Novel approaches in clinical development of cannabinoid drugs

Klumpers, L.E.

CHAPTER III

Manipulating brain connectivity with Δ^9-tetrahydrocannabinol: a pharmacological resting state fMRI study

Neuroimage (2012) volume 63, issue 3, pages 1701-1711

Linda E. Klumpers[1], David M. Cole[2-3-4], Najmeh Khalili-Mahani[2-3-5], Roelof P. Soeter[2-3-5], Erik T. te Beek[1], Serge A. R. B. Rombouts[2-3-5], Joop M. A. van Gerven[1]

1 Centre for Human Drug Research, Leiden
2 Department of Radiology, Leiden University Medical Center, Leiden
3 Leiden Institute for Brain and Cognition (LIBC), Leiden University
4 Centre for Neuroscience, Division of Experimental Medicine, Imperial College London, UK
5 Institute of Psychology, Leiden University

ABSTRACT

AIM Resting state-functional magnetic resonance imaging (RS-FMRI) is a neuroimaging technique that allows repeated assessments of functional connectivity in resting state. While task-related FMRI is limited to indirectly measured drug effects in areas affected by the task, resting state can show direct CNS effects across all brain networks. Hence, RS-FMRI could be an objective measure for compounds affecting the CNS. Several studies on the effects of cannabinoid receptor type 1 (CB_1)-receptor agonist Δ^9-tetrahydrocannabinol (THC) on task-dependent FMRI have been performed. However, no studies on the effects of cannabinoids on resting state networks using RS-FMRI have been published. Therefore, we investigated the effects of THC on functional brain connectivity using RS-FMRI.

METHODS Twelve healthy volunteers (9 male, 3 female) inhaled 2, 6 and 6 mg THC or placebo with 90-minute intervals in a randomized, double blind, cross-over trial. Eight RS-FMRI scans of 8 minutes were obtained per occasion. Subjects rated subjective psychedelic effects on a visual analogue scale after each scan, as pharmacodynamic effect measures. Drug-induced effects on functional connectivity were examined using dual regression with FSL software (FMRIB Analysis Group, Oxford). Eight maps of voxel-wise connectivity throughout the entire brain were provided per RS-FMRI series with eight predefined resting-state networks of interest. These maps were used in a mixed effects model group analysis to determine brain regions with a statistically significant drug-by-time interaction. Statistical images were cluster-corrected, and results were Bonferroni-corrected across multiple contrasts.

RESULTS THC administration increased functional connectivity in the sensorimotor network, and was associated with dissociable lateralised connectivity changes in the right and left dorsal visual stream networks. The brain regions showing connectivity changes included the cerebellum and dorsal frontal cortical regions. Clear increases were found for feeling high, external perception, heart rate and cortisol, whereas prolactin decreased.

CONCLUSIONS This study shows that THC induces both increases and (to a lesser extent) decreases in functional brain connectivity, mainly in brain regions with high densities of CB_1-receptors. Some of the involved regions could be functionally related to robust THC-induced CNS-effects that have been found in previous studies (Zuurman et al, 2008), such as postural stability, feeling high and altered time perception.

INTRODUCTION

Ideally, early clinical phase drug development for neurological and psychiatric indications should use tests that measure effects in an objective way and repeatedly over time across different species. These tests should also be able to distinguish unique effect profiles for different classes of drugs. Traditionally, measurements of drug effects on the central nervous system (CNS) in healthy volunteers include cognitive tasks, various questionnaires, neurophysiological measurements, and increasingly also neuroimaging. The wide diversity of these tests and their numerous variations limits their applicability for decision making in clinical practice or drug development. In addition, pharmacological studies can only include a limited number of pre-defined pharmacodynamic tests, which can easily miss drug effects in CNS domains that are not tested. Moreover, most CNS effects are influenced by various functions like attention and motor coordination, and therefore do not provide direct information on an exact site of drug action.

Imaging techniques have the advantage of objectively assessing direct effects in the body. However, positron emission tomography (PET) studies have radiation dose restrictions that limit repeated measurements within subjects, and the targeted pharmacological or functional system is restricted by the availability of an appropriate imaging agent. Functional magnetic resonance imaging (FMRI) on the other hand is a non-invasive imaging technique based on blood-oxygen-level-dependent (BOLD) measurements that represent brain activity. Until recently, FMRI was applicable in task-related designs only, in which pharmacologically induced changes in BOLD signals were measured in response to a specific task. The application of FMRI in drug development has several restrictions, imposed by the need for a pre-defined hypothesis about how the drug affects the task, and by limitations related to the scanning environment and to repetitive testing.

Resting state (RS) FMRI is a recently developed imaging technique that measures spontaneous BOLD changes of subjects who are in a resting state, without the interference of any task or specific stimulus. This means that RS-FMRI can be applied in studies without *a priori* hypotheses on action site. The fact that RS-FMRI is non-invasive and not affected by variability or limitations of task performance and that it can be frequently and rapidly repeated, could make it a highly valuable technique in CNS drug development. Although experience is still limited, RS-FMRI could be applied in pre-clinical animal studies, healthy volunteers and patients, which could make it a suitable translational instrument in drug development.

Previous studies found that coherent resting state BOLD fluctuations form spatially correlated brain maps, or resting-state networks (RSNS) (Beckmann et al., 2005; Biswal et al., 2010). RSNS have shown to be consistently present across human subjects, and could represent brain regions that are anatomically and functionally connected, and related to behavioural outcomes and clinical conditions (De Luca et al., 2006; Greicius et al., 2004; Fox et al., 2007; Quigley et al., 2003; Smith et al., 2009; Damoiseaux et al., 2006). A previous study by Mennes et al. suggested that inter-individual differences in RS-FMRI could predict the response to task-induced BOLD activity (Mennes et al., 2010). Only a few studies investigated the effects of pharmacologically active CNS compounds on the functional topography of RSNS. We recently conducted a study where RS-FMRI was repeated while plasma levels of morphine and alcohol were kept stable (Khalili-Mahani et al., 2011). In order to develop a broad basis for this technique by investigating reliability and reproducibility, more studies using different drug classes should be performed. This would provide important methodological information and reference data for the use of RS-FMRI as a biomarker for CNS drug research (Wise and Preston, 2010).

In the current study we investigated the effects of Δ^9-tetrahydrocannabinol (THC) on the brain using RS-FMRI. THC is a major pharmacologically active constituent of the plant *Cannabis sativa* L. In the body, THC

binds to two cannabinoid receptors (CB_1 and CB_2) of which CB_1 receptors are predominantly present in various brain areas (Herkenham, 1992). The action of THC on the CB_1 receptors is generally considered responsible for the commonly known pharmacodynamic effects, such as feeling high and postural instability (Zuurman et al., 2008).

Previous PET and FMRI studies with THC that investigated regional cerebral blood flow and BOLD signal fluctuation found THC-induced effects on the limbic system (thalamus, amygdala, hippocampus, parahippocampal gyrus, cingulate cortex) and connected areas (basal ganglia, frontal cortex), which are involved in reward, emotion, memory, awareness, pain, and executive functions (Bhattacharyya et al., 2009; Mathew et al., 1998; Mathew et al., 1999; Mathew et al., 2002; Stokes et al., 2010; van Hell et al., 2011). THC also affects areas of sensory (insula, postcentral gyrus, superior temporal gyrus), and motor coordination systems (cerebellum). The functions associated with these regions are related to the behavioural effects after THC or cannabis use (Zuurman et al., 2009).

The primary aim of this study was to investigate the effects of THC on task-independent RS-FMRI functional connectivity patterns using repetitive measures in healthy volunteers. Based on previous studies using other psychopharmacological manipulations (Khalili-Mahani et al., 2011) we hypothesised that THC would induce changes in brain connectivity compared to placebo. In addition, we measured the plasma concentrations of THC and its active metabolite 11-HYDROXY-THC (11-OH-THC) as well as a number of well-known THC-related CNS effects. Based on our previous studies, we expected to measure clear THC and metabolite plasma concentration profiles, and prominent pharmacodynamic effects, other than RS-FMRI (Strougo et al., 2008; Zuurman et al., 2008).

METHODS

Design

This was a double-blind, randomized, placebo-controlled, two-way cross-over study with a wash-out period of at least 2 weeks.

Subjects

Healthy, right-handed male and female volunteers aged 18 to 45 years with a body mass index of 18.0 to 28.5 kg/m^2 were included in the study. Subjects with a history of psychiatric or neurological illness, or with a history of hereditary psychiatric illness in first degree relatives or neurological illness in first- or second degree relatives were excluded from participation. Subjects had to be cannabis users for at least 1 year with use frequency of no more than once a week, and had to be able to refrain from using cannabinoids from at least 2 weeks prior to the first treatment period up to the end of the study. They had to refrain from nicotine and caffeinated products on study days. Subjects were excluded if they used medication other than contraceptives, and if they were pregnant (as assessed by HCG urine test). They were not allowed to have a positive alcohol breath test or drug urine test at the screening visit or at the start of a study day, neither a history of alcohol or drug dependence. Subjects could not participate if they had metal body implants or claustrophobia.

As this was an explorative study, no sample size calculation could be performed. We planned a sample size of 12 volunteers (6 male and 6 female) who completed two occasions, since in all drug studies that we have performed so far, numbers of 12 subjects were found to be sufficient (Strougo et al., 2008; Desmond and Glover, 2002), and a similar number was also mentioned in a study about the power of FMRI and RS-FMRI. Subjects who were not able to complete two occasions would be replaced.

Procedure

Subjects gave written informed consent before any study-specific procedure was performed. Eligible subjects were enrolled in the study after a general health screen within three weeks before the first study day. Subjects were acquainted with the visual analogue scales questionnaire and the inhalation procedure using THC vehicle. At each study day, THC or placebo was administered at 0m, 1h30m and 3h00m. Pharmacodynamic (PD) and pharmacokinetic (PK) measurements were frequently performed on all study days at fixed time points, as chronologically indicated in Figure 1. At the beginning of each study day a venflon cannula was inserted intravenously for all blood samples that were drawn on both study days. Subjects were fasted for at least 4 hours at arrival, and standardized meals were provided pre-dose, and at 3h40m and after the last study day activity at 6h47m. The wash-out period between study days was at least two weeks. The study protocol was approved by the Medical Ethics Review Board of Leiden University Medical Center and complied with the principles of ICH-GCP, the Helsinki declaration and Dutch laws and regulations.

Treatments

Each study day, subjects received three doses of THC (2-, 6-, and 6 mg) or placebo via inhalation with 1.5 h intervals. Two mg purified THC was dissolved in 200 µl 100% ethanol. The THC dosages were selected to reach and maintain clear central nervous system effects as predicted by PK-PD models that were based on a previous study (Strougo et al., 2008). Procedures for vaporizing the solution and inhalation of the vapour were done according to a method as previously described (Zuurman et al., 2008). In addition to this procedure, the current study used a nose clip during the THC or placebo administrations to prevent nasal exhalation, in order to reduce pharmacokinetic variability by minimizing undetected loss of THC vapour.

Outcome measures

A schematic representation of the time points of the study day activities is given in Figure 1. The precision of all activities is imposed by the tight time schedule.

PHARMACOKINETIC MEASUREMENTS AND BIO-ANALYSIS

To determine THC plasma concentration, venous blood samples were collected in 4 ml EDTA tubes at 5, 20 and 88 min after each administration and at 178 min after the third administration only. After collection, the tubes were kept on ice water in aluminium foiled containers and centrifuged within one hour for 10 minutes at 2000G at 4 ^{0}C. THC samples were handled sheltered from light. Plasma samples were stored at –20 ^{0}C and sent to Analytisch Biochemisch Laboratorium (ABL, Assen) for analysis. Plasma THC as well as metabolite concentrations (11-HYDROXY-THC and 11-NOR-9 CARBOXY-THC) were determined using tandem mass spectrometry with a lower limit of quantification of 1.00 ng/ml.

PHARMACODYNAMIC ASSESSMENTS

IMAGING – Resting state functional magnetic resonance imaging (RS-FMRI) scans were made pre-dose and at 10 and 70 min after the first and second THC administrations, and 10, 100 and 190 min after the third administration. The differences in time points of measurements performed after the first and second administration versus after the third THC administration were chosen to investigate a more extended time course of THC and metabolite plasma concentrations, and pharmacodynamics. As the interval of the THC dosing schedule was 90 minutes, the time frame in which measurements could be performed that were related to the previous THC administration was limited

to 90 minutes. Subjects were asked not to move or talk and to look at a fixation cross during scanning to improve the subject's comfort in THC conditions, and to minimize the risk of falling asleep during scanning. Four chest electrodes and the scanner's flexible pressure belt were used to record heart rate and respiration rate during scanning. A 3T Achieva scanner (Philips Medical System, Best) was used for image acquisition. RS-FMRI scans were T2*-weighted and consisted of 220 gradient echo 'echo planar imaging' (EPI) volumes (repetition time interval = 2180 ms; echo time interval = 30 ms; flip angle = 80^0; 38 axial slices; 64x64x38 isotropic resolution 3.44 mm; scan time = 8.1 min). For anatomical registration, a T1-weighted scan was obtained for each subject at the end of each study day.

VISUAL ANALOGUE SCALES (VAS) – VAS by Bond and Lader is a 16-item subjective assessment of subjective effect on alertness (composition of items alert/drowsy, strong/feeble, muzzy/clear-handed, well coordinated/clumsy, lethargic/energetic, mentally slow/quick-witted, attentive/dreamy, incompetent/proficient, and interested/bored), on mood (composition of items contended/discontented, troubled/tranquil, happy/sad, antagonistic/amicable, and withdrawn/gregarious), and calmness (composition of items calm/excited, and tense/relaxed) (Bond and Lader, 1974). The adapted version of VAS by Bowdle et al. (1998) is a 13-item assessment of subjective effects on item 'feeling high' and on factors 'internal perception' and 'external perception', which are both compositions of items that are affected differently by THC as previously described (Zuurman et al., 2008). VAS were included in this study to provide a positive control for THC-induced pharmacodynamic effects, as previous studies showed clear effects on the VAS (Zuurman et al., 2008; Zuurman et al., 2008; Zuurman et al., 2009). The measurements were taken twice pre-dose, and at time points: 29 and 59 min, 1h23min, 1h59min, 2h29min, 2h53min, 3h35min, 4h29min, 4h57min, 5h59min, and 6h42min.

HEART RATE AND BLOOD PRESSURE – Heart rate and blood pressure were taken as safety measurements using a Nihon-Koden (Lifescope EC, Tokyo, Japan) blood pressure apparatus. Heart rate measurements were used as an objective measure for treatment effects, as previous studies showed clear heart rate effects (Zuurman et al., 2008; Zuurman et al., 2008; Zuurman et al., 2009). Heart rate measurements were taken 3 minutes after each time point of VAS measurements as mentioned in the previous paragraph. Blood pressure was measured pre-dose, and at 6h45min.

HORMONES – Prolactin levels (µgr/l) were measured as a biomarker for dopaminergic activity (de Visser et al., 2001). Cortisol (µmol/ml), luteinizing hormone (LH, ng/ml) and follicle-stimulating hormone (FSH, U/l) were measured as exploratory biomarkers of hypothalamic-pituitary activity (Chen et al., 2010). Due to the diurnal rhythm of cortisol, the two study days of each subject were consistently scheduled at the same time of the day. Blood samples for LH, FSH, prolactin and cortisol were collected twice pre-dose, at 20 and 1h28min after each THC administration and an additional sample was taken at 5h58min. Serum was separated by centrifugation (2000g at 4^{0}C for 10 min) within 1 h of collection. The samples were analyzed by the Central Clinical Chemistry Laboratory (Leiden University Medical Center, Leiden) using an electrochemoluminiscence-immunoassay for prolactin and cortisol, and a fluoro-immunoassay for LH and FSH.

METABOLIC BLOOD MEASURES – The study was also used to perform an exploratory analysis of several metabolic effects of THC. Glucose (mmol/l), high-density lipoprotein (HDL) cholesterol (mmol/l), leptin (µg/l) and triglycerides (mmol/l) serum samples were analyzed by the Central Clinical Chemistry Laboratory (Leiden University Medical Center, Leiden). For description of serum collection and time points, see 'hormones' section.

Statistical analyses

CLINICAL EFFECTS

For vital signs [heart rate (HR) in beats per minute (bpm) and blood pressure (mmHg)], raw data and changes from baseline were analyzed by type of measurement and parameter and treatment using descriptive statistics. HR and PR-, QRS-, and QT-intervals, corrected QT (QTC) (all in ms) from automatic reading were analyzed as raw parameter value and change from baseline (for HR and QTC only). Adverse events were coded according to the Medical Dictionary for Regulatory Activities (MedDRA version 13.0).

PHARMACOKINETICS

All concentrations and maximal concentration (C_{MAX}), time of maximal concentration (T_{MAX}), area under the curve from zero to infinity ($AUC_{0-\infty}$), and terminal half-life ($t_{1/2}$) of THC and its metabolites 11-OH-THC, and THC-COOH were summarized by mean, standard deviation (SD), standard error of the mean (SEM), coefficient of variation (CV%), and number of available observations. Also, a population pharmacokinetic analysis was performed based on a previously described two-compartmental model (Strougo et al., 2008), with the addition of the active metabolite 11-OH-THC in a separate compartment. A post hoc analysis on gender differences was performed using a linear mixed effect model with treatment, period, time and treatment by time as fixed effects, subjects and subject by treatment as random effects and with baseline value as covariate (SAS for windows V9.1.2 ; SAS Institute, Inc., Cary, NC, USA).

PHARMACODYNAMICS

Resting State FMRI data processing was carried out using the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL 4.1, Oxford, UK), using the same analysis techniques for pharmacological RS-FMRI as reported previously (Khalili-Mahani et al., 2011).

For preprocessing the following standard procedure was performed: head motion correction, brain extraction, Gaussian smoothing with a 5 mm FWHM kernel, grand-mean scaling of each BOLD FMRI dataset by a single multiplicative factor; high-pass temporal filtering (FWHM = 100s). After preprocessing, the EPI data were affine-registered to the anatomical T1-weighted scan, and the anatomical scan was subsequently affine-registered to the MNI 152 standard space (Montreal Neurological Institute, Montreal, Canada). FMRI images in MNI space were interpolated to 2x2x2 mm voxels.

RSN functional connectivity was determined as similarity of the BOLD fluctuations in each brain voxel in relation to characteristic fluctuation in eight predefined networks of interest (NOIS). These networks were obtained from a published model-free analysis of the spatio-temporal structure of the resting-state BOLD fluctuations (Beckmann et al., 2005). The template NOIS include over 80% of the total brain volume and comprise the following networks: medial and lateral visual (NOIS 1 and 2, respectively, including primary visual areas), auditory and somatosensory (NOI 3, including areas involved with hearing), sensorimotor (NOI 4), default mode [NOI 5, of which is hypothesized that these regions are associated with the representation of the world around us and spatial attention (Miller and Cohen, 2001)], executive control (NOI 6, these areas have been hypothesized to provide bias signals to other areas of the brain in order to implement cognitive control), and right and left-lateralized fronto-parietal dorsal visual [NOIS 7 and 8, probably representing information relevant for (visual) attention, but related to visuospatial and verbal attention respectively] (Beckmann et al., 2005; Laird et al., 2011). The predefined networks, as determined by the weighted template NOIS, were calculated for the study sample.

Connectivity to each of the 8 NOIS, for each voxel, was measured using dual-regression (Filippini et al., 2009; Beckmann et al., 2005) followed by

a mixed effects model group analysis. Dual-regression analysis generated whole-brain statistical maps of z-scores representing voxel-wise functional connectivity across all regions with the characteristic activity in each of the NOIs (12 subjects x 8 scans x 2 occasions x 8 NOIs). The higher the absolute value of the z-score, the stronger the connectivity to a given NOI.

Variations in heart rate and respiratory rate could be induced pharmacologically by THC administrations (Zuurman et al., 2008; Zuurman et al., 2008), and these fluctuations could induce variance in the resting-state BOLD signal unrelated to functional CNS-effects (Beckmann and Smith, 2005; Birn et al., 2008; Chang et al., 2009). It has been shown that BOLD signal fluctuations measured in the white matter (WM) and cerebrospinal fluid (CSF) are reliable representations of non-neuronal physiological noise in RS-FMRI data (Birn, 2012). Therefore, we included separate WM and CSF confounds, as well as six motion parameters, as nuisance variables in the second stage of the dual regression analysis for each scan. These separate WM and CSF confounds were measured for each scan by calculating tissue-specific segmentations of each subject's high-resolution T1 structural scan (segmented using FSL FAST) (Zhang et al., 2001), transforming the resulting WM and CSF maps to the corresponding subject's EPI space and subsequently extracting mean time series from that functional scan within the space of each of these tissue-specific maps.

For group analyses, treatment and time were used as fixed factors and subject was used as a random factor. Average respiration and heart rates per RS-FMRI scan were also added as nuisance covariates (Khalili-Mahani et al., 2011). Within-subject average z-maps were modelled with separate fixed factors, to allow the model to estimate the correlation between z-maps. Permutation-based statistical inference was used (5000 repeated permutations) on the treatment by time interactions (Nichols and Holmes, 2002). Higher-level analyses were restricted to study population-specific grey matter regions by registration of the grey matter volumes resulting from FAST segmentation to MNI space and subsequent summing across subjects. Significant THC effects on functional connectivity were defined using threshold-free cluster enhancement ($p < 0.05$, family-wise error-corrected) (Smith and Nichols, 2009). Correction for 16 multiple comparisons was done using Bonferroni correction. The multiple comparisons consisted of 2 comparisons (either connectivity increase or decrease after THC administration) for 8 NOIs.

VAS and heart rate were analyzed using a linear mixed effect model with treatment, period, time and treatment by time as fixed effects, subjects and subject by treatment as random effects and with baseline value as covariate (SAS for windows V9.1.2; SAS Institute, Inc., Cary, NC, USA). From this model, pair wise differences and corresponding 95% confidence intervals were estimated to verify the effects of THC. Measurements from VAS Bowdle (e.g. feeling high, external and internal perception) were log (VAS score+2) transformed for statistical analysis and reported in 'units' (U).

RESULTS

Subject characteristics

Twenty-two healthy volunteers (eleven male, eleven female) were randomized and treated, twelve of whom completed two occasions and were included in the pharmacodynamic and pharmacokinetic analysis. One of these subjects missed the last two scans on the placebo occasion due to nausea and vomiting. For safety analysis, all treated subjects were included. Eight female subjects and one male subject dropped out from the study due to adverse events during THC occasions. Details on the nature of the adverse events can be found in section 0. One male subject discontinued the study after the first occasion with THC treatment, for personal reasons. Also, this subject had an incomplete first THC administration (2 mg) due to leakage of the vaporizer. Details on subject demographics can be found in Table 1.

Adverse effects

Nine subjects dropped out due to adverse effects during THC occasions only. Two subjects dropped out due to a vasovagal collapse, one female 22 min after the first THC inhalation, and one male 27 min after the second THC inhalation. One female subject discontinued due to nausea that started 4 min after the second THC inhalation, and another female became nauseous after the third THC inhalation. One female dropped out because of nausea and anxiety that started 12 min after the second THC inhalation. Four other females discontinued due to anxiety: two at 12 and 21 minutes after the first THC administration, and two at 10 and 12 minutes after the second. Most adverse events that were observed in this study were typically related to THC use. The most occurring treatment related adverse effects were feeling high (7/22 subjects), nausea (7/22), and anxiety (6/22).

Pharmacokinetics

Five minutes after each THC administration, a peak plasma concentration was observed (Figure 2). Mean peak plasma concentrations were: 29.5 ng/ml (SD 11.6) after the first administration (2 mg THC), 139.9 ng/ml (SD 42.1) after the second administration (6 mg THC) and 109.1 ng/ml (SD 55.3) after the third administration (6mg THC). After each peak, a rapid decline in plasma concentration was observed. An overview of the pharmacokinetic parameters of THC and 11-OH-THC is given in Table 2.

GENDER

The unexpectedly large number of THC-related adverse events in females raised questions about potential sex-related pharmacokinetic differences. Therefore, a post hoc analysis of THC and metabolite plasma concentrations was performed in males and females. In Figure 3 the THC concentration curves of males and females are given. When compared graphically, the average plasma concentration for females was higher compared to males. A reliable statistical analysis could not be performed for subjects who completed the entire study, since only three females received all treatments. However, THC concentrations were significantly higher in the eleven females who inhaled the first dose of THC 2 mg (42.3 ng/ml), than in the nine males (26.3 ng/ml; difference 61.0%, 95% CI 13.3-128.7, $p = 0.0087$).

Resting State Connectivity

Each of the 8 NOIs showed treatment effects on connections with several brain regions (Table 3). After Bonferroni correction, treatment-related connectivity differences were observed within the sensorimotor and right and left dorsal visual stream networks (NOI 4, 7, 8), which are depicted in Figure 4. Most changes occurred in connectivity patterns of the right dorsal visual stream network (NOI 7). After THC administration,

connectivity of this network increased with the left and bilateral frontal pole and dorsomedial prefrontal cortex, and with the left superior pre-frontal cortex (t = 5.69 with 149 voxels; t = 4.97 with 130 voxels respectively, Bonferroni corrected), with an extension into the left superior frontal gyrus. Also, a connectivity decrease (t = 5.44; 53 voxels) was found in the right and dorsal visual stream network (NOI 7). This decrease was observed in the area covering the superior frontal pole, middle and inferior frontal gyrus, and dorsolateral prefrontal cortex, with all regions being lateralized to the right hemisphere. An increase of connectivity was found between the cerebellum and the sensorimotor network (NOI 4) after THC administration (t = 6.36; 6101 voxels, Bonferroni corrected). The area including the occipital pole and lateral occipital cortex showed an increased connectivity (t = 5.01; 52 voxels) with the left dorsal visual stream network (NOI 8).

Other pharmacodynamic parameters

Graphs of feeling high and heart rate plotted against time are given in Figure 5. Treatment comparison of the pharmacodynamic effects other than FMRI measurements demonstrated significant increases after THC administration on VAS external perception (0.225 (U); 95%CV 0.054 - 0.396; p = 0.0149), feeling high (0.768 (U); 95%CV 0.578 - 0.957; p = <.0001), and heart rate (10.3 bpm; 95%CV 4.4 - 16.2; p = 0.0026). The centrally mediated external perception and feeling high scores increased after the first and second THC administration, but not after the third THC inhalation. The decrease of these effects was relatively slow. Heart rate remained stable during placebo treatment; whereas THC induced acute heart rate elevations that declined relatively rapidly after each dose (Figure 5B). Stress-hormone cortisol showed a 32.2% increase (95%CV 11.9 - 56.3; p = 0.0051) after THC, whereas prolactin decreased with 21.0% (95%CV -33.0 -/-7.0; p = 0.0100). The THC effect on cortisol was maximal around the third THC administration. The first prolactin measurement after the first THC administration showed no significant differences between THC and placebo treatment, however, as time progressed, concentration differences increased by a continuously decreasing prolactin concentration after THC compared to placebo. The mean glucose concentration over time increased by 7.2% after THC treatment (95%CV 0.1 – 14.8; p = 0.0468). Visual inspection indicated that this difference was solely caused by a larger glucose increase in the THC arm after a standardized meal (at t = 4.28 h, 48 min after lunch and 1h28m after the third THC administration) (6.19 mmol/l in the placebo group and 8.34 mmol/l in the THC treated group). No significant changes were seen for HDL cholesterol, leptin and triglycerides. An overview of the pharmacodynamic parameters can be found in Table 4.

DISCUSSION

This study demonstrated that THC induced changes in RSN functional connectivity. As predicted by the PK-PD models that were based on a previous study (Strougo et al., 2008) the THC and 11-OH-THC concentrations were within the effective range, inducing significant effects on external perception and feeling high from VAS Bowdle and on heart rate measures.

Connectivity and function

THC induced significant effects on functional connectivity between various brain areas and the sensorimotor and right and left dorsal visual stream networks. In general, an increase of network connectivity was found after Bonferroni-correction, with one area showing decreased connectivity in the left dorsal visual stream cortex. The areas that were found to be most affected by THC in this study were comparable to findings from previous THC studies using PET, in which changes in resting-state blood flow or [11C]-raclopride binding potential were found, including the cerebellum, frontal pole, left superior frontal gyrus, right middle frontal gyrus (Mathew et al., 1998; Stokes et al., 2010).

NOI 4

The areas displaying THC-induced connectivity changes in the sensorimotor network could be associated with the functional changes that are observed after THC administration, such as the increase of external perception as seen in this study. The cerebellum, which showed connectivity increase, is associated with motor coordination and time perception (which is one aspect of external perception) and has high CB_1 receptor density (Nyberg et al., 2010; Stoodley and Schmahmann, 2009; Romero et al., 2002). A previous study with THC reported a correlation between altered time perception and cerebellar blood flow (Mathew et al., 1998). Also, the cerebellum is likely to be involved in THC-induced postural stability changes as previously observed (Zuurman et al., 2008; Zuurman et al., 2008; Zuurman et al., 2009). A possible correlation between the increase of external perception (a composite scale including the item 'altered time perception') and cerebellar connectivity changes should be further explored in a future study using PK-PD modelling.

NOI 7

The bilateral and left DMPFC, and the left frontal pole and left superior frontal gyrus (SFG) had an increased connectivity to the right dorsal visual stream network, whereas the right superior frontal pole, right dorsolateral PFC (DLPFC), and the right inferior and middle frontal gyri had a decreased connectivity. The DMPFC and frontal pole are functionally associated with decision making and cognitive control, such as subserving the monitoring of action outcomes and cognitive branching, the ability to put on hold an alternative course of action during the concurrent performance of the ongoing one (Venkatraman et al., 2009; Daw et al., 2006; Koechlin et al., 1999). We have not studied these functions in our study, but the literature includes a few studies of the effects of THC/cannabis on complex problem solving and planning tasks (Tinklenberg et al., 1972; Crockett et al., 1976), which could be attributed to fronto-polar PFC changes.

The SFG is involved in higher cognitive functions, such as the executive functions of working memory processing, and is suggested to be associated with the excitatory and inhibitory influences on craving, as found in a lesion study and a study with tobacco cigarettes (du Boisgueheneuc et al., 2006; Rose et al., 2011). In human brain tissue, CB_1 receptors are present in the SFG (Eggan and Lewis, 2007), suggesting effects of cannabinoids on the higher cognitive functions. However, in healthy subjects, THC demonstrated no effects on cognitive functions such as plan-

ning and reasoning in the very limited available literature (Ramaekers et al., 2009; Morrison et al., 2009). The right inferior and middle frontal gyri are involved in risk attitudes and contingency awareness (Carter et al., 2006). These behaviours are affected by THC (Foltin et al., 1990), but are much dependent on the exact type of behaviour that is tested (McDonald et al., 2003; Zuurman et al., 2009). One FMRI-study, for example, showed that THC attenuated activity in the right inferior frontal and anterior cingulate gyri when performing the Go/No-Go task for response inhibition, but no difference was seen on the task performance itself (Borgwardt et al., 2008). The DLPFC is involved with organization of working memory (Jha et al., 2006), which can also be affected by THC use (Bocker et al., 2010).

NOI 8

This study showed that the right posterior pole and lateral occipital cortex had an increased connectivity with the left visual dorsal stream network. The occipital regions are functional visual areas (Hine, 1918; Kolmel, 1988). Previous studies found that THC influences several aspects of vision that could be attributed to visual cortex changes (Koethe et al., 2006; Emrich et al., 1991; Winton-Brown et al., 2011). In this study, THC had clear effects on VAS external perception, which includes several scales of changes of colours and shapes.

In summary, the different NOIs show significant connectivity effects on brain areas that are functionally related and that have been found to be significantly affected by THC administration in previous studies. This suggests that connectivity changes that are found with RS-FMRI could be related to functional alterations. Since similar conclusions were previously reached with morphine and ethanol (Khalili-Mahani et al., 2011), RS-FMRI can possibly be a useful technique for prediction of drug effects, although more studies are needed to understand the potential role of this technique in drug development.

Other pharmacodynamic parameters and gender effects

CORTISOL

The cortisol increase, or reduced decrease (which occurs during daytime due to the diurnal rhythm), is consistent with previous findings (Goodwin et al., 2011; Ranganathan et al., 2009). Pre-clinical studies found that the cannabinoid-induced hypothalamic-pituitary axis activity increase is caused by cannabinoid action in the paraventricular nuclei in the hypothalamus and the pituitary gland, where CB_1 and corticotrophin releasing hormone receptors are co-expressed (Corchero et al., 1999; Dewey et al., 1970; Wenger et al., 1999). No clear connectivity changes were found in hypothalamic regions. The question whether connectivity changes could be expected between, for example, the hypothalamus and limbic regions after a THC-induced cortisol increase remains unanswered, as the relationship between connectivity changes and functional changes is unknown and should be further investigated. Possibly, the THC-induced enhancement of postprandial glucose elevations was due to a THC-induced cortisol increase, which may have induced gluconeogenesis. No comparable studies to our study have been reported, but similar findings have been reported in pre-clinical studies and a clinical study in fasting conditions (Kim et al., 2011; Benowitz et al., 1976). As the subjects were served a standardized meal, glucose elevation due to larger carbohydrate intake is unlikely. Future studies may reveal interesting information about the circuitry involved in adaptive regulation of the brain-body function.

FEELING HIGH

Most subjects experienced the familiar feelings of subjective ‘high’ after administration of THC. This raises the question of which networks

could be associated with these psychomimetic effects. The answer to this question cannot be given easily, since THC causes many different effects with very similar time profiles (Strougo et al., 2008). Consequently, it is difficult if not impossible to distinguish the network activities that are uniquely associated with feeling high, from those related to other THC-effects like upright postural instability or sedation. As our database of similar pharmacological studies with other psychomimetic drugs expands, we may be able to address the question of neural correlates of 'feeling high' by integration and, for example, factor analyses of data from different drugs in the future.

PHYSIOLOGICAL VARIATIONS

We have found a significant effect of THC on heart rate. Because physiological pulsations may cause movement of large vessels, various retrospective processing techniques are proposed to correct for correlated physiological noise. As recently recommended in (Birn, 2012) we have used the BOLD fluctuations within individual's CSF and WM masks as an indirect measure of physiological noise. Furthermore, we have used average physiological variables as covariates at the higher-level group analysis. Previously, it has been shown that the functional connectivity of the default mode network (NOI 5) *in particular* is susceptible to heart pulsations (Chang et al., 2009). However, the impact of such corrections is likely to vary depending on the method used for estimating functional connectivity (e.g. ICA, dual-regression or seed-based) without any significant impact at the group level analysis (Starck et al., 2010). Because the aim of our study is to localize drug effects in the brain, we have refrained from performing any physiological correction that assumes a hemodynamic response function for the respiration and heart rate variations. Therefore, our results should be interpreted with the caveat that some of the regional drug effects might be confounded with signal change due to vascular motion.

Gender

The post-hoc analysis on pharmacokinetic gender differences showed a higher THC plasma concentration in females compared to males after the 2 mg dose. This study did not anticipate gender differences, which have rarely been examined in the literature. We administered a fixed dose using a nose clip to prevent surreptitious exhalation, whereas during recreational use (as cannabis), individual titration to the subjective effect could easily obscure most gender differences. Possible explanations for the pharmacokinetic gender differences found in this study are differences in height, weight, body composition, metabolism, hormones, or frequency of habitual cannabis use. This could not be explored further in this study, which was not designed to examine pharmacokinetic or pharmacodynamic gender differences. This would require future studies with a larger sample size and adequate considerations of other sex-associated confounders.

Concluding remarks and future directions

In line with previous findings, this study confirms that RS-FMRI seems a promising technique for clinical pharmacological studies and drug development (Khalili-Mahani et al., 2011; Cole et al., 2010). The possible THC concentration-effect relationship including the active metabolite 11-OH-THC needs to be further studied using PK-PD modelling. This would allow the quantitative examination of THC-induced effects on connectivity, including changes at low concentrations that might be observed without pronounced behavioural effects.

REFERENCES

Beckmann, C. F., DeLuca, M., Devlin, J. T., & Smith, S. M. (2005). Investigations into resting-state connectivity using independent component analysis. *Philos.Trans.R.Soc. Lond.B.Biol.Sci., 360,* 1001-1013.

Beckmann, C. F. & Smith, S. M. (2005). Tensorial extensions of independent component analysis for multisubject fMRI analysis. *Neuroimage, 25,* 294-311.

Benowitz, N. L., Jones, R. T., & Lerner, C. B. (1976). Depression of growth hormone and cortisol response to insulin-induced hypoglycemia after prolonged oral delta-9-tetrahydrocannabinol administration in man. *J Clin Endocrinol.Metab., 42,* 938-941.

Bhattacharyya, S., Fusar-Poli, P., Borgwardt, S., Martin-Santos, R., Nosarti, C., O'Carroll, C. et al. (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. *Arch.Gen.Psychiatry, 66,* 442-451.

Birn, R. M. (2012). The role of physiological noise in resting-state functional connectivity. *Neuroimage., Epub ahead of print*.

Birn, R. M., Smith, M. A., Jones, T. B., & Bandettini, P. A. (2008). The respiration response function: the temporal dynamics of fMRI signal fluctuations related to changes in respiration. *Neuroimage, 40,* 644-654.

Biswal, B. B., Mennes, M., Zuo, X. N., Gohel, S., Kelly, C., Smith, S. M. et al. (2010). Toward discovery science of human brain function. *Proc.Natl.Acad.Sci.U.S.A., 107,* 4734-4739.

Bocker, K. B., Hunault, C. C., Gerritsen, J., Kruidenier, M., Mensinga, T. T., & Kenemans, J. L. (2010). Cannabinoid modulations of resting state EEG theta power and working memory are correlated in humans. *J Cogn Neurosci., 22,* 1906-1916.

Bond, A. & Lader, M. (1974). The use of analogue scales in rating subjective feelings. *Br J Med Psychol, 47,* 211-218.

Borgwardt, S. J., Allen, P., Bhattacharyya, S., Fusar-Poli, P., Crippa, J. A., Seal, M. L. et al. (2008). Neural basis of Delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. *Biol. Psychiatry, 64,* 966-973.

Bowdle, T. A., Radant, A. D., Cowley, D. S., Kharasch, E. D., Strassman, R. J., & Roy-Byrne, P. P. (1998). Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. *Anesthesiology, 88,* 82-88.

Carter, R. M., O'Doherty, J. P., Seymour, B., Koch, C., & Dolan, R. J. (2006). Contingency awareness in human aversive conditioning involves the middle frontal gyrus. *Neuroimage, 29,* 1007-1012.

Chang, C., Cunningham, J. P., & Glover, G. H. (2009). Influence of heart rate on the BOLD signal: the cardiac response function. *Neuroimage, 44,* 857-869.

Chen, C. L., Willis, B. A., Mooney, L., Ong, G. K., Lim, C. N., Lowe, S. L. et al. (2010). Cortisol response to individualised graded insulin infusions: a reproducible biomarker for CNS compounds inhibiting HPA activation. *Br.J Clin Pharmacol, 70,* 886-894.

Cole, D. M., Beckmann, C. F., Long, C. J., Matthews, P. M., Durcan, M. J., & Beaver, J. D. (2010). Nicotine replacement in abstinent smokers improves cognitive withdrawal symptoms with modulation of resting brain network dynamics. *Neuroimage, 52,* 590-599.

Corchero, J., Fuentes, J. A., & Manzanares, J. (1999). Chronic treatment with CP-55,940 regulates corticotropin releasing factor and proopiomelanocortin gene expression in the hypothalamus and pituitary gland of the rat. *Life Sci., 64,* 905-911.

Crockett, D., Klonoff, H., & Clark, C. (1976). The effects of marijauna on verbalization and thought processes. *J.Pers.Assess., 40,* 582-587.

Damoiseaux, J. S., Rombouts, S. A., Barkhof, F., Scheltens, P., Stam, C. J., Smith, S. M. et al. (2006). Consistent resting-state networks across healthy subjects. *Proc Natl.Acad Sci U.S.A, 103,* 13848-13853.

Daw, N. D., O'Doherty, J. P., Dayan, P., Seymour, B., & Dolan, R. J. (2006). Cortical substrates for exploratory decisions in humans. *Nature., 441,* 876-879.

De Luca, M., Beckmann, C. F., De Stefano, N., Matthews, P. M., & Smith, S. M. (2006). fMRI resting state networks define distinct modes of long-distance interactions in the human brain. *Neuroimage, 29,* 1359-1367.

de Visser, S. J., van der Post, J., Pieters, M. S., Cohen, A. F., & van Gerven, J. M. (2001). Biomarkers for the effects of antipsychotic drugs in healthy volunteers. *Br.J.Clin Pharmacol, 51,* 119-132.

Desmond, J. E. & Glover, G. H. (2002). Estimating sample size in functional MRI (fMRI) neuroimaging studies: statistical power analyses. *J Neurosci.Methods., 118,* 115-128.

Dewey, W. L., Peng, T. C., & Harris, L. S. (1970). The effect of 1-trans-delta 9-tetrahydrocannabinol on the hypothalamo-hypophyseal-adrenal axis of rats. *Eur.J Pharmacol, 12,* 382-384.

du Boisgueheneuc, F., Levy, R., Volle, E., Seassau, M., Duffau, H., Kinkingnehun, S. et al. (2006). Functions of the left superior frontal gyrus in humans: a lesion study. *Brain., 129,* 3315-3328.

Eggan, S. M. & Lewis, D. A. (2007). Immunocytochemical distribution of the cannabinoid CB1 receptor in the primate neocortex: a regional and laminar analysis. *Cereb.Cortex., 17,* 175-191.

Emrich, H. M., Weber, M. M., Wendl, A., Zihl, J., von, M. L., & Hanisch, W. (1991). Reduced binocular depth inversion as an indicator of cannabis-induced censorship impairment. *Pharmacol Biochem.Behav, 40,* 689-690.

Filippini, N., MacIntosh, B. J., Hough, M. G., Goodwin, G. M., Frisoni, G. B., Smith, S. M. et al. (2009). Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. *Proc.Natl.Acad.Sci.U.S.A, 106,* 7209-7214.

Foltin, R. W., Fischman, M. W., Brady, J. V., Bernstein, D. J., Capriotti, R. M., Nellis, M. J. et al. (1990). Motivational effects of smoked marijuana: behavioral contingencies and low-probability activities. *J.Exp Anal.Behav, 53,* 5-19.

Fox, M. D., Snyder, A. Z., Vincent, J. L., & Raichle, M. E. (2007). Intrinsic fluctuations within cortical systems account for intertrial variability in human behavior. *Neuron., 56,* 171-184.

Goodwin, R. S., Baumann, M. H., Gorelick, D. A., Schwilke, E., Schwope, D. M., Darwin, W. D. et al. (2011). CB1 - Cannabinoid Receptor Antagonist Effects on Cortisol in Cannabis-Dependent Men. *Am J DrugAlcohol.Abuse.*.

Greicius, M. D., Srivastava, G., Reiss, A. L., & Menon, V. (2004). Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. *Proc Natl.Acad Sci U.S.A, 101,* 4637-4642.

Herkenham, M. (1992). Cannabinoid receptor localization in brain: relationship to motor and reward systems. *Ann.N.Y.Acad Sci, 654,* 19-32.

Hine, M. L. (1918). The recovery of fields of vision in concussion injuries of the occipital cortex. *Br.J Ophthalmol., 2,* 12-25.

Jha, A. P., Ranucci, M. B., & Giuliani, N. R. (2006). Organization of mnemonic and response operations within prefrontal cortex. *Brain.Res, 1097,* 133-141.

Khalili-Mahani, N., Zoethout, R. M., Beckmann, C. F., Baerends, E., de Kam, M. L., Soeter, R. P. et al. (2011). Effects of morphine and alcohol on functional brain connectivity during "resting state": A placebo-controlled crossover study in healthy young men. *Hum Brain.Mapp.*.

Kim, W., Doyle, M. E., Liu, Z., Lao, Q., Shin, Y. K., Carlson, O. D. et al. (2011). Cannabinoids inhibit insulin receptor signaling in pancreatic beta-cells. *Diabetes, 60,* 1198-1209.

Koechlin, E., Basso, G., Pietrini, P., Panzer, S., & Grafman, J. (1999). The role of the anterior prefrontal cortex in human cognition. *Nature., 399,* 148-151.

Koethe, D., Gerth, C. W., Neatby, M. A., Haensel, A., Thies, M., Schneider, U. et al. (2006). Disturbances of visual information processing in early states of psychosis and experimental delta-9-tetrahydrocannabinol altered states of consciousness. *Schizophr.Res, 88,* 142-150.

Kolmel, H. W. (1988). Pure homonymous hemiachromatopsia. Findings with neuro-ophthalmologic examination and imaging procedures. *Eur.Arch.Psychiatry Neurol.Sci., 237,* 237-243.

Laird, A. R., Fox, P. M., Eickhoff, S. B., Turner, J. A., Ray, K. L., McKay, D. R. et al. (2011). Behavioral interpretations of intrinsic connectivity networks. *J Cogn Neurosci., 23,* 4022-4037.

Mathew, R. J., Wilson, W. H., Chiu, N. Y., Turkington, T. G., DeGrado, T. R., & Coleman, R. E. (1999). Regional cerebral blood flow and depersonalization after tetrahydrocannabinol administration. *Acta Psychiatr. Scand., 100,* 67-75.
Mathew, R. J., Wilson, W. H., Turkington, T. G., & Coleman, R. E. (1998). Cerebellar activity and disturbed time sense after THC. *Brain. Res, 797,* 183-189.
Mathew, R. J., Wilson, W. H., Turkington, T. G., Hawk, T. C., Coleman, R. E., DeGrado, T. R. et al. (2002). Time course of tetrahydrocannabinol-induced changes in regional cerebral blood flow measured with positron emission tomography. *Psychiatry Res, 116,* 173-185.
McDonald, J., Schleifer, L., Richards, J. B., & de, W. H. (2003). Effects of THC on behavioral measures of impulsivity in humans. *Neuropsychopharmacology, 28,* 1356-1365.
Mennes, M., Kelly, C., Zuo, X. N., Di, M. A., Biswal, B. B., Castellanos, F. X. et al. (2010). Inter-individual differences in resting-state functional connectivity predict task-induced BOLD activity. *Neuroimage, 50,* 1690-1701.
Miller, E. K. & Cohen, J. D. (2001). An integrative theory of prefrontal cortex function. *Annu. Rev Neurosci., 24,* 167-202.
Morrison, P. D., Zois, V., McKeown, D. A., Lee, T. D., Holt, D. W., Powell, J. F. et al. (2009). The acute effects of synthetic intravenous Delta9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. *Psychol. Med, 39,* 1607-1616.
Nichols, T. E. & Holmes, A. P. (2002). Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum. Brain. Mapp., 15,* 1-25.
Nyberg, L., Kim, A. S., Habib, R., Levine, B., & Tulving, E. (2010). Consciousness of subjective time in the brain. *Proc. Natl. Acad. Sci. U S. A., 107,* 22356-22359.
Quigley, M., Cordes, D., Turski, P., Moritz, C., Haughton, V., Seth, R. et al. (2003). Role of the corpus callosum in functional connectivity. AJNR. *Am. J. Neuroradiol., 24,* 208-212.
Ramaekers, J. G., Kauert, G., Theunissen, E. L., Toennes, S. W., & Moeller, M. R. (2009). Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *J. Psychopharmacol., 23,* 266-277.
Ranganathan, M., Braley, G., Pittman, B., Cooper, T., Perry, E., Krystal, J. et al. (2009). The effects of cannabinoids on serum cortisol and prolactin in humans. *Psychopharmacology. (Berl.), 203,* 737-744.
Romero, J., Hillard, C. J., Calero, M., & Rabano, A. (2002). Fatty acid amide hydrolase localization in the human central nervous system: an immunohistochemical study. *Brain. Res Mol. Brain. Res, 100,* 85-93.
Rose, J. E., McClernon, F. J., Froeliger, B., Behm, F. M., Preud'homme, X., & Krystal, A. D. (2011). Repetitive Transcranial Magnetic Stimulation of the Superior Frontal Gyrus Modulates Craving for Cigarettes. *Biol. Psychiatry*.
Smith, S. M., Fox, P. T., Miller, K. L., Glahn, D. C., Fox, P. M., Mackay, C. E. et al. (2009). Correspondence of the brain's functional architecture during activation and rest. *Proc. Natl. Acad. Sci. U S. A., 106,* 13040-13045.
Smith, S. M. & Nichols, T. E. (2009). Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage, 44,* 83-98.
Stokes, P. R., Egerton, A., Watson, B., Reid, A., Breen, G., Lingford-Hughes, A. et al. (2010). Significant decreases in frontal and temporal [11C]-raclopride binding after THC challenge. *Neuroimage, 52,* 1521-1527.
Stoodley, C. J. & Schmahmann, J. D. (2009). Functional topography in the human cerebellum: a meta-analysis of neuroimaging studies. *Neuroimage, 44,* 489-501.
Strougo, A., Zuurman, L., Roy, C., Pinquier, J. L., van Gerven, J. M., Cohen, A. F. et al. (2008). Modelling of the concentration-effect relationship of THC on central nervous system parameters and heart rate - insight into its mechanisms of action and a tool for clinical research and development of cannabinoids. *J Psychopharmacol, 22,* 717-726.
Tinklenberg, J. R., Kopell, B. S., Melges, F. T., & Hollister, L. E. (1972). Marihuana and alcohol, Time production and memory functions. *Arch. Gen. Psychiatry, 27,* 812-815.
van Hell, H. H., Bossong, M. G., Jager, G., Kristo, G., van Osch, M. J., Zelaya, F. et al. (2011). Evidence for involvement of the insula in the psychotropic effects of THC in humans: a double-blind, randomized pharmacological MRI study. *Int. J. Neuropsychopharmacol.,* 1-12.
Venkatraman, V., Rosati, A. G., Taren, A. A., & Huettel, S. A. (2009). Resolving response, decision, and strategic control: evidence for a functional topography in dorsomedial prefrontal cortex. *J. Neurosci., 29,* 13158-13164.
Wenger, T., Fernandez-Ruiz, J. J., & Ramos, J. A. (1999). Immunocytochemical demonstration of CB_1 cannabinoid receptors in the anterior lobe of the pituitary gland. *J Neuroendocrinol., 11,* 873-878.
Winton-Brown, T. T., Allen, P., Bhattacharyya, S., Borgwardt, S. J., Fusar-Poli, P., Crippa, J. A. et al. (2011). Modulation of auditory and visual processing by delta-9-tetrahydrocannabinol and cannabidiol: an FMRI study. *Neuropsychopharmacology, 36,* 1340-1348.
Wise, R. G. & Preston, C. (2010). What is the value of human FMRI in CNS drug development? *Drug Discov. Today., 15,* 973-980.
Zhang, Y., Brady, M., & Smith, S. (2001). Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. CAMS. *Trans. Med Imaging, 20,* 45-57.
Zuurman, L., Ippel, A. E., Moin, E., & van Gerven, J. M. (2009). Biomarkers for the effects of cannabis and THC in healthy volunteers. *Br J Clin Pharmacol, 67,* 5-21.
Zuurman, L., Roy, C., Schoemaker, R., Amatsaleh, A., Guimaeres, L., Pinquier, J. et al. (2008). Inhibition of THC-induced effects on the central nervous system and heart rate by a novel CB_1 receptor antagonist AVE1625. *J Psychopharmacol*.
Zuurman, L., Roy, C., Schoemaker, R., Hazekamp, A., den Hartigh, J., Bender, J. et al. (2008). Effect of intrapulmonary tetrahydrocannabinol administration in humans. *J Psychopharmacol, 22,* 707-716.

TABLE 1 Demographics of the subjects that were included for pharmacodynamic and pharmacokinetic analyses. SD = standard deviation

Gender	Variable	N	Mean	SD
All	Age (year)	12	22.17	2.95
	BMI (kg/m²)	12	22.36	2.55
	Height (m)	12	1.82	0.09
	Weight (kg)	12	74.33	13.17
Female	Age (year)	3	23.33	2.52
	BMI (kg/m²)	3	22.07	0.32
	Height (m)	3	1.70	0.06
	Weight (kg)	3	63.83	4.93
Male	Age (year)	9	21.78	3.11
	BMI (kg/m²)	9	22.46	2.97
	Height (m)	9	1.86	0.05
	Weight (kg)	9	77.82	13.32

TABLE 2 Pharmacokinetic parameters of THC as assessed by non-compartmental pharmacokinetic analysis. CV = coefficient of variation, IIV = interindividual variability, Cl = clearance, F = bioavailability, V = distribution volume, Q= intercompartmental clearance, $t_{1/2}$ = initial half-life, NA = not applicable.

Parameter Units	Median	Uncertainty (%CV)	IIV (%CV)
CL/F (L/hr)	145	11.2	37.4
V/F (L)	20.1	12.5	37.4
V peripheral (L)	78.6	15.8	37.4
Q (L/hr)	95.6	15.1	37.4
$t_{1/2}$ (hr)	0.986	4.91	NA

TABLE 3 Overview of the significant decreases and increases of connectivity (p<0.05, threshold-free cluster enhancement corrected). The areas in grey are significant regions after Bonferroni correction.

Networks		Region (Harvard-Oxford)	t-value[a]	X	Y	Z	Voxel number	THC effect[b]
NO11: Medial visual	L	Superior and medial frontal gyrus (premotor cortex)	5.32	-24	-2	46	26	-
	B	Dorsal ACC	5.02	2	12	34	20	-
	L	Temporal occipital fusiform cortex	4.9	-30	-48	-18	17	-
NO12: Lateral visual	L	Temporal occipital fusiform cortex (extending into para-hippocampal gyrus & hippo-campus)	4.91	-30	-48	-12	163	+
	L	Ventromedial cerebellum	5.03	-22	-60	-44	36	+
	R	Temporal occipital fusiform cortex (extending into parahip-pocampal gyrus)	4.16	26	-38	-18	22	+
	R	Middle frontal gyrus, dlPFC	5.34	28	20	44	14	+
	B	Posterior precuneous cortex	4.8	2	-74	42	203	-
	R	occipital pole, lateral occipital cortex	4.3	34	-92	-10	27	-
	R	Precentral gyrus	5.54	46	-10	58	12	-
NO13: Auditory	R	Parahippocampal gyrus (extending into hippocampus)	4.66	40	-34	-10	132	+
	B	PCC, retrosplenial cortex	5.04	0	-46	2	20	+
	R	Caudate	5.86	20	32	2	16	+
	B	Supramarginal gyrus, superior/ medial/inferior temporal gyri, temporal pole, parahippocampal gyrus, lateral OFC	6.07	62	-30	28	25075	-
	B	Superior frontal gyrus, dmPFC	4.83	0	50	28	262	-
	R	Frontal pole, dmPFC	4.13	10	60	22	104	-
	L	Precentral gyrus, superior parietal cortex	3.85	-10	-16	64	52	-
	B	Mid-cingulate cortex	4.26	-4	-8	34	38	-
	L	Precentral gyrus, superior mid-cingulate cortex	4.11	-4	-20	50	30	-
	R	vmPFC	4.12	16	54	6	28	-
	R	Middle frontal gyrus	3.36	46	10	40	19	-
	R	Precentral gyrus, superior mid-cingulate	4.12	6	-26	54	17	-

(Table continues on next page)

Networks		Region (Harvard-Oxford)	t-value[a]	X	Y	Z	Voxel number	THC effect[b]
NO14: Sensorimotor	L	Midbrain	3.92	-8	-28	-18	22	+
	B	**Cerebellum (more extensive in right hemisphere)**	**6.36**	**14**	**-70**	**-50**	**6101**	+
	L	**Cerebellum (antero-ventral)**	**5.53**	**-20**	**-48**	**-54**	**47**	+
	L	**Cerebellum (ventromedial)**	**5.15**	**-10**	**-64**	**-50**	**5.15**	+
	R	Postcentral gyrus	5.09	40	-32	62	184	-
	R	Precuneous cortex	4.28	14	-46	44	169	-
	R	Superior posterior parietal cortex	4.39	20	-56	62	142	-
	L	Superior posterior parietal cortex	3.73	-22	-54	50	99	-
	L	Postcentral gyrus, superior parietal cortex	4.39	-18	-40	64	71	-
	R	Superior posterior parietal cortex (mid-superior)	4.24	30	-42	64	57	-
NO15: Default mode	L	Frontal pole, dorsal PFC	5.17	-28	46	16	35	+
	L	Intracalcarine (visual) cortex	5.63	-18	-80	10	17	+
NO16: Executive/ salience	L	Precuneous Cortex.	5.19	-8	-58	36	17	-
NO17: Right dorsal visual stream	**B**	**Frontal pole, dmPFC**	**5.69**	**-12**	**66**	**10**	**149**	+
	L	**Frontal pole, dmPFC**	**4.97**	**-12**	**54**	**30**	**130**	+
	L	**dmPFC, frontal pole, superior frontal gyrus**	**4.53**	**-2**	**52**	**30**	**15**	+
	R	**Superior frontal pole, middle frontal gyrus, dlPFC, inferior frontal gyrus**	**5.44**	**38**	**38**	**24**	**53**	-
	R	Superior frontal pole, inferior and medial frontal gyrus, dlPFC	5.44	38	38	24	324	-
	R	Superior frontal pole, inferior frontal gyrus (partial)	4.16	32	50	14	138	-
	R	Frontal pole (inferior), ventrolateral PFC	4.61	44	52	-6	109	-
NO18: Left dorsal visual stream	R	Occipital pole, lateral occipital cortex	5.01	42	-92	2	770	+
	L	Pre and post-central gyrus, central sulcus	4.74	-44	-16	40	29	+
	R	**Occipital pole, lateral occipital cortex**	**5.01**	**42**	**-92**	**2**	**52**	+
	B	PCC	4.7	-2	-38	24	105	-

a Uncorrected peak t-value
b The minus (-) indicates a connectivity decrease after THC, and the plus (+) an increase.
Abbreviations: L - left, R - right, B - bilateral, ACC/PCC - anterior/posterior cingulated cortex, PFC - prefrontal cortex, dlPFC - dorso-lateral PFC, dmPFC - dorso-medial PFC, vmPFC - ventro-medial PFC, OFC - orbito-frontal cortex

TABLE 4 Overview of the non-FMRI pharmacodynamic parameters

	LSM Treatment			Contrasts	LSM change from baseline	
Parameter	Placebo	THC	P-value	THC vs Placebo	Placebo	THC
VAS Alertness (mm)	52.5	45.9	0.0646	-6.6 (-13.7, 0.5)	1.3	-5.3
VAS Calmness (mm)	53.9	55	0.2248	1.1 (-0.8, 3.0)	0.3	1.4
VAS Mood (mm)	55	55	0.9787	0.1 (-4.6, 4.8)	-0.6	-0.5
VAS External log (mm)	0.32	0.545	0.0149*	0.225 (0.054, 0.396)	0.008	0.233
VAS Internal log (mm)	0.308	0.346	0.0718	0.037 (-.004, 0.079)	0.004	0.041
VAS feeling high log (mm)	0.285	1.053	<.0001*	0.768 (0.578, 0.957)	-0.02	0.748
Heart rate (BPM)	66.7	77	0.0026*	10.3 (4.4, 16.2)	-1.8	8.4
FSH (U/L)	2.327	2.291	0.5601	-1.56% (-7.11%, 4.33%)	-3.87	-5.37
LH (ng/ml)	4.16	3.15	0.0935	-24.20% (-45.9%, 6.12%)	2.02	-22.71
Cortisol (µmol/ml)	0.36	0.47	0.0051*	32.21% (11.87%, 56.25%)	-29.24	-6.45
Prolactin (µgr/l)	8.41	6.64	0.0100*	-21.00% (-33.0%, -7.01%)	-28.68	-43.69
Glucose (mmol/l)	4.7	5.1	0.0468*	7.2% (0.1%, 14.8%)	-4.64	2.25
HDL cholesterol (mmol/l)	1.12	1.12	0.8933	0.63% (-9.21%, 11.53%)	0.93	1.57
Leptin (µg/l)	3.6	3.7	0.8328	1.95% (-16.6%, 24.62%)	-2.94	-1.04
Triglycerides (mmol/l)	0.98	0.97	0.9086	-0.70% (-13.2%, 13.64%)	3.47	2.74

* Statistically significant values

FIGURE 1 Visual representation of the chronological study day activities after each THC inhalation. The horizontal axis represents the time line and should be read from left to right. The vertical lines connected to the dots represent the relative time points for the activities indicated in the boxes. The grey lines represent measurements that were only performed after the third THC inhalation. The time points are given in hours and minutes relative to the THC administration, and refer to THC administration and RS-MRI measurements. At the first blood sample (1) for each cycle, only a PK sample was taken.

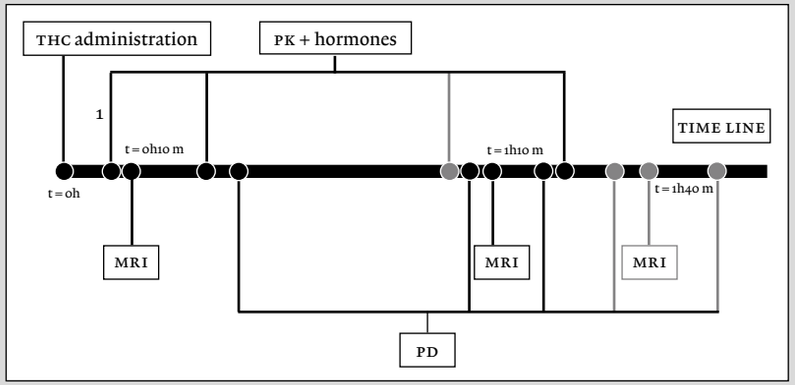


FIGURE 2 Mean THC plasma concentration (+ standard deviation) graph. THC was administered at 0 min (2 mg), 90 min (6 mg), and 180 min (6 mg).

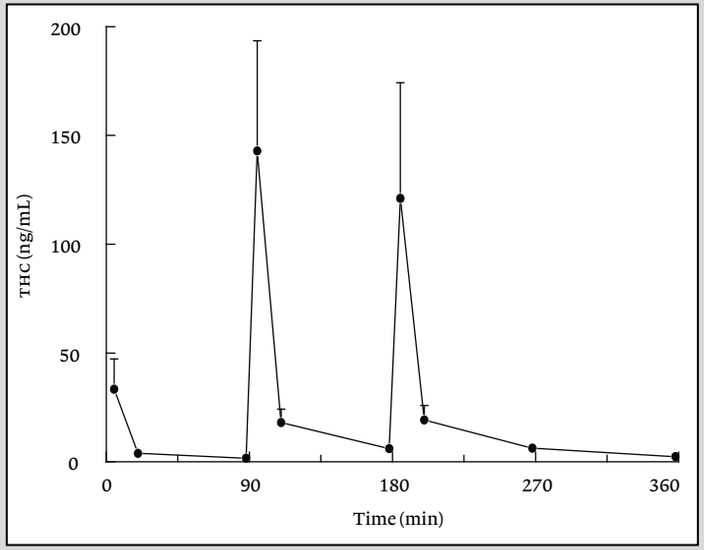


FIGURE 3 Mean THC plasma concentration (+ standard deviation) graph by gender. Dots = males; Circles = females.

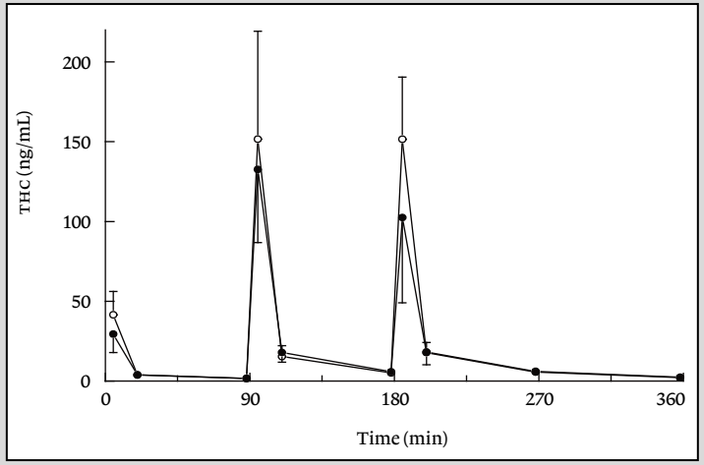
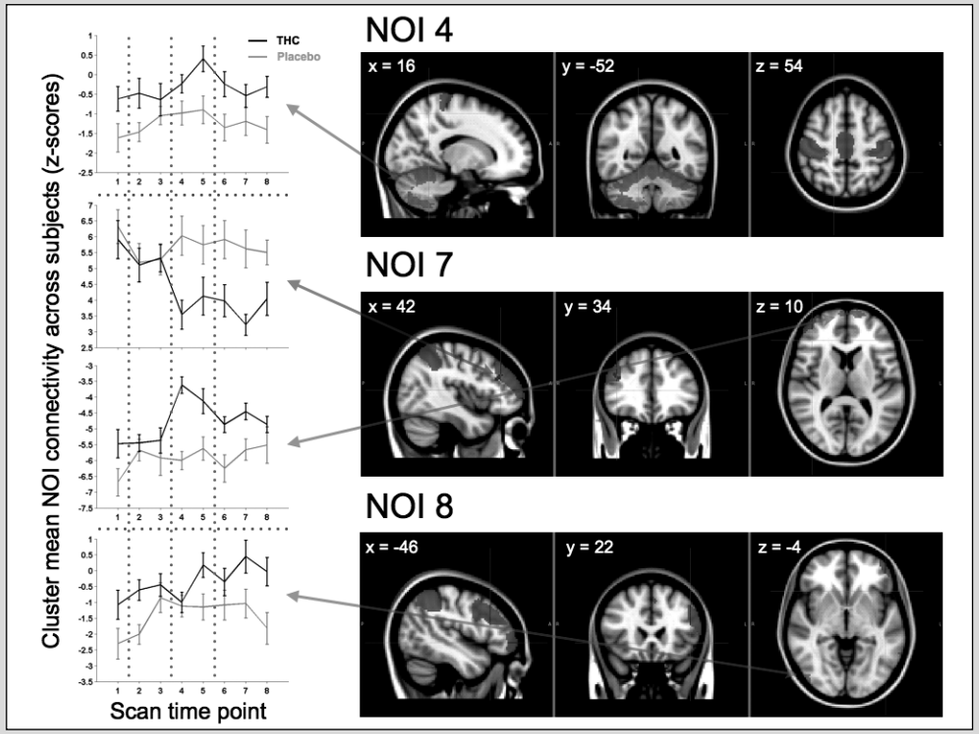


FIGURE 4 Brain regions showing clusters of significant differences (Bonferroni corrected) in NOI functional connectivity following THC relative to placebo. Spatial maps (right): Axial and coronal slices are displayed in radiological convention such that left=right. Green = NOI, Red = Connectivity increase after THC relative to placebo, Blue = Connectivity decrease after THC relative to placebo; crosshairs indicate position of displayed slices. Connectivity changes across time (left): plots visualise z-scores resulting from each significant contrast (Bonferroni corrected) only, split by scan time point and averaged across clusters and subjects, separately for placebo (grey) and THC (black) conditions. Error bars represent the standard error of the mean. Vertical green dotted lines indicate the three points at which a dose was inhaled. Red and blue arrows link the associated spatial and temporal information.



(see inside cover for this figure in colour)

FIGURE 5 Graphs of pharmacodynamic effects, with VAS feeling high (figure A) average scores of log (mm) + standard deviations (SD), and mean heart rate + SD (figure B). Open circle: THC, closed circle: placebo. THC inhalations were given at time points 0, 90, and 180 min.