Journal of Psychopharmacology

Dissociable effects of cannabis with and without cannabidiol on the human brain's resting-state functional connectivity.

Journal:	*Journal of Psychopharmacology*
Manuscript ID	JOP-2018-3591.R1
Manuscript Type:	Original Paper
Date Submitted by the Author:	21-Jan-2019
Complete List of Authors:	Wall, Matthew B; Invicro London, Clinical Applications; Imperial College London, Division of Brain Sciences; University College London, Department of Clinical, Educational and Health Psychology Pope, Rebecca; University College London, Department of Clinical, Educational and Health Psychology Freeman, Tom; University College London, Clinical Psychopharmacology Unit; University of Bath, Department of Psychology; Kings College London, Institure of Psychiatry, Psychology, and Neuroscience Kowalczyk, Olivia; Institute of Psychiatry, Psychology & Neuroscience, Centre for Neuroimaging Sciences, Demetriou, Lysia; Invicro London, Clinical Applications; Imperial College London, Division of Brain Sciences Mokrysz, Claire; University College London, Clinical Psychopharmacology Unit Hindocha, Chandni; University College London, Department of Clinical, Educational and Health Psychology Lawn, Will; University College London, Department of Clinical, Educational and Health Psychology Bloomfield, Michael; University College London, Department of Clinical, Educational and Health Psychology; University College London, Division of Psychiatry; Imperial College London, MRC London Institute of Clinical Sciences Freeman, Abigail; University College London, Department of Clinical, Educational and Health Psychology Feilding, Amanda; The Beckley Foundation, . Nutt, David; Imperial College , Neuropychopharmacology Curran, H V; University College London, Department of Clinical, Educational and Health Psychology
Please list at least 3 keywords which relate to your manuscript::	Cannabis, fMRI, Resting-State, Cannabidiol, THC
Abstract:	Background: Two major constituents of cannabis are Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is the main psychoactive component; CBD may buffer the user against the harmful effects of THC. Aims: We examined the effects of two strains of cannabis and placebo on the human brain's resting-state networks using fMRI.

	Methods: 17 healthy volunteers (experienced with cannabis, but not regular users) underwent three drug treatments and scanning sessions. Treatments were cannabis containing THC (Cann-CBD; 8mg THC), cannabis containing THC with CBD (Cann+CBD; 8mg THC + 10mg CBD), and matched placebo cannabis. Seed-based resting-state functional-connectivity analyses were performed on three brain networks: the default mode (DMN; defined by positive connectivity with the posterior cingulate cortex: PCC+), executive control (ECN; defined by negative connectivity with the posterior cingulate cortex: PCC-) and salience (SAL; defined by positive connectivity with the anterior insula: AI+) network. Results: Reductions in functional connectivity (relative to placebo) were seen in the DMN (PCC+) and SAL (AI+) networks for both strains of cannabis, with spatially dissociable effects. Across the entire salience network (AI+) Cann-CBD reduced connectivity relative to Cann+CBD. The PCC in the DMN was specifically disrupted by Cann-CBD and this effect correlated with subjective drug effects including feeling 'stoned', and 'high'. Conclusions: THC disrupts the default mode network and the PCC is a key brain region involved in the subjective experience of THC intoxication. CBD restores disruption of the salience network by THC, which may explain its potential to treat disorders of salience such as psychosis and addiction.

Dissociable effects of cannabis with and without cannabidiol on the human brain's resting-state functional connectivity.

Matthew B. Wall[1,2,3], Rebecca Pope[2], Tom P. Freeman[2,4,5], Oliwia S. Kowalczyk[6], Lysia Demetriou[1,3], Claire Mokrysz[2], Chandni Hindocha[2], Will Lawn[2], Michael A. P. Bloomfield[2,7,8], Abigail M. Freeman[2], Amanda Feilding[9], David J. Nutt[3], & H. Valerie Curran[2]

[1]Invicro London, Burlington Danes Building, Hammersmith Hospital, Du Cane Road, London, W12 0NN, UK

[2]Clinical Psychopharmacology Unit, University College London, 1-19 Torrington Place, London, WC1E 7HB, UK.

[3]Division of Brain Sciences, Imperial College London, Hammersmith Campus, Du Cane Road, London, W12 0NN, UK

[4]Addiction and Mental Health Group (AIM), Department of Psychology, University of Bath, Claverton Down, Bath BA2 7AY

[5]National Addiction Centre, Institute of Psychiatry, Psychology & Neuroscience, London SE5 8BB

[6]Centre for Neuroimaging Sciences, Institute of Psychiatry, Psychology and Neuroscience, Kings College London, De Crespigny Park, Camberwell, London, SE6 8AF, UK.

[7]Division of Psychiatry, University College London, B Wing, 6th Floor, Maple House, 149 Tottenham Court Road, London, W1T 7NF, UK

[8]Psychiatric Imaging, MRC London Institute of Clinical Sciences, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN

[9]The Beckley Foundation, Beckley Park, Oxford, UK.

Short Title: Resting-state fMRI of different strains of cannabis

Keywords: Cannabis, cannabidiol, THC, fMRI, Resting-State, marijuana, Default mode network, Salience network.

Corresponding Author:

Matthew Wall
Invicro London
Burlington Danes Building
Hammersmith Hospital
Du Cane Road
London, W12 0NN
United Kingdom
matt.wall@invicro.co.uk

Abstract

Background: Two major constituents of cannabis are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is the main psychoactive component; CBD may buffer the user against the harmful effects of THC.

Aims: We examined the effects of two strains of cannabis and placebo on the human brain's resting-state networks using fMRI.

Methods: 17 healthy volunteers (experienced with cannabis, but not regular users) underwent three drug treatments and scanning sessions. Treatments were cannabis containing THC (Cann-CBD; 8mg THC), cannabis containing THC with CBD (Cann+CBD; 8mg THC + 10mg CBD), and matched placebo cannabis. Seed-based resting-state functional-connectivity analyses were performed on three brain networks: the default mode (DMN; defined by positive connectivity with the posterior cingulate cortex: PCC+), executive control (ECN; defined by negative connectivity with the posterior cingulate cortex: PCC-) and salience (SAL; defined by positive connectivity with the anterior insula: AI+) network.

Results: Reductions in functional connectivity (relative to placebo) were seen in the DMN (PCC+) and SAL (AI+) networks for both strains of cannabis, with spatially dissociable effects. Across the entire salience network (AI+) Cann-CBD reduced connectivity relative to Cann+CBD. The PCC in the DMN was specifically disrupted by Cann-CBD and this effect correlated with subjective drug effects including feeling 'stoned', and 'high'.

Conclusions: THC disrupts the default mode network and the PCC is a key brain region involved in the subjective experience of THC intoxication. CBD restores disruption of the salience network by THC, which may explain its potential to treat disorders of salience such as psychosis and addiction.

Declaration of interest and funding

This study was funded by Drug Science, Channel 4 Television, and the Beckley Foundation. Author AF is involved with a cannabis-related business: Beckley Canopy Therapeutic. All other authors declare no relevant conflicts of interest.

Introduction

Cannabis has been used by humans for thousands of years for medical, spiritual, and recreational purposes. Two of the main psychoactive ingredients of cannabis are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). As well as making people "stoned", THC produces amnestic, anxiogenic, and psychotomimetic effects (including perceptual distortions, paranoia, disruptions of cognitive functions, and euphoria; D'Souza et al., 2004), by acting as an agonist at endocannabinoid 1 (CB1) receptors (Pertwee, 2008). CBD's effects have been less well studied, but early findings suggest it may have somewhat opposite effects, being anti-psychotic (Leweke et al., 2012), and perhaps anxiolytic (Bergamaschi et al., 2011). CBD is non-intoxicating, and has a more complex neuropharmacological profile, including reducing the cellular reuptake and hydrolysis of anandamide, antagonism of the orphan receptor GPR55 and the 5-HT1A receptor, and antagonism of the CB1 receptor with a low affinity (Pertwee, 2008).

THC is also largely responsible for providing many of the subjective effects of intoxication that recreational users seek (Curran et al., 2002). Concern has recently been raised about the high levels of THC found in modern cannabis, alongside minimal, if any, levels of CBD (ElSohly et al., 2016; Niesink et al., 2015). This high-strength cannabis (often referred to as 'skunk') is popular with users, but is also hypothesised to be responsible for the dramatic increase in reporting of cannabis-related health issues in recent years; most notably addiction, and cannabis-induced psychosis (Di Forti et al. 2009; Freeman et al., 2018; Freeman and Winstock, 2015). Because of its putatively opposing psychological and pharmacological effects, cannabis that contains higher levels of CBD may be a safer option on the basis that CBD may buffer the user against the main negative effects of THC (Curran et al., 2016; Englund et al., 2013; Hindocha et al., 2015; Niesink and van Laar, 2013).

As cannabis transitions to legal/decriminalised status in many jurisdictions, understanding the neural effects of different strains of cannabis (with different levels of THC and CBD) is now a priority for public health. Functional Magnetic Resonance Imaging (fMRI) is a popular method for indexing drug effects (Bourke and Wall, 2015; Iannetti and Wise, 2007), with resting-state fMRI (Fox and Raichle, 2007; Luca et al., 2006) particularly useful, as it can derive results from multiple brain systems, and provides a sensitive index of drug effects (e.g. Carhart-Harris et al., 2015; Kaelen et al., 2016). The DMN is perhaps the most prominent and well-studied resting-state network and its activity increases in periods of

wakeful rest, and during internally-focussed states such as autobiographical memory retrieval (Buckner et al., 2008). In contrast, its complementary network (the Executive Control Network, or ECN) is most active when subjects are engaged on an external task (Fox et al., 2005). The Salience network (Seeley et al., 2007) is involved in the detection of emotional and sensory stimuli, and may be responsible for the switch between internally-focussed states supported by the DMN, and externally-focussed states supported by the ECN (Goulden et al., 2014). Unfortunately the differential effects of herbal cannabis with different concentrations of THC and CBD on these networks is largely unknown. Most previous neuroimaging studies using an acute drug challenge have focussed on the effects of synthetic THC (e.g. Klumpers et al., 2012). Bossong and colleagues (2013) demonstrated acute disruptive effects of synthetic THC on the Default Mode Network (DMN), but in the context of an executive function task, with less effect on task-related brain regions. A recent study has also found similar results (reduction in default mode function) using the CB1 neutral antagonist tetrahydrocannibivarin (THCv; Rzepa et al., 2016). Another set of studies has compared oral synthetic THC and CBD, and found opposite effects of the two treatments on a range of functional and perceptual tasks, including differing effects on brain regions involved in salience processing (Bhattacharyya et al., 2010, 2012, 2014; Winton-Brown et al., 2011). Further studies have focussed on other resting-state connectivity networks, including corticostriatal connectivity (Grimm et al., 2018; Ramaekers et al., 2016), and the insula and frontal lobe (van Hell et al., 2011)

Our aim was to use fMRI to directly investigate the effects of different strains of herbal cannabis on resting-state functional connectivity, using one strain containing high levels of THC but negligible levels of CBD (Cann-CBD), and another strain containing more balanced levels of THC and CBD (Cann+CBD). Both treatments were matched for total THC content, and were compared to placebo cannabis (containing neither compound), which was well matched for terpene content and therefore had the same smell and appearance as active treatments. We hypothesized that the Cann-CBD treatment would induce more disruption (i.e. reductions in functional connectivity measures) in resting-state networks than the Cann+CBD strain.

Methods

Design and Participants

A randomised, crossover, placebo-controlled, double-blind design was used to compare cannabis containing both THC and CBD (Cann+CBD), cannabis containing THC but no CBD (Cann-CBD), and matched placebo cannabis containing neither compound. Participants were randomly assigned to one of three treatment order conditions, based on a Latin Square design. In order to eliminate potential carry-over effects, scanning sessions were separated by wash-out periods of at least one week, which is more than three times the elimination half-life of THC (Hindocha et al., 2014, 2015). Additional data from this study have been published elsewhere (Freeman, Pope, Wall, Bisby, Luijten, Hindocha, Mokrysz, Lawn, Bloomfield, et al., 2017; Lawn et al., 2016).

Participants were 17 (9 female) healthy volunteers. Inclusion criteria were age between 18-70, cannabis use $\leq$ 3 times per week and ≥4 times in the last year, and fluency in English. Exclusion criteria were previous negative experiences with cannabis, alcohol use >5 times per week, other illicit drug use > twice per month, current/history of psychosis, current/history of psychosis in an immediate family member, colour blindness, any other physical health problems deemed clinically significant, and general MRI contraindications. The mean age of subjects was 26.2 (SD = 7.1), and they reported using cannabis an average of 8.1 days per month (SD = 5.5). Full demographic data and information about current drug use for the group is provided in the supplementary material (Table S1). The study was approved by the University College London (UCL) Ethics Committee and was conducted in accordance with the Declaration of Helsinki. Subjects provided written informed consent, were reimbursed £7.50/hour, and could also win extra money via completion of other tasks (not reported here).

Drug Administration

Cannabis was sourced from Bedrocan (The Netherlands) and stored in foil-sealed pouches at -20°C, and then at ambient temperature immediately prior to administration. All three varieties of cannabis were well matched in terms of appearance and smell, and the same amount of cannabis (133.4mg) was administered in each session (see (Lawn et al., 2016) for full details of the dosing regime). Target doses were 8mg THC and 10 mg CBD (in the

Cann+CBD treatment) and 8mg THC (in the Cann-CBD treatment). This is equivalent to roughly 25% of an average UK joint, assuming a roughly 10% THC content (Freeman et al., 2014). Doses were vaporized in a Volcano Medic Vaporizer (Storz and Bickel, Tuttlingen, Germany) at 210ºC, and the resulting vapour was collected in two balloons. These were inhaled sequentially at the participants' own pace, with each inhalation held in the lungs for eight seconds, until the balloons were empty. This administration protocol using a vaporizer and inhaled balloons was similar to previous studies that have produced clear behavioural and brain effects with similar dosages (Bossong et al., 2009; Hindocha et al., 2015; Mokrysz et al., 2016).

Procedure

Participants completed a baseline/screening session consisting of task training (outside of the MRI scanner), video training for the vaporizer protocol, heart rate and blood pressure readings, and trait measures (BDI, TEPS, SDS, drug history). Subjects were asked to refrain from drug and alcohol use for 24 hours before each test session, and each session began with a urine screen to confirm recently reported drug use. Approximately 30 minutes following drug administration, participants were situated in the MRI scanner, and completed an approximately one-hour scanning session. The scanning session included standard anatomical scans, a music listening task (Freeman et al., 2017) a memory task, and a resting-state scan (reported herein). Ratings of subjective effects using Visual Analogue Scales (VAS) were administered immediately before the drug dosing, approximately five minutes after drug dosing, and approximately 90 minutes after drug dosing (after the MRI scan). These consisted of the following items: "Alert", "Happy", "Anxious", "Paranoid", "Mentally impaired", "Stoned", "High", "Feel drug effect", "Like drug effect", "Dry mouth", "Enhanced colour perception", "Enhanced sound perception", "Want to listen to music", "Want food", and "Want more cannabis". Analysis of the VAS scores has been reported elsewhere (Freeman et al., 2017; Lawn et al., 2016). Following the MRI scan subjects completed a number of additional behavioural tests and questionnaires; these are also fully reported elsewhere (Lawn et al., 2016).

MRI Acquisition and Analysis

Data were acquired on a Siemens Avanto 1.5T MRI scanner (Erlangen, Germany) using a 32-channel phased-array head-coil. At the beginning of the scan session standard MPRAGE

(Magnetization Prepared RApid Gradient Echo) anatomical scans were acquired (TR = 2730ms; TE = 3.57ms; matrix = 176 x 256 x 256; 1mm isotropic voxels; flip angle = 7°; bandwidth = 190Hz/pixel; parallel imaging acceleration factor = 2). The resting-state functional images were acquired with a gradient-echo Echo-Planar Imaging (EPI) sequence with a repetition time (TR) of 2800 ms, 32 slices with 3.2mm isotropic voxels, an echo-time (TE) of 43ms, and a flip-angle of 90°. A total of 260 volumes were acquired, for a total scan length of 12 minutes and 8 seconds.

All analyses were performed with FSL 5.0.4 (except where noted below). Pre-processing of the data consisted of head-motion correction, spatial smoothing with a 6mm FWHM (Full-Width, Half-Maximum) Gaussian kernel, high-pass temporal filtering (100s), and registration to a standard template (MNI152). Anatomical data were skull-stripped with FSL's Brain Extraction Tool (BET) and segmented into grey/white matter and CSF (Cerebro-Spinal Fluid) masks using FMRIB's Automated Segmentation Tool (FAST).

Seed-based functional connectivity analyses were conducted using the general methodological approach previously used by Demetriou et al. (2018) and (Comninos et al., 2018). Regions Of Interest (ROIs) were defined in the posterior cingulate cortex (PCC) and anterior insula (AI) as seed-regions (see supplementary figure S1). These regions were derived from automated meta-analytic data on http://neurosynth.org/, using the 'default mode' and 'salience' terms. These meta-analysis maps were thresholded, and the PCC and anterior insula clusters were isolated and binarised for use as image masks. These masks were co-registered to each individual participant's functional image space, thresholded (at 0.5), and time-series from these resulting mask images were extracted and used as the regressor of interest in separate first-level analysis models. Additional regressors modelled noise effects and were derived from the mean white matter and CSF anatomical masks (also co-registered to individual functional space, and thresholded at 0.5). Group-level analyses used FSL's FLAME-1 mixed-effects model and results were thresholded at $Z > 2.3$ ($p < 0.05$, cluster-corrected for multiple comparisons). Separate group-level models were produced in order to model mean functional connectivity effects (all subjects, all scans) and voxelwise comparisons between the three treatment conditions. The group mean functional connectivity results were used to produce image masks (thresholded at Z=5) in order to quantify the treatment effects across the entire network(s).

This procedure of defining resting-state networks using a single seed-region is an established method (Comninos et al., 2018; Passow et al., 2015; Seeley et al., 2007), however networks can also be defined by Independent Components Analysis (ICA), multi-seed region analysis, and various other more exotic methods (see Cole et al., 2010 for a review). The single-seed region method has benefits in that it is strongly hypothesis driven, and generally produces robust patterns of connectivity, which bear a strong relationship to the canonical networks derived from large-scale ICA analyses (e.g. Biswal et al., 2010; Smith et al., 2009). However, this is dependent on the selection of a suitable seed-region, and the main drawback of this method is potential bias and/or error in region selection. For this reason, and for the sake of absolute precision, we will henceforth refer to these networks as DMN (PCC+; positive connectivity with the PCC), ECN (PCC-; negative connectivity with the PCC), and the salience network or SAL (AI+; positive connectivity with the anterior insula).

Significant clusters resulting from these whole-brain analyses were defined as ROIs, and data from these ROIs was used to perform correlation analyses with VAS measures rated outside the scanner. A False Discovery Rate (FDR) correction for multiple comparisons (Benjamini and Hochberg, 1995) was applied to the *p* values resulting from these analyses within each brain region.

Results

Seed-based functional connectivity analyses

Group mean (all subjects, all scans) analyses of seed-based functional connectivity showed brain networks similar to those reported previously for the DMN and ECN (using the PCC seed region; e.g. Fox et al., 2005) and the salience network (using the anterior insula seed region; e.g. Seeley et al., 2007). There was also strong concordance between the observed networks and the meta-analytic maps available on http://neurosynth.org/ from which the original seed-regions were derived. These group mean connectivity maps are included in the supplementary material (see Figure S3).

Treatment effects on the mean connectivity across the entire network(s) are shown in Figure 1. Both treatments (relative to placebo) had similarly disruptive effects on the DMN (PCC+) network (Cann+CBD: $t[16] = 2.46$, $p = 0.026$; Cann-CBD: $t[16] = 2.22$, $p = 0.041$), and non-significant effects on the ECN (PCC-) network (all $p > 0.1$). In the SAL (AI+) network the Cann-CBD treatment caused a reduction in connectivity (relative to Cann+CBD; $t[16]=3.18$, $p = 0.005$), however neither of the two drug treatments were significantly different to placebo.

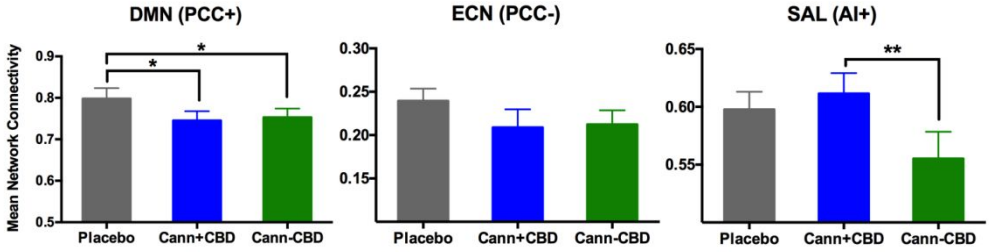

Figure 1. Treatment effects on the mean connectivity across the three networks; Default Mode Network (DMN; PCC+, left), Executive Control Network (ECN; PCC-, middle) and the Salience Network (SAL, AI+, right). * $p < 0.05$, ** $p < 0.005$. Error bars are standard errors.

Voxelwise comparison of the treatment conditions revealed that in the DMN (PCC+) network, both strains caused a decrease in functional connectivity in the right inferior parietal lobe, and the hippocampus, though effects were restricted to the right hippocampus for the Cann-CBD strain, and were bilateral for the Cann+CBD strain. There was also a specific effect of Cann-CBD cannabis in the PCC/precuneus region (see Figure 2).

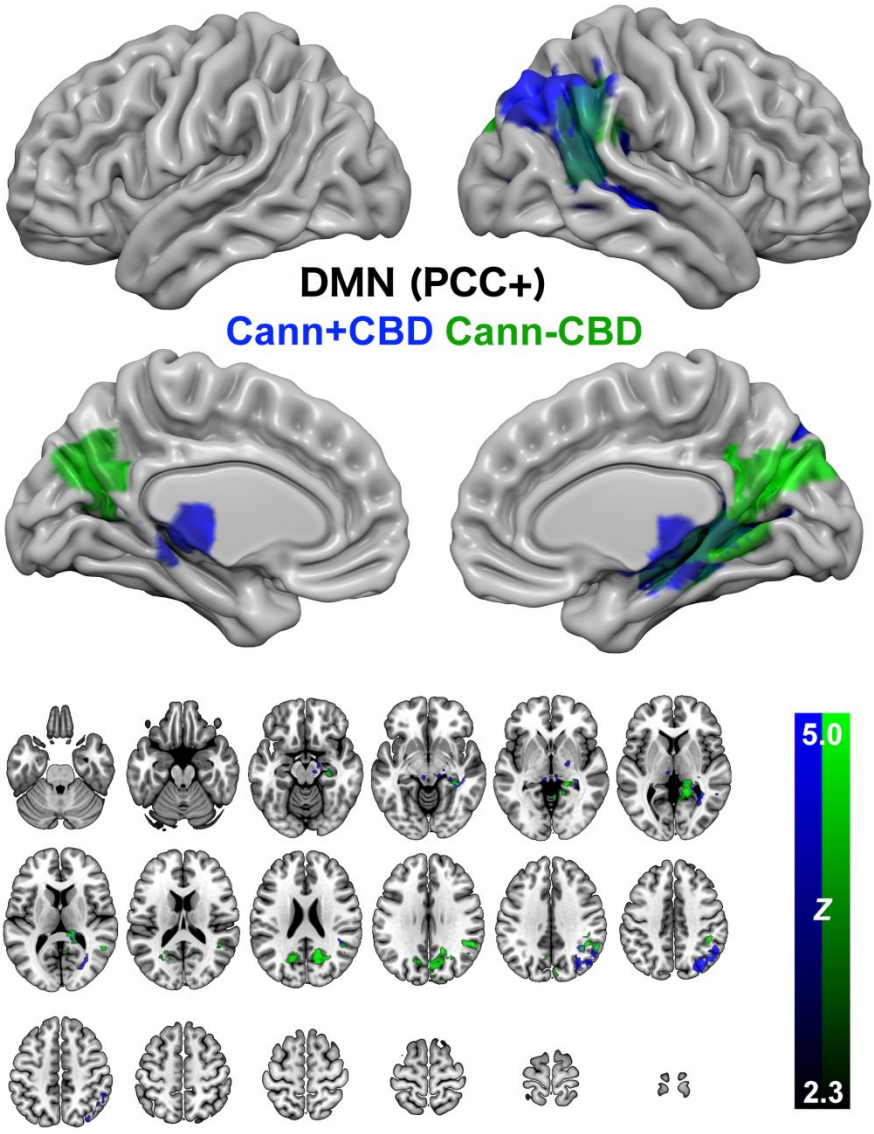

Figure 2. Drug treatment effects on the DMN (PCC+) network. All contrasts are placebo > drug, therefore significant ($Z = 2.3$, $p < 0.05$, cluster corrected for multiple comparisons) clusters represent relative decreases in functional connectivity in the drug condition. The Cann+CBD treatment session is shown in the blue scale, and the Cann-CBD treatment session is shown in the green scale.

Disruptions of functional connectivity in the ECN (PCC-) network induced by both active treatments were relatively minimal, with effects restricted to the left frontal lobe. The two strains produced spatially dissociable effects however, with Cann+CBD showing most effect in the inferior frontal gyrus, and Cann-CBD showing most effect in ventro-lateral prefrontal cortex. See Figure 3.

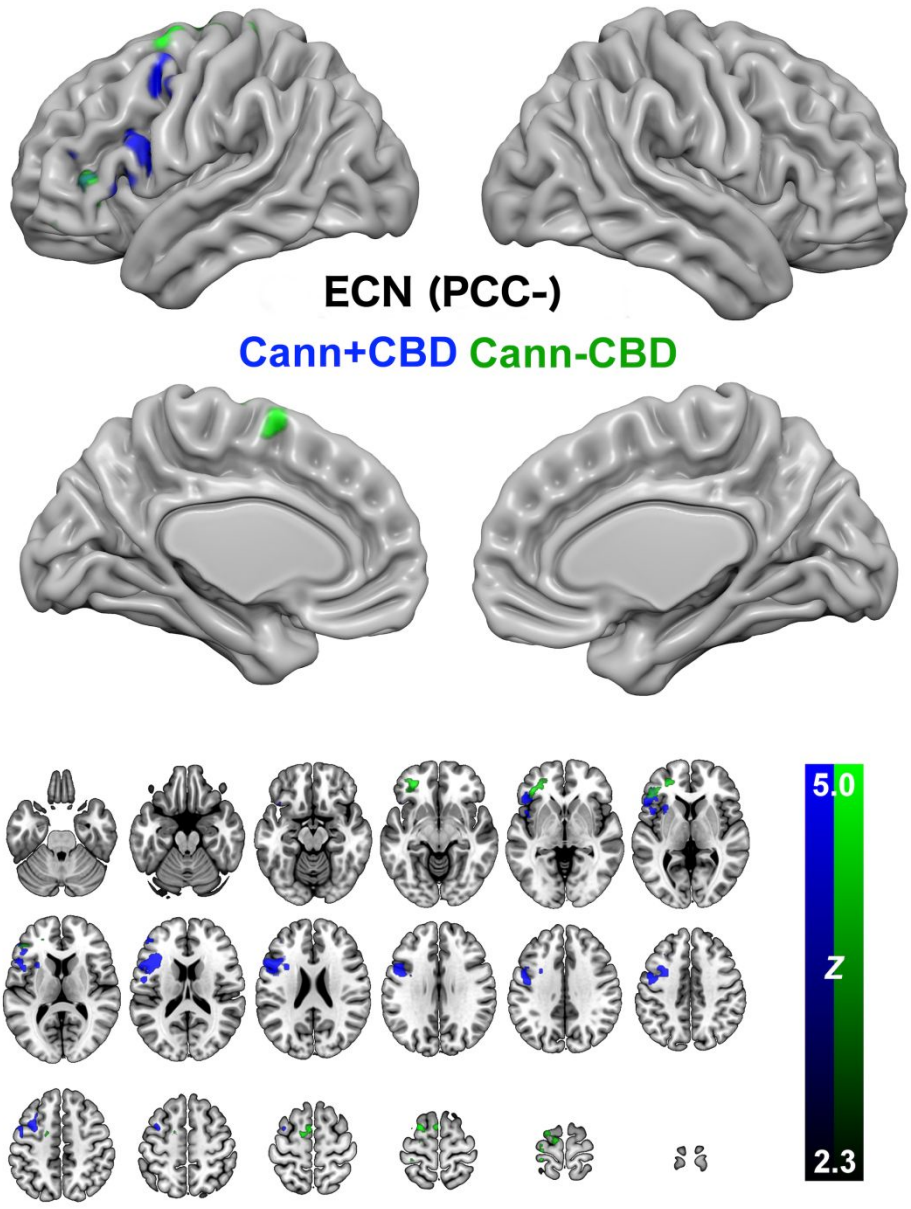

Figure 3. Drug treatment effects on the ECN (PCC-) network. All contrasts are placebo > drug, therefore significant ($Z = 2.3$, $p < 0.05$, cluster corrected for multiple comparisons) clusters represent relative decreases in functional connectivity in the drug condition. The Cann+CBD treatment session is shown in the blue scale, and the Cann-CBD treatment session is shown in the green scale.

Effects on the SAL (AI+) network were also strongly dissociated, with only minimal disruption seen for the Cann+CBD treatment in the left hemisphere post-central gyrus and the frontal pole. However the Cann-CBD strain produced widespread disruptions (reductions) in functional connectivity in left frontal (dorsolateral prefrontal cortex, ventrolateral prefrontal cortex) and temporal (anterior superior temporal gyrus, posterior inferior temporal gyrus)

regions. Also present in the Cann-CBD treatment were bilateral effects in the putamen, the ventromedial prefrontal cortex, and the frontal pole. See Figure 4.

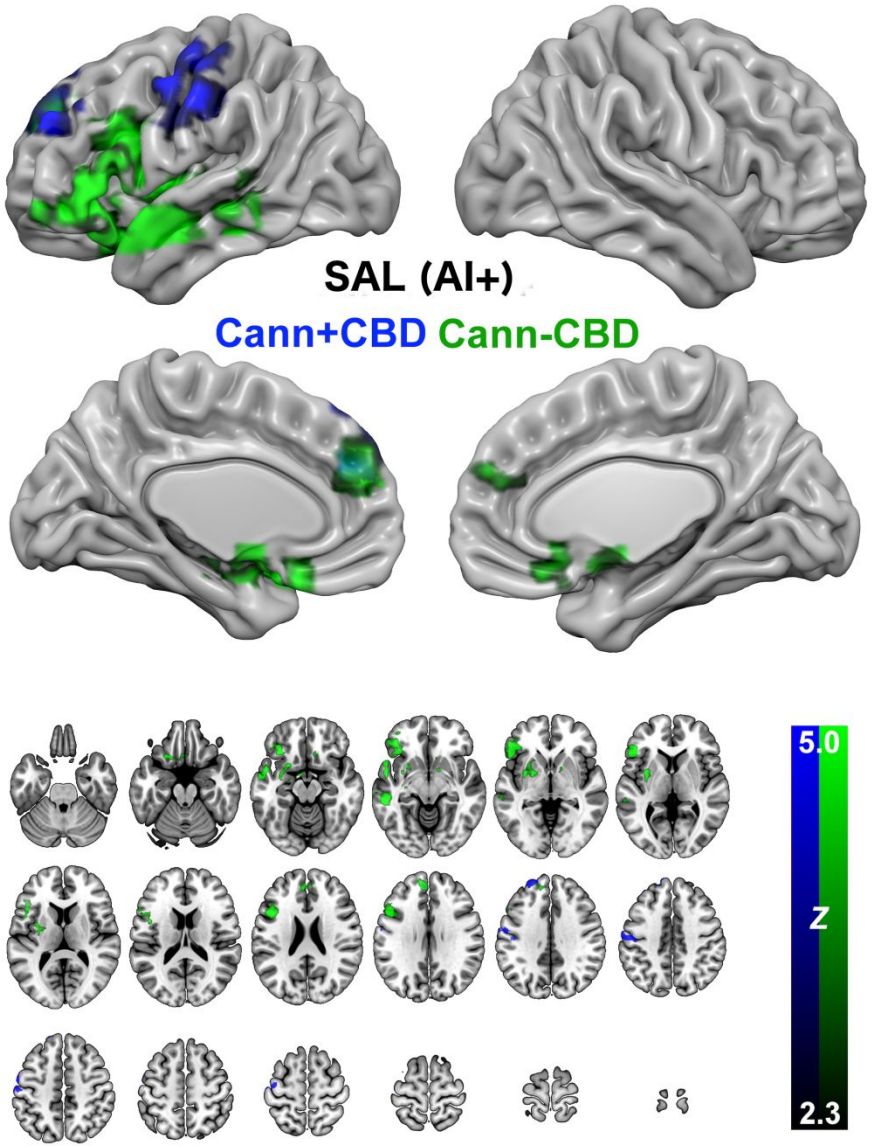

Figure 4. Drug treatment effects on the SAL (AI+) network. All contrasts are placebo > drug, therefore significant ($Z = 2.3$, $p < 0.05$, cluster corrected for multiple comparisons) clusters represent relative decreases in functional connectivity in the drug condition. The Cann+CBD treatment session is shown in the blue scale, and the Cann-CBD treatment session is shown in the green scale.

Group-level voxelwise comparisons between the two active treatment conditions (Cann-CBD vs. Cann+CBD) produced no significant clusters, in any of the three networks. Likewise there

were no significant clusters when increases in functional connectivity (relative to placebo) were examined; all observed effects were decreases, relative to placebo.

Each of the major clusters resulting from the analyses of treatment effects was defined as a ROI, and response amplitude data was extracted from these regions in order to perform cross-subject correlations with self-report response measures performed outside the scanner, immediately following the scan session. The majority of significant (FDR-corrected) correlations involved the Cann-CBD treatment and the region in the PCC that showed specific effects for this treatment in the DMN (PCC+) network analysis. The extent of disruption of connectivity in the PCC showed strong correlations with a number of subjective measures: 'Stoned', 'High', 'Feel drug effect', 'Dry mouth', 'Enhanced colour perception', and 'Enhanced sound perception'. See Figure 5 for scatterplots and correlation coefficients for this region and treatment. One additional significant correlation involved the frontal pole region seen in the salience network analysis; this region significantly negatively correlated with feelings of paranoia, again specifically in the Cann-CBD treatment (r = -0.674, p(FDR) = 0.048). All other correlations were non-significant (p > 0.05, FDR-corrected). See supplementary material for full tables of the correlation results.

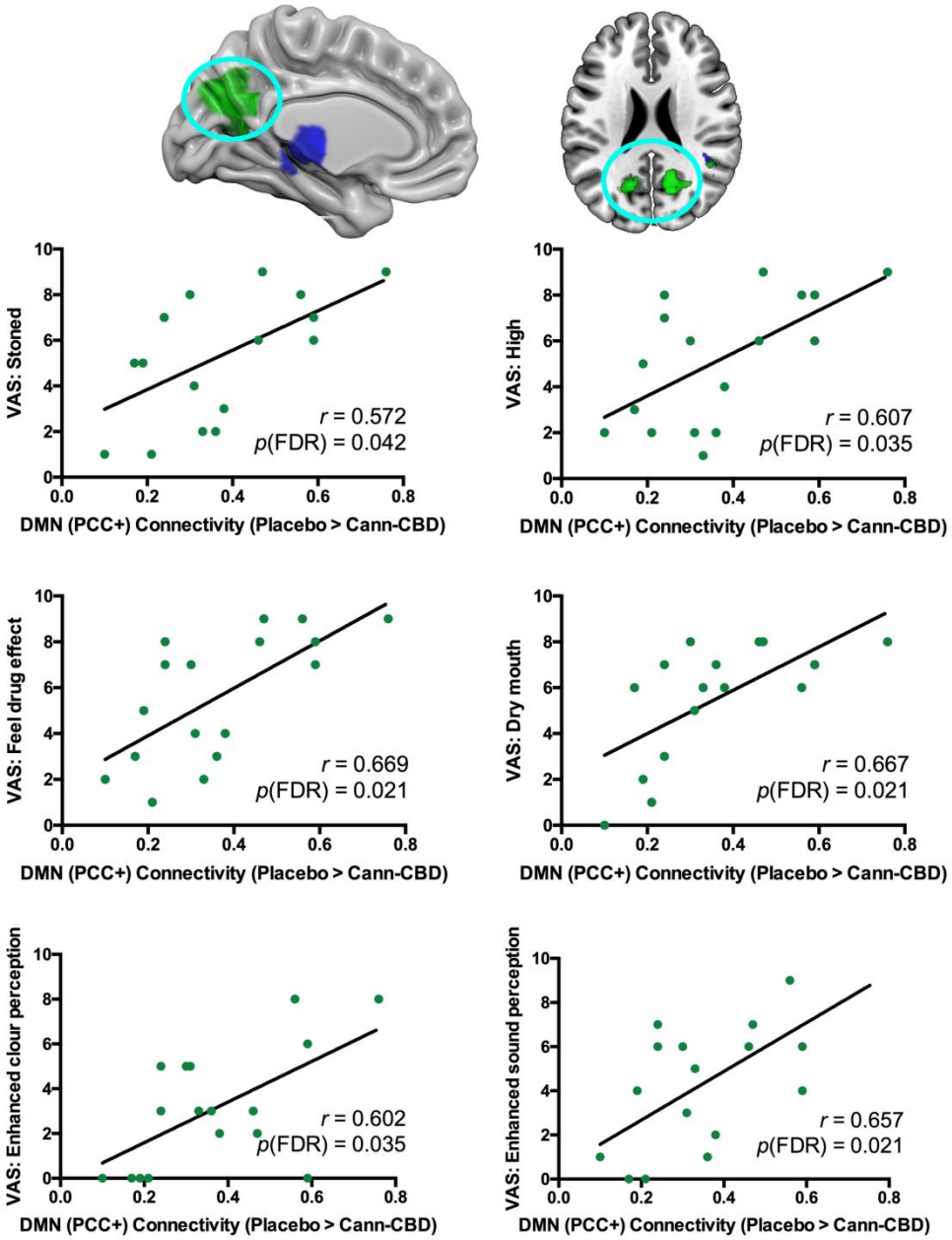

Figure 5. Correlations between the specific effect of Cann-CBD on the PCC in the DMN (PCC+) network analysis and Visual Analogue Scale (VAS) measures collected immediately after the MRI scanning session (approximately 90 minutes post-dosing). Correlations between the effect of Cann-CBD cannabis on the PCC cluster (top row, surface and slice-based visualisations of the region) and six separate VAS scales; feeling 'stoned', feeling 'high', feeling the drug effect, having a dry mouth, experiencing enhanced colour and sound perception. Pearson's *r* values and False Discovery Rate (FDR) corrected *p* values are included for each plot. See supplementary information for full statistical tables of *r*, *p*, and FDR-corrected *p* values.

Discussion

We have shown that cannabis reduces functional connectivity in a number of canonical resting-state brain networks, and furthermore that different strains of cannabis have dissociable effects on these networks. Effects on the DMN (PCC+) and SAL (AI+) networks are extensive, while effects on the ECN (PCC-) network appear relatively minor. Furthermore, effects of the THC without CBD strain (Cann-CBD) are more widespread in the DMN (PCC+) and SAL (AI+) networks, and the specific effect of this strain in the PCC region of the DMN (PCC+) is highly associated with classic subjective measures of the drug effect such as feeling 'stoned' and 'high' and having enhanced perception of both sounds and colours. Specific effects of the Cann-CBD strain were also seen in left frontal and temporal regions in the salience network.

These findings are broadly consonant with the few previous reports using cannabinoids and resting-state fMRI. One recent study (Rzepa et al., 2016) used the CB1 neutral antagonist THCV, and showed a pattern of disruption of the DMN strikingly similar to the present data, with selective effects in the PCC and right hemisphere parietal lobe. Another previous resting-state study (Klumpers et al., 2012) which used pure synthetic THC showed effects in the visual cortex, frontal lobe, cerebellum, and sensorimotor regions, though notably, in this study THC instead appeared to increase connectivity measures in the majority of regions. A third previous study (Bossong et al., 2013) also showed less deactivation (relative to placebo) in the DMN (particularly in the PCC) with pure synthetic THC treatment during a cognitive task. This deactivation of the PCC was also negatively correlated with task performance, suggesting that higher activation levels of the PCC during the task had a deleterious effect on task performance.

What these previous studies and the present data clearly demonstrate is that the PCC is a key brain structure involved in the neuropsychopharmacological effects of cannabinoids (including THCV, and pure THC). This is further reinforced by investigations using CB1-active radioligands and Positron Emission Tomography (PET) to image CB1 receptor distribution and function, which have shown a very high density of CB1 receptors in the PCC, visual cortex, putamen, and temporal lobe regions (Burns et al., 2007). A further PET study demonstrated that CB1 receptor distributions were down-regulated in daily cannabis smokers, most notably in the PCC/precuneus, visual cortex, and temporal and frontal lobes, and that this down-regulation was reversible after four weeks of abstinence (Hirvonen et al.,

2012). This is also consistent with findings that show reductions in endogenous cannabinoids in chronic cannabis use (Morgan et al., 2013). One other recent study (Orr et al., 2013) on cannabis dependent adolescents demonstrated *increased* PCC connectivity in the default mode network (while abstinent). These findings taken together therefore suggest a possible mechanism for the effect of cannabinoids (particularly THC) on the PCC. The acute effect is to disrupt PCC function (as demonstrated by (Bossong et al., 2013; Rzepa et al., 2016), and the present data), and regular use may lead to down-regulation of CB1 receptors in the region (Hirvonen et al., 2012). This longer-term impairment of PCC function may then lead to compensatory hyperactivation/hyperconnectivity of the PCC in long-term users (as seen in Orr et al., 2013). This proposed mechanism, while plausible, rests on results from only a few studies, and therefore requires much further substantiation. In addition, how these potential effects on the PCC are precisely related to issues associated with long-term use such as dependence, and cannabis-induced psychosis is a key question for future research.

In the present data, the PCC also emerged as the only region that was significantly related to subjective effects of the drug, and this was only true when administered cannabis which contained no CBD. This lends support to an emerging view that the effects of THC and CBD are in many ways oppositional, and that CBD may serve to buffer the user somewhat against the harmful long-term effects of THC (Curran et al., 2016; Demirakca et al., 2011; Morgan et al., 2012; Morgan and Curran, 2008; Niesink and van Laar, 2013; Yücel et al., 2016). The present data further suggest that CBD may also buffer the user against the *acute* effects of THC on the PCC and abolishes the relationship between functional disruption in this region and the subjective effects of intoxication. Adding this element to the potential physiological mechanism outlined above, dampening of the acute effects of THC by CBD may lead to less overall down-regulation of CB1 receptors with long-term use, and lessen the probability of the user developing dependence and/or psychosis (Morgan et al., 2010, 2012; Morgan and Curran, 2008). Two cross-sectional studies to date have also reported associations between chronic CBD exposure and protection of the hippocampus (Demirakca et al., 2011; Yücel et al., 2016), also a key DMN region with high CB1 receptor density.

The salience network has been proposed (Goulden et al., 2014; Sridharan et al., 2008) as the mechanism that switches between higher activity in the DMN (reflecting an internal focus, or a resting, relaxed state) and higher activity in the ECN (reflecting active engagement with a task, or focussed attention). Efficient function of the salience network therefore supports

the functions of the other networks in an important manner. Disruption of the salience network may therefore also underlie some of the acute phenomenology of cannabis intoxication, which include a variety of cognitive effects such as impairments in memory (Curran et al., 2002), executive function (Ramaekers et al., 2006), effort-related decision making (Lawn et al., 2016), and effects on salience processing (Bhattacharyya et al., 2012, 2014). Across the SAL (AI+) network as a whole, the reduction in connectivity produced by Cann-CBD was not seen in the treatment containing CBD. Regional disruption of the salience network was also much more evident and widespread in the Cann-CBD treatment, again suggesting that CBD buffers the user somewhat against the effects of THC on this network. Disruptions of salience attribution are also thought to play a key role in the development and maintenance of addiction (Robinson and Berridge, 1993, 2001) and psychosis (Kapur, 2003). This differential effect on the salience network may therefore be a potential neuro-protective mechanism for CBD, by which it prevents the development of such issues with chronic use. This finding is also consistent with previous behavioural evidence that cannabis without CBD acutely increases the salience of cannabis cues on an attentional bias task, while cannabis containing CBD reversed this effect so attention was directed away from cannabis-cues (Morgan et al., 2010).

Results have also been reported by Freeman et al. (2017) on a music-listening fMRI task conducted on the same cohort, in the same scan session, as the resting-state data presented here. These showed that the Cann-CBD treatment significantly dampened responses to music in the auditory cortex, and in limbic and striatal regions (amygdala, hippocampus, and right ventral striatum) while the Cann+CBD treatment had little effect. While it is difficult to make precise comparisons between the two sets of results, Cann-CBD produced more disruptions in function than Cann+CBD on this task, and this general pattern is consistent with the resting-state results presented here.

A major strength of the present study is that the treatments were administered by vaporiser inhalation, using the whole plant form rather than synthetic THC and CBD. Doing this in a placebo-controlled cross-over study gives our findings strong ecological validity and relevance in a time of increasing liberalisation of cannabis controls across many parts of the globe. However, given the somewhat exploratory nature of the study and the fact that some of the results (e.g. the correlations between VAS measures and the PCC) were unpredicted, the results require replication to be fully substantiated. Replication with a larger sample,

that included use of a 3 Tesla MRI scanner and further optimised acquisition protocols would certainly be useful. The use of a larger sample may also enable other factors to be considered, such as the relationship between the acute response to the drug and the subjects' regular usage patterns. Subjects in the current study were somewhat regular, though not heavy, cannabis users (< 3 times per week, > 4 times in the past year). A more strictly drug-naïve subject group may have been preferable; however this has to be balanced against the ethical issues associated with using drug-naïve subjects in pharmacological studies of this type. Also, subjects who are (semi-)regular users may be more representative of typical cannabis users than entirely naïve subjects. Other limitations are related to the study protocol. The resting-state scan was placed towards the end of the imaging protocol; approximately 70-75 minutes after dosing. Even though subjects still indicated strong subjective effects of cannabis intoxication after the scan session, it is likely the peak drug effect occurred somewhat earlier, before the resting state scan. Finally, blood samples were not acquired in this study protocol, so we have no information about plasma levels of cannabinoids; future studies should incorporate blood sampling in the protocol to address this.

To summarise, both low-CBD and high-CBD strains of cannabis have widespread effects on the brain's major resting state networks, but cannabis devoid of CBD appears to have more widespread effects, particularly on the DMN (PCC+) and SAL (AI+) networks. In particular, reductions of connectivity in the SAL (AI+) network produced by the Cann-CBD treatment were not evident in the presence of CBD. Strong and specific correlations were found only in the Cann-CBD treatment between PCC function in the DMN (PCC+) and subjective measures of drug effects, suggesting the PCC is a key region underlying the psychoactivity of THC. A productive avenue for future work on cannabis would be to examine potential changes in these networks (and the psychological processes that depend upon them) in a longitudinal study with individuals who use different strains of cannabis in differing frequencies and amounts.

References

Benjamini Y and Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society* 57(1). WileyRoyal Statistical Society: 289–300. DOI: 10.2307/2346101.

Bergamaschi MM, Queiroz RHC, Chagas MHN, et al. (2011) Cannabidiol Reduces the Anxiety Induced by Simulated Public Speaking in Treatment-Naïve Social Phobia Patients. *Neuropsychopharmacology* 36(6): 1219–1226. DOI: 10.1038/npp.2011.6.

Bhattacharyya S, Morrison PD, Fusar-Poli P, et al. (2010) Opposite Effects of Δ-9-Tetrahydrocannabinol and Cannabidiol on Human Brain Function and Psychopathology. *Neuropsychopharmacology* 35(3): 764–774. DOI: 10.1038/npp.2009.184.

Bhattacharyya S, Crippa JA, Allen P, et al. (2012) Induction of psychosis by ??9-tetrahydrocannabinol reflects modulation of prefrontal and striatal function during attentional salience processing. *Archives of General Psychiatry* 69(1): 27–36. DOI: 10.1001/archgenpsychiatry.2011.161.

Bhattacharyya S, Falkenberg I, Martin-Santos R, et al. (2014) Cannabinoid Modulation of Functional Connectivity within Regions Processing Attentional Salience. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* (2014). Nature Publishing Group: 1–10. DOI: 10.1038/npp.2014.258.

Biswal BB, Mennes M, Zuo X-N, et al. (2010) Toward discovery science of human brain function. *Proceedings of the National Academy of Sciences of the United States of America* 107(10): 4734–9. DOI: 10.1073/pnas.0911855107.

Bossong MG, Van Berckel BNM, Boellaard R, et al. (2009) Δ9-Tetrahydrocannabinol Induces Dopamine Release in the Human Striatum. *Neuropsychopharmacology* 34(3): 759–766. DOI: 10.1038/npp.2008.138.

Bossong MG, Jansma JM, van Hell HH, et al. (2013) Default Mode Network in the Effects of Δ9-Tetrahydrocannabinol (THC) on Human Executive Function. *PLoS ONE* 8(7): 1–10. DOI: 10.1371/journal.pone.0070074.

Bourke JH and Wall MB (2015) phMRI: methodological considerations for mitigating potential confounding factors. *Frontiers in Neuroscience* 9(May): 1–7. DOI: 10.3389/fnins.2015.00167.

Buckner RL, Andrews-Hanna JR and Schacter DL (2008) The brain's default network: Anatomy, function, and relevance to disease. *Annals of the New York Academy of*

Sciences 1124: 1–38. DOI: 10.1196/annals.1440.011.

Burns HD, Van Laere K, Sanabria-Bohórquez S, et al. (2007) [18F]MK-9470, a positron emission tomography (PET) tracer for in vivo human PET brain imaging of the cannabinoid-1 receptor. *Proceedings of the National Academy of Sciences of the United States of America* 104: 9800–5. DOI: 10.1073/pnas.0703472104.

Carhart-Harris RL, Kevin M, Robert L, et al. (2015) The Effects of Acutely Administered 3,4-Methylenedioxymethamphetamine on Spontaneous Brain Function in Healthy Volunteers Measured with Arterial Spin Labelling and Blood Oxygen Level-Dependent Resting-State Functional Connectivity. *Biological Psychiatry* 78(8): 554–562. DOI: 10.1016/j.biopsych.2013.12.015.

Cole DM, Smith SM and Beckmann CF (2010) Advances and pitfalls in the analysis and interpretation of resting-state FMRI data. *Frontiers in systems neuroscience* 4(April): 8. DOI: 10.3389/fnsys.2010.00008.

Comninos AN, Demetriou L, Wall MB, et al. (2018) Modulations of human resting brain connectivity by kisspeptin enhance sexual and emotional functions. *JCI Insight* 3(20): 0–10. Available at: https://doi.org/10.1172/jci.insight.121958.

Curran VH, Brignell C, Fletcher S, et al. (2002) Cognitive and subjective dose-response effects of acute oral Δ9-tetrahydrocannabinol (THC) in infrequent cannabis users. *Psychopharmacology* 164(1): 61–70. DOI: 10.1007/s00213-002-1169-0.

Curran H V, Freeman, T P, Mokrysz C, et al. (2016) Keep off the grass? Cannabis, cognition and addiction. *Nature Reviews Neuroscience* 17(5): 293–306. DOI: 10.1038/nrn.2016.28.

D'Souza DC, Perry E, MacDougall L, et al. (2004) The Psychotomimetic Effects of Intravenous Delta-9-Tetrahydrocannabinol in Healthy Individuals: Implications for Psychosis. *Neuropsychopharmacology* 29(8): 1558–1572. DOI: 10.1038/sj.npp.1300496.

Demetriou L, Kowalczyk OS, Tyson G, et al. (2018) A comprehensive evaluation of increasing temporal resolution with multiband-accelerated sequences and their effects on statistical outcome measures in fMRI. *NeuroImage* 176. Elsevier Ltd: 404–416. Available at: https://doi.org/10.1016/j.neuroimage.2018.05.011.

Demirakca T, Sartorius A, Ende G, et al. (2011) Diminished gray matter in the hippocampus of cannabis users: Possible protective effects of cannabidiol. *Drug and Alcohol Dependence* 114(2–3). Elsevier: 242–245. DOI: 10.1016/j.drugalcdep.2010.09.020.

Di Forti, Morgan, Dazzan, Pariente, Mondelli, Marques, Handley, Luzi, Russo, Paparelli, Butt, Stilo, Wiffen powell and M (2009) High potency cannabis and the risk of psychosis.

British Journal ofPsychiatry 195(195): 488–491. Available at: http://bjp.rcpsych.org/content/195/6/488.full (accessed 9 September 2017).

ElSohly MA, Mehmedic Z, Foster S, et al. (2016) Changes in cannabis potency over the last 2 decades (1995-2014): Analysis of current data in the United States. *Biological Psychiatry* 79(7): 613–619. DOI: 10.1016/j.biopsych.2016.01.004.

Englund A, Morrison PD, Nottage J, et al. (2013) Cannabidiol inhibits THC-elicited paranoid symptoms and hippocampal-dependent memory impairment. *Journal of Psychopharmacology* 27(1): 19–27. DOI: 10.1177/0269881112460109.

Fox MD and Raichle ME (2007) Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nature reviews. Neuroscience* 8(9): 700–11. DOI: 10.1038/nrn2201.

Fox MD, Snyder AZ, Vincent JL, et al. (2005) The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proceedings of the National Academy of Sciences of the United States of America* 102(27): 9673–8. DOI: 10.1073/pnas.0504136102.

Freeman TP and Winstock AR (2015) Examining the profile of high-potency cannabis and its association with severity of cannabis dependence. *Psychological Medicine* 45(15): 3181–3189. DOI: 10.1017/S0033291715001178.

Freeman TP, Morgan CJA, Hindocha C, et al. (2014) Just say 'know': how do cannabinoid concentrations influence users' estimates of cannabis potency and the amount they roll in joints? *Addiction (Abingdon, England)* 109(10): 1686–1694. DOI: 10.1111/add.12634.

Freeman TP, Pope RA, Wall MB, Bisby JA, Luijten M, Hindocha C, Mokrysz C, Lawn W, Bloomfield MAP, et al. (2017) Cannabis dampens the effects of music in brain regions sensitive to reward and emotion. *International Journal of Neuropsychopharmacology*. DOI: 10.1093/ijnp/pyx082/4102982/Cannabis-dampens-the-effects-of-music-in-brain.

Freeman TP, van der Pol P, Kuijpers W, et al. (2018) Changes in cannabis potency and first-time admissions to drug treatment: a 16-year study in the Netherlands. *Psychological Medicine* (February): 1–7. DOI: 10.1017/S0033291717003877.

Goulden N, Khusnulina A, Davis NJ, et al. (2014) The salience network is responsible for switching between the default mode network and the central executive network: replication from DCM. *NeuroImage* 99: 180–90. DOI: 10.1016/j.neuroimage.2014.05.052.

Grimm O, Löffler M, Kamping S, et al. (2018) Probing the endocannabinoid system in healthy

volunteers: Cannabidiol alters fronto-striatal resting-state connectivity. *European Neuropsychopharmacology* 28(7): 841–849. DOI: https://doi.org/10.1016/j.euroneuro.2018.04.004.

Hindocha C, Wollenberg O, Carter Leno V, et al. (2014) Emotional processing deficits in chronic cannabis use: A replication and extension. *Journal of Psychopharmacology* 28(5). SAGE PublicationsSage UK: London, England: 466–471. DOI: 10.1177/0269881114527359.

Hindocha C, Freeman TP, Schafer G, et al. (2015) Acute effects of delta-9-tetrahydrocannabinol, cannabidiol and their combination on facial emotion recognition: A randomised, double-blind, placebo-controlled study in cannabis users. *European Neuropsychopharmacology* 25(3): 325–334. DOI: 10.1016/j.euroneuro.2014.11.014.

Hirvonen J, Goodwin RS, Li C-T, et al. (2012) Reversible and regionally selective downregulation of brain cannabinoid CB1 receptors in chronic daily cannabis smokers. *Molecular Psychiatry* 17(6): 642–649. DOI: 10.1038/mp.2011.82.

Iannetti GD and Wise RG (2007) BOLD functional MRI in disease and pharmacological studies: room for improvement? *Magnetic resonance imaging* 25(6): 978–88. DOI: 10.1016/j.mri.2007.03.018.

Kaelen M, Roseman L, Kahan J, et al. (2016) LSD modulates music-induced imagery via changes in parahippocampal connectivity. *European Neuropsychopharmacology* (April). DOI: 10.1016/j.euroneuro.2016.03.018.

Kapur S (2003) Psychosis as a state of aberrant salience: A framework linking biology, phenomenology, and pharmacology in schizophrenia. *American Journal of Psychiatry* 160(1): 13–23. DOI: 10.1176/appi.ajp.160.1.13.

Klumpers LE, Cole DM, Khalili-Mahani N, et al. (2012) Manipulating brain connectivity with δ^9-tetrahydrocannabinol: a pharmacological resting state FMRI study. *NeuroImage* 63(3). Elsevier Inc.: 1701–11. DOI: 10.1016/j.neuroimage.2012.07.051.

Lawn W, Freeman TP, Pope RA, et al. (2016) Acute and chronic effects of cannabinoids on effort-related decision-making and reward learning: an evaluation of the cannabis 'amotivational' hypotheses. *Psychopharmacology*. Psychopharmacology. DOI: 10.1007/s00213-016-4383-x.

Leweke FM, Piomelli D, Pahlisch F, et al. (2012) Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Translational Psychiatry* 2(3): e94. DOI: 10.1038/tp.2012.15.

Luca M De, Beckmann CF, Stefano N De, et al. (2006) fMRI resting state networks define distinct modes of long-distance interactions in the human brain. *NeuroImage* 29(4): 1359–1367. DOI: 10.1016/j.neuroimage.2005.08.035.

Mokrysz C, Freeman TP, Korkki S, et al. (2016) Are adolescents more vulnerable to the harmful effects of cannabis than adults? A placebo-controlled study in human males. *Translational psychiatry* 6(11): e961. DOI: 10.1038/tp.2016.225.

Morgan CJA and Curran HV (2008) Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *British Journal of Psychiatry* 192(4): 306–307. DOI: 10.1192/bjp.bp.107.046649.

Morgan CJA, Freeman TP, Schafer GL, et al. (2010) Cannabidiol attenuates the appetitive effects of Δ9- tetrahydrocannabinol in humans smoking their chosen cannabis. *Neuropsychopharmacology* 35(9). Nature Publishing Group: 1879–1885. DOI: 10.1038/npp.2010.58.

Morgan CJA, Gardener C, Schafer G, et al. (2012) Sub-chronic impact of cannabinoids in street cannabis on cognition, psychotic-like symptoms and psychological well-being. *Psychological Medicine* 42(2): 391–400. DOI: 10.1017/S0033291711001322.

Morgan CJA, Page E, Schaefer C, et al. (2013) Cerebrospinal fluid anandamide levels, cannabis use and psychotic-like symptoms. *British Journal of Psychiatry* 202(5): 381–382. DOI: 10.1192/bjp.bp.112.121178.

Niesink RJM and van Laar MW (2013) Does Cannabidiol Protect Against Adverse Psychological Effects of THC? *Frontiers in Psychiatry* 4(October): 1–8. DOI: 10.3389/fpsyt.2013.00130.

Niesink RJM, Rigter S, Koeter MW, et al. (2015) Potency trends of Δ9-tetrahydrocannabinol, cannabidiol and cannabinol in cannabis in the Netherlands: 2005-15. *Addiction* 110(12): 1941–1950. DOI: 10.1111/add.13082.

Orr C, Morioka R, Behan B, et al. (2013) Altered resting-state connectivity in adolescent cannabis users. *The American journal of drug and alcohol abuse* 39(6): 372–81. DOI: 10.3109/00952990.2013.848213.

Passow S, Specht K, Adamsen TC, et al. (2015) Default-mode network functional connectivity is closely related to metabolic activity. *Human Brain Mapping* 36(6). John Wiley & Sons, Ltd: 2027–2038. DOI: 10.1002/hbm.22753.

Pertwee RG (2008) The diverse CB_1 and CB_2 receptor pharmacology of three plant cannabinoids: Δ^9-tetrahydrocannabinol, cannabidiol and Δ^9-tetrahydrocannabivarin. *British Journal of Pharmacology* 153(2): 199–215. DOI: 10.1038/sj.bjp.0707442.

Ramaekers JG, Kauert G, Van Ruitenbeek P, et al. (2006) High-potency marijuana impairs executive function and inhibitory motor control. *Neuropsychopharmacology* 31(10): 2296–2303. DOI: 10.1038/sj.npp.1301068.

Ramaekers JG, Wel JH Van, Spronk D, et al. (2016) Cannabis and cocaine decrease cognitive impulse control and functional corticostriatal connectivity in drug users with low activity DBH genotypes. *Brain Imaging and Behavior*. Brain Imaging and Behavior: 1254–1263. DOI: 10.1007/s11682-015-9488-z.

Robinson T and Berridge K (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain research reviews* 8: 247–291. Available at: http://www.sciencedirect.com/science/article/pii/016501739390013P (accessed 7 July 2014).

Robinson TE and Berridge KC (2001) Incentive-sensitization and addiction. *Addiction* 96(1): 103–14. DOI: 10.1080/09652140020016996.

Rzepa E, Tudge L and McCabe C (2016) The CB1 neutral antagonist tetrahydrocannabivarin reduces default mode network and increases executive control network resting state functional connectivity in healthy volunteers. *International Journal of Neuropsychopharmacology* 19(2): 1–7. DOI: 10.1093/ijnp/pyv092.

Seeley WW, Menon V, Schatzberg AF, et al. (2007) Dissociable intrinsic connectivity networks for salience processing and executive control. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 27(9): 2349–56. DOI: 10.1523/JNEUROSCI.5587-06.2007.

Smith SM, Fox PT, Miller KL, et al. (2009) Correspondence of the brain's functional architecture during activation and rest. *Proceedings of the National Academy of Sciences of the United States of America* 106(31): 13040–5. DOI: 10.1073/pnas.0905267106.

Sridharan D, Levitin DJ and Menon V (2008) A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. 105(34).

van Hell HH, Bossong MG, Jager G, et al. (2011) Evidence for involvement of the insula in the psychotropic effects of THC in humans : a double-blind, randomized pharmacological MRI study. *International Journal of Neuropsychopharmacology* 14: 1377–1388. DOI: 10.1017/S1461145711000526.

Winton-Brown TT, Allen P, Bhattacharrya S, et al. (2011) Modulation of Auditory and Visual Processing by Delta-9-Tetrahydrocannabinol and Cannabidiol: an fMRI Study. *Neuropsychopharmacology* 36(7): 1340–1348. DOI: 10.1038/npp.2011.17.

Yücel M, Lorenzetti V, Suo C, et al. (2016) Hippocampal harms, protection and recovery following regular cannabis use. *Translational psychiatry* 6(November 2015): e710. DOI: 10.1038/tp.2015.201.

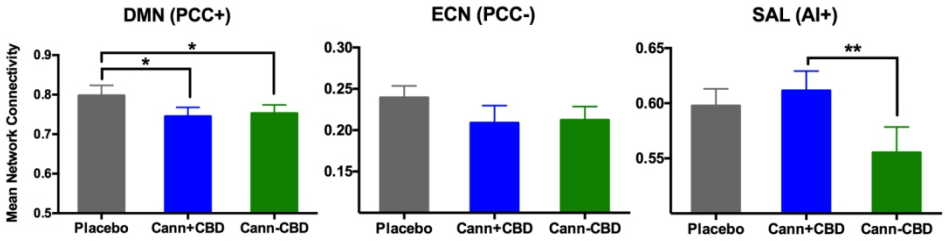

Figure 1. Treatment effects on the mean connectivity across the three networks; Default Mode Network (DMN; PCC+, left), Executive Control Network (ECN; PCC-, middle) and the Salience Network (SAL, AI+, right). * $p < 0.05$, ** $p < 0.005$. Error bars are standard errors.

138x36mm (300 x 300 DPI)

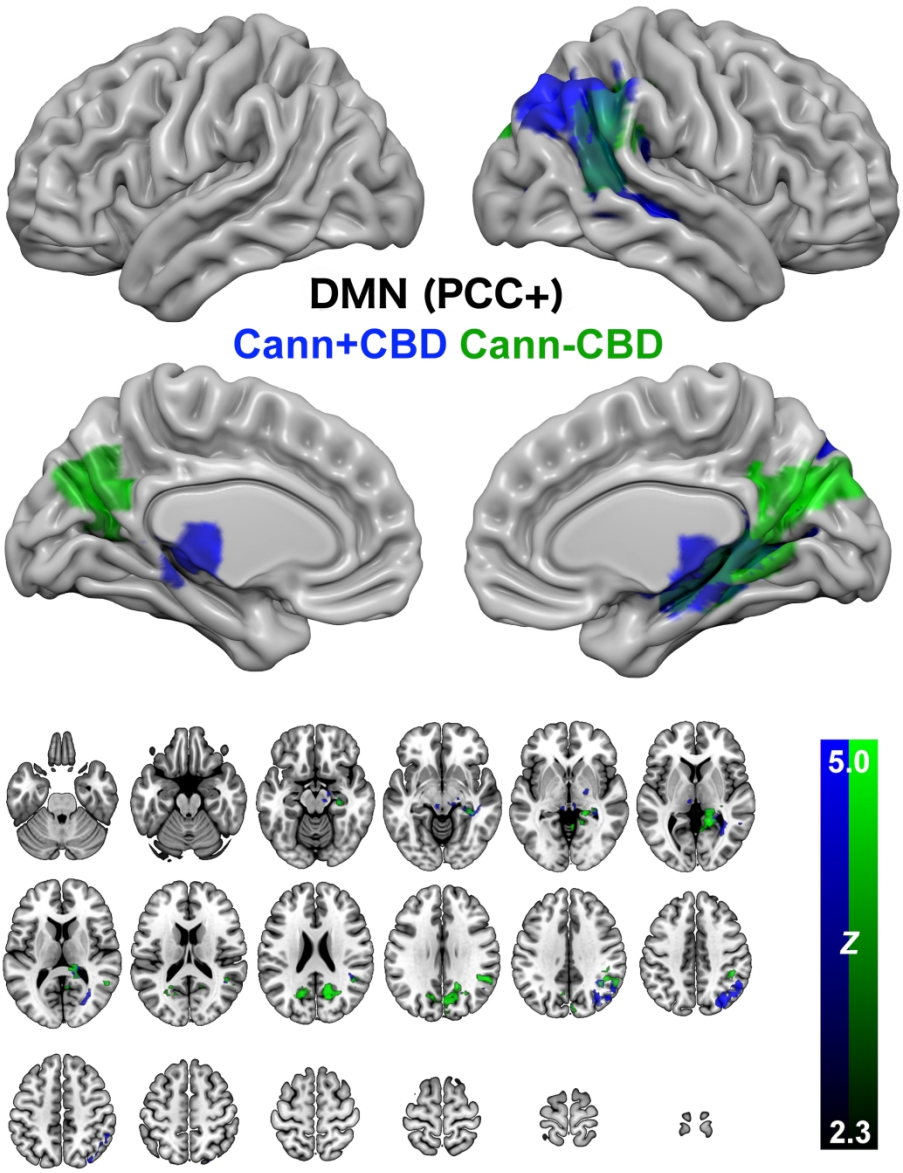

Figure 2. Drug treatment effects on the DMN (PCC+) network. All contrasts are placebo > drug, therefore significant (Z = 2.3, p < 0.05, cluster corrected for multiple comparisons) clusters represent relative decreases in functional connectivity in the drug condition. The Cann+CBD treatment session is shown in the blue scale, and the Cann-CBD treatment session is shown in the green scale.

805x1028mm (72 x 72 DPI)

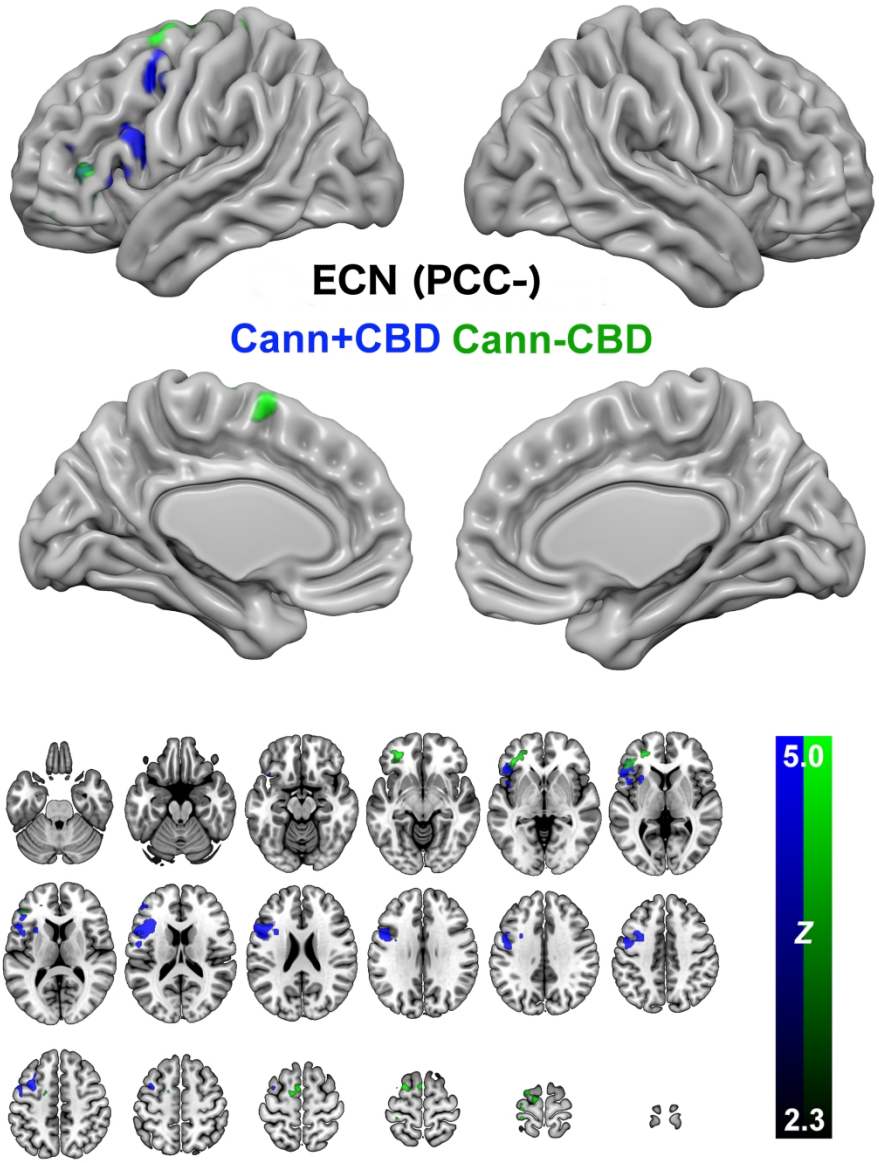

Figure 3. Drug treatment effects on the ECN (PCC-) network. All contrasts are placebo > drug, therefore significant (Z = 2.3, p < 0.05, cluster corrected for multiple comparisons) clusters represent relative decreases in functional connectivity in the drug condition. The Cann+CBD treatment session is shown in the blue scale, and the Cann-CBD treatment session is shown in the green scale.

805x1047mm (72 x 72 DPI)

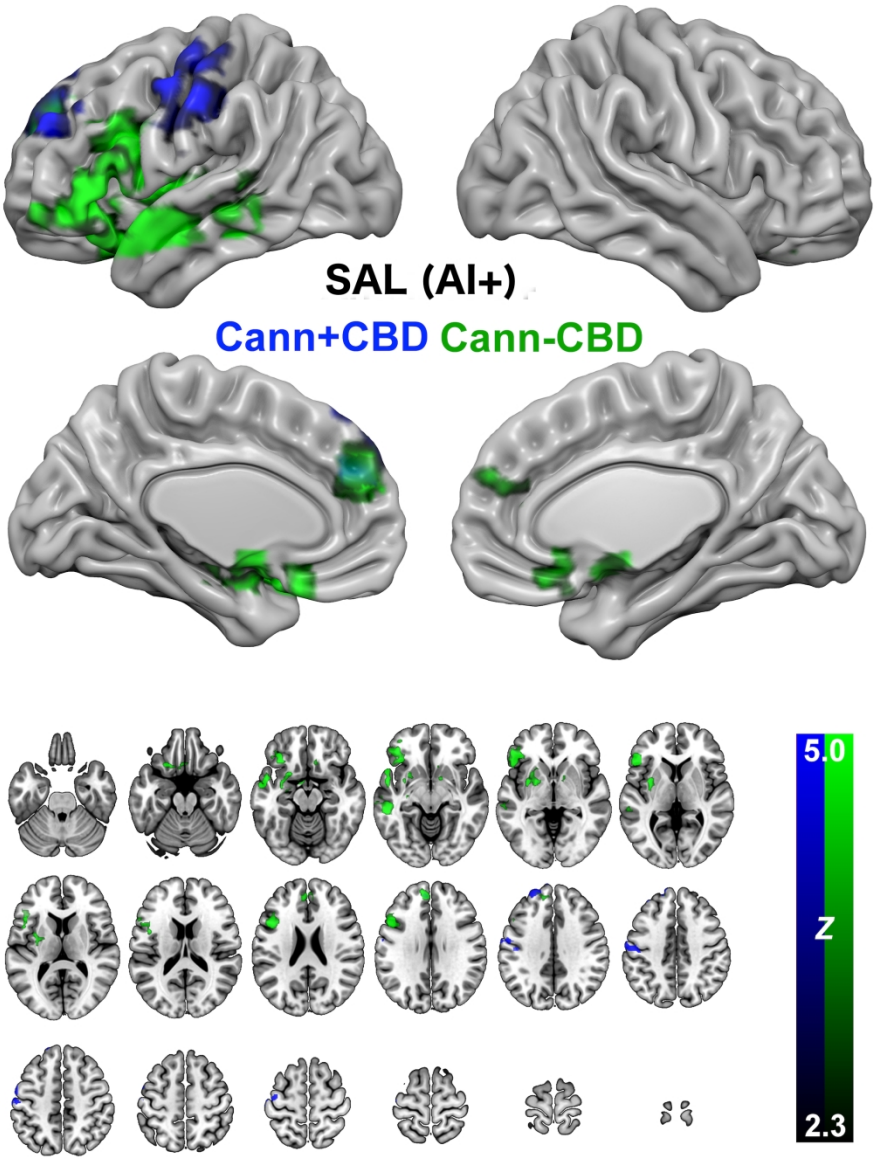

Figure 4. Drug treatment effects on the anterior insula network. All contrasts are placebo > drug, therefore significant (Z = 2.3, p < 0.05, cluster corrected for multiple comparisons) clusters represent relative decreases in functional connectivity in the drug condition. The Cann+CBD treatment session is shown in the blue scale, and the Cann-CBD treatment session is shown in the green scale.

801x1032mm (72 x 72 DPI)

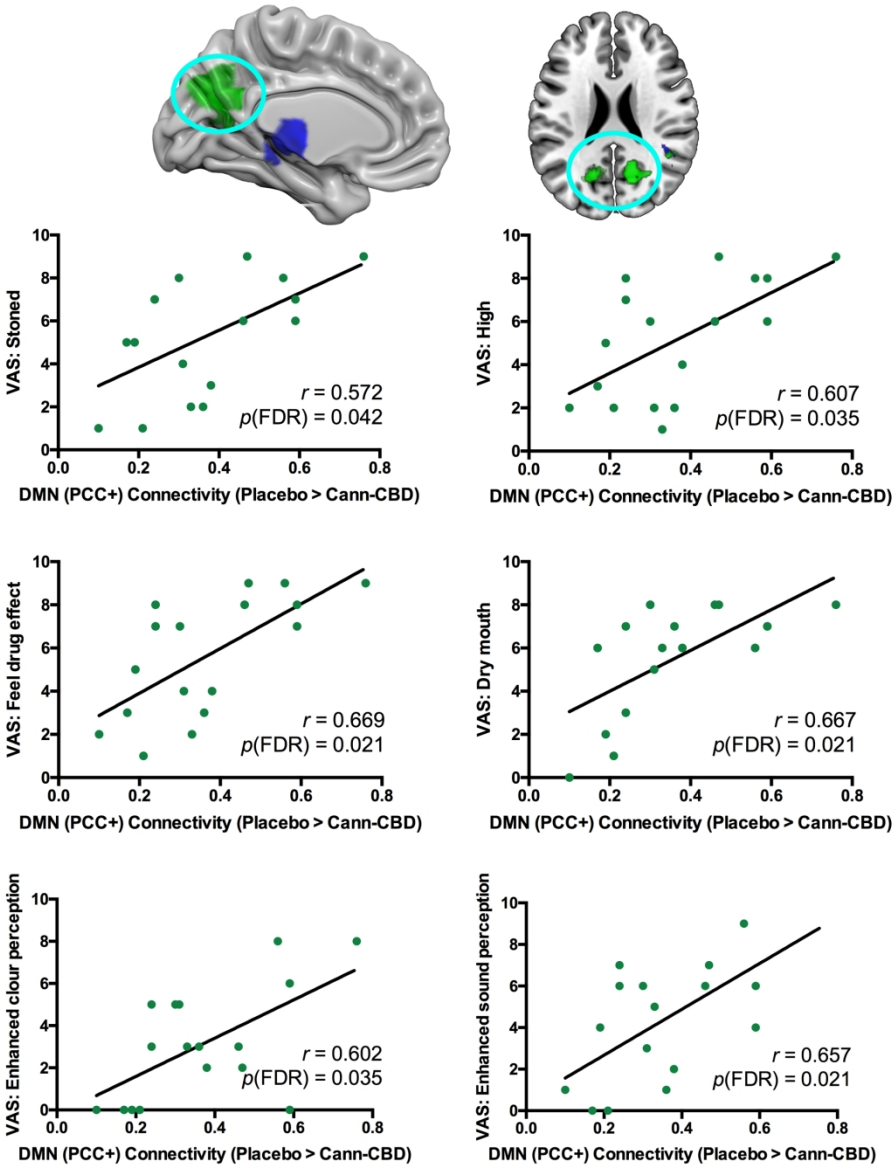

Figure 5. Correlations between the specific effect of Cann-CBD on the PCC in the DMN (PCC+) network analysis and Visual Analogue Scale (VAS) measures collected immediately after the MRI scanning session (approximately 90 minutes post-dosing). Correlations between the effect of Cann-CBD cannabis on the PCC cluster (top row, surface and slice-based visualisations of the region) and six separate VAS scales; feeling 'stoned', feeling 'high', feeling the drug effect, having a dry mouth, experiencing enhanced colour and sound perception. Pearson's r values and False Discovery Rate (FDR) corrected p values are included for each plot. See supplementary information for full statistical tables of r, p, and FDR-corrected p values.

165x216mm (300 x 300 DPI)

Supplementary Material

Methods

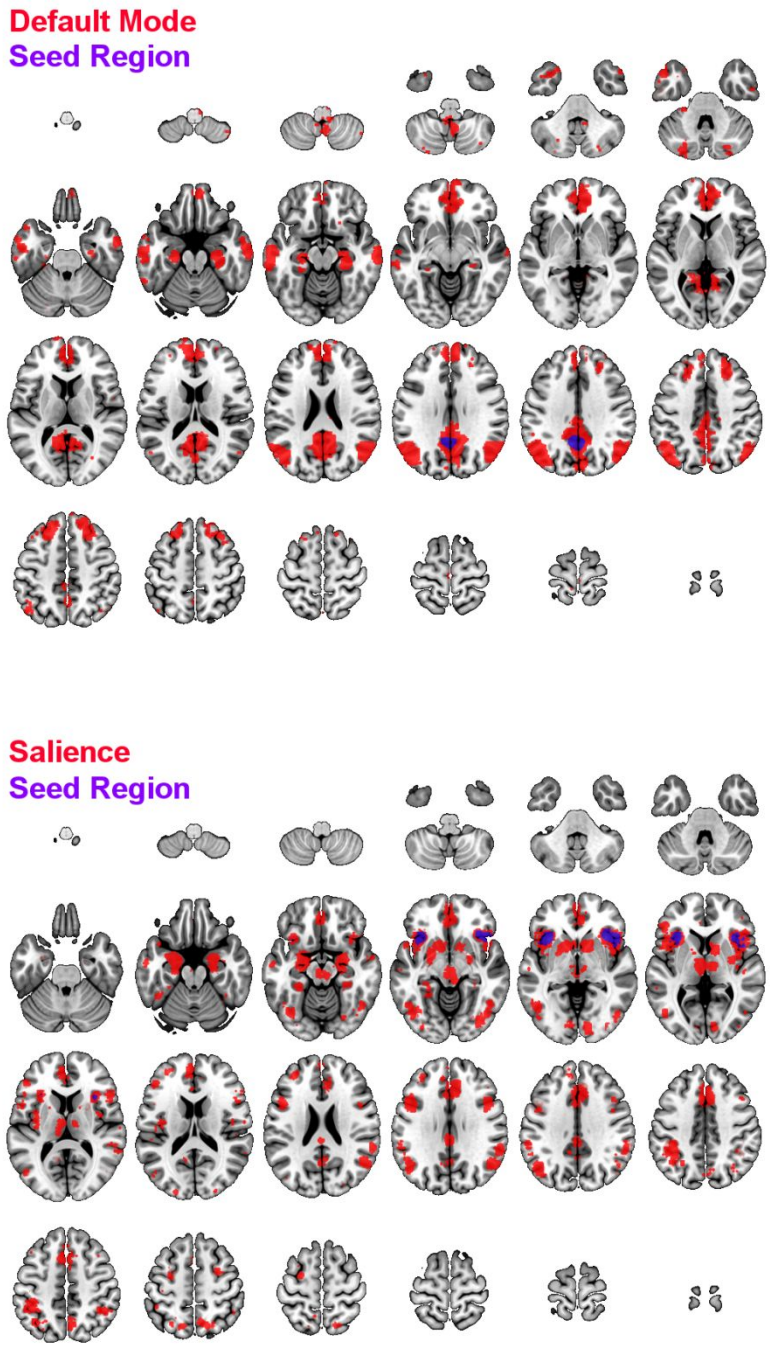

Figure S1. Masks and derived seed-regions used for the seed-based analyses. Masks were derived from automated meta-analytic data provided by Neurosynth (Yarkoni, Poldrack, Nichols, Van Essen, & Wager, 2011) using the 'default mode' (http://www.neurosynth.org/analyses/terms/default%20mode/) and 'salience'

(http://www.neurosynth.org/analyses/terms/salience/) terms. Posterior cingulate cortex and anterior insula ROIs were derived from these maps for use in the seed-based analyses.

	Participants
Age	26.18 (7.13)
Gender (m/f)	8/9
BDI	3.38 (3.12)
TEPS consummatory	43.50 (5.61)
TEPS anticipatory	42.06 (4.85)
TEPS total	86.56 (9.30)
Cannabis SDS	1.13 (1.26)
Alcohol ever used (y/n)	16/0
Alcohol use now (y/n)	16/0
Alcohol days per month	10.81 (4.86)
Alcohol units/session	5.93 (2.08)
Amphetamine ever used (y/n)	8/8
Amphetamine use now (y/n)	0/16
Amphetamine days per month	NA
Amphetamine grams/session	NA
Cannabis ever used (y/n)	16/0
Cannabis use now (y/n)	16/0
Cannabis days per month	8.06 (5.48)
Cannabis days to smoke an 8th	25.88 (33.73)
Cocaine ever used (y/n)	11/5
Cocaine use now (y/n)	3/13
Cocaine days per month	1.0 (0.0)
Cocaine grams/session	0.5 (0.0)
Heroin ever used (y/n)	0/16
Heroin use now (y/n)	0/16
Heroin days per month	NA
Heroin grams/session	NA
Ketamine ever used (y/n)	10/6
Ketamine use now (y/n)	2/14
Ketamine days per month	1.50 (0.71)
Ketamine grams/session	0.75 (0.35)
Mephedrone ever used (y/n)	7/9
Mephedrone use now (y/n)	0/16
Mephedrone days per month	NA
Mephedrone grams/session	NA
MDMA ever used (y/n)	14/2
MDMA use now (y/n)	6/10
MDMA days per month	1.50 (0.84)
MDMA grams/session	0.31 (0.19)
Tobacco ever used (y/n)	15/1
Tobacco use now (y/n)	15/1
Tobacco days per month	11.30 (10.27)
Tobacco cigs/day	3.63 (3.62)
Tobacco average cigs/day	2.16 (3.48)

Table S1. Means (S.D.) and frequencies for demographic data and drug use for participants. Data was missing for one participant for BDI, TEPS and drugs history. TEPS = Temporal Experience of Pleasure scale. BDI = Beck Depression Inventory. SDS = Severity of Dependence Scale.

Results

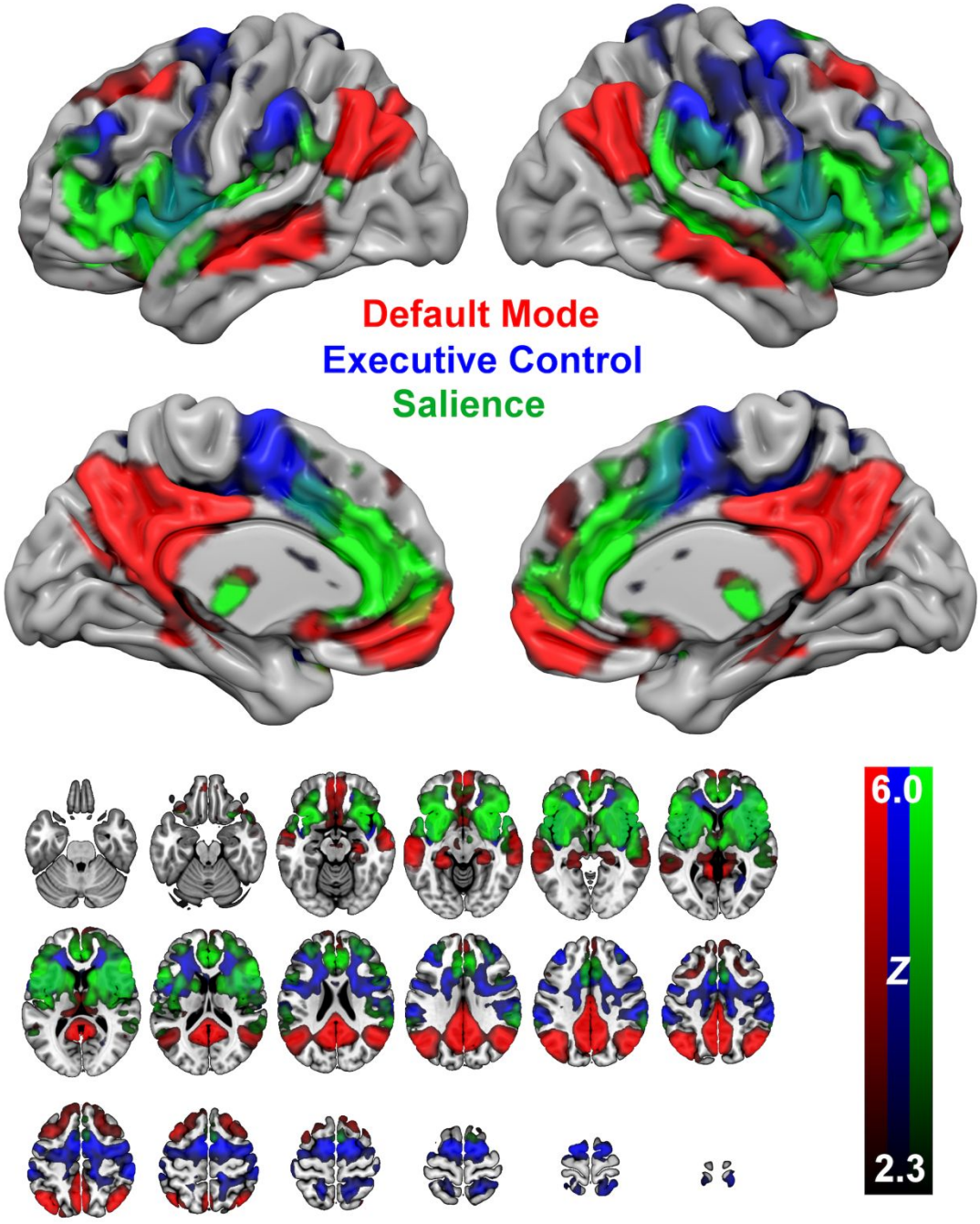

Figure S3. Mean (all subjects, all scans) functional connectivity maps from the seed-region analyses showing the Default Mode Network (DMN; defined by positive connectivity with the PCC seed region; red), the Executive Control Network (ECN; defined by negative connectivity with the PCC seed region; blue), and the Salience network (defined by positive connectivity with the anterior insula seed region; green).

Treatment	Analysis	Anatomical Location	Number of Voxels	Z-Max	P(corr.)	COG-X	COG-Y	COG-Z
Placebo vs. Cann+CBD:	**DMN (PCC+)**	Hippocampus (RH)	1420	-3.63	6.79E-06	20	-37	-1
		Parietal Lobe (RH)	1365	-4.21	1.04E-05	42	62	38
	ECN (PCC-)	Inferior frontal gyrus (LH)	2189	-3.92	5.96E-08	44	13	23
	SAL (AI+)	Precentral gyrus (LH)	724	-3.75	0.000955	-55	-11	48
		Frontal Pole	438	-3.97	0.0239	-18	53	40
Placebo vs. Cann-CBD:	**DMN (PCC+)**	Precuneous/PCC	1539	-3.64	2.80E-06	11	-55	15
		Parietal Lobe (RH)	457	-3.51	0.035	49	-47	31
	ECN (PCC-)	Superior frontal gyrus (RH)	564	-3.56	0.0113	-16	-4	68
		Inferior frontal gyrus (LH)	543	-3.42	0.0141	-36	40	-1
	SAL (AI+)	Inferior frontal lobe (LH)	3630	-4.52	2.96E-13	-39	12	-1
		Frontal pole (LH)	399	-4.4	0.0386	-6	49	28

Table S2. Coordinates of the major activation clusters shown in Figures 2, 3, and 4 of the main text. Z-Max = Maximum Z-score in cluster. LH = left Hemisphere, RH = Right Hemisphere. COG = Centre Of Gravity. Coordinates are in MNI space. Z values are negative as only reductions in connectivity (relative to placebo) were found.

VAS Item	Brainstem			Hippocampus			Lateral Parietal		
	r	*p*	*p* (FDR)	*r*	*p*	*p* (FDR)	*r*	*p*	*p* (FDR)
Alert	-0.148	0.57	0.820	-0.381	0.131	0.977	0.383	0.129	0.619
Happy	0.099	0.706	0.820	-0.018	0.945	0.977	-0.236	0.362	0.659
Anxious	0.077	0.769	0.820	-0.072	0.782	0.977	-0.063	0.811	0.927
Paranoid	0.213	0.412	0.820	-0.102	0.696	0.977	0.001	0.997	0.997
Mentally impaired	0.505	0.039	0.624	0.408	0.104	0.977	-0.04	0.88	0.939
Stoned	0.249	0.335	0.820	0.013	0.961	0.977	-0.207	0.425	0.659
High	0.335	0.188	0.820	0.151	0.562	0.977	-0.306	0.232	0.619
Feel drug effect	0.27	0.294	0.820	0.14	0.591	0.977	-0.409	0.103	0.619
Like drug effect	0.087	0.739	0.820	0.018	0.944	0.977	-0.195	0.453	0.659
Dry mouth	-0.226	0.384	0.820	-0.155	0.553	0.977	-0.207	0.426	0.659
Enhanced colour perception	0.126	0.631	0.820	0.067	0.799	0.977	-0.447	0.072	0.619
Enhanced sound perception	0.127	0.627	0.820	-0.018	0.946	0.977	-0.328	0.198	0.619
Want to listen to music	0.125	0.634	0.820	-0.038	0.885	0.977	-0.359	0.157	0.619
Want food	-0.104	0.692	0.820	0.008	0.977	0.977	-0.09	0.73	0.898
Want more cannabis balloon	-0.146	0.575	0.820	-0.107	0.683	0.977	-0.107	0.683	0.898
Want to smoke cannabis	0.022	0.933	0.933	-0.113	0.665	0.977	0.197	0.448	0.659

Table S3. Correlation coefficients between ROIs defined based on the results of the Cann+CBD treatment in the DMN (PCC+), and visual analogue scale scores of subjective effects, taken in the same treatment session. Tables show Pearson's *r*, the uncorrected *p* values, and FDR-corrected *p* values for each region.

VAS Item	Dorsolateral Prefrontal Cortex			Inferior Frontal			Medial Frontal Gyrus		
	r	*p*	*p* (FDR)	*r*	*p*	*p* (FDR)	*r*	*p*	*p* (FDR)
Alert	-0.050	0.848	0.987	-0.148	0.572	0.785	0.014	0.958	0.976
Happy	0.096	0.715	0.987	0.133	0.611	0.785	0.211	0.416	0.976
Anxious	0.106	0.686	0.987	-0.206	0.427	0.785	-0.191	0.463	0.976
Paranoid	0.086	0.743	0.987	-0.114	0.663	0.785	-0.015	0.955	0.976
Mentally impaired	-0.223	0.389	0.987	-	0.68	0.785	0.100	0.70	0.976

				0.106	7			4	
Stoned	0.006	0.983	0.987	0.280	0.276	0.785	-0.008	0.976	0.976
High	-0.018	0.947	0.987	0.279	0.279	0.785	0.071	0.787	0.976
Feel drug effect	0.004	0.987	0.987	0.204	0.431	0.785	-0.116	0.657	0.976
Like drug effect	-0.232	0.371	0.987	0.256	0.321	0.785	-0.037	0.888	0.976
Dry mouth	-0.075	0.776	0.987	0.071	0.788	0.829	-0.255	0.323	0.976
Enhanced colour perception	-0.102	0.697	0.987	0.198	0.446	0.785	-0.072	0.783	0.976
Enhanced sound perception	-0.100	0.704	0.987	0.133	0.611	0.785	-0.151	0.562	0.976
Want to listen to music	-0.058	0.824	0.987	0.488	0.047	0.752	0.231	0.373	0.976
Want food	0.184	0.481	0.987	0.316	0.217	0.785	-0.029	0.911	0.976
Want more cannabis balloon	-0.083	0.752	0.987	0.057	0.829	0.829	-0.215	0.408	0.976
Want to smoke cannabis	0.159	0.542	0.987	0.144	0.583	0.785	-0.022	0.934	0.976

Table S4. Correlation coefficients between ROIs defined based on the results of the Cann+CBD treatment in the ECN (PCC-), and visual analogue scale scores of subjective effects, taken in the same treatment session. Tables show Pearson's *r*, the uncorrected *p* values, and FDR-corrected *p* values for each region.

	LH Motor Cortex		
VAS Item	***r***	***p***	***p* (FDR)**
Alert	0.059	0.822	0.877
Happy	-0.416	0.097	0.585
Anxious	-0.213	0.411	0.750
Paranoid	-0.207	0.425	0.750
Mentally impaired	0.020	0.939	0.939
Stoned	-0.471	0.056	0.585
High	-0.362	0.154	0.585
Feel drug effect	-0.292	0.256	0.585
Like drug effect	-0.059	0.822	0.877

Dry mouth	-0.382	0.130	0.585
Enhanced colour perception	-0.318	0.213	0.585
Enhanced sound perception	-0.312	0.223	0.585
Want to listen to music	-0.131	0.617	0.849
Want food	-0.188	0.469	0.750
Want more cannabis balloon	0.124	0.637	0.849
Want to smoke cannabis	-0.092	0.727	0.877

Table S5. Correlation coefficients between ROIs defined based on the results of the Cann+CBD treatment in the SAL (AI+) network, and visual analogue scale scores of subjective effects, taken in the same treatment session. Tables show Pearson's *r*, the uncorrected *p* values, and FDR-corrected *p* values for each region.

	Hippocampus			Posterior Cingulate		
VAS Item	***r***	***p***	***p* (FDR)**	***r***	***p***	***p* (FDR)**
Alert	-0.035	0.895	0.990	-0.283	0.271	0.394
Happy	-0.157	0.547	0.990	0.059	0.823	0.933
Anxious	0.488	0.047	0.376	0.188	0.469	0.577
Paranoid	0.511	0.036	0.376	0.225	0.386	0.515
Mentally impaired	0.218	0.401	0.917	0.513	0.035	0.078
Stoned	0.300	0.241	0.771	0.573	0.016	**0.043**
High	0.381	0.131	0.699	0.607	0.010	**0.035**
Feel drug effect	0.308	0.228	0.771	0.669	0.003	**0.021**
Like drug effect	0.020	0.938	0.990	0.409	0.103	0.165
Dry mouth	-0.052	0.842	0.990	0.667	0.003	**0.021**
Enhanced colour perception	0.139	0.594	0.990	0.602	0.011	**0.035**
Enhanced sound perception	0.242	0.349	0.917	0.657	0.004	**0.021**
Want to listen to music	-0.075	0.774	0.990	0.022	0.933	0.933
Want food	0.034	0.898	0.990	-0.031	0.905	0.933
Want more cannabis balloon	0.003	0.990	0.990	0.466	0.060	0.107
Want to smoke cannabis	0.032	0.904	0.990	0.504	0.039	0.078

Table S6. Correlation coefficients between ROIs defined based on the results of the Cann-CBD treatment in the DMN (PCC+), and visual analogue scale scores of subjective effects, taken in the same treatment session. Tables show Pearson's *r*, the uncorrected *p* values, and FDR-corrected *p* values for each region. Significant (FDR-corrected) *p* values are highlighted in bold text.

	LH Supplementary Motor Area			LH Orbitofrontal Cortex		
VAS Item	***r***	***p***	***p* (FDR)**	***r***	***p***	***p* (FDR)**
Alert	0.147	0.573	0.813	-0.269	0.296	0.773
Happy	0.102	0.696	0.813	-0.339	0.183	0.773
Anxious	0.205	0.431	0.791	-0.010	0.971	0.985
Paranoid	0.217	0.403	0.791	-0.056	0.831	0.985
Mentally impaired	0.335	0.189	0.791	0.276	0.283	0.773
Stoned	0.227	0.382	0.791	0.011	0.965	0.985
High	0.103	0.695	0.813	0.190	0.466	0.773
Feel drug effect	0.229	0.378	0.791	0.206	0.427	0.773
Like drug effect	-0.259	0.316	0.791	-0.005	0.985	0.985
Dry mouth	-0.199	0.445	0.791	-0.051	0.847	0.985
Enhanced colour perception	0.162	0.536	0.813	0.283	0.272	0.773
Enhanced sound perception	0.079	0.762	0.813	0.189	0.468	0.773
Want to listen to music	0.080	0.759	0.813	0.183	0.483	0.773
Want food	-0.238	0.358	0.791	-0.324	0.205	0.773
Want more cannabis balloon	-0.045	0.863	0.863	-0.040	0.878	0.985

Want to smoke cannabis	0.291	0.257	0.791	-0.200	0.441	0.773

Table S7. Correlation coefficients between ROIs defined based on the results of the Cann-CBD treatment in the ECN (PCC-), and visual analogue scale scores of subjective effects, taken in the same treatment session. Tables show Pearson's *r*, the uncorrected *p* values, and FDR-corrected *p* values for each region.

VAS Item	Putamen			Dorsomedial Prefrontal Cortex			Dorsolateral Prefrontal Cortex		
	r	*p*	*p* (FDR)	*r*	*p*	*p* (FDR)	*r*	*p*	*p* (FDR)
Alert	-0.423	0.091	0.918	-0.260	0.314	0.625	-0.169	0.518	0.872
Happy	-0.104	0.691	0.918	-0.102	0.698	0.798	-0.160	0.538	0.872
Anxious	-0.304	0.236	0.918	-0.421	0.093	0.380	-0.298	0.246	0.872
Paranoid	-0.216	0.404	0.918	-0.674	0.003	**0.048**	-0.079	0.763	0.872
Mentally impaired	0.065	0.805	0.918	-0.418	0.095	0.380	0.120	0.646	0.872
Stoned	0.141	0.589	0.918	-0.206	0.427	0.625	-0.036	0.890	0.932
High	0.325	0.204	0.918	-0.182	0.484	0.645	0.022	0.932	0.932
Feel drug effect	0.131	0.616	0.918	-0.205	0.430	0.625	-0.085	0.746	0.872
Like drug effect	0.219	0.398	0.918	0.008	0.974	0.974	0.205	0.430	0.872
Dry mouth	0.201	0.440	0.918	0.160	0.539	0.663	-0.080	0.760	0.872
Enhanced colour perception	-0.030	0.908	0.918	-0.444	0.074	0.380	-0.093	0.722	0.872
Enhanced sound perception	0.083	0.753	0.918	-0.302	0.238	0.625	0.091	0.728	0.872
Want to listen to music	-0.031	0.906	0.918	-0.083	0.751	0.801	0.254	0.325	0.872
Want food	-0.027	0.918	0.918	-0.229	0.378	0.625	0.313	0.221	0.872
Want more cannabis balloon	-0.156	0.551	0.918	-0.291	0.257	0.625	-0.237	0.359	0.872
Want to smoke cannabis	-	0.78	0.918	-0.233	0.368	0.625	-0.257	0.319	0.872

0.071 7

	Frontal Operculum			Medial Orbitofrontal cortex		
VAS Item	***r***	***p***	***p* (FDR)**	***r***	***p***	***p* (FDR)**
Alert	0.319	0.212	0.436	-0.430	0.085	0.275
Happy	0.362	0.153	0.436	-0.276	0.284	0.499
Anxious	-0.241	0.352	0.481	-0.409	0.103	0.275
Paranoid	0.180	0.490	0.490	-0.510	0.036	0.203
Mentally impaired	0.395	0.117	0.436	-0.342	0.179	0.358
Stoned	0.243	0.347	0.481	-0.257	0.320	0.499
High	0.200	0.442	0.481	-0.139	0.595	0.680
Feel drug effect	0.233	0.369	0.481	-0.245	0.343	0.499
Like drug effect	0.421	0.092	0.436	-0.160	0.541	0.666
Dry mouth	0.221	0.394	0.481	-0.005	0.984	0.984
Enhanced colour perception	0.315	0.218	0.436	-0.615	0.009	0.144
Enhanced sound perception	0.436	0.080	0.436	-0.362	0.153	0.350
Want to listen to music	0.584	0.014	0.224	-0.088	0.736	0.785
Want food	0.329	0.198	0.436	-0.170	0.515	0.666
Want more cannabis balloon	0.200	0.441	0.481	-0.506	0.038	0.203
Want to smoke cannabis	0.291	0.257	0.791	-0.200	0.441	0.773

Table S8 and S9. Correlation coefficients between ROIs defined based on the results of the Cann-CBD treatment in the SAL (AI+) network, and visual analogue scale scores of subjective effects, taken in the same treatment session. Tables show Pearson's *r*, the uncorrected *p* values, and FDR-corrected *p* values for each region. Significant (FDR-corrected) *p* values are highlighted in bold text.

References

Yarkoni, T., Poldrack, R. A., Nichols, T. E., Van Essen, D. C., & Wager, T. D. (2011). Large-scale automated synthesis of human functional neuroimaging data. *Nature Methods*, *8*(8), 665–670. http://doi.org/10.1038/nmeth.1635