Cannabidiol attenuates insular activity during motivational salience processing in patients with early psychosis

***Supplement***

**Supplementary Methods**

Participants

All participants provided written informed consent prior to commencing the trial approved by the National Research Ethics Service Committee of London (Camberwell, St. Giles, ethics reference: 14/LO/1861). Patients with psychosis in the early stages of illness were recruited from early-intervention services in the South London and Maudsley NHS foundation trust, London, United Kingdom. Of 17 participants initially recruited into the study (1 did not meet inclusion criteria and another withdrew consent) 15 participants attended for 2 study days, and 14 completed two fMRI scanning sessions (1 patient requested to end the scanning session early). An experienced research psychiatrist confirmed the diagnosis of psychosis using the Structured Clinical Interview for DSM-IV (Bell, 1994). The patient inclusion criteria required a psychotic mental illness diagnosis (meeting criteria for schizophrenia, schizophreniform, or brief psychotic disorder, but no other Axis I diagnoses) within 5 years of illness onset. Nineteen healthy control (HC) participants, who were not administered CBD or placebo, were recruited by local and internet-based advertisements to permit comparison with psychosis patients given placebo.

Exclusion criteria included: a history of neurological disorders (other than a psychotic mental illness meeting the criteria for schizophrenia, schizophreniform, or brief psychotic disorder), current DSM IV diagnosis of substance use disorder (except cannabis in the patient group), acute intoxication with alcohol/ any other psychoactive substance on the day of experimentation, IQ of less than 70, lack of capacity to consent, urine drug screen positive for other known psychotogenic and psychedelic substances such as PCP, amphetamine or MDMA, severe intercurrent illness, pregnancy, and any contraindication to MRI. Additional exclusion criteria for the HC group included diagnosis of a mental illness, current/past recipients of psychiatric treatment, a first-degree relative who had experienced psychosis, or more than 10 instances of cannabis use throughout their lifetime.

Study Design

In patients with psychosis, we employed a within-subject, crossover, double-blind randomised placebo-controlled design over 2 sessions with a 1-week interval to allow for the washout of CBD. Randomisation and blinding of (CBD or placebo) was conducted at the Maudsley Pharmacy. On study days, participants were given a light standardised breakfast. One hundred and twenty minutes after breakfast, either 600mg CBD (approx. 99.9% pure, THC-Pharm, Frankfurt, Germany), or a visually identical gelatine placebo (PLB) capsule was administered. One hundred and eighty minutes post drug administration participants underwent fMRI scanning and performed the MIDT. Blood samples were acquired through intravenous cannulation in the non-dominant arm to assay CBD levels. Blood samples were obtained at three-time points: T1) 60 minutes before drug administration, T2) 60 minutes post drug administration, T3) 270 minutes post drug administration. The Positive and Negative Syndrome Scale (Kay, Fiszbein, & Opler, 1987) and the ‘state’ subscale of the State-Trait Anxiety Inventory (Spielberger, 2010) were used to assess psychopathology at timepoints T1 and T3.

Prior to the study, participants were advised to abstain from caffeine and alcohol intake for 12 and 24 hours respectively. Furthermore, all participants were asked to avoid using any recreational drugs (apart from cannabis amongst the patient group) for 2 weeks before the study day. Urine samples were obtained on each study day to screen for use of amphetamines, barbiturates, benzodiazepines cocaine, methamphetamine, morphine, methadone, phencyclidine (PCP), tricyclic antidepressants, and THC, using the Alere™ Drug Screen Urine Test Cup. Carbon monoxide breath levels were also measured using the Bedfont™ Smokerlyzer.

Power calculation

The data presented in this study is a subset of a larger study which utilised a number of neuroimaging approaches (O’Neill, Annibale, et al., 2021; O’Neill, Wilson, et al., 2021). For the overall study, an initial power calculation was conducted, however, this focused on medial temporal activation during a verbal learning task (O’Neill, Wilson, et al., 2021) rather than specifically estimating power to detect an effect on brain activation during the MIDT. This power calculation has been re-reported here for completeness.

To estimate the sample size for the healthy vs psychosis comparison, differences in brain activation were examined during a memory task that utilised both healthy and psychosis participants (Rasetti et al., 2014). The mean difference between hippocampal activation in healthy (-0.05 SD=0.1) and psychosis (0.1 SD=0.1) participants was used to estimate that a sample size of 9 participants per group would be suitable to investigate differences in brain activation (alpha (α)= 0.05, power= 80%).

At the time of planning the study, there was no data available of CBD effect on brain activation in psychosis patients. Therefore, we estimated the sample size for the PSY-PLB vs PSY-CBD comparison based on differences in brain activation from a previous study that investigated the acute effect of CBD, relative to placebo, on brain activation in healthy participants using a within subject design (Bhattacharyya et al., 2010). Based on this, we estimated that a sample size of 15, using a repeated-measures within-subject design would be adequate to detect brain activation differences between the drug conditions (difference in means= 0.037 (SD 0.04), alpha (α)= 0.05, power= 90%). In light of existing evidence on the antipsychotic potential of CBD (Rohleder, Müller, Lange, & Leweke, 2016) we assumed that any effect of CBD on functional brain activation would be greater in psychosis participants than that observed in healthy participants by Bhattacharyya et al and that our proposed sample would have adequate power.

Data Acquisition

The visual cue was displayed for 250ms and feedback was displayed for 1450ms. The initial target time presentation was 250ms. This time was individually adjusted ±10ms ranging from 150ms to 300ms, resulting in approximately 66% accuracy per participants. In accordance with previous work, responses <100ms after target presentation were recorded as a false-start (Wilson et al., 2019). Scanning of anticipation occurred during the interval between cue and target which varied from 3700 to 4500ms in duration (10s inter-trial interval) (Supplementary Figure 1).

SUPPLEMENTARY FIGURE 1

Images were acquired using a General Electric Signa HDx 3.0T MRI scanner. Structural images were acquired using a whole-brain sagittal T1-weighted scan based on Alzheimer’s Disease Neuroimaging Initiative parameters (TE=2.85ms, TR = 6.98ms, inversion time=400ms, flip angle=110, voxel size 1.0 x 1.0 x 1.2mm). 480 T2\*-weighted images were acquired in two 8-minute runs (TE=30ms, TR=2.0s, flip angle=75°, 39 x 3mm thick axial planes, 3.3mm inter-slice gap, in-plane voxel size 3.75 x 3.75mm).

**Supplementary References**

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**Supplementary Figures**

Supplementary Figure 1. MIDT visual cue sequence example