SUPPLEMENTARY INFORMATION FOR MANUSCRIPT "Cannabis affects people differently. Inter-subject variation in the psychotogenic effects of delta-9-tetrahydrocannabinol: An fMRI study with healthy volunteers"

This file contains supplementary methods, references, Table 1 (Mean reaction times and error rates by psychosis group and drug condition for Go/No-Go task), Figure 1 (Pulse, systolic and diastolic blood pressure in TP and NP groups in placebo and THC conditions) and Figure 2 (Other VAMS subscales; mental sedation, physical sedation, other, in placebo and THC conditions)

This study aimed to look at the both behavioural and neurophysiological effects of THC, in comparison to placebo, in those who developed transient psychosis and those who did not.

Participants' screening

Participants were screened for regular use of all substances using the Addiction Severity Index (McLellan, 1992) and a semi-structured interview to exclude personal and family history of psychiatric or physical illness for ethical reasons. None had a history of substance misuse or dependence, except for nicotine. They were asked to abstain from any illicit drug use during the study period, from alcohol and coffee 24 and 12 hours before respectively and cigarettes on the morning of each session. Each subject also received a urine drug screening analysis to ensure no traces of any drugs were present prior to sessions.

Behavioural measurements

The behavioural scales were chosen to examine the acute effects of THC in intoxication, anxiety, mood and psychosis and were evaluated at baseline (before drug administration), +1-hour (immediately before scanning), +2hour (immediately after scanning) and at +3-hour time points.

The Analogue Intoxication Scale (AIS) is an analogue scale when the participant is asked to indicate to express how intoxicated they feel by marking a line between two points of being 'not intoxicated' to 'extremely intoxicated' (Matthew et al. 1999). To further evaluate the subjective experience of the participant, we have also used the 48-item, shortened version of the Addictions Research Centre Inventory (ARCI), which is a specifically designed, standardized self-administered questionnaire for assessing the subjective effects of psychoactive drugs (Martin et al. 1971). This scale has yes/no statements describing the subjective effects of various classes of substances. The Spielberger State Anxiety Inventory (STAI Trait/State) is a 40-item, self-administered questionnaire to assess anxiety (Spielberger, 1983). In order to measure mood and anxiety, we have used Visual Analogue Mood Scale (VAMS), with four subscles: mental sedation, physical sedation, anxiety effects and other types of feelings or experiences (Folstein & Luria, 1973). The Positive and Negative Syndrome Scale (PANSS) is a 30-item questionnaire to assess psychotic symptoms (Kay et al. 1987). It is not normally used in healthy volunteers, however, as a universally recognized measure, it has also been

previously used in assessing transient psychosis in other drug challenge studies (D'Souza et al. 2004). It has three subsections: positive, negative, and general psychopathology, and the summation of these sections provides the total score. Each item score ranges from 1 (absent) to 7 (extreme).

As the focus of this paper is to explore the differences between those who become transiently psychotic under THC and those who do not, we mainly present the baseline and 2-hour measurements. But in this supplement we have also included some of the 3-hour measurements (Figure 1).

Behavioural Analyses

Data was recorded on SPSS version 18.0 and analyzed using Stata 11. Descriptive statistics were used to summarise the baseline variables. The age, years of education, cannabis, cigarette, alcohol and other drug use between the two groups were compared using a t-test (or equivalent non-parametric Mann Whitney U test (MWU)) or Fisher's Exact test. A multilevel model was used to assess the effect of THC on each outcome measure with subject included as a random effect and time as a fixed effect. A second multilevel model assessed the difference between the TP and NP groups. This model contained the main effects of time and group as well as their interaction. The small sample size lacked power to investigate an interaction with treatment and was not sufficient for an accurate estimation of three-way interaction parameters so, in both models, separate analyses were run conditioning on THC or placebo. The analysis

focused on baseline and 2 hours after drug administration, which were the main time points of interest.

Functional MRI paradigm – Go/No-go

Rapid, mixed trial, randomised presentation, event related fMRI design was used. Inter-stimulus-intervals (ISI) were randomly jittered between 1.6s and 2s and the appearance of target events was randomised to optimise statistical efficiency (Dale and Buckner, 1997; Dale, 1999). Subjects practiced the Go/No-go task once prior to scanning to ensure familiarity. The task involves motor response inhibition and selective attention (Rubia *et al.* 2005; Rubia *et al*. 2006). Subjects are required to either execute or inhibit a motor response according to the visual cues presented on a screen. Arrows appeared for 500 msec, followed by a blank screen, adding up to a mean inter-trial-interval of 1800 msec (range; 1600 – 2000 msec). When an arrow is pointing left or right, the participants were asked to press a left or right response button (Go trials) and when the arrow is pointing upwards they were asked not to press any buttons (No-Go trials). No-Go trials constituted 11% of the trials and required participants to inhibit their motor responses. In another 11% of the trials, arrows obliquely pointing left or right at a 23° angle appeared and subjects were instructed to respond to these the same as Go trials. These "Oddball" stimuli provided control for novelty effects associated with low frequency and different orientation of the No-go trials, relative to Go trials. 24 No-Go stimuli and 24 Oddball stimuli were pseudorandomly interspersed with 160 Go stimuli. In order to control for the attentional oddball effect of the more infrequent appearance of No-Go stimuli, the event-related analysis contrasted the activation related to successful No-Go trials with activation related to successful oddball trials

(No-Go – Oddball trials). The accuracy and speed of subjects' button press responses were recorded throughout by image acquisition. The main dependent variables of the task are the commission errors as an indicator of inhibitory capacity and the mean reaction time to go trials, reflecting the Go process of the task.

Data Processing and Analysis

A non-parametric approach (XBAM v4; http://www.brainmap.co.uk) was used to analyze the data, as this method does not assume that the population distribution is Gaussian. It is difficult to test this assumption with neuroimaging data in small groups and when tested, is often found to be violated (Rabe-Hesketh *et al*. 1997; Thirion *et al*. 2007). Instead, it uses median statistics to control outlier effects and employs permutation rather than normal theory based inference as recommended by Hayasaka & Nichols (2003). The test statistic is computed by standardising for individual difference in residual noise before embarking on second-level, multi-subject testing, using robust permutation-based methods, employing a mixed-effects. The group activation maps for each task condition were computed for THC and placebo by determining the median SSQ ratio at each voxel and then compared using non-parametric repeated measures analysis of co-variance (ANOVA), with a voxelwise threshold of p=0.05. The SSQ ratio maps for each participant were transformed into the standard space of Talairach (1988) using a two-stage warping procedure (Brammer *et al*. 1997). The clusterwise threshold was set such that the total number of false-positive clusters per brain volume was <1 per map and the p value at which this occurred was reported.

During the event-related first level analysis, No-Go and oddball trials were contrasted against the implicit baseline, which were the Go trials. To control for the oddball effect of low frequency appearance of No-go trials, brain activation during the successfully performed oddball trials, was then subtracted from brain activation during successful No-Go trials. This analysis was repeated for placebo and THC treatments separately. We used non-parametric 2^{nd} level ANOVA to identify main effects of group (TP vs. NP), drug (PLB vs. THC) and interaction effects (group X drug). SSQ ratios for each subject were extracted from all significant clusters obtained from the repeated measures ANOVA brain activation maps (cluster p = 0.01; placebo vs. delta-9-THC) to assess the phase of these activations. Results are reported with a voxelwise threshold of p = .05 and the clusterwise threshold set such that the total number of false-positive clusters per brain volume was < 1 per map and the p value at which this occurred is reported. Cluster-level testing provides greater sensitivity by incorporating information from more than one voxel in the test statistic and additionally it reduces the search volume or number of tests needed for a whole-brain analysis, which mitigates the multiple comparisons problem. This conservative method of analysis has already been employed in numerous peer-reviewed publications.

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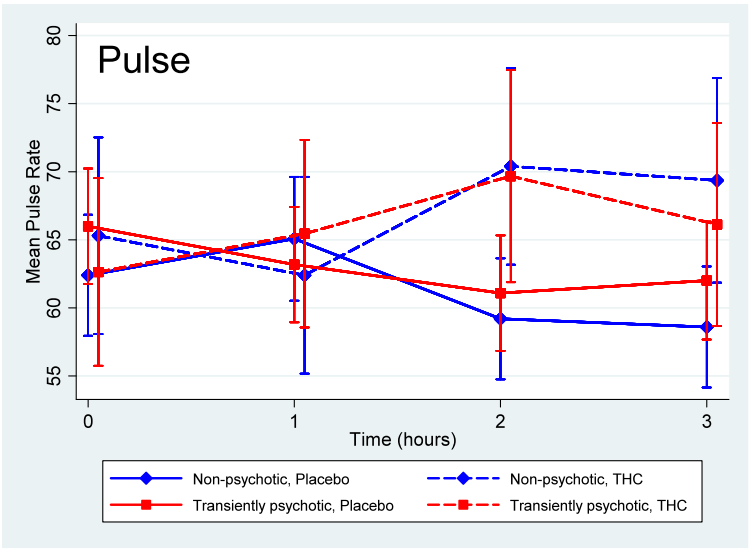
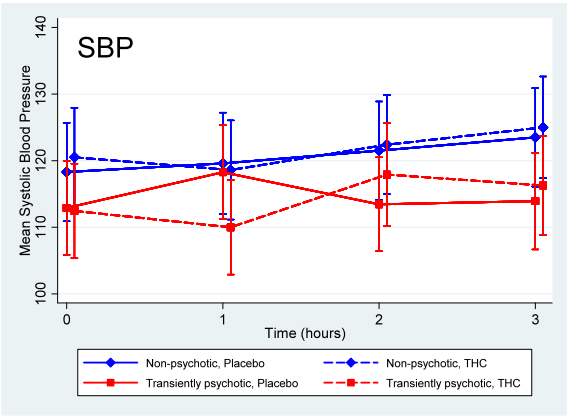
Table 1. Mean reaction times and error rates by psychosis group and drug condition for Go/No-Go task

	TP **Mean (SD)**		**NP** **Mean (SD)**	
	THC	**Placebo**	**THC**	**Placebo**
Reaction time to Go signals (msec)	0.39 (0.07)	0.43 (0.05)	0.43 (0.04)	0.44 (0.07)
Reaction time to Oddball signals (msec)	0.43 (0.11)	0.41 (0.17)	0.45 (0.06)	0.48 (0.07)
Inhibition error (%)	12.50 (9.77)	2.38 (2.23)	2.92 (4.41)	3.70 (4.86)

FIGURE 1 – Pulse, systolic (SBP) and diastolic blood (DBP) pressure in TP and NP groups in placebo and THC conditions





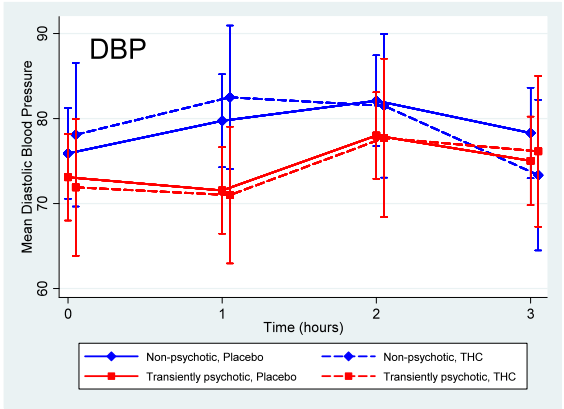


FIGURE 2: OTHER VAMS Subscales; mental sedation, physical sedation, other

THC CONDITION **PLACEBO CONDITION**