score) of the groups (TP vs NP and TA vs NA) were compared using two-sample *t*-tests while symptom data at 2

hours after THC and placebo administration were compared using Mann-whitney U tests as they did not fit normal distribution.

Imaging data were pre-processed and analysed (following previously reported approaches) using XBAMv4.1 (http://www.brainmap.co.uk), a non-parametric image analysis programme that minimises assumptions about the distribution of the data, which is important in fMRI where the data may not follow a Gaussian distribution(Thirion *et al.*, 2007) (see Supplementary Methods).

For each drug condition, we contrasted each of the active (encoding or recall) conditions of the verbal memory task against the baseline (fonts) condition at the individual subject level to generate contrast of interest map ('encoding minus baseline' and for 'recall minus baseline' conditions) for each subject, which were used for subsequent group-level analyses (TP vs NP and TA vs NA).

To investigate our primary hypothesis that 'TP PLB' group would show greater hippocampal activation compared to the 'NP PLB' group, analysis of variance compared the 'TP PLB' group and 'NP PLB' during the placebo condition in order to assess differences in functional activation during the contrast of interest (for 'encoding minus baseline' and for 'recall minus baseline' conditions, henceforth referred to as 'encoding' and 'recall' respectively unless otherwise specified) in the absence of THC. To test the robustness of these group differences in activation and whether they were driven by outliers, we carried out a 'leave one subject out' (LOSO) analysis, which involved repeating the ANOVA with a different subject from the TP group being left out on each repeat. A total of 14 repeat ANOVAs were carried out, once with each of the 14 'TP PLB' subjects being left out. We then carried out

exploratory analyses to examine whether these brain activation differences during the encoding and recall conditions were specific to the sub-groups classified according to sensitivity to the psychotomimetic effects of THC ('TP PLB' vs 'NP PLB') or were similar to that between subgroups classified based on sensitivity to anxiogenic effects of THC ('TA PLB' vs 'NA PLB'). One-way analysis of variance compared task-related brain activation differences (during encoding and recall conditions) between the 'TA PLB' and 'NA PLB' groups under the placebo condition to examine whether similar group differences exist between 'TA PLB' and 'NA PLB' groups as between 'TP PLB' and 'NP PLB' groups. To test our hypothesis that THC administration would modulate brain activation differently in the TP and NP groups, further, comparisons (using 2-way ANOVA) were then made between the drug given, TP and NP groups and the interaction of effects between them. Statistical values from differentially activated brain clusters (mean of all voxels in the cluster) were used to identify correlation with behavioural data to test our hypothesis that difference in brain activation under the placebo condition between TP and NP groups would be directly associated with the magnitude of THC-induced psychotomimetic effects in the same individuals. A similar approach was employed to compare 'TA PLB' and 'NA PLB' groups.

Results

Symptomatic and behavioural differences between TP and NP

Of the 36 subjects who participated in the study, 14 satisfied our pre-defined criteria on the basis of PANSS ratings following THC administration to be

classified to the TP group, whilst the remaining 22 subjects formed the NP group. These two groups were not significantly different in terms of their socio-demographic characteristics and estimated pre-morbid IQ ($p>0.5$) (Table 1) or in terms of symptoms experienced under the placebo condition (Mann-Whitney U-tests $p>0.4$; Figure 1; eTable 2). However, 2 hours after the administration of THC, the 'TP THC' group scored significantly higher on both the STAI ($p=0.008$) and AIS ($p=0.001$) ratings (Figure 1; Supplementary Table 2). As expected, following THC administration, the 'TP THC' group showed marked increases in all PANSS subscale scores (Figure 1) which were significantly higher than the NP group for all of the PANSS subscales (Mann-Whitney U-tests; all $p<0.001$; Supplementary Table 2). Both the TP ('TP THC' and 'TP PLB') and NP ('NP THC' and 'NP PLB') groups showed similar total recall scores under both THC (t-tests; $p= 0.85$) and placebo conditions ($p=0.22$) (Supplementary Table 2).

Of the total sample, 18 participants were assigned to the TA group and 18 were assigned to the NA group based on their STAI ratings following THC administration. The two groups were well matched on age and years of education, however there was a significant difference between NART-IQ scores between TA and NA groups ($p=0.04$; Table 1). The 'TA THC' group had higher STAI scores compared to the 'NA THC' group following THC administration ($p=0.016$; Supplementary Table 2). However, they were not significantly different ($p=0.18$) in terms of the severity of transient psychotic symptoms (PANSS-Positive subscale) induced by THC. Both groups also showed similar total recall scores under both placebo and THC (Supplementary Table 2).

Of the 14 TP participants, 8 were also in the TA group based on their anxiety ratings and 6 were in the NA group. Of the 22 NP participants, 10 were in the TA and 12 in the NA group. There was a positive correlation (0.41, p=0.01) between transient psychotic symptoms (PANSS-Positive subscale) and anxiety (STAI) measured 2 hours after administration of THC.

Brain regions engaged by the verbal memory task in all individuals

As expected, during both the encoding and recall conditions in all study participants, the verbal memory task was associated with engagement of brain regions previously implicated in memory processing, particularly the prefrontal and medial temporal cortices (encoding: Supplementary Table 3A; Supplementary Figure 2A; and recall: Supplementary Table 3B; Supplementary Figure 2B).

Differences in activation between the 'TP PLB' and 'NP PLB' groups under placebo

Investigation of our hypothesis that 'TP PLB' group would show greater hippocampal activation compared to the 'NP PLB' group showed that during the encoding condition, there was greater engagement of the left hippocampus, left anterior cingulate (ACC) and right superior temporal gyrus (STG) ($p<0.001$, corrected for <1 false positive cluster) in the 'TP PLB' group than the 'NP PLB' group, whereas the converse was true in the cerebellum bilaterally (Figure 2; Supplementary Table 4). Left hippocampal engagement remained significantly different between the 'TP PLB' and 'NP PLB' groups across all repeats of the leave one subject out (LOSO) analysis (which involved repeating the ANOVA with a different subject from the 'TP PLB'

group being left out on each repeat), whereas the clusters of activation in the STG and ACC did not consistently survive this analysis. As there was a direct correlation between the transient psychotic symptoms and anxiety induced following THC administration, post hoc, we compared the 'TP PLB' and 'NP PLB' groups during the encoding condition under placebo after controlling for the severity of anxiety symptoms induced under THC. This did not change the pattern and direction of results (data not shown, but available from the authors on request), particularly the difference in left hippocampal engagement between the 'TP PLB' and 'NP PLB' groups.

In order to examine whether the group difference in activation between the 'TP PLB' and 'NP PLB' groups truly represented a marker of sensitivity to the psychotomimetic effects of THC, we then tested whether activation in these regions under placebo condition was directly related to the severity of transient psychotic symptoms induced by THC in these individuals. Engagement of the left hippocampus under placebo condition showed a non-linear correlation with the increase in the severity of psychotic symptoms following administration of THC (spearman's rho = 0.44, p=0.008; Figure 2D).

During the recall condition, significant between-group differences ('TP PLB' vs 'NP PLB'') in activation were observed in the left medial frontal, right middle temporal gyrus (MTG) and anterior lobe of cerebellum, where the 'TP PLB' group showed greater engagement relative to the 'NP PLB' group, whereas the converse applied in the left inferior parietal lobule, the precentral gyrus bilaterally, the precuneus and cingulate gyrus on the right side and the posterior lobe of the cerebellum

(Supplementary Figure 3 & Supplementary Table 4). Group ('TP PLB' vs 'NP PLB') differences in activation in these regions did not always survive the LOSO analysis and were not investigated further.

Specificity of brain activation differences between the 'TP PLB' and 'NP PLB' groups under placebo

Exploratory one-way analysis of variance revealed significant differences in task-related activation between the 'TA PLB' and 'NA PLB' groups under the placebo condition during both the encoding (Supplementary Table 5; Supplementary Figure 4) and the recall (Supplementary Table 5; Supplementary Figure 5) conditions. However, these regions did not overlap with those that were differentially activated on contrasting the 'TP PLB' and 'NP PLB' groups.

Differences in activation between the TP and NP groups under THC

Investigation of our hypothesis that THC administration would modulate brain activation differently in the TP and NP groups, reflecting the different symptomatic effects with two-way ANOVA [Group (TP vs NP) by drug (THC vs Placebo)] revealed significant differences in the effect of THC on activation in the two groups in a number of areas during the encoding but not the recall condition (Figure 3; Supplementary Table 4). Under placebo during the encoding condition, there was greater engagement of the right middle frontal gyrus (MFG) extending to the precentral gyrus and in the left cingulate gyrus and cerebellum in

the ‘TP PLB’ group relative to the ‘NP PLB’ group. However, under the THC condition there was a reversal of engagement of these regions: THC attenuated their activation in the ‘TP THC’ group but augmented it in the ‘NP THC’ group, relative to placebo. The THC-induced change in activation of the right MFG inversely correlated with increase in the severity of psychotic symptoms following THC (spearman’s rho = -0.6, p=<0.001; Figure 3C). There was no significant group (TP vs NP) by drug (THC vs Placebo) interaction in the left hippocampus where there was a difference between the ‘TP PLB’ and ‘NP PLB’ groups under placebo condition, nor was a similar difference observed between ‘TP THC’ and ‘NP THC’ groups under THC alone.

Discussion

Here we investigated whether differences in hippocampal activation measured using BOLD fMRI under placebo conditions may distinguish between healthy males who are sensitive to the psychotomimetic effects of THC from those who are not. The results suggest that altered activation in the left hippocampus an area implicated in both normal memory processing and the neuropathology of psychosis, differentiated those who experience transient psychotic symptoms following a single dose of THC from those who do not.

These differences were not simply a result of differential levels of task performance in these two groups, nor were they related to group differences in socio-demographic characteristics or psychological ratings at the time the neuroimaging data were acquired. As predicted, increased encoding-related engagement of the left hippocampus, a region critical for encoding (Eichenbaum *et al.*, 2007), differentiated

the THC-sensitive group from those not sensitive to its psychotomimetic effects. Furthermore, left hippocampal engagement which reliably differentiated healthy individuals on the basis of their sensitivity to acute psychotomimetic effects of THC, was directly correlated with the severity of psychotic symptoms induced under the influence of THC, such that the greater the engagement of the left hippocampus under the placebo condition, greater was the severity of psychotic symptoms induced by THC. This was consistent with our hypothesis that difference in brain activation under the placebo condition between TP and NP groups would be directly associated with the magnitude of THC-induced psychotomimetic effects in the same individuals. Difference in hippocampal engagement distinguished individuals sensitive to the psychotomimetic effects of THC but not those who experienced anxiety under THC as revealed in exploratory analyses, suggesting the relationship was specific to psychotic symptoms. This difference in hippocampal engagement persisted even after controlling for the severity of THC-induced anxiety suggesting that difference in hippocampal activation under placebo condition between the 'TP PLB' and 'NP PLB' individuals was not a marker of differential sensitivity to THC-induced anxiety in the same individuals. Collectively, these findings suggest that increased left hippocampal engagement during word encoding may be a marker of sensitivity to the acute psychotomimetic effects of THC. This is consistent with evidence of increased resting hippocampal regional cerebral blood flow (Allen *et al.*, 2016) in those at high clinical risk of psychosis and reduced hippocampal volume in those with established schizophrenia (Nelson *et al.*, 1998). However, it is worth noting that it is regular rather than acute cannabis use that has been linked to schizophrenia. Hence, while altered left hippocampal engagement may be a marker of sensitivity to the acute

psychotomimetic effects of THC, it may not necessarily be a marker of sensitivity to the development of schizophrenia following regular cannabis use.

By definition, the TP and NP groups had different responses to THC in relation to their levels of acute psychotic symptoms. Consistent with our hypothesis that THC administration would modulate brain activation differently in the TP and NP groups, our second major finding was that this difference in psychotic symptom generation was associated with a difference in the neurophysiological effects of THC in the two groups. There was a significant interaction between drug and group in the middle frontal gyrus (MFG), a region involved in the organization of memory (Simons and Spiers, 2003). Attenuation of lateral prefrontal activity by THC in our study correlated with the increase in psychotic symptoms induced by it, and is consistent with a similar attenuation of lateral prefrontal activity(Bhattacharyya *et al.*, 2015a) by THC that correlated with the severity of psychotic symptoms(Bhattacharyya *et al.*, 2015a) induced by it as well as genetic moderation(Bhattacharyya *et al.*, 2014) of the effects of THC in this region in the context of a cognitive activation task that engaged inhibitory control processes. Altered brain activity in this region has also been shown in the context of inhibitory and related motor control tasks in cannabis users both under acute THC challenge condition(Weinstein *et al.*, 2008) and in its absence(Eldreth *et al.*, 2004, Tapert *et al.*, 2007). The lateral prefrontal cortex is rich in CB1 receptors(Elphick and Egertova, 2001), the main target of THC in the brain(Pertwee, 2008), and results presented here suggest that the effects of THC in this region may be involved in the generation of paranoia under its influence consistent with dorsolateral prefrontal hypoactivity reported in schizophrenia (Callicott *et al.*, 2000) and role of altered lateral prefrontal activity in the pathophysiology of psychotic symptoms in schizophrenia(Shergill *et al.*, 2000).

We have previously reported that the normal pattern of medial temporal engagement while learning new information is altered by an acute THC challenge (Bhattacharyya *et al.*, 2009). The present study extends this by establishing that altered hippocampal engagement during a memory task distinguishes those healthy individuals who are particularly sensitive to the acute psychotomimetic effects of THC from those who are not.

Limitations

The results presented here should be considered preliminary in light of certain limitations. An important caveat relates to the generalizability of these results under laboratory conditions to the small proportion of real-world cannabis users who may be sensitive to the psychotomimetic effects of THC, which may be manifest on a continuum from mild transient paranoia to frank schizophreniform disorder. It is worth noting that psychotic-like symptoms experienced by participants in this study were transient and self-limited unlike those observed in established psychosis, but not dissimilar to the transient paranoia experienced by large number of cannabis users. We ensured that the psychotic symptoms experienced by participants classified as part of the transiently psychotic group were qualitatively similar to overt psychotic symptoms such as delusions and hallucinations and not merely a result of behavioural disorganization, by setting a cut-off threshold identical to that employed in clinical practice. It is also worth noting the present study does not account for other factors such as genetic (Bhattacharyya *et al.*, 2012a, Bhattacharyya *et al.*, 2014, Di Forti *et al.*, 2012, van Winkel *et al.*, 2011) and personality and familial factors (Henquet *et al.*, 2005, McGuire *et al.*, 1995, Stirling *et al.*, 2008) as well as the composition

(Bhattacharyya *et al.*, 2010) and dose (Schoeler *et al.*, 2016c) of cannabis that may also underlie differential sensitivity to the effects of cannabis.

It may also be argued that the articulation of verbal responses during the task may have resulted in head movement, which would have affected brain activation that in turn may have influenced our results. An effect of articulation seems unlikely because the findings in our study were obtained from comparisons of repetitions of the same condition between groups or between the effects of drugs on the same conditions in the two groups. As the verbal responses in these comparisons were identical, even if articulation had affected the fMRI signal, it would have had to have a systematically different effect between the two groups (TP vs NP) or in the presence of one drug versus another. This seems unlikely, as there was no change in the demands on articulation between the two groups or between the drug conditions, and there was no significant difference in the performance of the task between the groups under the two drug conditions. We thus think that it is highly unlikely that head movement due to verbal responses during the task significantly affected the results.

Similarly, one may suggest that expectation or memory of the psychotomimetic effects of THC may partly account for the brain activation differences between the TP and NP groups. However, this is also unlikely to fully account for these findings, as such an effect on brain activation should have been similarly evident on comparison of the TA and NA groups, which it was not. Furthermore, because of the very nature of this study, individuals with marked psychosis-like effects during previous cannabis use may have been less likely to volunteer for such a study, suggesting that any effect of expectation or memory of psychotomimetic effects of THC is unlikely to have been substantial. It is also worth noting that participants in this study had limited previous exposure to cannabis. Hence, even if such an effect had been present, it is likely that

this would have been cancelled out on comparison of two groups with minimal previous exposure to cannabis (TP vs NP).

It is also important to note that this study cannot establish whether association between hippocampal activation and sensitivity to the psychotomimetic effects of THC was specific to the use of a verbal paired associate learning task as opposed to cognitive paradigms that engage other cognitive processes affected by THC, as we did not investigate this. Future systematic investigation in this area may be warranted. Finally, the relatively modest sample size of the present cohort should also be noted, highlighting the need for independent replication in larger samples.

Collectively, our results suggest altered hippocampal activation may underlie sensitivity to the acute psychotomimetic effects of THC under experimental conditions in occasional cannabis users. While one may speculate that altered hippocampal activation may also predict sensitivity to the onset of psychotic disorders or a relapse of psychosis following regular cannabis use, this was not tested here and will require further investigation in prospective studies.

Authors Contributions: Dr Bhattacharyya had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Bhattacharyya

Acquisition, analysis or interpretation of data: Bhattacharyya, Sainsbury, Allen, Nosarti, Atakan, Giampietro, Brammer, McGuire

Drafting of the manuscript: Bhattacharyya, Sainsbury

Critical revision of the manuscript for important intellectual content: Bhattacharyya, Allen, Nosarti, Atakan, Giampietro, Brammer, McGuire

Statistical analysis: Bhattacharyya, Sainsbury, Giampietro, Brammer

Obtained funding: Bhattacharyya, McGuire

Administrative, technical, or material support: Bhattacharyya, Nosarti, Atakan, Giampietro, Brammer, McGuire

Study supervision: Bhattacharyya

ACKNOWLEDGEMENTS: Glynis Ivin, BPharm, provided assistance with the masking procedure, as well as with storage and dispensing of the drugs.

REFERENCES:

Allen, P., Chaddock, C. A., Egerton, A., Howes, O. D., Bonoldi, I., Zelaya, F., Bhattacharyya, S., Murray, R. & McGuire, P. (2016). Resting Hyperperfusion of the Hippocampus, Midbrain, and Basal Ganglia in People at High Risk for Psychosis. *American Journal of Psychiatry* **173**, 392-9.

Atakan, Z., Bhattacharyya, S., Allen, P., Martin-Santos, R., Crippa, J. A., Borgwardt, S. J., Fusar-Poli, P., Seal, M., Sallis, H., Stahl, D., Zuardi, A. W., Rubia, K. & McGuire, P. (2013). Cannabis affects people differently: inter-subject variation in the psychotogenic effects of Delta9-tetrahydrocannabinol: a functional magnetic resonance imaging study with healthy volunteers. *Psychological Medicine* **43**, 1255-67.

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J. A., Kambeitz, J., Malhi, S., Giampietro, V., Williams, S., Brammer, M., Rubia, K., Collier, D. A. & McGuire, P. K. (2015a). Impairment of inhibitory control processing related to acute psychotomimetic effects of cannabis. *European Neuropsychopharmacology* **25**, 26-37.

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J. A., Kambeitz, J., Prata, D., Williams, S., Brammer, M., Collier, D. A. & McGuire, P. K. (2012a). Preliminary report of biological basis of sensitivity to the effects of cannabis on psychosis: AKT1 and DAT1 genotype modulates the effects of delta-9-tetrahydrocannabinol on midbrain and striatal function. *Molecular Psychiatry* **17**, 1152-5.

Bhattacharyya, S., Crippa, J. A., Allen, P., Martin-Santos, R., Borgwardt, S., Fusar-Poli, P., Rubia, K., Kambeitz, J., O'Carroll, C., Seal, M. L., Giampietro, V., Brammer, M., Zuardi, A. W., Atakan, Z. & McGuire, P. K. (2012b). Induction of psychosis by Delta9-tetrahydrocannabinol reflects modulation of prefrontal and striatal function during attentional salience processing. *Archives of General Psychiatry* **69**, 27-36.

Bhattacharyya, S., Egerton, A., Kim, E., Rosso, L., Riano Barros, D., Hammers, A., Brammer, M., Turkheimer, F. E., Howes, O. D. & McGuire, P. (2017). Acute induction of anxiety in humans by delta-9-tetrahydrocannabinol related to amygdalar cannabinoid-1 (CB1) receptors. *Scientific Reports* **7**, 15025-39.

Bhattacharyya, S., Falkenberg, I., Martin-Santos, R., Atakan, Z., Crippa, J. A., Giampietro, V., Brammer, M. & McGuire, P. (2015b). Cannabinoid modulation of functional connectivity within regions processing attentional salience. *Neuropsychopharmacology* **40**, 1343-52.

Bhattacharyya, S., Fusar-Poli, P., Borgwardt, S., Martin-Santos, R., Nosarti, C., O'Carroll, C., Allen, P., Seal, M. L., Fletcher, P. C., Crippa, J. A., Giampietro, V., Mechelli, A., Atakan, Z. & McGuire, P. (2009). Modulation of mediotemporal and ventrostriatal function in humans by Delta9-tetrahydrocannabinol: a neural basis for the effects of Cannabis sativa on learning and psychosis. *Archives of General Psychiatry* **66**, 442-51.

Bhattacharyya, S., Iyegbe, C., Atakan, Z., Martin-Santos, R., Crippa, J. A., Xu, X., Williams, S., Brammer, M., Rubia, K., Prata, D., Collier, D. A. & McGuire, P. K. (2014). Protein kinase B (AKT1) genotype mediates sensitivity to cannabis-induced impairments in psychomotor control. *Psychological Medicine* **44**, 3315-28.

Bhattacharyya, S., Morrison, P. D., Fusar-Poli, P., Martin-Santos, R., Borgwardt, S., Winton-Brown, T., Nosarti, C., O' Carroll, C. M., Seal, M., Allen, P., Mehta, M. A., Stone, J. M., Tunstall, N., Giampietro, V., Kapur, S., Murray, R. M., Zuardi, A. W., Crippa, J. A., Atakan, Z. & McGuire, P. K. (2010). Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology* **35**, 764-74.

Callicott, J. H., Bertolino, A., Mattay, V. S., Langheim, F. J., Duyn, J., Coppola, R., Goldberg, T. E. & Weinberger, D. R. (2000). Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. *Cerebral Cortex* **10**, 1078-92.

Curran, H. V., Brignell, C., Fletcher, S., Middleton, P. & Henry, J. (2002). Cognitive and subjective dose-response effects of acute oral Delta 9-tetrahydrocannabinol (THC) in infrequent cannabis users. *Psychopharmacology (Berl)* **164**, 61-70.

D'Souza, D. C., Abi-Saab, W. M., Madonick, S., Forselius-Bielen, K., Doersch, A., Braley, G., Gueorguieva, R., Cooper, T. B. & Krystal, J. H. (2005). Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biological Psychiatry* **57**, 594-608.

D'Souza, D. C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y. T., Braley, G., Gueorguieva, R. & Krystal, J. H. (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology* **29**, 1558-72.

Di Forti, M., Iyegbe, C., Sallis, H., Kolliakou, A., Falcone, M. A., Paparelli, A., Sirianni, M., La Cascia, C., Stilo, S. A., Marques, T. R., Handley, R., Mondelli, V., Dazzan, P., Pariante, C., David, A. S., Morgan, C., Powell, J. & Murray, R. M. (2012). Confirmation that the AKT1 (rs2494732) genotype influences the risk of psychosis in cannabis users. *Biological Psychiatry* **72**, 811-6.

Eggan, S. M. & Lewis, D. A. (2007). Immunocytochemical distribution of the cannabinoid CB1 receptor in the primate neocortex: a regional and laminar analysis. *Cerebral Cortex* **17**, 175-91.

Eichenbaum, H., Yonelinas, A. P. & Ranganath, C. (2007). The medial temporal lobe and recognition memory. *Annual Review of Neuroscience* **30**, 123-52.

Eldreth, D. A., Matochik, J. A., Cadet, J. L. & Bolla, K. I. (2004). Abnormal brain activity in prefrontal brain regions in abstinent marijuana users. *Neuroimage* **23**, 914-20.

Elphick, M. R. & Egertova, M. (2001). The neurobiology and evolution of cannabinoid signalling. *Philosophical Transactions of Royal Society London B Biological Science* **356**, 381-408.

Hannula, D. E. & Ranganath, C. (2008). Medial temporal lobe activity predicts successful relational memory binding. *Journal of Neuroscience* **28**, 116-24.

Henquet, C., Krabbendam, L., Spauwen, J., Kaplan, C., Lieb, R., Wittchen, H. U. & van Os, J. (2005). Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. *The British Medical Journal* **330**, 11-15.

Henquet, C., Rosa, A., Krabbendam, L., Papiol, S., Fananas, L., Drukker, M., Ramaekers, J. G. & van Os, J. (2006). An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. *Neuropsychopharmacology* **31**, 2748-57.
Heyser, C. J., Hampson, R. E. & Deadwyler, S. A. (1993). Effects of delta-9-tetrahydrocannabinol on delayed match to sample performance in rats: alterations in short-term memory associated with changes in task specific firing of hippocampal cells. *Journal of Pharmacology & Experimental Therapeutics* **264**, 294-307.
Jacobson, N. S. & Truax, P. (1991). Clinical significance: a statistical approach to defining meaningful change in psychotherapy research. *Journal of Consulting & Clinical Psychology* **59**, 12-9.
Kay, S. R., Fiszbein, A. & Opler, L. A. (1987). The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin* **13**, 261-76.
Lepage, M., Hawco, C. & Bodnar, M. (2015). Relational Memory as a Possible Neurocognitive Marker of Schizophrenia. *Journal of the American Medical Association Psychiatry* **72**, 946-7.
Lodge, D. J. & Grace, A. A. (2007). Aberrant hippocampal activity underlies the dopamine dysregulation in an animal model of schizophrenia. *Journal of Neuroscience* **27**, 11424-30.
Lodge, D. J. & Grace, A. A. (2011). Hippocampal dysregulation of dopamine system function and the pathophysiology of schizophrenia. *Trends in Pharmacological Science* **32**, 507-13.
Mathew, R. J., Wilson, W. H., Humphreys, D. F., Lowe, J. V. & Wiethe, K. E. (1992). Regional cerebral blood flow after marijuana smoking. *Journal of Cerebral Blood Flow & Metabolism* **12**, 750-8.
McGuire, P. K., Jones, P., Harvey, I., Williams, M., McGuffin, P. & Murray, R. M. (1995). Morbid risk of schizophrenia for relatives of patients with cannabis-associated psychosis. *Schizophrenia Research* **15**, 277-81.
McLellan, A. T., Luborsky, L., Woody, G. E. & O'Brien, C. P. (1980). An improved diagnostic evaluation instrument for substance abuse patients. The Addiction Severity Index. *Journal of Nervous & Mental Disease* **168**, 26-33.
Moore, T. H., Zammit, S., Lingford-Hughes, A., Barnes, T. R., Jones, P. B., Burke, M. & Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *The Lancet* **370**, 319-28.
Morrison, P. D., Nottage, J., Stone, J. M., Bhattacharyya, S., Tunstall, N., Brenneisen, R., Holt, D., Wilson, D., Sumich, A., McGuire, P., Murray, R. M., Kapur, S. & Ffytche, D. H. (2011). Disruption of frontal theta coherence by Delta9-tetrahydrocannabinol is associated with positive psychotic symptoms. *Neuropsychopharmacology* **36**, 827-36.
Nelson, M. D., Saykin, A. J., Flashman, L. A. & Riordan, H. J. (1998). Hippocampal volume reduction in schizophrenia as assessed by magnetic resonance imaging: a meta-analytic study. *Archives of General Psychiatry* **55**, 433-40.
Patel, R., Wilson, R., Jackson, R., Ball, M., Shetty, H., Broadbent, M., Stewart, R., McGuire, P. & Bhattacharyya, S. (2016). Association of cannabis use with hospital admission and antipsychotic treatment failure in

first episode psychosis: an observational study. *British Medical Journal Open* **6**, e009888.
Pertwee, R. G. (2008). Ligands that target cannabinoid receptors in the brain: from THC to anandamide and beyond. *Addiction Biology* **13**, 147-59.
Ragland, J. D., Ranganath, C., Harms, M. P., Barch, D. M., Gold, J. M., Layher, E., Lesh, T. A., MacDonald, A. W., 3rd, Niendam, T. A., Phillips, J., Silverstein, S. M., Yonelinas, A. P. & Carter, C. S. (2015). Functional and Neuroanatomic Specificity of Episodic Memory Dysfunction in Schizophrenia: A Functional Magnetic Resonance Imaging Study of the Relational and Item-Specific Encoding Task. *Journal of the American Medical Association Psychiatry* **72**, 909-16.
Ranganathan, M. & D'Souza, D. C. (2006). The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berl)* **188**, 425-44.
Robbe, D., Montgomery, S. M., Thome, A., Rueda-Orozco, P. E., McNaughton, B. L. & Buzsaki, G. (2006). Cannabinoids reveal importance of spike timing coordination in hippocampal function. *Nature Neuroscience* **9**, 1526-33.
Schaefer, J., Giangrande, E., Weinberger, D. R. & Dickinson, D. (2013). The global cognitive impairment in schizophrenia: consistent over decades and around the world. *Schizophrenia Research* **150**, 42-50.
Schoeler, T. & Bhattacharyya, S. (2013). The effect of cannabis use on memory function: an update. *Substance Abuse & Rehabilitation* **4**, 11-27.
Schoeler, T., Kambeitz, J., Behlke, I., Murray, R. & Bhattacharyya, S. (2016a). The effects of cannabis on memory function in users with and without a psychotic disorder: findings from a combined meta-analysis. *Psychological Medicine* **46**, 177-88.
Schoeler, T., Monk, A., Sami, M. B., Klamerus, E., Foglia, E., Brown, R., Camuri, G., Altamura, A. C., Murray, R. & Bhattacharyya, S. (2016b). Continued versus discontinued cannabis use in patients with psychosis: a systematic review and meta-analysis. *The Lancet Psychiatry* **3**, 215-25.
Schoeler, T., Petros, N., Di Forti, M., Klamerus, E., Foglia, E., Ajnakina, O., Gayer-Anderson, C., Colizzi, M., Quattrone, D., Behlke, I., Shetty, S., McGuire, P., David, A. S., Murray, R. & Bhattacharyya, S. (2016c). Effects of continuation, frequency, and type of cannabis use on relapse in the first 2 years after onset of psychosis: an observational study. *The Lancet Psychiatry* **3**, 947-953.
Schoeler, T., Petros, N., Di Forti, M., Pingault, J. B., Klamerus, E., Foglia, E., Small, A., Murray, R. & Bhattacharyya, S. (2016d). Association Between Continued Cannabis Use and Risk of Relapse in First-Episode Psychosis: A Quasi-Experimental Investigation Within an Observational Study. *Journal of the American Medical Association Psychiatry* **73**, 1173-1179.
Shergill, S. S., Brammer, M. J., Williams, S. C., Murray, R. M. & McGuire, P. K. (2000). Mapping auditory hallucinations in schizophrenia using functional magnetic resonance imaging. *Archives of General Psychiatry* **57**, 1033-8.
Simons, J. S. & Spiers, H. J. (2003). Prefrontal and medial temporal lobe interactions in long-term memory. *Nature Review Neuroscience* **4**, 637-48.
Solowij, N., Stephens, R. S., Roffman, R. A., Babor, T., Kadden, R., Miller, M., Christiansen, K., McRee, B., Vendetti, J. & Marijuana Treatment

Project Research, G. (2002). Cognitive functioning of long-term heavy cannabis users seeking treatment. *Journal of the American Medical Association* **287**, 1123-31.
Spielberger, C. D. (1983). *Manual for the state/trait anxiety inventory (form Y) : (self evaluation questionnaire)*. Consulting Psychologists Press: Palo Alto.
Stirling, J., Barkus, E. J., Nabosi, L., Irshad, S., Roemer, G., Schreudergoidheijt, B. & Lewis, S. (2008). Cannabis-induced psychotic-like experiences are predicted by high schizotypy. Confirmation of preliminary results in a large cohort. *Psychopathology* **41**, 371-8.
Tapert, S. F., Schweinsburg, A. D., Drummond, S. P., Paulus, M. P., Brown, S. A., Yang, T. T. & Frank, L. R. (2007). Functional MRI of inhibitory processing in abstinent adolescent marijuana users. *Psychopharmacology (Berl)* **194**, 173-83.
Thirion, B., Pinel, P., Meriaux, S., Roche, A., Dehaene, S. & Poline, J. B. (2007). Analysis of a large fMRI cohort: Statistical and methodological issues for group analyses. *Neuroimage* **35**, 105-20.
Valli, I., Stone, J., Mechelli, A., Bhattacharyya, S., Raffin, M., Allen, P., Fusar-Poli, P., Lythgoe, D., O'Gorman, R., Seal, M. & McGuire, P. (2011). Altered medial temporal activation related to local glutamate levels in subjects with prodromal signs of psychosis. *Biological Psychiatry* **69**, 97-9.
van Winkel, R., Genetic, R. & Outcome of Psychosis, I. (2011). Family-based analysis of genetic variation underlying psychosis-inducing effects of cannabis: sibling analysis and proband follow-up. *Archives of General Psychiatry* **68**, 148-57.
Weinstein, A., Brickner, O., Lerman, H., Greemland, M., Bloch, M., Lester, H., Chisin, R., Mechoulam, R., Bar-Hamburger, R., Freedman, N. & Even-Sapir, E. (2008). Brain imaging study of the acute effects of Delta9-tetrahydrocannabinol (THC) on attention and motor coordination in regular users of marijuana. *Psychopharmacology (Berl)* **196**, 119-31.
Zammit, S., Allebeck, P., Andreasson, S., Lundberg, I. & Lewis, G. (2002). Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. *The British Medical Journal* **325**, 1199-1203.

Figure Legends

Figure 1. Changes in anxiety, intoxication and psychotic symptoms under the THC condition

Line graphs show anxiety (A: state-trait anxiety inventory- state; STAI) and intoxication (B: analogue intoxication scale; AIS) ratings for both the transiently psychotic (TP THC) and non-psychotic (NP THC) group recorded before (0 hour) and one, two and three hours after drug administration. Line graphs show ratings on positive (C), negative (D), general psychopathology (E) subscales and total score (F) of the positive and negative syndrome scale (PANSS for both the transiently psychotic group (TP) and non-psychotic group (NP) following the administration of THC (TP THC and NP THC) and placebo (TP PLB and NP PLB) recorded before (0 hour) and one, two and three hours after drug administration. Error-bars represent standard error of the mean.

Figure 2. Brain activation differences between those sensitive to the psychotomimetic effects of THC (TP PLB) versus those who were not (NP PLB) under placebo during the encoding condition of the verbal learning task. Brain sections on the left column show greater engagement of the left hippocampus (A), left anterior cingulate (B) and right superior temporal gyrus (C) in the transiently psychotic (TP PLB) compared to the non-psychotic (NP PLB) group during encoding under the placebo condition. Bar charts on the right column display the mean brain activation (error bars represent standard error of mean; SEM) values (arbitrary units) from the corresponding brain regions.. All results are significant at $p<0.008$ (cluster *p* values corrected to yield <1 false positive cluster). Left side of the brain is shown on the left side of the brain images.

Scatter-plot (D) displays the non-linear correlation between engagement of the left hippocampus under placebo condition with the increase in severity of psychotic symptoms following administration of THC (spearman's rho = 0.44, p=0.008).

Figure 3. Brain activation differences between those sensitive to the psychotomimetic effects of THC (TP) versus those who were not (NP) under THC relative to placebo treatment during the encoding condition of the verbal learning task.

Brain section (A) displays a cluster in the right middle frontal gyrus (MFG) extending to the precentral gyrus (cross-hair) and a cluster in the left cingulate, where there was greater engagement (shown in bar chart B; mean brain activation and error bars represent standard error of mean, SEM; all values in arbitrary units) under placebo treatment in the TP group relative to the NP group, which was reversed under THC treatment condition. Left side of the brain is shown on the left side of the brain image.

THC-induced change in activation of the right MFG inversely correlated (scatter-plot C) with increase in severity of psychotic symptoms under THC (spearman's rho = -0.6, p=<0.001).

Table 1: Socio-demographic variables

	Transiently psychotic (*n=14*)	**Non-psychotic (*n=22*)**	***p* value**
Mean age, years (S.D.)	25.86 (5.14)	26.05 (5.96)	0.92
Mean NART Score (S.D.)	97.07 (8.32)	98.24 (5.37)	0.65
Mean years in education (S.D.)	16.58 (4.06)	17.41 (4.40)	0.59
	Transiently anxious (*n=18*)	**Non-anxious (*n=18*)**	***p* value**
Mean age, years (S.D.)	26.56 (6.00)	25.39 (5.24)	0.53
Mean NART Score (S.D.)	100 (4.38)	95.61 (7.70)	0.04
Mean years in education (S.D.)	16.94 (3.21)	17.28 (5.07)	0.81

S.D. is standard deviation reported within brackets

Figure 1A_B

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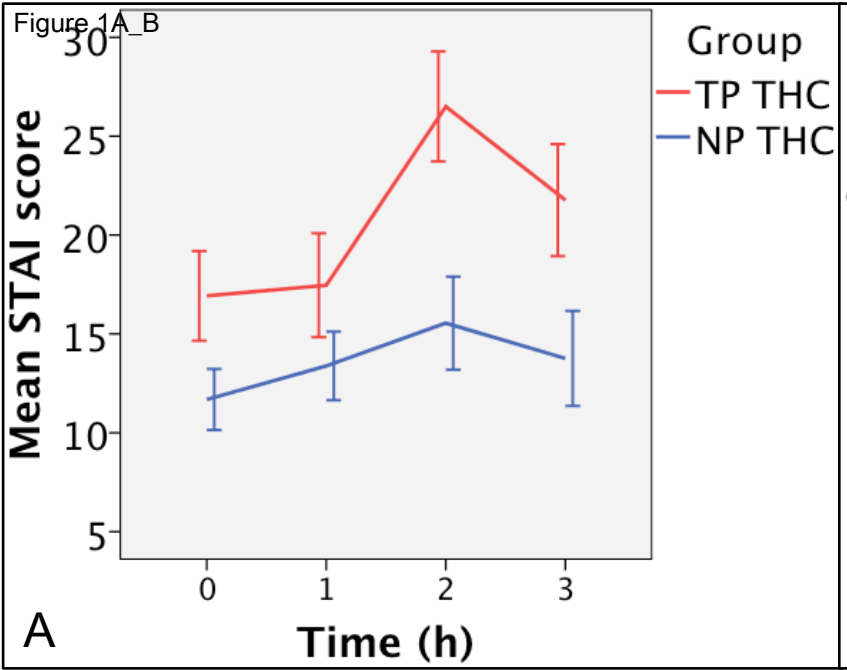

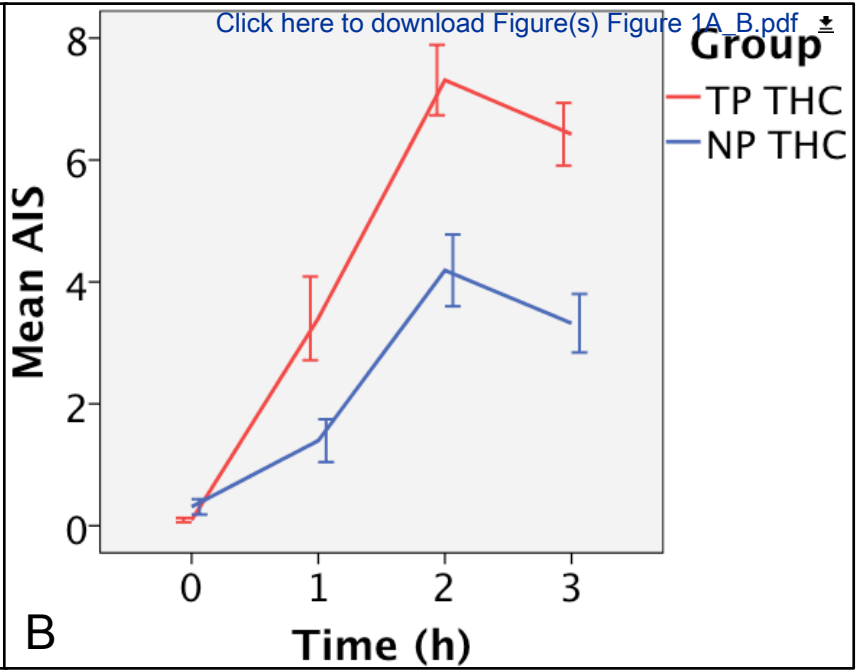

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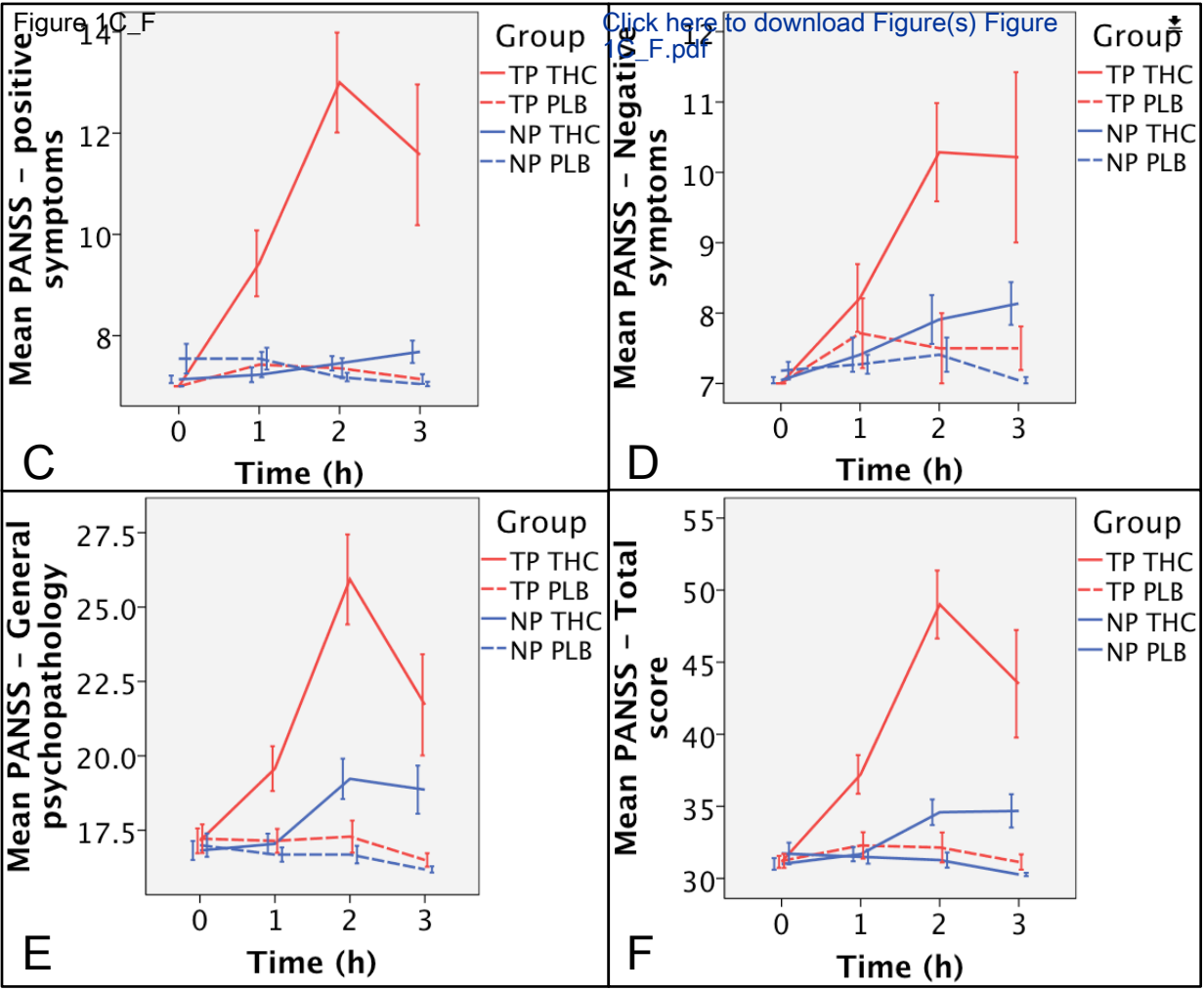

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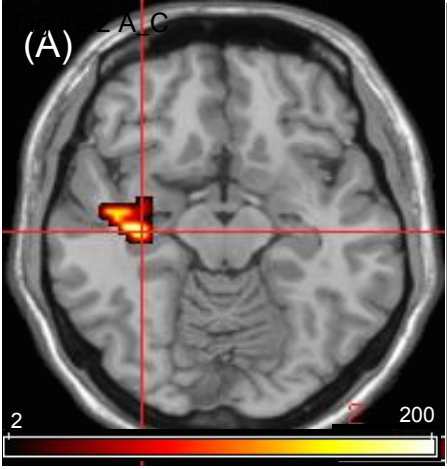

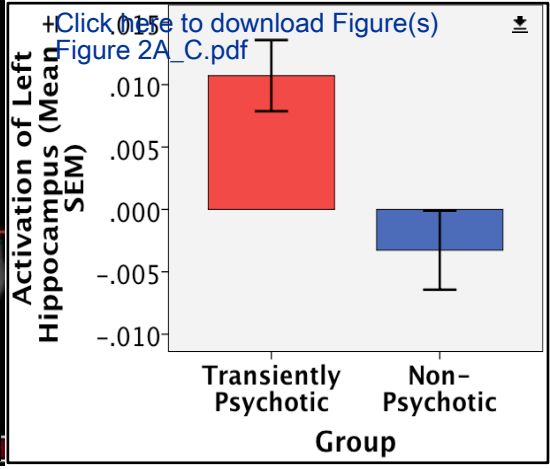

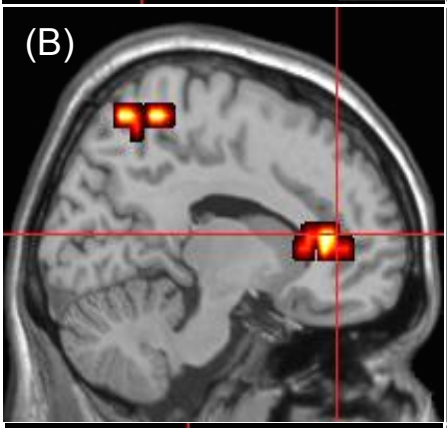

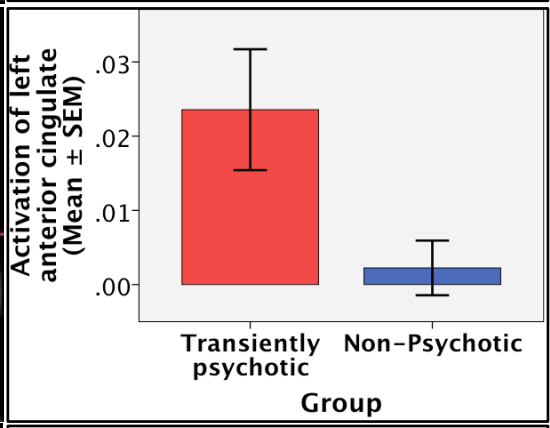

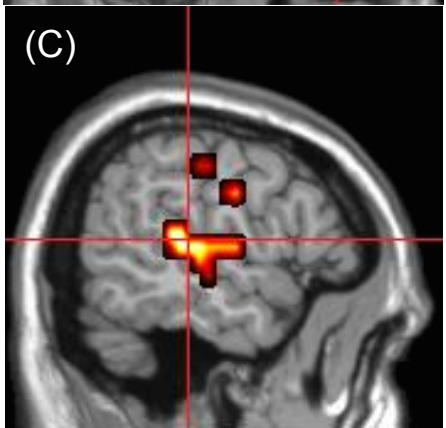

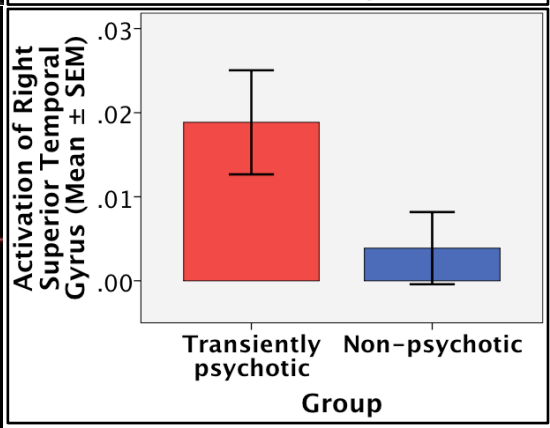

Figure 2 D

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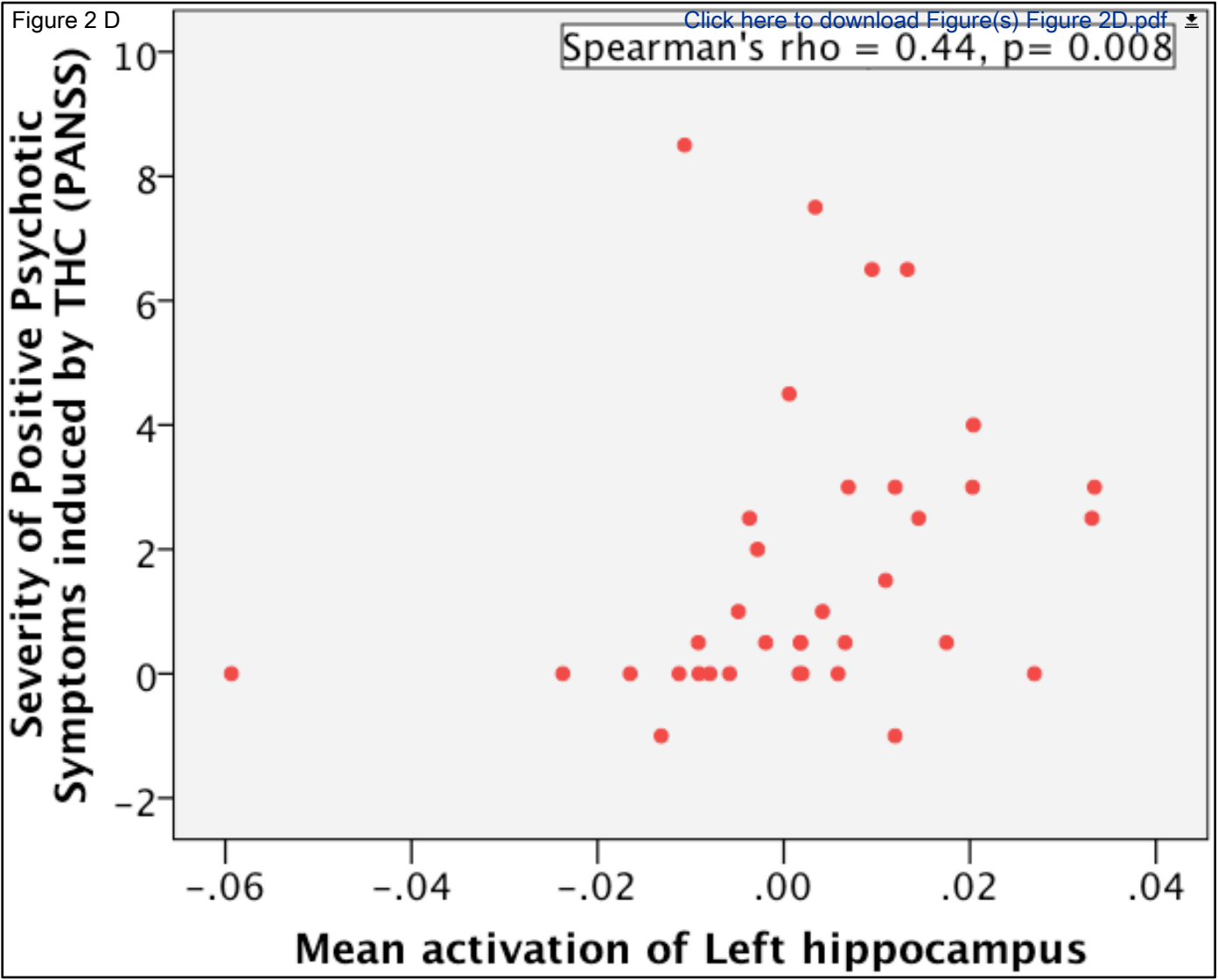

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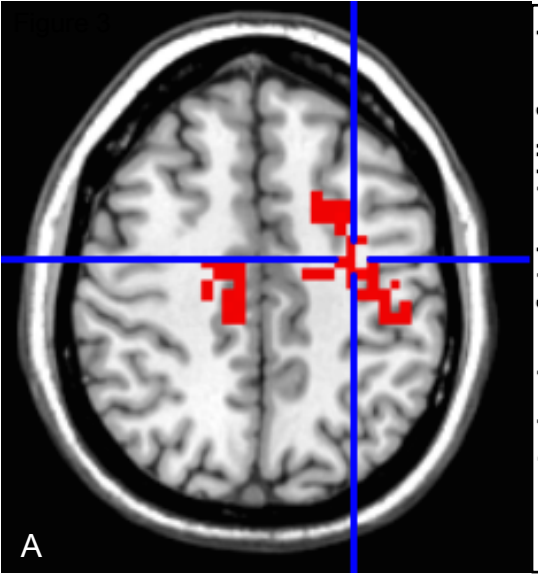

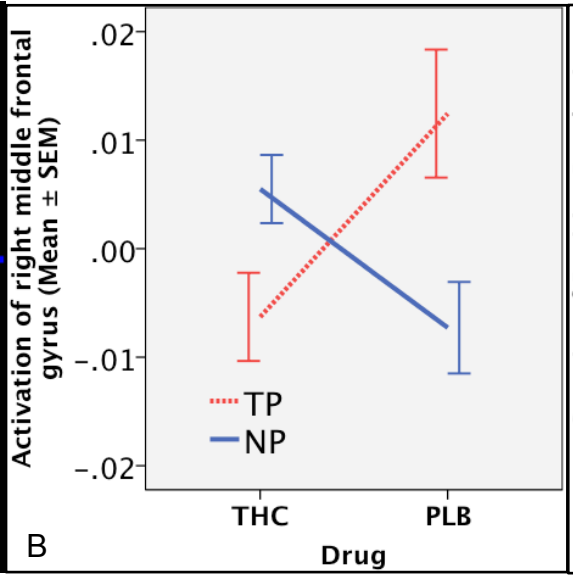

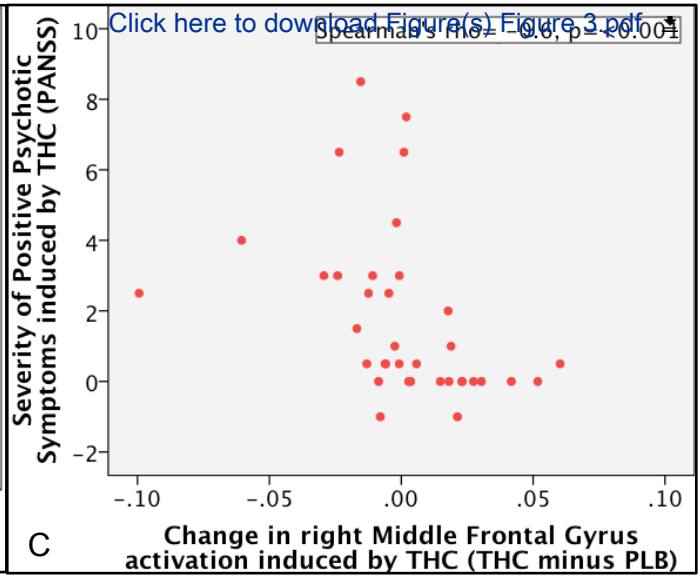

Other Supplementary Material
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Increased hippocampal engagement during learning as a marker of sensitivity to transient psychotomimetic effects of delta-9-THC

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Supplementary Material

Supplementary Methods, Supplementary Tables & Supplementary Figures

Supplementary Methods

The study was conducted in accordance with the declaration of Helsinki and was granted ethical approval by the joint South London and Maudsley and IOP NHS research committee. All participants gave informed written consent to be part of the study.

Participants

Out of a total of 39 healthy volunteers who took part in the study, 2 dropped out after the first session and another could not continue because of adverse drug reactions, resulting in a final sample of 36 healthy volunteers for whom all data was available. All of them were right-handed English-speaking males. None of them had a lifetime personal or family history (in first-degree relatives) of mental illness. History of mental illness was assessed on the basis of a standard psychiatric interview by an experienced psychiatrist. The mean age of this sample was 25.97±5.58 and they had a mean National adult reading test (NART) score of 97.7±6. Use of alcohol, cannabis and other illicit drugs was assessed using the Addiction Severity index (McLellan *et al.*, 1980). All of the subjects had used cannabis more than once but upto 25 times within their lifetime. In addition all of the participants drank less than 21 units/week of alcohol and none used any other illicit drugs on a regular basis (eTable 1). Participants were asked to abstain from all recreational drugs for the duration of the study and one month prior to it.

Experimental design

Each participant attended two sessions that were separated by at least a month interval. At each of these sessions either a single dose of 10mg of THC (approximately 99.6% pure, THC-pharm, Frankfurt, Germany) or a placebo (a gelatine capsule matched in weight and appearance to the THC capsule) was administered to the subjects employing a double-blind design. The order of drug administration was pseudo-randomised to ensure that an equal number of participants received either drug at each session. During each of the sessions participants were required to complete a paired associate verbal learning task while their brain activity (as indexed using blood oxygen level-dependent haemodynamic response; BOLD) was measured using functional Magnetic Resonance Imaging (fMRI).

Prior to these sessions, participants were asked to abstain from smoking for 4 hours, drinking alcohol for 24 hours and taking caffeine for the 12 hours before each session. On the night before the session all subjects were asked to get at least 6 hours sleep. They ate a standardised light breakfast on the morning of the scan after overnight fast. Each participant then passed a negative urine drug screen (using immunometric assay kits) on the morning of each session for opiates, cocaine, amphetamines, benzodiazepines and THC to ensure that no traces of these drugs were in their systems. Each session began (prior to drug administration) with psychopathological ratings and a venous blood sample. This blood sample was taken via the insertion of an indwelling catheter into a subcutaneous vein of the non-dominant forearm of the subject. Psychological ratings and blood samples were subsequently repeated 1, 2, and 3 hours after drug administration. Psychological ratings and blood sampling were carried out at each of the time points outside the MRI scanner.

Blood samples from pilot studies demonstrated that the concentration of THC in blood plateaued at approximately between 1 and 2 hours after ingestion of the drug giving a stable concentration for scans to be conducted. MRI scans were therefore performed starting 1 hour after ingestion of the drug and these lasted no longer than 60 minutes. Participants were asked to complete a verbal paired associate task lasting about 12.5 minutes while they were scanned. This task has previously been useful in fMRI studies investigating the effect of THC on verbal memory (Bhattacharyya *et al.*, 2009).

The Verbal Paired Associate Task

The verbal paired associate learning task (Bhattacharyya *et al.*, 2009) consisted of three different conditions (encoding, recall and baseline) that were presented sequentially and involved visual presentation of stimuli. During the encoding condition participants were presented with two words presented visually. To promote encoding, participants were required to decide whether these words went well together in terms of their meaning. Their answers, either ‘yes’ or ‘no’, were communicated verbally and then noted down by the researcher.

For the recall condition, participants were presented with a single word from one of the pairs that had previously been presented in the encoding condition. The missing word from that pair was replaced with a question mark. Participants were required to recall and articulate the missing word. If the subject could not recall the missing word then they were required to say ‘pass’.

During the baseline condition, participants were presented with pairs of words printed with identical or different fonts. These words were different to those that had been presented during the encoding and recall conditions and these word pairs were not repeated across baseline blocks. This ensured that learning was kept to a minimum to allow the effect of the encoding and recall conditions on neural activity to be identified through comparison of the baseline fMRI data with that of the encoding and recall conditions.

The stimuli for all of the conditions were presented in 40-second blocks. Each of the blocks consisted of 8 word pairs and the three conditions were presented sequentially in the same order (encoding then recall followed by the baseline condition). The appearance of the different word pairs in each block was randomised. Preceding each block, participants were reminded of the task for that block by a visual prompt. For the encoding condition this was 'Do these words go well together?', for the recall condition 'Which word was associated with this?' and for the baseline condition 'Are these fonts the same?'. Participants practiced the task beforehand using a set of word pairs that were different to those that were then presented during the actual fMRI task. Recall scores were recorded as a measure of task performance.

All words used in this task were drawn from the Medical Research Council Psycholinguistic Database (Coltheart, 1981). These are words that have been matched for frequency of use, number of letters, familiarity and comprehension (Kučera and Francis, 1967).

Whilst 4 blocks were presented, only the results from the first 3 blocks are reported here. This is because an analysis of task performance revealed that the participants stopped learning after the 3 blocks and almost all scored maximally on the recall task.

Behavioural data acquisition.

An experienced clinical researcher determined the psychotomimetic effects of THC using the Positive and Negative Symptom Scale (PANSS) (Kay *et al.*, 1987). Whilst PANSS is usually employed in clinical trials of schizophrenia to rate positive and negative symptoms of psychosis as well as other symptoms that are commonly present in those with psychosis (general psychopathology), it has also been used in a number of other studies investigating the transient psychotomimetic effects of THC

(Atakan *et al.*, 2013, Bhattacharyya *et al.*, 2012a, Bhattacharyya *et al.*, 2012b, Bhattacharyya *et al.*, 2009, D'Souza *et al.*, 2005, D'Souza *et al.*, 2004)

In addition to psychotic symptoms, participants' state of anxiety and intoxication were assessed using the State-Trait Anxiety Inventory (STAI) (Spielberger, 1983)and the Analogue Intoxication scale (AIS) (Mathew *et al.*, 1992) respectively. All psychological ratings including PANSS were carried out while participants were outside the scanner.

Classification of participants on the basis of sensitivity to THC

For the purpose of this investigation, we established *a priori* criteria to define transient psychosis induced by THC, which were used to classify the participants into those who experienced transient psychotomimetic effects (TP) and those who did not (NP). Classification was carried out following completion of all data acquisition. Participants were identified as having experienced transient psychotic symptoms and allocated to the TP group if they scored 3 or more on any of the PANSS positive subscale items that measured psychotic symptoms (Delusions, Hallucinations, Suspiciousness/ Persecution) during any of the time-points when ratings were obtained following THC administration (Atakan *et al.*, 2013). Each item of PANSS (Kay *et al.*, 1987) is scored on a 7-point Likert scale, with a score of 1 denoting that the item being measured is "absent", a score of 2 denoting that it is "minimal" (indicating "questionable or subtle or suspect pathology" and a score of 3 denoting "mild" (indicating "a symptom whose presence is clearly established but not pronounced"). A score of 3 was used as the cut-off as this is the threshold used in the clinical setting to indicate clear, unambiguous presence of a psychotic symptom (Kay *et al.*, 1987). While PANSS does not describe the score of '3' as a threshold, in practice the score of '3' becomes a threshold for denoting presence of a symptom, as a score of '2' indicates "Questionable pathology; may be at the upper extreme of normal limits". This is also the scoring threshold used in the clinical setting, especially in the context of clinical trials of antipsychotic medications to indicate clear, unambiguous presence of these symptoms. For the item 'Delusions', a score of 3 on PANSS refers to "Mild- Presence of one or two delusions that are vague, uncrystallized, and not tenaciously held. Delusions do not interfere with thinking, social relations, or behavior." For "Hallucinatory Behaviour", a score of 3 denotes "Mild- One or two clearly formed but infrequent hallucinations, or else a number of vague abnormal perceptions which do not result in distortions of thinking or behaviour.", while for "Suspiciousness/

Persecution", a score of 3 indicates "Mild - Presents a guarded or even openly distrustful attitude, but thoughts, interactions and behaviour are minimally affected." Higher scores on each of these items indicate greater severity, while a score of 2 indicates "Questionable pathology; may be at the upper extreme of normal limits". Psychotic symptoms scored in these participants were otherwise comparable to that observed in a clinical situation except that they were transient in nature, a characteristic that is typical of psychotic symptoms observed under the experimental THC challenge condition. In order to classify participants between those who experienced transient anxiety (TA) and those who did not (NA) under the influence of THC, we used the change in their STAI score over time in response to THC administration. Using the Reliable Change Index (Jacobson and Truax, 1991), we estimated that a 4-point change in STAI score (by deducting baseline STAI score from the peak STAI score following THC) would reliably differentiate those who experienced anxiety from those who did not experience anxiety following THC administration. Therefore any participant, who had over a 4-point change in their STAI, when baseline STAI score was deducted from their peak post-THC STAI score, was allocated to the TA group, while participants who had less than a 4-point change in their score were in the NA group.

Image Acquisition

Functional MRI images were acquired at the Maudsley hospital using a 1.5 Tesla GE Signa system (GE Medical Systems, Milwaukee, WI, USA). During the verbal learning task a gradient-echo sequence was used to acquire one hundred and forty-eight T2* weighted images at 16 near-axial planes (7 mm thick, inter-slice gap 0.7 mm) parallel to the inter-commissural (AC-PC) plane with a repetition time (TR) of 5000msec (image volume acquisition over 1500msec and period between clustered acquisition of image volumes 3500 msec), TE of 40 msec and flip angle of 70˚ (FOV 24 x 24 cm and matrix 64^2). The inter-stimulus interval was 5000msec. The first 4 (dummy) volumes were discarded to allow for T1 equilibration effects. During the first 1500msec of the TR images were acquired, for the remaining 3500msec the scanner was silent. Each of the visual stimuli that were presented to the subject during the verbal learning task were shown to the subject at the beginning of each silent period. This allowed for each trial to be performed and verbal response to be recorded without the interference of scanner noise. We employed this strategy of using an acquisition sequence in which image

acquisition was compressed into the initial part of the each TR, thereby creating a 'silent' period when images were not being acquired and the scanner did not produce acoustic noise(Amaro *et al.*, 2002), in order to minimise the potential effect of articulation of verbal responses during the task on brain activation. As verbal responses during the task were restricted to these 'silent' periods, any head movement associated with articulation occurred outside the time when the images were being acquired, reducing the likelihood of motion-correlated artifacts(Bullmore *et al.*, 1999a). Furthermore, in the absence of acoustic scanner noise, participants did not need to shout their responses. An inversion recovery EPI dataset (TR 3000msec, TE 40 msec, flip angle 90˚, near-axial slices, 3mm thick, inter-slice gap 0.3mm, in-plane resolution 1.5mm) was acquired to facilitate anatomic localization of the functional data.

Statistical analysis

Image analysis

XBAM_v4.1 (http://www.brainmap.co.uk), a non-parametric data analysis software package developed at the Institute of Psychiatry, Psychology & Neuroscience (King's College London) was used for analysing fMRI data. The non-parametric approach minimises assumptions about the distribution of the data. This is important in the analysis of fMRI data because the distribution of data may not necessarily follow a normal Gaussian distribution (Brammer *et al.*, 1997)(Thirion *et al.*, 2007). By using medians rather than averages as a test statistic, XBAM is less sensitive to the effects of outlier values misrepresenting the distribution of the data (Hayasaka and Nichols, 2003). The test statistic is computed in this method by standardizing for individual differences in residual noise before embarking on a second- level, multi-subject testing, using robust permutation-based methods, employing a mixed-effects approach.

Images were first realigned to correct for head motion (Bullmore *et al.*, 1999a). This involved the computation of a 3D volume consisting of the average intensity at each voxel over the whole experiment, which was used as a template. The 3D image volume at each time-point was then realigned to this template by computing the combination of rotations (around the x, y and z axes) and translations (in x, y and z) that maximised the correlation between the image intensities of the volume in question

and the template 3D volume. The data were then smoothed by the application of a 7.2mm full-width-at-half-maximum Gaussian filter to average the relative intensities of neighbouring voxels. Activation maps were created for each individual by modelling the BOLD signal using 2 gamma-variate functions, peaking at 4 and 8 seconds to allow for variability in haemodynamic delay. Then, using the constrained BOLD effects model, a best fit between the weighted sum of these convolutions and the change over time at each voxel was computed (Friman *et al.*, 2003). This reduces the possibility of the model-fitting procedure giving rise to mathematically plausible, but physiologically implausible results. Following the least squares fitting of this model to the data, the sum of squares (SSQ) ratio (ratio of the SSQ of deviations from the mean image intensity due to the model component over the whole time series to the SSQ of deviations due to the residuals) was estimated for each voxel and this was followed by permutation testing to determine which voxels were significantly activated (Bullmore *et al.*, 2001). This addresses the problem associated with the use of the F statistic that the residual degrees of freedom are often unknown in fMRI time series due to the presence of coloured noise in the signal. Data were permuted by the wavelet-based method described and characterized previously (Bullmore *et al.*, 2001), which permits data driven calculation of the null distribution of SSQ under the assumption of no experimentally-determined response. This distribution can then be used to threshold the activation maps at any desired type 1 error rate. Activated voxels were then grouped into clusters using a method described before (Bullmore *et al.*, 1999b), which has been shown to give excellent cluster-wise type 1 error control. SSQ ratio maps for each individual were transformed into standard stereotactic space (Talairach and Tournoux, 1988) using a two-stage warping procedure (Brammer *et al.*, 1997) for the purpose of localization of activations. As a first step, an average image intensity map for each individual over the course of the experiment was computed. We then computed the transformations required to map this image to the structural scan for each individual and then from 'structural space' to the Talairach template by maximizing the correlation between the images at each stage. The SSQ ratio and BOLD effect size maps were then transformed into Talairach space using these transformations. Group activation maps were computed for each group (TP vs NP or TA vs NA) in each drug condition by determining the median SSQ ratio at each voxel (over all individuals) in the observed and permuted data maps. Medians were used to minimize outlier effects. The distribution of median SSQ ratios over all intracerebral voxels from the permuted data was then used to derive the null distribution of SSQ ratios. This allows group activation maps to be thresholded at the desired voxel or

cluster-level type 1 error rate. This gave group activation maps for each condition that could be compared against each other using non-parametric repeated-measure analysis of variance (ANOVA) (Brammer *et al.*, 1997). The voxel-wise statistical threshold was set at p=0.05 and the cluster-wise thresholds were adjusted to ensure that the number of false positive clusters per brain would be <1 (regions that survive this critical statistical threshold and the corresponding p values are reported). This excluded any areas of activation, which did not meet this threshold of significance. By conducting analyses at a cluster-level, data from more than one voxel is integrated into the test statistic giving greater sensitivity and it also allows for a reduction in the search volume or overall number of required tests for whole brain analysis. In comparison to analysis at the voxel level, cluster level analyses thereby helps mitigate the multiple comparisons problem.

For each drug condition, we contrasted each of the active (encoding or recall) conditions of the verbal memory task against the baseline (fonts) condition at the individual subject level to generate contrast of interest map ('encoding minus baseline' and for 'recall minus baseline' conditions) for each subject, which were used for subsequent group-level analyses. As the baseline condition of the task was designed to keep learning to a minimum this analysis was used to exclude those areas that were involved in the completion of a task involving verbal stimuli but that were not crucial to learning and memory.

Analysis of variance compared the TP group and NP during the placebo condition in order to assess differences in functional activation during the contrast of interest (for 'encoding minus baseline' and for 'recall minus baseline' conditions) in the absence of THC. Henceforth, for the purposes of simplicity, 'encoding minus baseline' and 'recall minus baseline' contrasts will be referred to as 'encoding' and 'recall' respectively, unless otherwise specified. To test the robustness of these group differences in activation and whether they were driven by outliers, we carried out a 'leave one subject out' (LOSO) analysis, which involved repeating the ANOVA with a different subject from the TP group being left out on each repeat. A total of 14 repeat ANOVAs were carried out, once with each of the 14 TP subjects being left out. We then examined whether these neural activation differences during the encoding and recall conditions were specific to the sub-groups classified according to sensitivity to the psychotomimetic effects of THC (TP vs NP) or were similar to that between subgroups classified based on sensitivity to anxiogenic effects of THC (TA vs NA). One-way analysis of variance compared

task-related neural activation differences (during encoding and recall conditions) between the TA and NA groups under the placebo condition to examine whether similar group differences exist between TA and NA groups as between TP and NP groups. Further, comparisons (using 2-way ANOVA) were then made between the drug given, TP and NP groups and the interaction of effects between them. The statistical values of the brain regions (clusters) differentially activated in these analyses, which were the mean of the SSQ ratio values of all the voxels in the respective clusters, were extracted to an IBM SPSS version 22 (IBM Corp. Released 2013; IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.), where they could be plotted into graphs accompanying the brain activation maps. They were also used to identify correlation with behavioural data. A similar approach was employed to compare TA and NA groups.

Social demographic and behavioural analysis

Behavioural data was recorded and analysed using SPSS version 22 (see above). Comparisons between the socio-demographic characteristics of the two groups (such as age, NART score and number of years in education) and task performance (recall score) were carried out using two-sample *t*-tests. Differences in symptomatic data between the groups at 2 hours after THC and placebo administration did not fit normal distribution. Mann-whitney U tests were therefore used to assess differences in the means. We used the 2-hour time point because this is around the time that THC level peaked in peripheral blood and because scans were acquired closer to this time point (between 1-2hrs).

REFERENCES:

Amaro, E., Jr., Williams, S. C., Shergill, S. S., Fu, C. H., MacSweeney, M., Picchioni, M. M., Brammer, M. J. & McGuire, P. K. (2002). Acoustic noise and functional magnetic resonance imaging: current strategies and future prospects. *Journal of Magnetic Resonance Imaging* **16**, 497-510.

Atakan, Z., Bhattacharyya, S., Allen, P., Martin-Santos, R., Crippa, J. A., Borgwardt, S. J., Fusar-Poli, P., Seal, M., Sallis, H., Stahl, D., Zuardi, A. W., Rubia, K. & McGuire, P. (2013). Cannabis affects people differently: inter-subject variation in the psychotogenic effects of Delta9-tetrahydrocannabinol: a functional magnetic resonance imaging study with healthy volunteers. *Psychological Medicine* **43**, 1255-67.

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J. A., Kambeitz, J., Prata, D., Williams, S., Brammer, M., Collier, D. A. & McGuire, P. K. (2012a). Preliminary report of biological basis of sensitivity to the effects of cannabis on psychosis: AKT1 and DAT1 genotype modulates the effects of delta-9-tetrahydrocannabinol on midbrain and striatal function. *Molecular Psychiatry* **17**, 1152-5.

Bhattacharyya, S., Crippa, J. A., Allen, P., Martin-Santos, R., Borgwardt, S., Fusar-Poli, P., Rubia, K., Kambeitz, J., O'Carroll, C., Seal, M. L., Giampietro, V., Brammer, M., Zuardi, A. W., Atakan, Z. & McGuire, P. K. (2012b). Induction of psychosis by Delta9-tetrahydrocannabinol reflects modulation of prefrontal and striatal function during attentional salience processing. *Archives of General Psychiatry* **69**, 27-36.

Bhattacharyya, S., Fusar-Poli, P., Borgwardt, S., Martin-Santos, R., Nosarti, C., O'Carroll, C., Allen, P., Seal, M. L., Fletcher, P. C., Crippa, J. A., Giampietro, V., Mechelli, A., Atakan, Z. & McGuire, P. (2009). Modulation of mediotemporal and ventrostriatal function in humans by Delta9-tetrahydrocannabinol: a neural basis for the effects of Cannabis sativa on learning and psychosis. *Archives of General Psychiatry* **66**, 442-51.

Brammer, M. J., Bullmore, E. T., Simmons, A., Williams, S. C., Grasby, P. M., Howard, R. J., Woodruff, P. W. & Rabe-Hesketh, S. (1997). Generic brain activation mapping in functional magnetic resonance imaging: a nonparametric approach. *Magnetic Resonance Imaging* **15**, 763-70.

Bullmore, E., Long, C., Suckling, J., Fadili, J., Calvert, G., Zelaya, F., Carpenter, T. A. & Brammer, M. (2001). Colored noise and computational inference in neurophysiological (fMRI) time series analysis: resampling methods in time and wavelet domains. *Human Brain Mapping* **12**, 61-78.

Bullmore, E. T., Brammer, M. J., Rabe-Hesketh, S., Curtis, V. A., Morris, R. G., Williams, S. C., Sharma, T. & McGuire, P. K. (1999a). Methods for diagnosis and treatment of stimulus-correlated motion in generic brain activation studies using fMRI. *Human Brain Mapping* **7**, 38-48.

Bullmore, E. T., Suckling, J., Overmeyer, S., Rabe-Hesketh, S., Taylor, E. & Brammer, M. J. (1999b). Global, voxel, and cluster tests, by theory and permutation, for a difference between two groups of structural MR images of the brain. *IEEE Transactions in Medical Imaging* **18**, 32-42.

Coltheart, M. (1981). The MRC Psycholinguistic Database. *Quarterly Journal of Experimental Psychology* **33A**, 497-505.
D'Souza, D. C., Abi-Saab, W. M., Madonick, S., Forselius-Bielen, K., Doersch, A., Braley, G., Gueorguieva, R., Cooper, T. B. & Krystal, J. H. (2005). Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biological Psychiatry* **57**, 594-608.
D'Souza, D. C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y. T., Braley, G., Gueorguieva, R. & Krystal, J. H. (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology* **29**, 1558-72.
Friman, O., Borga, M., Lundberg, P. & Knutsson, H. (2003). Adaptive analysis of fMRI data. *Neuroimage* **19**, 837-45.
Hayasaka, S. & Nichols, T. E. (2003). Validating cluster size inference: random field and permutation methods. *Neuroimage* **20**, 2343-56.
Jacobson, N. S. & Truax, P. (1991). Clinical significance: a statistical approach to defining meaningful change in psychotherapy research. *Journal of Consulting & Clinical Psychology* **59**, 12-9.
Kay, S. R., Fiszbein, A. & Opler, L. A. (1987). The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin* **13**, 261-76.
Kučera, H. & Francis, W. N. (1967). *Computational analysis of present-day American English*. Dartmouth Publishing Group.
Mathew, R. J., Wilson, W. H., Humphreys, D. F., Lowe, J. V. & Wiethe, K. E. (1992). Regional cerebral blood flow after marijuana smoking. *Journal of Cerebral Blood Flow & Metabolism* **12**, 750-8.
McLellan, A. T., Luborsky, L., Woody, G. E. & O'Brien, C. P. (1980). An improved diagnostic evaluation instrument for substance abuse patients. The Addiction Severity Index. *Journal of Nervous & Mental Disease* **168**, 26-33.
Spielberger, C. D. (1983). *Manual for the state/trait anxiety inventory (form Y) : (self evaluation questionnaire)*. Consulting Psychologists Press: Palo Alto.
Talairach, J. & Tournoux, P. (1988). *[Co-planar Stereotaxic Atlas of the Human Brain.]*. Thieme Medical New York.
Thirion, B., Pinel, P., Meriaux, S., Roche, A., Dehaene, S. & Poline, J. B. (2007). Analysis of a large fMRI cohort: Statistical and methodological issues for group analyses. *Neuroimage* **35**, 105-20.

Supplementary Tables:

Supplementary Table 1A: Previous exposure to psychoactive substances in study participants

Lifetime Illicit drug use	
Cannabis	<5 times: 12 subjects; 5-25 times: 24 subjects
Amphetamines	5 subjects*/¥ (4 subjects had experimented a few times and 1 had used small quantities from time to time)
LSD/ Psilocybin	10 subjects ‡ (all had experimented a few times)
Cocaine	3 subjects (all had experimented a few times)
Opiate	2 subjects (both had experimented a few times)
MDMA	11 subjects**/***; (all of them had experimented a few times)
Other psychoactive substances (current use)	
Nicotine	9 subjects; Mean number of cigarettes smoked/ day- 1.19 (SD-3.18) (range 0-15/ day); 2 subjects smoked >10 cigarettes/day lifetime; only 1 subject smoked at that level at the time of the study.
Caffeine	33 subjects; Mean number of cups of coffee, tea or caffeinated drinks/ day- 2.42 (SD- 1.86) (range 0-11)

* 1 subject had experimented with both amphetamines and LSD/Psilocybin
¥ 1 subject had used amphetamines once a day for 4 weeks about 4 years before study
‡ 1 subject had experimented with both opiates & Psilocybin
** 1 subject had experimented with both LSD/ Psilocybin and MDMA
*** 1 subject had experimented with both MDMA and opiates.

Supplementary Table 1B: Previous exposure to psychoactive substances in the two subgroups (Transiently psychotic: TP; Non-psychotic: NP) of study participants

Lifetime Alcohol and Illicit drug use				
Psychoactive substance	**Pattern of use (n)**	**TP**	**NP**	***p* value**
Alcohol	Drinks only at weekends (moderate amounts)	8	8	p>0.1
	Drinks everyday in moderate amounts	2	5	
	Drinks occasionally	3	8	
	Drinks everyday moderately, some days is drunk	1	1	
Amphetamines	No use	10	21	p>0.1
	Experimental use	3	1	
	Occasional use (small quantities from time to time)	1	0	
LSD/ Psilocybin	No use	11	15	p>0.1
	Experimental use	3	7	
	Occasional use (small quantities from time to time)	0	0	
Cocaine	No use	13	20	p>0.1
	Experimental use	1	2	
	Occasional use (small quantities from time to time)	0	0	
Opiate	No use	13	21	p>0.1
	Experimental use	1	1	
	Occasional use (small quantities from time to time)	0	0	
MDMA	No use	10	15	p>0.1
	Experimental use	4	7	
	Occasional use (small quantities from time to time)	0	0	
Other psychoactive substances (current use)				
Nicotine (number of cigarettes/ day) (Mean±SD)		1.36 (3.97)	1.09 (2.66)	0.81
Cannabis (number of times lifetime) (Mean±SD)		12.28 (7.67)	14.16 (7.02)	0.47
Caffeine (number of cups of coffee) (Mean±SD)		2.45 (2.18)	2.40 (1.68)	0.95

Supplementary Table 2. Symptomatic and task performance data.
Table showing the mean and standard deviation (SD) values for the positive and negative symptom subscale (PANSS) total score, each of the PANSS subscales, State-trait anxiety inventory- state subscale (STAI), Analogue intoxication scale (AIS) and recall scores. Data shown is for 2 hours after ∆9-tetrahydrocannabinol (THC) and placebo (PLB) administration.

	Transiently psychotic Mean (SD)	**Non-psychotic Mean (SD)**	***p* value**	**Transiently anxious Mean (SD)**	**Non-anxious Mean (SD)**	***p* value**
PANSS positive symptoms						
2 hrs after PLB treatment	7.36 (0.75)	7.55 (1.01)	0.77	7.22 (0.54)	7.28 (0.57)	0.79
2 hrs after THC treatment	13.00 (3.70)	7.23 (0.69)	<0.001	10.28 (4.04)	8.94 (3.03)	0.18
PANSS negative symptoms						
2 hrs after PLB treatment	7.50 (1.87)	7.41 (1.14)	0.64	7.50 (1.24)	7.39 (1.65)	0.44
2 hrs after THC treatment	10.29 (2.61)	7.91 (1.63)	<0.001	9.56 (2.17)	8.11 (2.34)	0.037
PANSS general psychopathology score						
2 hrs after PLB treatment	17.29 (2.01)	16.68 (1.39)	0.911	17.00 (1.53)	16.83 (1.82)	0.50
2 hrs after THC treatment	25.93 (5.66)	19.23 (3.18)	<0.001	23.22 (5.81)	20.44 (4.65)	0.097
PANSS total score						
2 hrs after PLB treatment	32.14 (3.90)	31.27 (2.47)	0.42	31.72 (2.82)	31.50 (3.4)	0.52
2 hrs after THC treatment	49.00 (8.84)	34.59 (4.14)	<0.001	43.06 (9.84)	37.33 (8.45)	0.05
STAI state score						
2 hrs after PLB treatment	15.71 (8.94)	10.18 (7.67)	0.83	10.33 (6.87)	14.33 (9.66)	0.22
2 hrs after THC treatment	26.79 (11.99)	15.55 (11.03)	0.008	24.72 (11.81)	15.11 (11.63)	0.016
AIS score						
2 hrs after PLB treatment	2.08 (2.69)	1.27 (1.55)	0.40	2.03 (2.54)	1.27 (1.34)	0.88
2 hrs after THC treatment	7.44 (2.08)	4.24 (2.82)	0.001	7.03 (1.80)	3.81 (2.97)	<0.001
Recall score						
Between 1-2 hrs after PLB treatment	29.28 (4.82)	30.68 (1.70)	0.22	30.66 (2.08)	29.61 (4.17)	0.34
Between 1-2 hrs after THC treatment	30.00 (3.50)	30.18 (2.51)	0.85	30.22 (2.66)	30.00 (3.18)	0.22

Supplementary Table 3A. Brain regions engaged by the encoding condition of the verbal learning task independent of drug condition.
Regions survive critical threshold of <1 false positive cluster

	Talairach Coordinates			Cluster size	
Area	**x**	**y**	**z**	**No. of voxels**	***p* value**
Superior frontal gyrus	0	4	48	58	0.0035
Precentral gyrus	51	-7	37	196	0.0035
	54	-4	15	267	0.0035
	-47	-15	42	181	0.001
	-54	-7	9	6	0.001
Postcentral gyrus	-47	-11	15	46	0.001
	-43	-19	37	37	0.001
Inferior frontal gyrus	-29	30	-7	21	0.001
	22	30	9	126	0.0035
Insula	-36	15	4	128	0.001
	36	19	-2	9	0.0035
Claustrum	29	19	4	59	0.0035
Anterior cingulate/ medial prefrontal cortex	-4	7	42	100	0.001
Hippocampus	-29	-41	4	16	0.001
	-25	-15	-13		0.001
	36	-33	4	71	0.0035
Parahippocampal gyrus / Amygdala	25	-7	-13	15	0.0035
Parahippocampal gyrus	-25	-15	-7	11	0.001
Superior temporal gyrus	43	-26	-2	54	0.0035
	43	-26	-7	51	0.0035
	58	-30	9	4	0.0035
Fusiform gyrus	22	-81	-13	23	0.0035
	-25	-44	15	57	0.001
	-18	-85	-13		0.001
Lingual gyrus	-11	-78	4	26	0.001
	18	-81	4	84	0.0035
Middle Occipital gyrus	25	-85	-7	74	0.0035
Posterior cingulate	-29	-41	9	12	0.001
	18	-67	9	4	0.0035
Cuneus	-4	-78	15	12	0.001
	11	-78	15	30	0.0035
Striatum	-18	-7	-2	24	0.001
	-22	-37	20	7	0.001
Cerebellum	-36	-67	-18	37	0.001
	36	-67	-24	22	0.0035
	25	-81	-18		0.0035

Supplementary Table 3B. Brain regions engaged by the recall condition of the verbal learning task independent of the drug condition.

Regions survive critical threshold of <1 false positive cluster

	Talairach Coordinates			Cluster size	
Area	**x**	**y**	**z**	**No. of voxels**	***p* value**
Precentral gyrus	-51	-7	9	280	0.001
	-40	-19	37		0.001
	-43	-15	48		0.001
	47	-11	31	137	0.003
	58	0	15		0.003
	51	-7	37		0.003
Postcentral gyrus	-43	-19	42	109	0.001
Inferior frontal gyrus	-43	15	-13	139	0.001
Insula	43	-22	-2	44	0.003
	-29	26	4	89	0.001
Superior Temporal Gyrus	54	-30	4	127	0.003
	36	-33	15		0.003
	-54	-4	4	62	0.001
Middle Temporal Gyrus	-54	-44	9	42	0.001
Parahippocampal gyrus-Hippocampus	-22	-19	-13	10	0.008
Parahippocampal gyrus- Amygdala	-25	0	-13		0.008
Fusiform gyrus	22	-81	-13	8	0.003
Lingual gyrus	-11	-81	-7	95	0.008
	11	-74	-7	13	0.003
Middle Occipital gyrus	29	-81	-7	6	0.003
Precuneus	-25	-63	48	131	0.008
Cuneus	-14	-74	15	55	0.008
	4	-74	20	13	0.003
Thalamus	-11	-19	15	87	0.008
Caudate	18	-11	20	61	0.003
	18	-11	26		0.003
Midbrain- substantia nigra	-14	-19	-2	11	0.008
Cerebellum	4	-67	-24	50	0.008
	4	-63	-13		0.008
	-4	-63	-18	48	0.008
	-32	-63	-13		0.008

Supplementary Table 4. Brain regions differentially engaged in those sensitive to psychotomimetic effects of THC (TP) versus those who were not (NP) during the encoding and recall conditions of the verbal learning task under placebo and under THC.

Unless otherwise stated, regions survive critical threshold of <1 false positive cluster. [a] These clusters did not survive the threshold to yield <1 false positive cluster.

	Talairach Coordinates			Cluster size	
Area	**x**	**y**	**z**	**No. of voxels**	***p* value**
Group effect on task under placebo condition					
Encoding condition					
(TP>NP)					
L. Hippocampus	-29	-11	-13	59	0.001
R. Superior Temporal Gyrus	58	-26	4	43	0.004
L. Anterior cingulate	-11	41	-2	33	0.006
R. Precentral gyrus	36	-15	31	39	0.004
L. Paracentral lobule	-18	-41	48	58	0.002
(NP>TP)					
R. Cerebellum, posterior lobe	14	-59	-13	50	0.004
L. Cerebellum, posterior lobe	-33	-67	-13	121	0.0002
Recall condition					
(TP>NP)					
R. Cerebellum, anterior lobe	7	-52	-24	32	0.01
R. Middle Temporal gyrus	47	-15	-13	43	0.002
L. Medial Frontal gyrus,	0	48	31	33	0.01
(NP>TP)					
R. Cerebellum, posterior lobe	33	-74	-18	67	<0.001
R. Precuneus	25	-56	31	75	<0.001
R. Precentral gyrus	51	0	26	24	0.005
L. Precentral gyrus	-33	11	31	34	0.006
L. Inferior Parietal lobule	-29	-41	26	46	0.006
R. Cingulate gyrus	14	-37	20	69	0.003
Group (TP vsNP) X drug Interaction (THC vs Placebo)					
Encoding condition					
L. cerebellum, anterior lobe	-18	-59	-24	118	0.003
R. Middle frontal gyrus	32	-4	42	67	0.001
R. Precentral gyrus	32	-7	37	33	0.001
L. Cingulate gyrus	-4	-15	37	106	0.001
Recall condition [a]					
R. Cerebellum, posterior lobe	4	-44	-40	57	<0.05
R. Superior temporal gyrus	51	0	4	26	<0.05
L. Insula	-29	22	4	26	<0.05
R. Lingual gyrus	7	-70	4	32	<0.05
L. Superior Frontal gyrus	-25	48	20	31	<0.05
R. Precentral gyrus	51	-15	31	33	<0.05
L. Inferior Parietal lobule	-43	-52	37	11	<0.05
R. Insula	32	-41	26	39	<0.05
L. Precentral gyrus	-43	-4	42	11	<0.05
L. cingulate gyrus	7	4	31	49	<0.05
L. Precuneus	-7	-70	48	20	<0.05

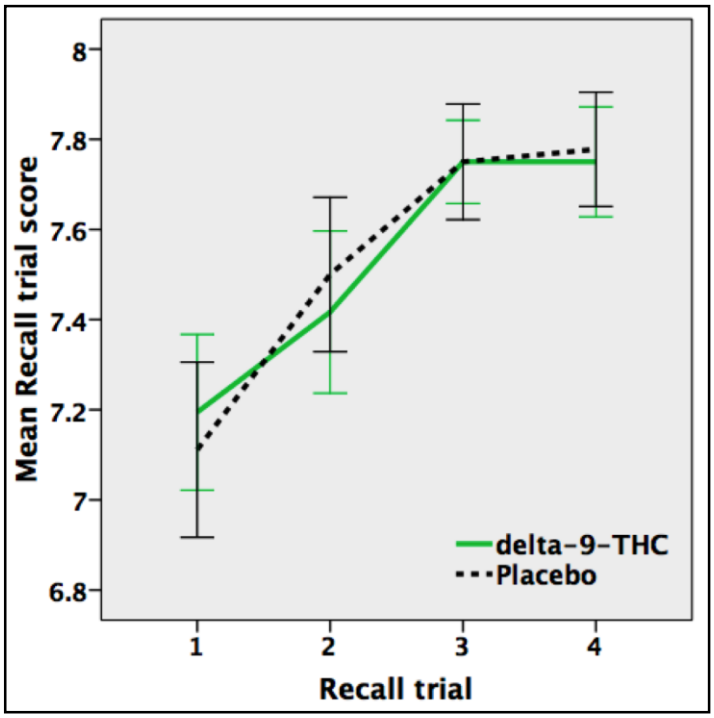
Supplementary Table 5. Brain regions differentially engaged in those sensitive to anxiogenic effects of THC (TA) versus those who were not (NA) during the encoding and recall conditions of the verbal learning task

Regions survive critical threshold of <1 false positive cluster.

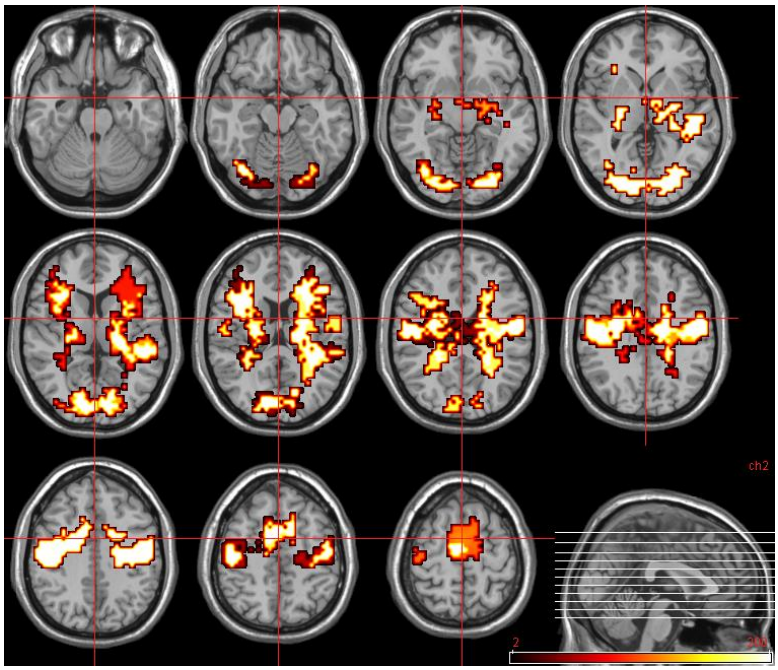
	Talairach Coordinates			Cluster size	
Area	**x**	**y**	**z**	**No. of voxels**	***p* value**
Group effect on task in the absence of THC					
Encoding condition (TA>NA)					
L. Fusiform gyrus	-25	-74	-13	38	0.002
L. Precuneus	-25	-67	37	36	0.005
L. Middle Frontal gyrus	-32	15	42	23	0.006
Recall condition (TA>NA)					
R. Fusiform gyrus/ Cerebellum	43	-63	-13	32	0.006
(NA>TA)					
L. Caudate	-14	4	20	65	0.002
L. Cingulate Gyrus	-11	-26	37	46	0.003

Supplementary Figures:

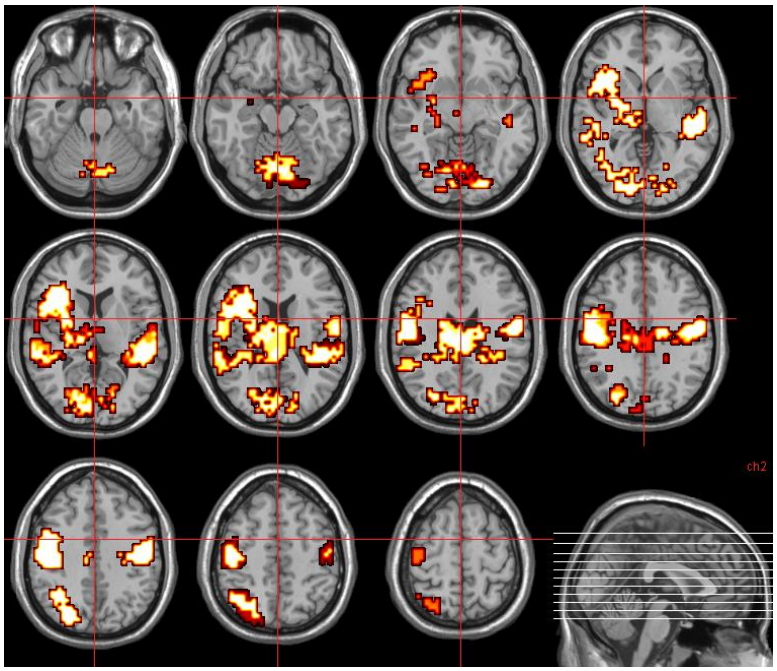
Supplementary Figure 1: Task performance: effect of drug
Line graphs showing recall task performance (Mean; error-bars represent standard error of mean) over repeated trials of the verbal learning task under the placebo (dashed black line) and THC (continuous green line) conditions.



Supplementary Figure 2A: Brain regions activated by the encoding condition of the verbal paired associate task independent of repetition and drug condition (display threshold: cluster p <0.01)



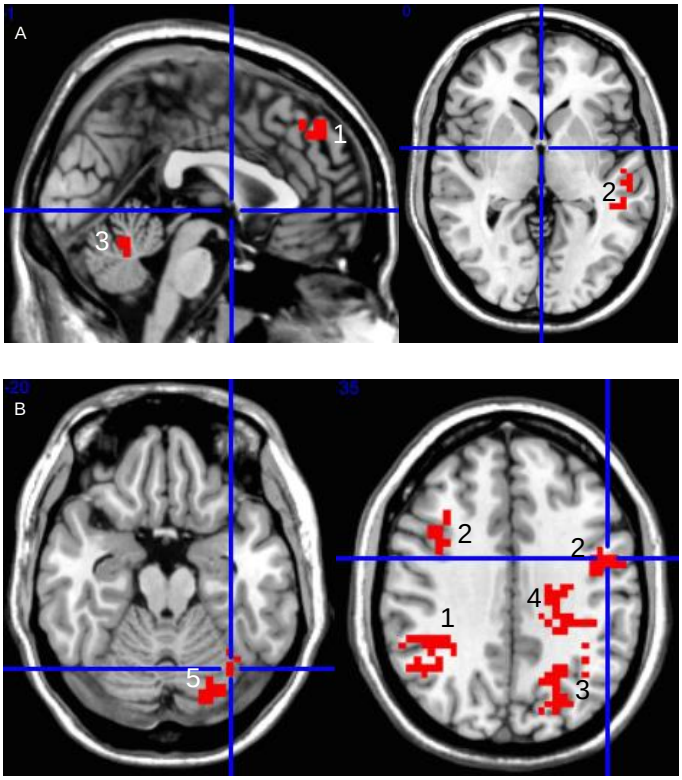
Supplementary Figure 2B: Brain regions activated by the recall condition of the verbal paired associate task independent of repetition and drug condition (display threshold: cluster p <0.01)



Supplementary Figure 3: Significant differences in brain activity between TP and NP groups in the placebo condition during the recall condition of the verbal paired associate task

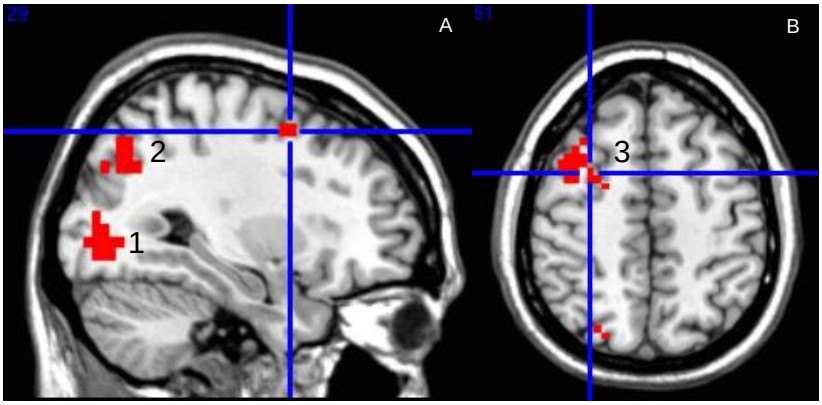
A. Greater engagement in the TP group relative to the NP group in the left medial frontal (1) and right middle temporal (2) gyri and cerebellum (3); (display threshold: cluster p <0.015).

B. Greater engagement in the NP group relative to the TP group in the left inferior parietal lobule (1), precentral gyrus (2) bilaterally, precuneus (3) and cingulate (4) gyrus on the right side and cerebellum (5); (display threshold: cluster p <0.007).



Supplementary Figure 4: Significant differences in brain activity between TA and NA groups in the placebo condition during the encoding condition of the verbal paired associate task

Greater engagement (shown in red) in the TA group relative to the NA group (A & B) in the left fusiform gyrus (1), precuneus (2) and middle frontal gyrus (3) (cross-hair); (display threshold: cluster p <0.01).



Supplementary Figure 5: Significant differences in brain activity between TA and NA groups in the placebo condition during the recall condition of the verbal paired associate task

A. Greater engagement (shown in red) in the TA group relative to the NA group in the right fusiform gyrus (cross-hair; 1) extending to the cerebellum; (display threshold: cluster p <0.01).
B. Greater engagement (shown in blue) in the NA group relative to the TA group in the body of caudate (cross-hair; 2) and cingulate gyrus on the left side; (display threshold: cluster p <0.01).