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Individual and combined effects of cannabidiol and Δ^9-tetrahydrocannabinol on striato-cortical connectivity in the human brain

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Abstract

Background: Cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) are the two major constituents of cannabis with contrasting mechanisms of action. THC is the major psychoactive, addiction-promoting, and psychotomimetic compound, while CBD may have opposite effects. The brain effects of these drugs alone and in combination are poorly understood. In particular, the striatum is implicated in the pathophysiology of several psychiatric disorders, but it is unclear how THC and CBD influence striato-cortical connectivity.

Aims: To examine effects of THC, CBD, and THC + CBD on functional connectivity of striatal sub-divisions (associative, limbic and sensorimotor).

Method: Resting-state functional Magnetic Resonance Imaging (fMRI) was used across two within-subjects, placebo-controlled, double-blind studies, with a unified analysis approach.

Results: Study 1 ($N = 17$; inhaled cannabis containing 8 mg THC, 8 mg THC + 10 mg CBD or placebo) showed strong disruptive effects of both THC and THC + CBD on connectivity in the associative and sensorimotor networks, but a specific effect of THC in the limbic striatum network which was not present in the THC + CBD condition. In Study 2 ($N = 23$, oral 600 mg CBD, placebo), CBD increased connectivity in the associative network, but produced only relatively minor disruptions in the limbic and sensorimotor networks.

Outcomes: THC strongly disrupts striato-cortical networks, but this effect is mitigated by co-administration of CBD in the limbic striatum network. Oral CBD administered has a more complex effect profile of relative increases and decreases in connectivity. The insula emerges as a key region affected by cannabinoid-induced changes in functional connectivity, with potential implications for understanding cannabis-related disorders, and the development of cannabinoid therapeutics.

Keywords
Cannabinoids, fMRI, resting-state, CBD, THC, cannabis

Introduction

Cannabis is a prevalent and commonplace drug that has been used by humans for thousands of years for recreational, spiritual, and medical purposes. The pharmacology of cannabis is complex, with almost 150 known cannabinoid compounds present in naturally occurring cannabis plant matter (Hanuš et al., 2016). The two major naturally occurring cannabinoids are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is the major psychoactive compound and is responsible for the majority of the subjective and cognitive effects (Curran et al., 2002), including feeling 'stoned', amnesia, anxiety, and psychotomimetic effects (D'Souza et al., 2004). THC is thought to exert its effects primarily by partial agonism at the CB1 receptor (Pertwee, 2008). CBD has less well understood and more complex pharmacological effects, including negative allosteric modulation at the CB1 receptor (Chesney et al., 2020), reducing reuptake of anandamide, and action on GPR55, μ-opioid and 5-HT1A receptors

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(Pertwee, 2008). CBD has antipsychotic (Leweke et al., 2012; McGuire et al., 2018), anxiolytic (Bergamaschi et al., 2011) and anti-addictive (Freeman et al., 2020; Hindocha et al., 2018; Hurd et al., 2019) properties, that are broadly oppositional to THC (Curran et al., 2016; Gunasekera et al., 2021). Experimental studies co-administering THC and CBD have produced mixed results, but the most common finding is that CBD reduces the effects of THC (Freeman et al., 2019a).

Cannabis is currently moving towards a decriminalised or fully legal status in a number of jurisdictions. There is also renewed interest in the medical uses of cannabinoids, with growth in their medical licencing (Freeman et al., 2019b; Hasin et al., 2017; Lucas and Walsh, 2017), particularly for the treatment of chronic and neuropathic pain (Leung, 2011) and mental health conditions (Walsh et al., 2017). As use of cannabinoids in medical contexts becomes more widespread, it is vital to understand the intricate pharmacological and physiological mechanisms behind their potential therapeutic effects. One brain system known to be strongly affected by both acute and chronic use of cannabis of particular relevance to therapeutic, recreational, and harmful effects is the dopaminergic system and associated brain regions, principally the striatum (Bloomfield et al., 2019). The density of CB1 receptors is medium to high in striatal regions (Glass et al., 1997) and previous work has shown reductions in striatal dopamine function in cannabis users (Bloomfield et al., 2014; Tomasi et al., 2015; Van de Giessen et al., 2017), and selective dopamine release in the limbic sub-division of the striatum with an acute THC challenge (Bossong et al., 2015). Functional and behavioural data have also shown that cannabis can acutely modulate striatal responses to hedonic stimuli (Freeman et al., 2018), and impair reward learning (Lawn et al., 2016). Multiple lines of evidence implicate the striatum in the pathophysiology of psychotic disorders (e.g. Howes et al., 2011; Karcher et al., 2019) and the nucleus accumbens in the limbic striatum in particular is the central region in influential theories of addiction (e.g. Everitt and Robbins, 2013; Robbins and Everitt, 2002). Recent work examining the effects of cannabinoids on striato-cortical connectivity has shown a mix of effects, particularly in terms of the directionality (i.e. increases or decreases in connectivity). Both Grimm et al. (2018) and Crane and Phan (2021) report increases (with CBD and THC, respectively) while others have reported decreases (Mason et al., 2019; Ramaekers et al., 2016). These inconsistencies in previous studies may arise from differences in the doses, the method of administration, the analysis methods used or a number of other methodological factors. Fully characterising and clarifying the effects of THC and CBD on the striatum is therefore vitally important for understanding its role in the pathophysiology of disorders, and as a means to evaluate potential cannabinoid treatments.

We therefore sought to investigate the effects of cannabinoids on functional connectivity of the striatum, using resting-state functional Magnetic Resonance Imaging (fMRI). First, we examined the effects of vaporised herbal cannabis with and without CBD on connectivity in three striatal sub-divisions. In a second study, to isolate the effects of CBD, we investigated the effects of oral CBD versus placebo in the same regions. Our first hypothesis was that THC will disrupt/reduce striato-cortical functional connectivity particularly in the limbic striatal sub-division. Our second hypothesis was that CBD would ameliorate these effects when delivered in combination with THC, so any significant reductions in connectivity seen would be less spatially extensive in the brain. Our third hypothesis was that CBD administered alone would also reduce connectivity in these networks, but likely with a qualitatively different pattern of functional modulations of brain regions to THC or THC + CBD.

Experimental procedures

Study 1

Additional data from this study have been published elsewhere (Freeman et al., 2018; Lawn et al., 2016). The current data are a re-analysis of the resting-state data reported in Wall et al., (2019); this previous report did not focus on striato-cortical connectivity.

Study design. This study included three drug conditions: cannabis containing both THC and CBD (THC + CBD), high-THC cannabis without CBD (THC), and placebo cannabis (without either THC or CBD). These three conditions were used in a randomised, crossover, placebo-controlled, double-blind design. A Latin Square design was used to randomly assign participants to one of three condition orders. To avoid carry-over effects, the scanning sessions were separated by at least 1 week. Data on cannabinoid half-lives vary, but the typical elimination half-life of THC is around 22 h and perhaps somewhat longer in heavy or regular users (Foster et al., 2019).

Participants. Full demographic and drug-history information is shown in Supplementary Table 1. Seventeen healthy volunteers (nine women) between 18 and 36 years old were recruited (mean age = 26.2, standard deviation (SD) = 7.1). The recruitment followed the inclusion criteria for cannabis use of ⩽3 times per week and ⩾4 times in the past year. The participants reported on average 8.1 (SD = 5.5) days/month of cannabis use.

Volunteers were excluded if there was current or past history of psychosis in themselves or an immediate family member and if there were any other medical problems considered clinically significant for the study. In addition, drug-related exclusion criteria were previous negative experiences with cannabis, alcohol use >5 times per week and use of any other illicit drug >twice per month. The study was conducted in accordance with the Declaration of Helsinki and was approved by the University College London (UCL) Ethics Committee. Participants provided written informed consent prior to the first study session, and they were reimbursed for their time.

Drug administration. All three varieties of cannabis were sourced from Bedrocan (The Netherlands), and were matched for appearance and smell. In each session, the same amount of cannabis was administered (133.4 mg). The THC and CBD doses for this study were determined based on the previous experiments that used similar vaporisation methods (Bossong et al., 2009; Hindocha et al., 2015) and Bedrocan product potencies (Niesink et al., 2015). The dose was 8 mg THC in both cannabis conditions (THC, THC + CBD) and 10 mg of CBD in the THC + CBD condition. The THC (8 mg) dose has produced subjective, cognitive and psychotomimetic effects in previous studies and reflects 1.6 standard units of THC at 5 mg (Freeman and Lorenzetti, 2020).

All the cannabis was used within 6 months of purchase and was stored in foil-sealed pouches at −20°C and then at ambient temperature immediately prior to administration.

Each cannabis dose was administered using a Volcano Medic Vaporiser (Storz & Bickel, Tuttlingen, Germany) in line with previous studies (Bossong et al., 2009; Hindocha et al., 2015; Mokrysz et al., 2016). The drug was vaporised at 210°C and the product was collected in two balloons. Participants were asked to inhale the drug from the balloons at their own pace and hold each inhalation for 8 s.

Procedure. Participants completed a telephone screening and then attended a screening visit to assess eligibility, drug history, and complete trait questionnaires. In addition, they received task training for tasks reported elsewhere (Freeman et al., 2018; Lawn et al., 2016) and a video training of the drug inhalation process. Prior to each study visit, participants were asked to abstain from drug and alcohol use for 24 h. At the beginning of each visit, a urine test was used to verify the participant's self-reported drug use and screen for pregnancy. Then, the drug was administered and 30 min post-administration the MRI scanning session commenced, which lasted approximately 1 h. Following the MRI session, participants received a top-up administration and completed a battery of behavioural tasks (reported in Lawn et al., 2016, and Mokrysz et al., 2020). Blood samples for measurement of drug concentrations in the plasma were not collected in this experiment.

MRI acquisition. A Siemens Avanto 1.5T scanner (Erlangen, Germany) using a 32-channel phased-array head-coil was used to acquire the MRI data. The resting-state functional images were acquired with a T2* gradient-echo echo-planar imaging (EPI) sequence with (repetition time (TR) = 2800 ms, 32 slices, 3.2 mm isotropic voxels, time to echo (TE) = 43 ms, flip angle = 90°). Volumes at the beginning of the scan were automatically discarded by the scanner to account for T1-equilibration effects. The scan duration was 12 min and 8 s, with a total of 260 volumes. At the beginning of the scan session, standard MPRAGE (Magnetisation Prepared RApid Gradient Echo) T1-weighted anatomical scans were also acquired for the purposes of co-registration of the functional images (TR = 2730 ms; TE = 3.57 ms; matrix = 176 × 256 × 256; 1 mm isotropic voxels; flip angle = 7°; bandwidth = 190 Hz/pixel; parallel imaging acceleration factor = 2).

Study 2

Additional data from this study have been published elsewhere; these previous reports did not investigate resting-state striato-cortical connectivity (Bloomfield et al., 2020a; Lawn et al., 2020).

Study design. The study used a double-blind, randomised, placebo-controlled, repeated-measures (within-subjects) design to compare the effects of oral CBD 600 mg (pure synthetic (-)-CBD) with matched placebo (PBO) in identical capsules at two sessions. The within-subjects design meant that subjects attended for three separate visits, and all subjects completed all three dosing conditions. Drug order was completely concealed from participants and experimenters until data collection, entry, and analysis had been completed. To avoid carry-over effects, the scanning sessions were separated by at least 1 week, which is more than three times the elimination half-life of THC (Hindocha et al., 2014). The order of drug was block randomised and stratified for sex. This study was conducted in accordance with Good Clinical Practice and the Helsinki Declaration (UCL Research Ethics Committee 3325/002). Participants provided written informed consent and received an honorarium for participation (£10 per hour).

Drug administration. Synthetic CBD (99.9% purity) was obtained from STI Pharmaceuticals (Brentwood, UK) and manufactured by Nova Laboratories (Leicester, UK). Size 2 gelatin capsules contained microcrystalline cellulose filler and CBD. Matched placebo capsules contained lactose filler. The CBD was formulated in 50 mg capsules. Participants swallowed all 12 capsules at their own pace under invigilation of the experimenter. The 600 mg dose was chosen as it produces an increase in plasma concentrations after acute administration (Babalonis et al., 2017; Englund et al., 2013), is well tolerated in humans (Grotenhermen et al., 2017), has been found to produce a significant anxiolytic effect (Bergamaschi et al., 2011), and has opposing effects to THC on the striatum during fMRI (Bhattacharyya et al., 2010). Previous research suggests that CBD reaches the peak level of plasma concentration after approximately 2.5 h (Babalonis et al., 2017).

Participants. Participants were recruited through online adverts, posters and word-of-mouth. We tested 28 healthy participants. Four participants did not complete both study visits, and one additional subject attended both visits but did not complete the scanning session, so their resting-state data was incomplete. These five subjects were excluded which meant 23 complete sets of data were available for analysis. Subjects ranged in age between 19 and 36 (mean = 23.8, SD = 4.3), all had normal body mass index (BMI) (mean = 22.4, SD = 3.6) and had sub-clinical scores on the Beck Depression Inventory (BDI) (mean = 2.2, SD = 4.9) and Beck Anxiety Inventory (BAI) (mean = 2.6, SD = 3.2). No participant showed any evidence of alcohol or nicotine dependence as measured by the Alcohol Use Disorders Identification Test (AUDIT; mean = 2.2, SD = 2.8) and the Fagerström Test for Nicotine Dependence (FTND; mean = 0, SD = 0). All participants included were right-handed and aged 18–70 years. Exclusion criteria were as follows: (1) current use of psychotropic agents; (2) current or past use of cannabis or CBD; (3) previous use of any psychoactive (recreational) drug on > 5 occasions; (4) current or previous mood disorder, psychosis, anxiety disorder or substance abuse disorder according to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria; (5) current nicotine dependence (defined by FTND score of > 4; Heatherton et al., 1991; Huang et al., 2008); (6) score > 7 on the AUDIT (Saunders et al., 1993); (7) pregnancy; (8) impaired mental capacity; (9) allergy to CBD or placebo excipients; (10) claustrophobia or other contraindications to MRI.

Procedure. Participants completed a screening on the telephone during which initial eligibility criteria (drug use, FTND, AUDIT, MRI contraindications, allergies, medical information and handedness) were assessed and basic participant details were recorded.

Participants who appeared eligible on the phone were invited to attend experimental sessions. Participants were asked to fast from midnight the day before both sessions, and refrain from smoking tobacco and consuming alcohol for 24 h before the start of the sessions. Upon arrival, participants underwent urine tests to verify they were not pregnant (if female) and they had not recently taken recreational drugs. They also completed breath tests for alcohol and carbon monoxide. Eligible participants then completed two 7-h experimental sessions, when they received CBD or placebo on the first session, and the other drug condition on the second session. The MRI scanning session commenced 2.5 h after drug administration and lasted approximately 1.5 h.

Plasma CBD concentrations. We performed venipuncture immediately after MRI scanning to measure CBD concentrations. Blood samples were collected in EDTA vacutainers and were immediately centrifuged to plasma for storage at −80°C. Samples were analysed using the Gas Chromatography coupled with Mass Spectrometry with a lower limit of quantification of 0.5 ng/mL.

MRI acquisition. MRI data were collected using a 3-Tesla Siemens Prisma MRI Scanner at the Robert Steiner MR unit at Hammersmith Hospital, London. Functional imaging used a gradient-echo T2*-weighted EPI sequence with 42 slices per volume (TR ms; TE ms; in-plane matrix = 64 × 64; 3 mm isotropic voxels; flip angle = 62°; bandwidth = 1594 Hz/pixel; 304 volumes; a slice thickness of 3 mm; field of view = 192 × 192 mm). The phase encoding direction was from anterior to posterior. Volumes at the beginning of the scan were automatically discarded by the scanner to account for T1-equilibration effects. For structural acquisition, a T1-weighted structural volume was acquired for all participants using a MPRAGE scan (TR = 2300 ms; TE = 2.28 ms, TI = 900 ms, flip angle = 9°, field of view = 256 mm, image matrix = 256 with 1 mm isotropic voxels; bandwidth = 200 Hz/pixel).

Statistical analysis (Study 1 and Study 2)

Image analyses were performed using FSL 5.0.4. The functional data were pre-processed using spatial smoothing with a 6 mm FWHM (full-width, half-maximum) Gaussian kernel, high-pass temporal filtering (100 s), head-motion correction using MCFLIRT and non-linear registration to a standard template (MNI152). The anatomical data were skull-stripped using FSL's brain extraction tool (BET) and segmented using FMRIB's automated segmentation tool (FAST), into grey/white matter and cerebro-spinal fluid (CSF).

Striatal networks: seed-based analysis (Study 1 and Study 2). Brain masks for the three striatal networks (associative, limbic and sensorimotor) were defined according to the original tripartite definition by Joel and Weiner (2000) and Martinez et al. (2003) and using the atlas provided by Tziortzi et al. (2014). The associative mask included the precommissural dorsal caudate, the precommissural dorsal putamen and postcommissural caudate. The limbic mask included the ventral pallidum and substantia nigra; the sensorimotor mask comprised the postcommissural putamen.

A set of seed-based analyses were conducted using methods similar to previous reports (Comninos et al., 2018; Demetriou et al., 2016; Wall et al., 2019). The standard-space striatal brain masks were co-registered to each participant's functional image space, and time-series were extracted from these regions that were subsequently used in the first-level analysis models as regressors of interest. In addition, the white matter and CSF time-series from each participant were included in the analysis models as regressors of no interest, along with head-motion regressors. The inclusion of white-matter and CSF regressors is a principled and effective method of noise-mitigation (similar to the 'CompCor' method; Behzadi et al., 2007) which models both subject-related (e.g. head-motion and physiological signals) and non-subject-related noise (e.g. scanner thermal noise). First-level models included use of FSL's FILM algorithm to correct for auto-correlation in the time-series. Higher-level analyses were performed using FSL's FLAME-1 mixed-effects model, and the results were cluster-corrected for multiple comparisons at $Z > 2.3$, $p < 0.05$. Separate group-level models were produced in order to model mean functional connectivity effects (all subjects, all scans) for each study, and voxelwise comparisons between the drug conditions (three comparisons in Study 1, two in Study 2). To quantify the treatment effects across each striatal network, the group-mean (all subjects, all scans) functional connectivity results were used to produce image masks (thresholded at $Z = 5$) from which numeric data were extracted for each subject/scan. Drug effects on mean network connectivity were assessed using standard statistical methods (repeated-measures analyses of variance (ANOVAs) with post hoc tests using Tukey's correction for multiple comparisons in Study 1, and standard *t*-tests in Study 2).

Results

Study 1

Both the doses of cannabis used produced clear subjective effects on visual analogue scales of drug effects, relative to placebo ($p < 0.001$ for all time-points other than baseline (pre-drug administration); see Lawn et al. (2016) for details).

Seed-based functional connectivity analyses. There were no effects seen in the active drug conditions > placebo contrasts, in any of the analyses, meaning the conditions did not significantly increase connectivity relative to placebo. When administered alone, THC significantly disrupted (placebo > active conditions) mean connectivity between the limbic striatum and the bilateral insula and frontal opercular cortex as shown in Figure 1. By contrast, when THC was co-administered with CBD, there was no evidence for disruption of connectivity between the limbic striatum and any brain region.

Administration of the THC + CBD condition reduced connectivity of the associative striatum with the dorsal anterior cingulate as well as a large lateral region covering part of frontal opercular cortex and sensorimotor regions in the left hemisphere (more restricted in the right hemisphere). The THC condition showed a broadly similar, although somewhat more widespread, distribution with the regions affected covering more of the frontal operculum and extending downwards into the insula (see Figure 2).

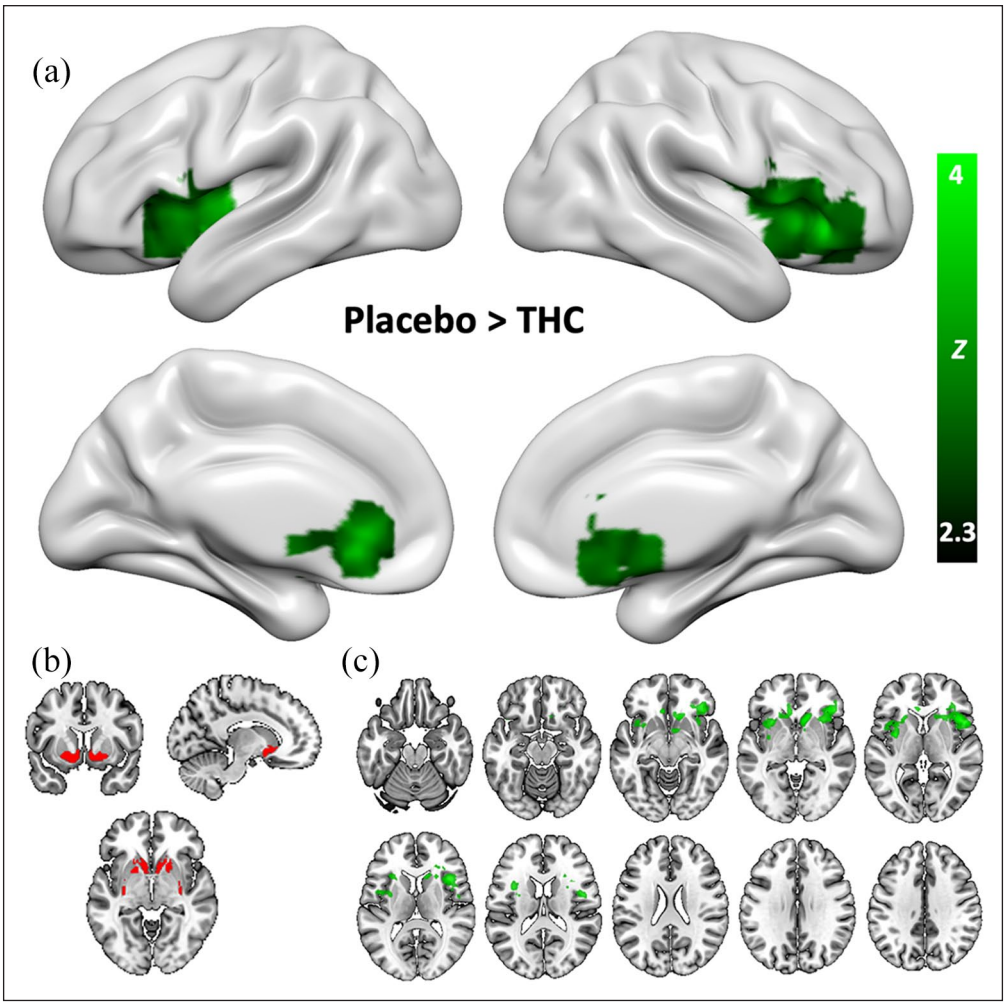

Figure 1. Drug effects on brain-wide connectivity with the limbic striatum in study 1. Contrast is placebo > THC. Clusters represent a significant decrease in functional connectivity with the limbic striatum in the active drug condition. (a) Significant clusters on 3D cortical surface renders. (b) The limbic striatum seed-region. (c) Significant clusters on axial slices. The THC + CBD condition showed no significant effects for this seed-region.

Connectivity with the sensorimotor striatum was the most strongly disrupted of the striatal networks in this study. The THC + CBD condition reduced activity within many sensory-motor–associated areas such as the parietal operculum cortex, central opercular cortex and the post central gyrus. Language and auditory–associated areas also had reduced connectivity including the supramarginal gyrus, planum temporale and Heschl's gyrus. There was also some reduction seen in the motor cortex. Similar disruptions were seen in the THC condition, the most notable differences are larger portion of Heschl's gyrus disrupted as well as secondary somatosensory cortex on the medial surface of both hemispheres (Figure 3).

For all three networks, direct voxelwise comparisons of the two active drug conditions (i.e. THC vs THC + CBD) showed no significant clusters. The overall mean connectivity of each network under each drug condition (relative to placebo) was also examined using thresholded versions of the group-mean connectivity maps as mask images. A repeated-measures ANOVA with a single three-way factor (drug treatment) of the associative striatum data showed no significant results. In the limbic striatum network, a marginal main effect of drug treatment was seen ($F(2,32)=3.25$, $p=0.052$), and post hoc tests showed a significant effects in the placebo versus THC comparison ($t(16)=2.69$, $p=0.040$). In the sensorimotor striatum network, there was a strong main effect of drug treatment ($F(2,32)=7.57$, $p=0.002$), and significant differences in the post hoc tests between both drug treatments and placebo (THC + CBD: $t(16)=2.93$, $p=0.025$, and THC: $t(16)=3.07$, $p=0.019$). All post hoc test p-values here are based on Tukey's tests and are therefore corrected for multiple comparisons. All other comparisons (including direct comparisons between the two active drug conditions) were non-significant (Figure 4).

Study 2

Blood plasma results showed higher CBD levels in CBD sessions (median = 6.01 ng/mL, inter-quartile range = 4.24) compared to the placebo sessions (median = 0 ng/mL, inter-quartile range = 0). The Wilcoxon signed-rank test is as follows: $z=4.1$, $p<0.001$, $r=0.88$.

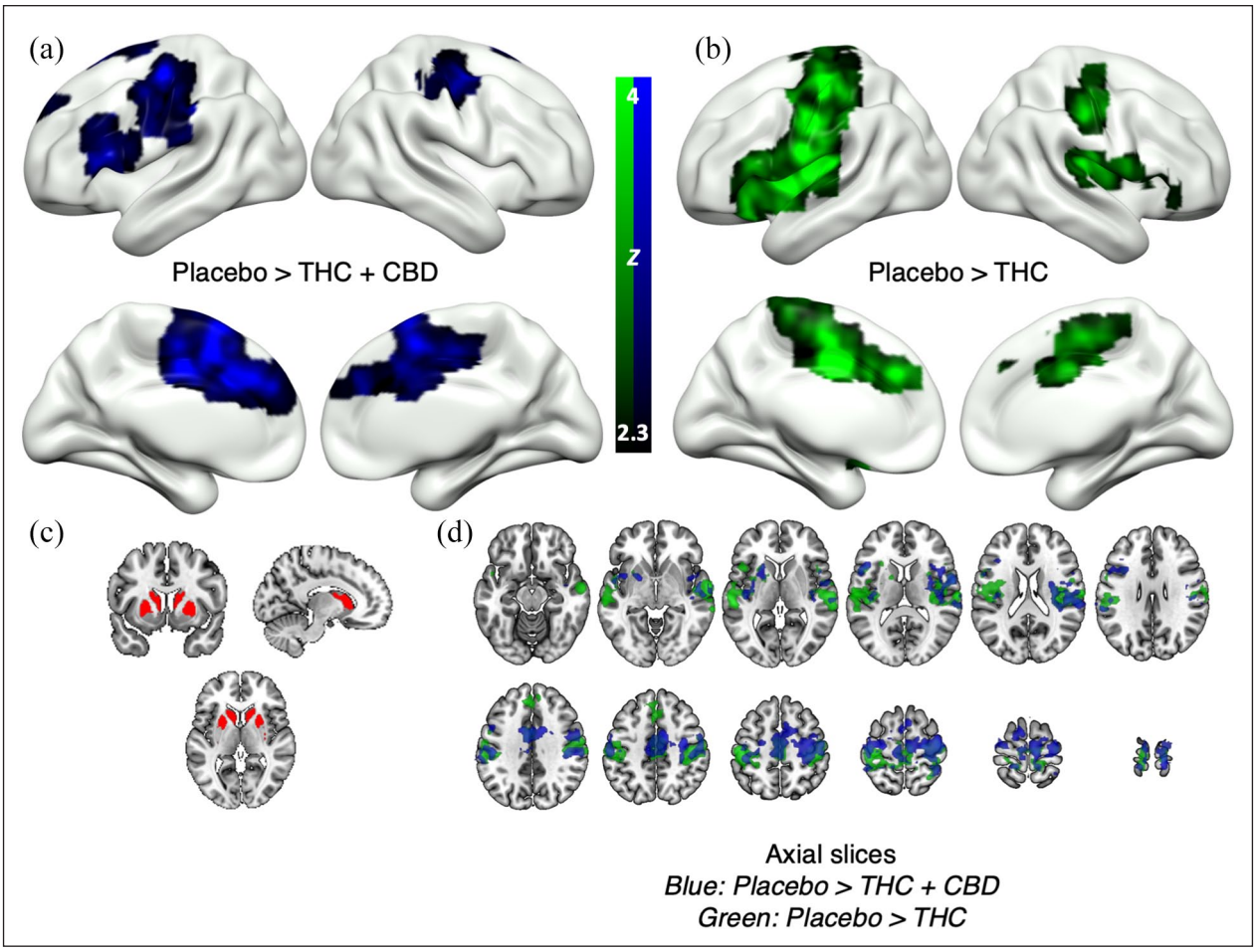

Figure 2. Drug effects on brain-wide connectivity with the associative striatum in Study 1. Contrasts are placebo > active drug. Clusters represent a significant decrease in functional connectivity with the associative striatum in the active drug conditions. (a) Significant clusters (blue) in the placebo > THC + CBD comparison on 3D cortical renders. (b) Significant clusters (green) in the placebo > THC comparison on 3D cortical renders. (c) The associative striatum seed-region. (d) Significant clusters in both comparisons ((a) and (b); same colours) overlaid together on axial slices.

Imaging results from Study 2 showed a markedly different effect of oral CBD on striatal functional connectivity. Figure 5 shows results from all three analyses (using associative, limbic, and sensorimotor sub-divisions as seed-regions) for the CBD condition versus placebo. Connectivity analyses with the associative sub-division showed drug effects in bilateral areas in the posterior parietal lobes, extending medially into the parieto-occipital sulcus and into the posterior cingulate in the left hemisphere. It is important to note that this result is the opposite contrast to the results found in Study 1 (both comparisons vs placebo, and in fact, the other two results described below from Study 2), and is in fact CBD > placebo, implying a relative *increase* in functional connectivity between these regions and the associative striatum, under the CBD condition. No areas showing significant relative decreases (placebo > CBD) were found in this analysis. For the limbic striatum seed-region, an area of decreased connectivity (placebo > CBD) was found in the right hemisphere insula and lateral frontal cortex. For the sensorimotor seed-region, significant clusters of relatively decreased connectivity (placebo > CBD) were seen in the left cerebellum. For these latter two analyses, no areas showing significant relative increases (CBD > placebo) were found. There were also no significant effects in the Region of Interest (ROI) mask analyses which compared mean connectivity between each treatment condition (all $p > 0.3$), for each network (see Supplementary Information for statistical results).

Discussion

The present data (from two studies, with different subjects, dosing and administration routes) collectively demonstrate extensive effects of cannabinoids on striatal functional connectivity networks. In Study 1, effects on the limbic striatum were specific to the inhaled THC condition, with disruptions (relative decreases in connectivity with the active drug condition) seen in the anterior insula, and areas of the striatum itself. Effects of the different drug conditions on associative striatal connectivity were both widespread, and somewhat dissociated, with both strains disrupting dorsal regions (anterior cingulate cortex (ACC) and motor

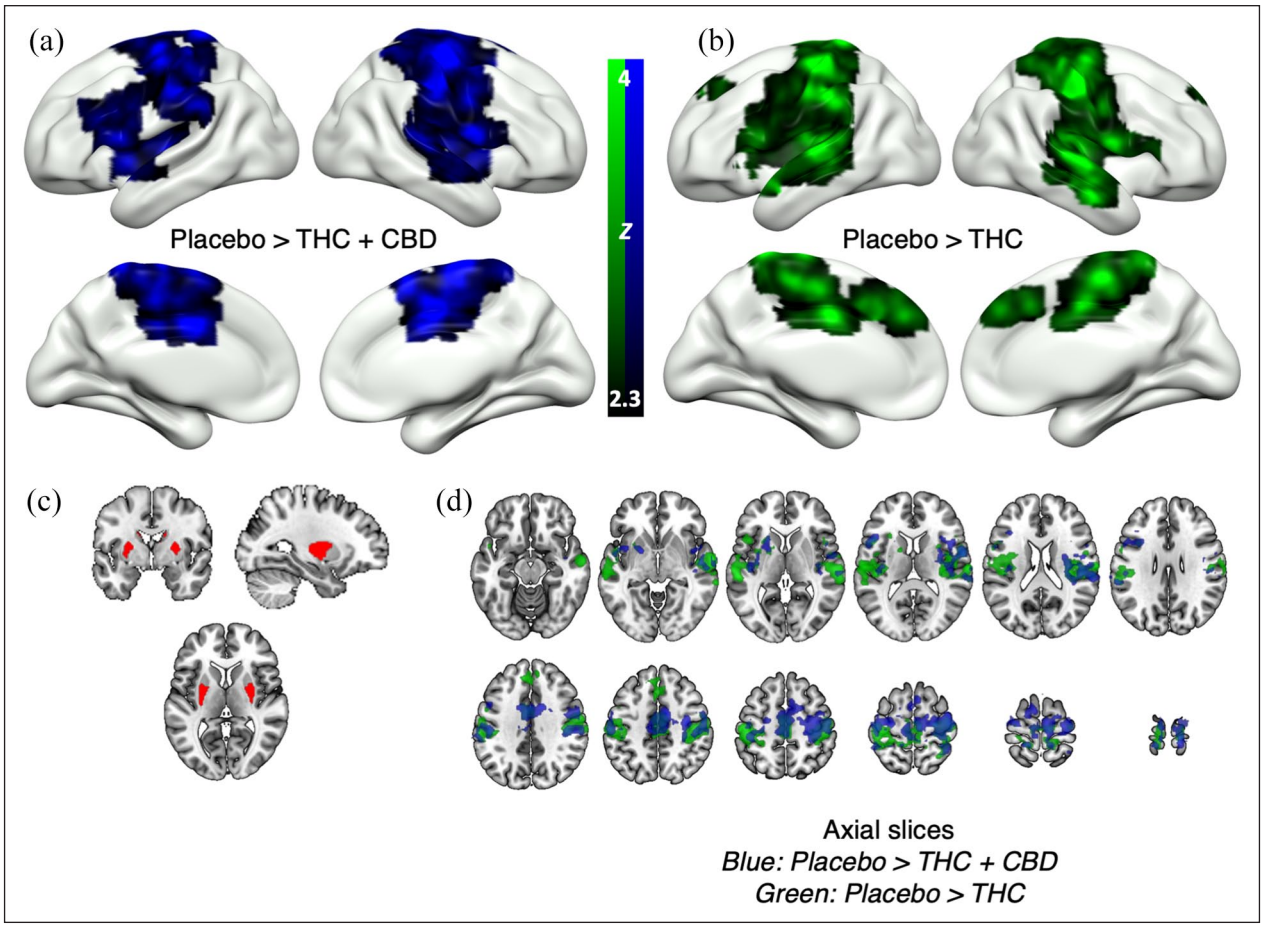

Figure 3. Drug effects on brain-wide connectivity with the sensorimotor striatum in study 1. Contrasts are placebo > active drug. Clusters represent a significant decrease in functional connectivity with the sensorimotor striatum in the active drug conditions. (a) Significant clusters (blue) in the placebo > THC + CBD comparison on 3D cortical renders. (b) Significant clusters (green) in the placebo > THC comparison on 3D cortical renders. (c) The sensorimotor striatum seed-region. (d) Significant clusters in both comparisons ((a) and (b); same colours) overlaid together on axial slices.

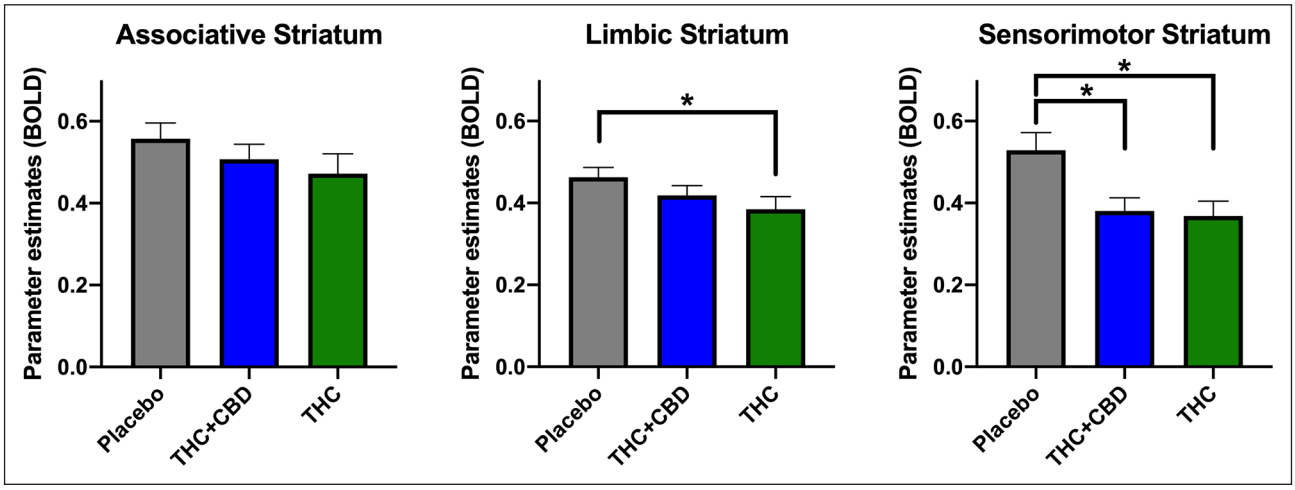

Figure 4. Mean connectivity within each network, across the three drug conditions. Significant effects were seen in the limbic striatum network (placebo vs THC: $t(16) = 2.69$, $p = 0.04$) and in the sensorimotor striatum network (placebo vs THC + CBD: $t(16) = 2.93$, $p = 0.025$; placebo vs THC: $t(16) = 3.07$, $p = 0.019$).
*$p < 0.05$ (Tukey's-corrected for multiple comparisons).

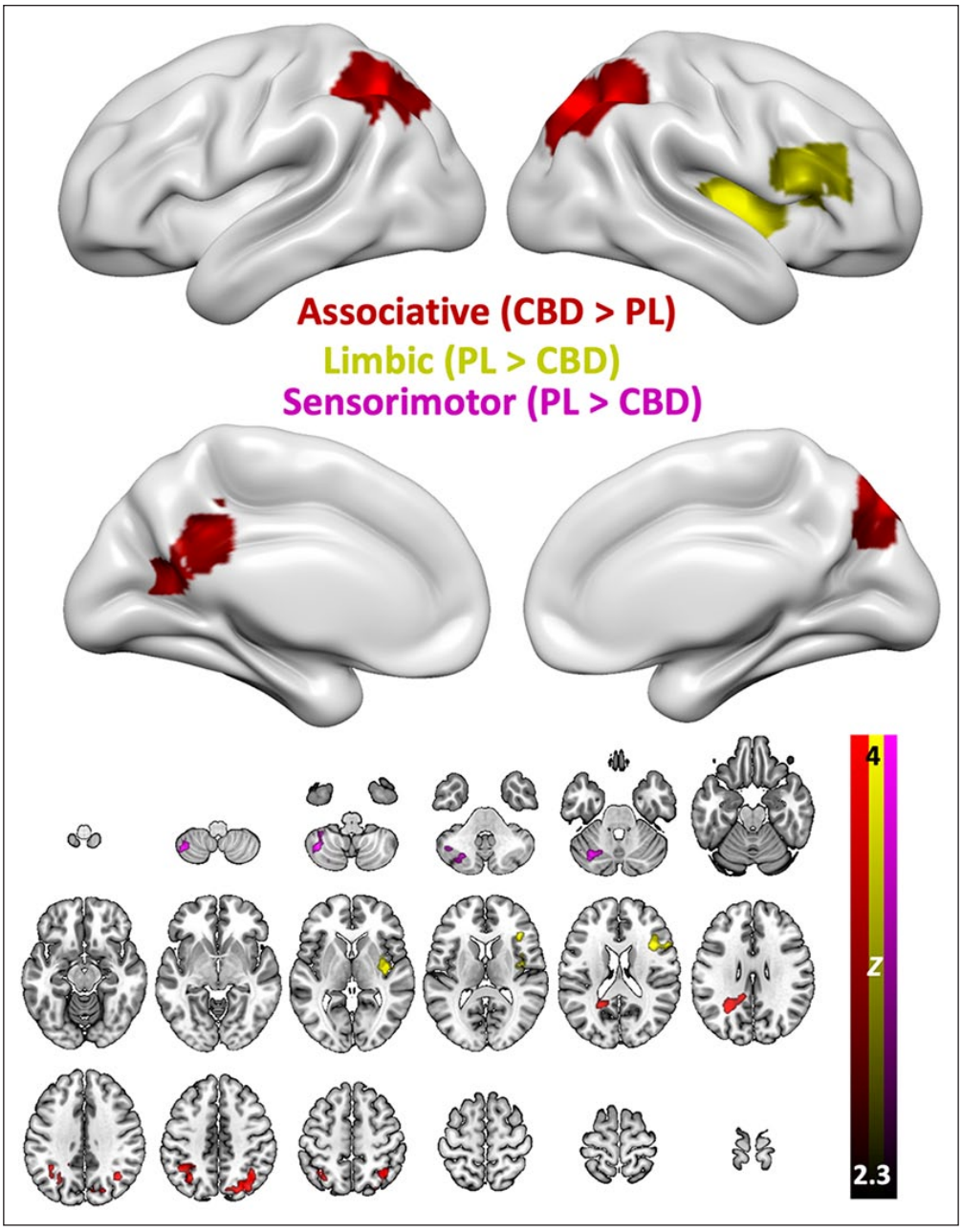

Figure 5. Drug effects on brain-wide connectivity with the associative (red), limbic (yellow) and sensorimotor (pink) striatum in Study 2.
CBD: cannabidiol; PL: placebo.
Both relative increases (CBD > PL) and decreases (PL > CBD) are shown, depending on the pattern of significant results in the three analyses. Effects on sensorimotor striatum connectivity were only seen in the left cerebellum, and are therefore not visible on the top panel, which only shows inflated views of the cortex.

cortex), but the inhaled THC condition also specifically affecting more ventral regions (frontal operculum and insula). Regions affected in the sensorimotor striatum analysis were somewhat similar, with perhaps less of a dorsal/ventral dissociation between the two conditions. In Study 2, the effect of 600 mg oral CBD is noticeably weaker and less widespread, with disruption of connectivity in the analyses of limbic and sensorimotor seed-regions only seen in localised regions in one hemisphere (the insula/lateral frontal lobe and the cerebellum, respectively). Intriguingly, the analysis of the associative striatum connectivity in Study 2 showed a result of opposite polarity; a relative increase, or enhancement of connectivity, in parietal regions as a result of the drug administration.

Overall, it is clear cannabinoids (in particular, inhaled THC) can have profoundly disruptive effects on striatal functional connectivity. The effects of 600 mg oral CBD are relatively minor when administered alone, but inhaled CBD effectively blocks or attenuates the effects of THC when administered together at a ratio of 1.25:1, in the limbic striatum. Effects of the two active treatments in the associative striatum network are broadly similar,

with the main difference being a selective effect of the THC-only condition in the insula, suggesting CBD may attenuate the effect of THC specifically in the insula. In the sensorimotor striatum network, the distribution of regions affected by the two treatments is not obviously different. The finding in Study 2 that CBD actually increases associative striatum connectivity is consistent with the result in Study 1 of an ameliorating effect of the CBD on the disruptive effects of THC in the associative striatum, when administered together. The oppositional effect of CBD (i.e. increasing functional connectivity in this network) is perhaps not sufficient to fully counter the disruptive effect of THC, at least with the dosing ratio used in Study 1. The specific effect of the inhaled pure-THC (THC) condition on the limbic striatum here is mirrored by a key previous result (Bossong et al., 2015) which showed that only the limbic striatum showed reliable dopamine release with a THC challenge, indexed by [^{11}C]raclopride Positron Emission Tomography (PET). This study used synthetic (therefore, pure) THC as the acute challenge; the present data therefore extend this result by suggesting that CBD may potentially block the release of dopamine produced by THC in the limbic striatum in cannabis containing THC and CBD. A complementary result is provided by Mason et al. (2019) who showed that THC increased glutamate concentrations in the striatum, and this was closely related to reductions in striato-cortical functional connectivity. CBD alone may also have effects on limbic striatum connectivity, as seen in Study 2, where the (right) insula is also significantly modulated by the oral CBD condition. This result is perhaps inconsistent with the interpretation developed above, but may reflect differences in the pharmacodynamics of CBD when it is administered alone compared to in combination with THC; there may be complex, perhaps synergistic, effects when administered together. Alternatively, it may reflect differences in the dosing, administration method, subjects, or other factors which differ between the two studies (see discussion of limitations below).

This may be significant, as the limbic striatum consists of the nucleus accumbens and the head of the caudate. The nucleus accumbens is one of the primary substrates known to be heavily involved in the formation and maintenance of addiction (Robbins and Everitt, 2002; Robinson and Berridge, 1993, 2001; Volkow et al., 2007). The increasing concentration of THC in modern cannabis (which also often has relatively low levels of CBD; Freeman and Lorenzetti, 2020; Niesink et al., 2015) is thought to be a major factor in the increase of cannabis related-health issues, in particular dependence (Freeman and Winstock, 2015). The finding here that CBD blocks the disruptive effect on limbic striatum connectivity is also consistent with previous behavioural work showing that CBD attenuates the appetitive and incentive-salience effects of THC and other drugs (Hindocha et al., 2018; Morgan et al., 2010). Taken together, these various findings suggest a possible physiological mechanism whereby THC promotes dopamine release in the ventral striatum (possibly via the increase in glutamate concentrations reported by Mason et al., 2019), making users who consume relatively pure-THC strains vulnerable to addiction. However, in users of more balanced strains containing CBD, the acute dopaminergic and addiction-promoting effects of THC on the ventral striatum may be ameliorated, or perhaps blocked entirely. This 'buffering' effect of CBD is also consistent with the previous results reported from this (Study 1) cohort (Freeman et al., 2018; Lawn et al., 2016; Wall et al., 2019).

The finding of a relative increase in connectivity with the CBD condition (in the associative striatum analysis; Study 2) is mirrored by a recent similar finding in Grimm et al. (2018), which also used oral administration and the same dose as the present data (600 mg oral CBD). These authors showed a relative increase in frontal-striatal connectivity with CBD, and speculate that this might account for the antipsychotic effects of CBD, as fronto-striatal connectivity effects are a common finding in studies of schizophrenic patients (e.g. Fornito et al., 2013). Another converging result is that of Rzepa et al. (2016) which used the CB1 neutral antagonist tetrahydrocannabivarin (THCV). This study showed increased connectivity within the executive control network; usually conceived as a network sub-serving attentional and cognitive processes involved in task engagement. CBD also may be a negative allosteric modulator at CB1 receptors (Chesney et al., 2020; Laprairie et al., 2015), and here we show increases in connectivity in the associative striatum; the region of the striatum most associated with cognitive functions and brain regions. Recent studies in clinical populations also support this narrative, showing normalisation of striatal connectivity with a single dose of CBD in schizophrenia patients (O'Neill et al., 2021), and partial normalisation of brain responses in task fMRI paradigms with CBD in a group at high-risk for psychosis (Bhattacharyya et al., 2018; Davies et al., 2020; Wilson et al., 2019). One limitation of this interpretation is that the regions of the associative network which show increases with CBD in Study 2 (the posterior cingulate and posterior parietal lobe) are not the same regions which show decreases in connectivity in Study 1 (sensorimotor cortex and areas in the frontal and temporal lobes; all more anterior). Again, this inconsistency may be because of complex effects of the two compounds when co-administered, or because of methodological differences between the two studies.

We also see marked effects on the insula, across all three networks examined in Study 1, and for the limbic striatum network in Study 2. The insula is a key hub in the salience network (Goulden et al., 2014; Seeley et al., 2007; Uddin, 2014), and recent work using combined PET and fMRI methods has identified a link between mesolimbic dopamine systems and the salience network (McCutcheon et al., 2019b). Previous work on the data from Study 1 (Wall et al., 2019) also showed a reassuringly convergent finding of connectivity from an insula seed-region (salience network) to part of the putamen. Connectivity between the striatum and the salience network has also been shown to be affected in psychotic disorders (Karcher et al., 2019), and striatal-salience network connectivity has been shown to be increased in individuals exposed to chronic psychosocial stressors (a key hypothesised factor in the development of psychosis; McCutcheon et al., 2019a). Previous reported work on this cohort (Study 1) has also shown that both strains of cannabis increase the self-report of psychotic-like symptoms (including thought and perceptual distortion, and cognitive disorganisation; Mokrysz et al., 2020). Subjective effects of the CBD dose in Study 2 were minimal (Bloomfield et al., 2020b), and no measures of psychotic-like effects were acquired. Prominent theories of schizophrenia highlight the role of (striatal) dopamine in aberrant salience attribution (Howes and Nour, 2016), and this has been supported by experimental evidence from patients (Ceaser and Barch, 2016). Taken together, these findings suggest a role for striatal-salience network connectivity in the pathophysiology of psychotic disorders, and further suggest that

compounds that specifically target these systems (such as CBD) may be useful therapeutically.

The insula is a large anatomical region, and has also been implicated in a wide variety of functions apart from salience processing, including interoception, pain, emotional and empathic functions, time-perception, decision-making and auditory/speech functions (for a review, see Uddin et al., 2017). Its role in interoception is particularly well-described (Craig, 2002, 2011) and may provide a plausible alternative interpretation of the effects seen here; namely that they are related to the subjects' interoceptive awareness of the physiological effects of the drug. This interpretation may seem somewhat more prosaic; however, interoceptive and time-perception dysfunctions in the insula may also play a role in a number of disorders (Vicario et al., 2020), suggesting the findings may still be of useful clinical or therapeutic significance.

To the authors' knowledge, this is the first report in human subjects of data from THC, THC + CBD and CBD acute challenges, achieved using a unified set of analysis methods, and with all comparisons performed in a placebo-controlled, double-blind design. These are important strengths as they serve to eliminate at least some of the divergence of methods used in previous studies in this area which have provided somewhat inconsistent results (Grimm et al., 2018; Mason et al., 2019). However, as the data come from two separate studies, a direct comparison between each of the treatment conditions is compromised by the use of different cohorts of subjects, and different routes of administration (inhalation in Study 1, oral dosing in Study 2), formulations (cannabis-based on Study 1, synthetic in Study 2), and doses. The different doses and administration procedures almost certainly led to differences in pharmacokinetics and overall bio-availability of the drugs. Other differences between studies were scanner model and field strength (1.5 Tesla in Study 1, 3 Tesla in study 2), data acquisition protocol and length of the scan. For these reasons, we opted not to make direct statistical comparisons between the two studies. This means that no explicit controls for these extraneous factors (scanner field strength, dosing, etc.) are required; however, it does render any comparisons between the two studies strictly qualitative. For the participants, Study 1 aimed to recruit subjects who were occasional or light cannabis users in order to minimise anxiety or other adverse reactions to the acute dose used in the study. In comparison, Study 2 sought to recruit subjects who were relatively inexperienced with cannabis or other recreational drugs (<5 occasions previous use). Clearly, these are somewhat different samples, and this further constrains cross-study comparisons. Sample sizes in both studies were also relatively small, but the within-subjects design of both studies is an advantageous feature. The age of subjects in both studies is also somewhat restricted, with young adults recruited in the opportunity samples used (mean ages of 26 years and 24 years in Study 1 and Study 2, respectively). While this places limits on the generalisability of the findings to other age groups, it does accurately reflect the natural history of cannabis use, which tends to peak in late adolescence and early adulthood, and decline from the mid-20s (Degenhardt et al., 2016). No explicit consideration of extraneous factors such as age, sex, alcohol and/or nicotine use was attempted; the small sample size in both studies meant that, for example, splitting the data by sex and conducting a between-groups comparison would be impractical. Clearly, these factors would be important for future larger studies to consider.

Cannabinoids exert a major acute effect on striato-cortical functional connectivity, with effects on striatal connectivity with the insula particularly evident across all three drug conditions. These effects on the limbic striatum in particular, and its connectivity with the insula (and by implication, the salience network), are likely a crucial finding in our evolving understanding of the acute brain effects of cannabinoids. A key question for future research is understanding how these acute effects translate into longer-term effects in chronic users, what role these striato-cortical connections may have in the pathophysiology of cannabis use disorder and cannabis-related psychosis, and what therapeutic options might usefully target them. These questions will grow increasingly more urgent as cannabis seems likely to continue its transition to quasi-legal or fully legal status in a growing number of jurisdictions.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship and/or publication of this article: M.B.W. and N.E.'s primary employer is Invicro LLC., a private company which performs contract research work for the pharmaceutical and biotechnology industries. L.D. was also previously employed by Invicro while some of the work in this submission took place but is now in an academic position at the University of Oxford. C.H. was employed by GW Pharmaceuticals during the revise and resubmit process. Her substantive contribution to this publication occurred before employment at GW pharmaceuticals. M.A.P.B. is a director of Bloomfield Health Limited, a mental health company which is not involved in the cannabinoid industry. All other authors declare no other conflicts of interest.

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Data and materials availability

All data, analysis code and other materials used in this work are available to other researchers on request.

Supplemental material

Supplemental material for this article is available online.

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