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

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

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

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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 $\geq$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.

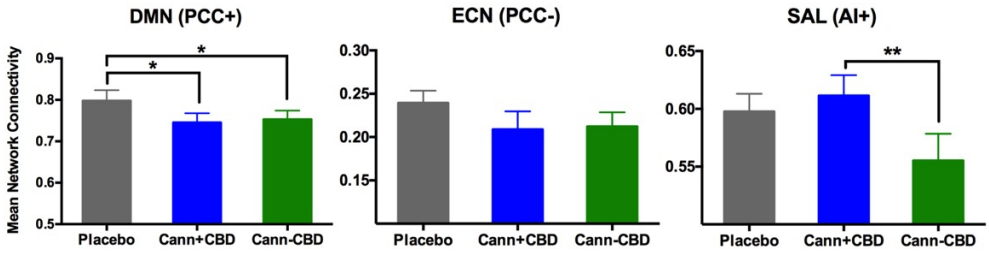

Figure 1. Treatment effects on the mean connectivity across the three networks; Default Mode Network (DMN; PCC+, left), Executive Control Network (ECN; PCC-, middle) and the Salience Network (SAL, AI+, right). * $p < 0.05$, ** $p < 0.005$. Error bars are standard errors.

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).

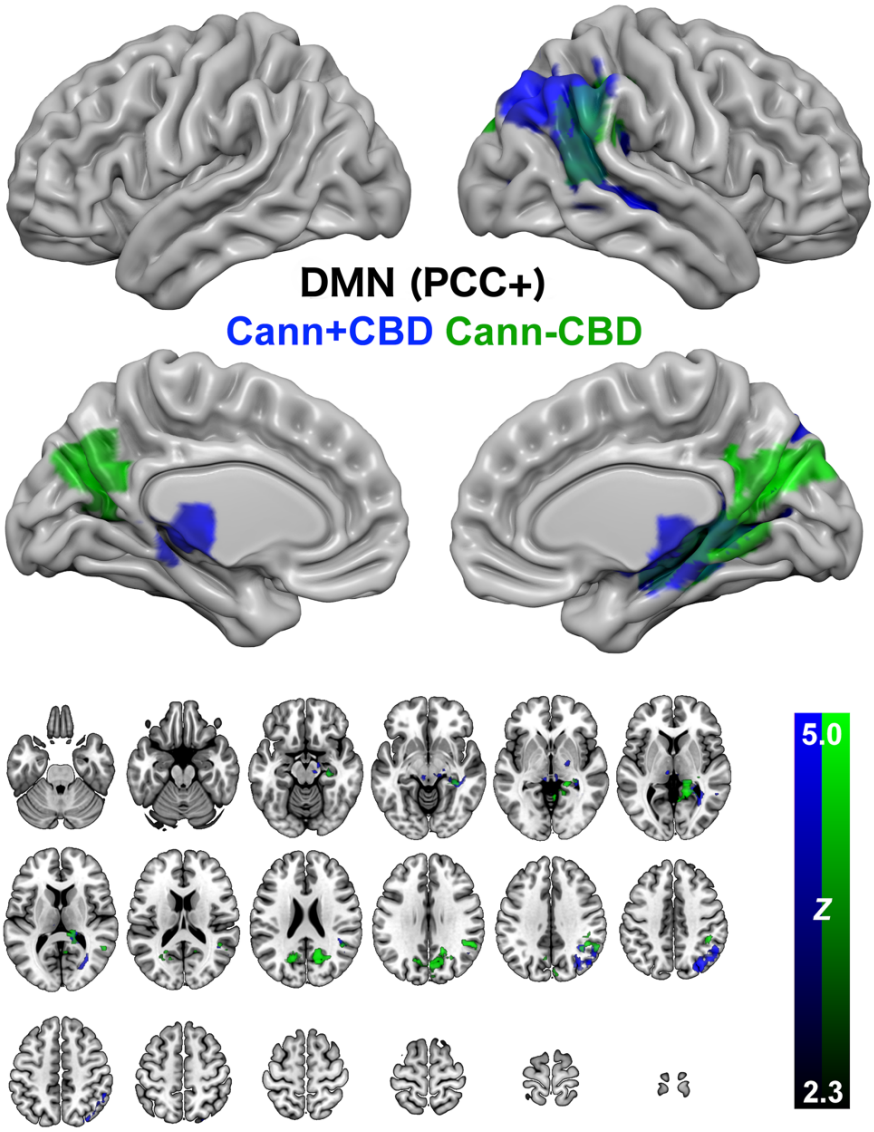

Figure 2. Drug treatment effects on the DMN (PCC+) network. All contrasts are placebo > drug, therefore significant ($Z = 2.3$, $p < 0.05$, cluster corrected for multiple comparisons) clusters represent relative decreases in functional connectivity in the drug condition. The Cann+CBD treatment session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

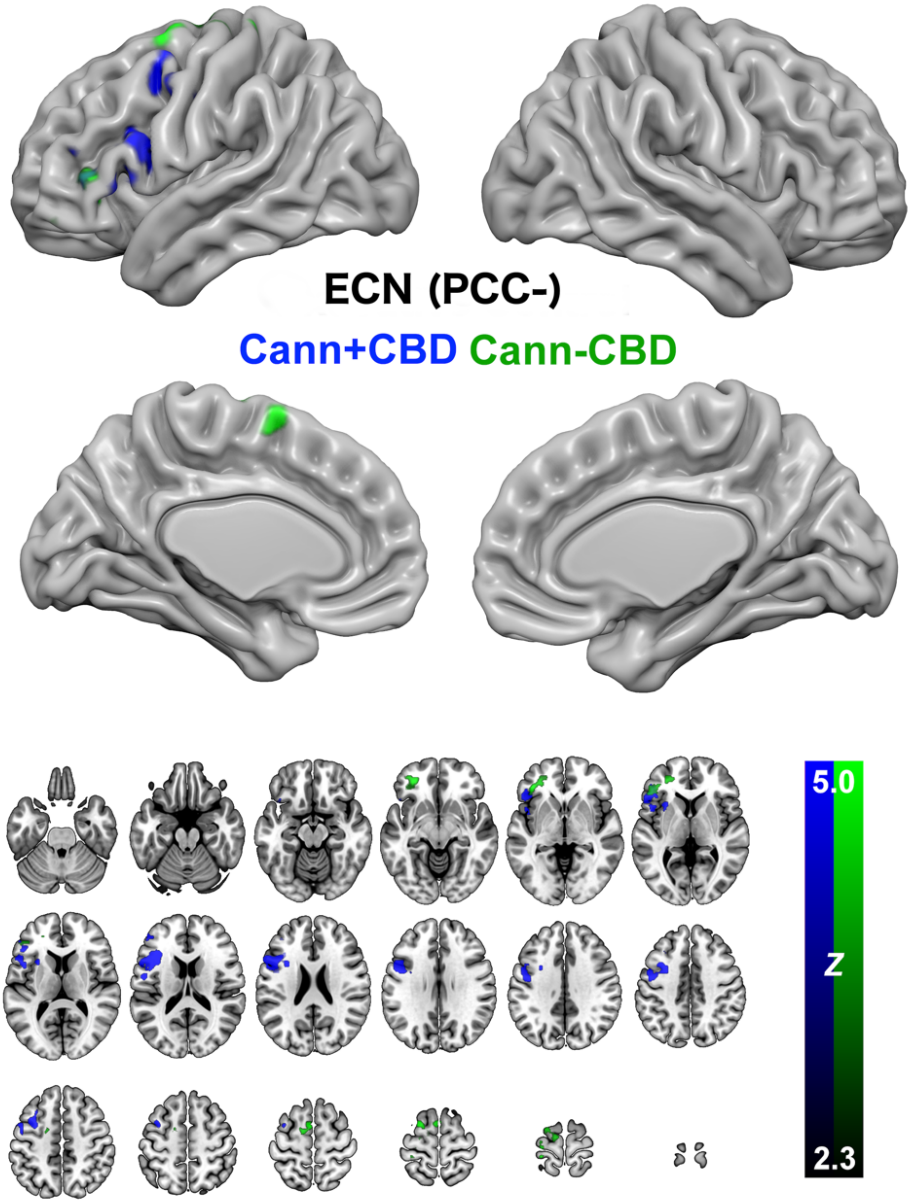

Figure 3. Drug treatment effects on the ECN (PCC-) network. All contrasts are placebo > drug, therefore significant ($Z = 2.3$, $p < 0.05$, cluster corrected for multiple comparisons) clusters represent relative decreases in functional connectivity in the drug condition. The Cann+CBD treatment session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

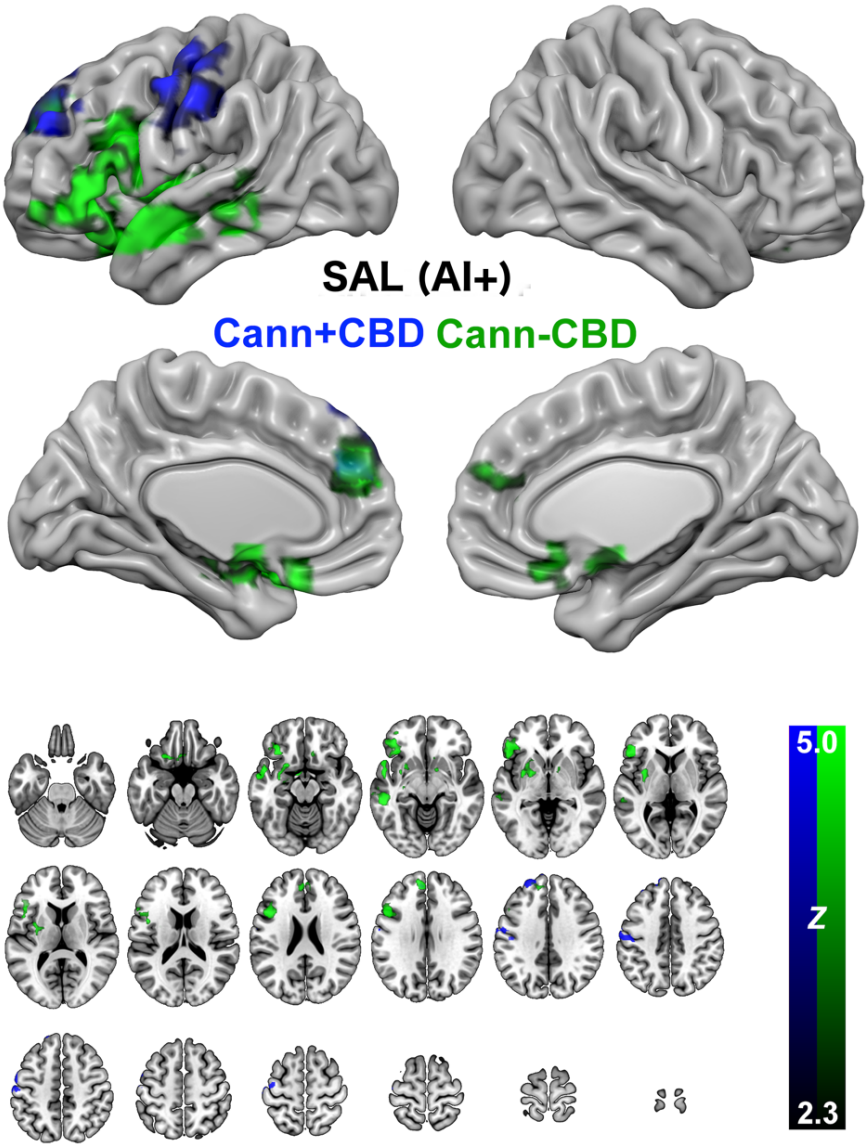

Figure 4. Drug treatment effects on the SAL (AI+) network. All contrasts are placebo > drug, therefore significant ($Z = 2.3$, $p < 0.05$, cluster corrected for multiple comparisons) clusters represent relative decreases in functional connectivity in the drug condition. The Cann+CBD treatment session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

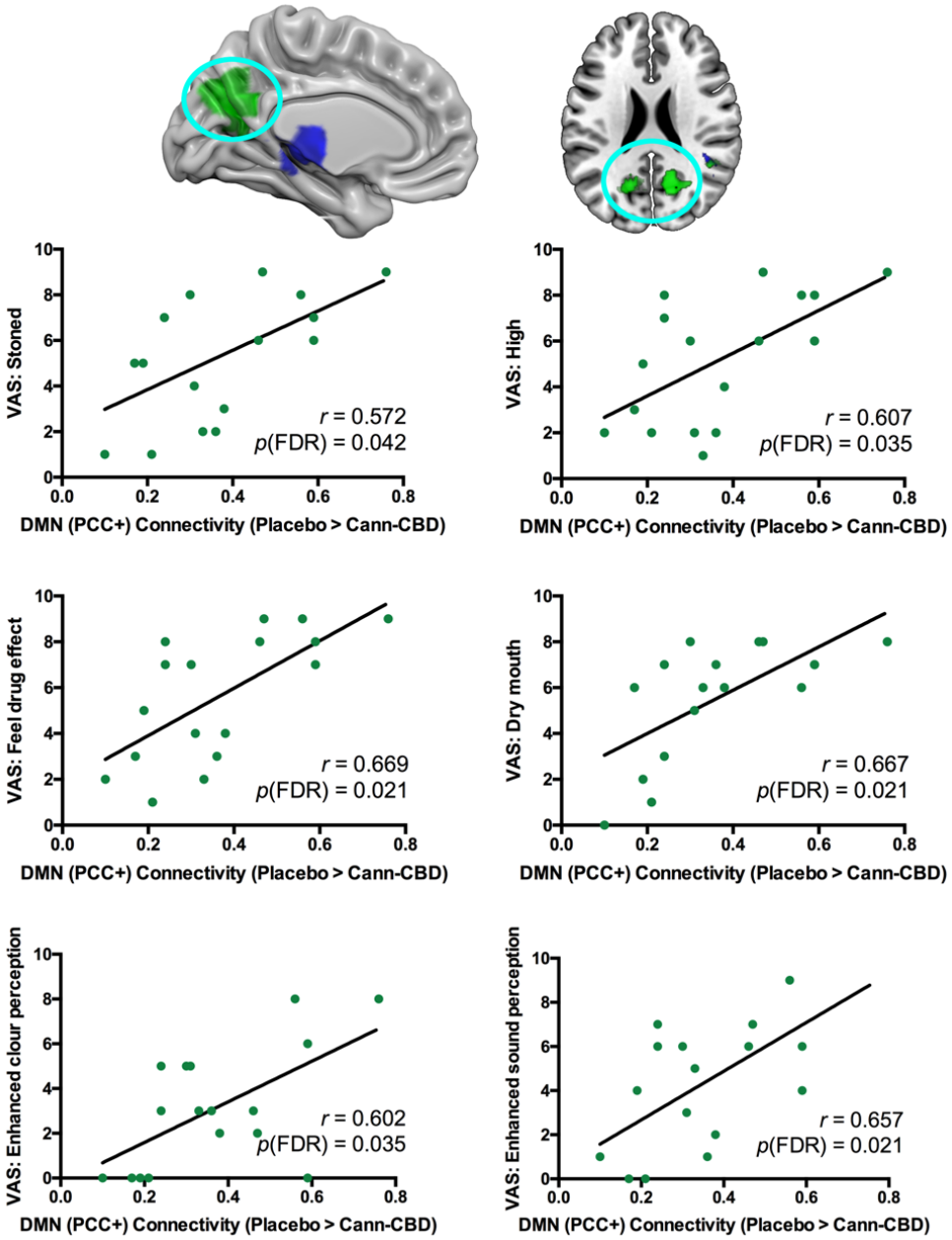

Figure 5. Correlations between the specific effect of Cann-CBD on the PCC in the DMN (PCC+) network analysis and Visual Analogue Scale (VAS) measures collected immediately after the MRI scanning session (approximately 90 minutes post-dosing). Correlations between the effect of Cann-CBD cannabis on the 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.

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