Hippocampal Glutamate, Resting Perfusion and the Effects of Cannabidiol in Psychosis Risk

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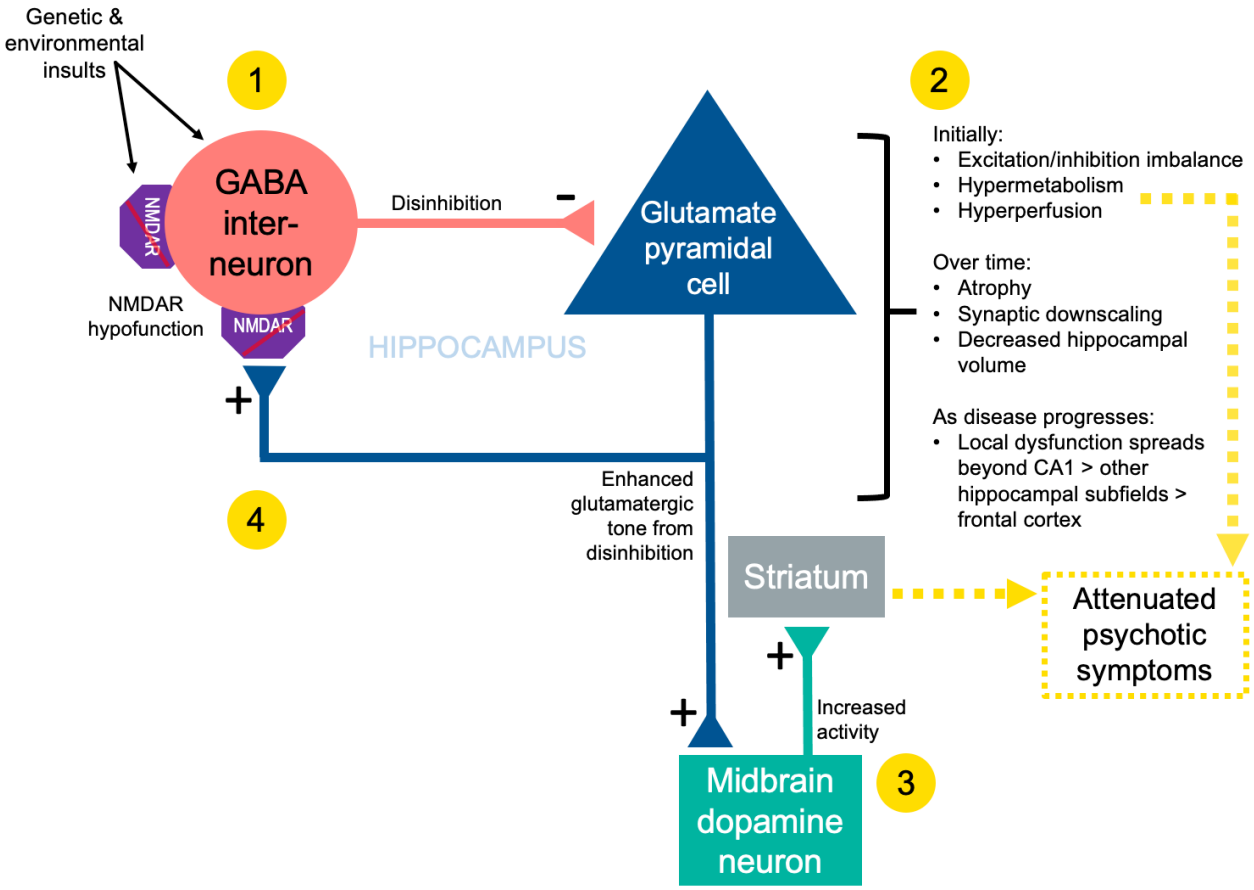
SUPPLEMENTARY MATERIAL

FIGURE S1. Schematic of proposed neural circuit mechanisms of hippocampal dysfunction in the pathophysiology underlying psychosis onset. In (1), low glutamate signal/input from hypofunctioning NMDARs (akin to faulty homeostatic sensors) prompts GABAergic interneurons to homeostatically increase excitation by reducing inhibition (disinhibition) of glutamatergic pyramidal cells. However, by disinhibiting pyramidal cells (and thus increasing glutamate signalling) in this dysfunctional neural environment, the potential homeostatic adaptation becomes allostatic, with enhanced excitatory drive inducing (2) hypermetabolism and hyperperfusion (elevated blood flow to meet increased metabolic demand), and (3) an overdrive in the responsivity of midbrain dopamine neurons, which project to the associative striatum. Note that the connection between hippocampal pyramidal cells and midbrain dopamine neurons is presented as monosynaptic but is in fact polysynaptic via the ventral striatum and ventral pallidum. Completing the (simplified) circuit, local glutamatergic tone is increased in (4) but is not detected as such by hypofunctioning NMDARs on GABAergic interneurons. Figure reproduced and adapted with permission (CCBY 4.0) from[1]. For original diagrams and discussion of evidence for this proposed circuit, see[2–6]. *Abbreviations:* Glu, glutamate; NMDAR, N-methyl-D-aspartate receptor; CA1, Cornu Ammonis 1.



SUPPLEMENTARY METHODS

Participants

Thirty-three antipsychotic-naive CHR individuals, aged 18–35, were recruited from specialist early detection services in the United Kingdom. CHR status was determined using the Comprehensive Assessment of At-Risk Mental States (CAARMS) criteria.[7] Briefly, subjects met one or more of the following subgroup criteria: (a) attenuated psychotic symptoms, (b) brief limited intermittent psychotic symptoms (BLIPS, psychotic episode lasting <1 week, remitting without treatment), or (c) either schizotypal personality disorder or first-degree relative with psychosis, all coupled with functional decline.[7] Participants were required to abstain from cannabis for 96h, other recreational substances for 2 weeks, alcohol for 24h and caffeine and nicotine for 6h before attending. A 96-hour abstinence period was considered sufficient to rule out any acute effects of cannabis intoxication and withdrawal, as previous evidence suggests that mean plasma elimination half-life of THC is around 21.5 hours[8] and most withdrawal symptoms last for around 4 days.[9]

Design, Materials, Procedure

The 600mg dose of CBD was selected based on previous findings that doses of 600-800 mg/day are effective in established psychosis[10] and anxiety.[11] The 180 min interval between drug administration and MRI acquisition was selected based on previous findings describing peak plasma concentrations at 180 min following oral administration.[12,13]

^{1}H-MRS Data Processing

Spectra were analysed using LCModel/6.3-0A[14] using the standard basis set of 16 metabolites (L-alanine, aspartate, creatine, phosphocreatine, GABA, glucose, glutamine, glutamate, glycerophosphocholine [choline], glycine, myo-inositol, L-lactate, N-acetylaspartate, N-acetylaspartylglutamate, phosphocholine, and taurine) acquired at the same field strength (3T), localisation sequence (PRESS), and echo time (30ms) as the ^{1}H-MRS spectra in the current study. Model metabolites and concentrations used in the basis set are detailed in the LCModel manual (http://s-provencher.com/pub/LCModel/manual/manual.pdf).

We calculated and corrected for ^{1}H-MRS voxel tissue content using SPM8 and in-house scripts to (a) segment the T1-weighted structural images into grey matter, white matter, and cerebrospinal fluid (CSF) using SPM8 in Matlab R2017a, (b) locate and map the coordinates of each voxel to the segmented T1 images, and (c) provide the tissue content proportions. Metabolite values were corrected for voxel tissue content using the formula: M_{corr}= M×([GM×1.21] +WM+ [CSF×1.55]) / (WM+GM), where M is the uncorrected metabolite value

and GM/WM/CSF are proportions of grey matter, white matter and CSF, respectively. The formula assumes a CSF water concentration of 55,556 mol/m^3 and the LCModel default brain water concentration of 35,880 mol/m^3.[15,16] Apart from assuming T_2 = 80 ms for tissue water, no corrections were applied for metabolite and water relaxation times.

Arterial Spin Labelling Acquisition & Processing

ASL Image Acquisition

For ASL image registration, a high resolution T2-weighted Fast Spin Echo (FSE) image (TE= 54.58ms, TR= 4380ms, Flip angle= 90deg, FoV= 240, Matrix size= 320 x 320, slice thickness= 2mm, 72 spatial locations) was acquired and used alongside the T1-weighted Spoiled Gradient Recalled (SPGR) images (detailed in the main text). Resting Cerebral Blood Flow (CBF) was measured using 3D pseudo-Continuous Arterial Spin Labelling (CASL) scans acquired with a 3D Fast Spin Echo (FSE) spiral multi-shot readout, following a post-labelling delay of 1.5s. The spiral acquisition used a short (10ms) TE, and 8 spiral arms (interleaves) with 512 points in each arm. FSE TE= 32.26ms, TR = 5500ms. 64 slices of 3mm thickness were obtained and the in-plane FoV was 240×240mm. Three pairs of tagged-untagged images were collected. The whole ASL pulse sequence, including the acquisition of calibration images, was performed in 6:08min.

ASL Image Processing

Data were preprocessed using FMRIB Software Library (FSL) 6.0.2 using the following procedure: (1) T1 and T2 images were skull-stripped and corresponding brain-only binary masks created; (2) original CBF images were coregistered to the T2 images and (3) multiplied by the binary T2 mask to create a skull-stripped CBF image in T2 space; (4) skull-stripped T2 was coregistered to skull-stripped T1; (5) skull-stripped T1 was first linearly coregistered to the MNI152 T1 2mm brain template, before non-linear registration (FNIRT) of the original T1 to MNI space; (6) original T2 images were registered to the MNI template (via T1 space) in a single concatenated step, using the T2-to-T1 transformation matrix (from step 4) and T1-to-MNI warp (from step 5); (7) skull-stripped CBF images (already in T2 space) were registered to the MNI template using the concatenated procedure in step 6; (8) normalised CBF images were spatially smoothed with a 6mm Gaussian kernel. All images were visually inspected for preprocessing errors.

SUPPLEMENTARY RESULTS

Power Calculation

The overarching study (https://doi.org/10.1186/ISRCTN46322781) was primarily powered for fMRI BOLD signal and not for the ^{1}H-MRS or ASL analyses reported here, and thus we did not do a formal power calculation *a priori* based on hippocampal glutamate (original power calculation is available in the supplement of our previous publication in the same sample[17]).

Post-hoc Sensitivity Power Calculations for Hippocampal Glutamate

Given that the original power calculation was not specifically tailored to analyses of glutamate levels, we conducted post-hoc sensitivity power calculations to facilitate consideration of our results in the context of our sample size. Sensitivity power calculations were computed for independent t-tests (two-tailed; 80% power; a=.05) with our sample sizes (CBD=16, placebo=17, controls=19). The associated percent change in glutamate levels for the effect sizes (determined by the sensitivity power calculation) were estimated using the mean and SD of control metabolite values from a previous study by Stone et al[18], and as previously conducted in our earlier work[19] (here for independent t-tests; two-tailed; 80% power; a=.05; assumed SD and sample size equal for both groups).

Post-hoc power calculations suggested that for the placebo vs control group contrast, the minimum effect size (Cohen's d) for a between-group difference in glutamate (detectable at 80% power when a=.05, with our sample size) was d=0.96 (~22.4% difference in glutamate levels).

For the CBD vs placebo group contrast, where we did not find significant results, post-hoc power calculations indicated that the minimum effect size (Cohen's d) for between-group difference in glutamate (detectable at 80% power when a=.05, with our sample size) was d=1.00 (~23.4% difference in glutamate levels). Therefore, it is possible that CBD had (between-group) effects of smaller magnitude but we were unable to detect them with our sample sizes. Future studies with larger samples are therefore needed. It is also worth noting that this study administered only a single acute dose of CBD, and it remains possible that sustained dosing would produce a detectable effect at the current sample size.

Effects of Sex and Cannabis Use on Group Differences in Hippocampal Glutamate

Inclusion of mean-centred sex in an ANCOVA showed that for the placebo vs control contrast, the effect of sex was not significant ($F(1,33)= 0.25$, $p=.62$), but the effect of group remained significant ($F(1,33)= 5.78$, $p=.022$), with significantly lower glutamate in the placebo relative to the control group: estimated marginal means M (SE) in placebo = 7.44 (0.29) and controls = 8.39 (0.27). Inclusion of mean-centred sex in an ANCOVA for the CBD vs placebo contrast showed no significant effect of sex ($F(1,30)= 0.16$, $p=.70$), and the effect of group remained non-significant ($F(1,30)= 0.550$, $p=.46$): estimated marginal means M (SE) in the placebo = 7.44 (0.34) and CBD group = 7.81 (0.35).

For the effects of drug (cannabis) use in the placebo vs control contrast, because cannabis partially covaries with group status it was not possible to adjust for this within the analysis. However, as an alternative approach to explore this, we repeated our initial analysis (t-test) restricted to current non-users only, which showed that the placebo vs control difference in hippocampal glutamate was not significant but was in the same direction as our key results ($t(27)= 1.76$, $p=.089$) which, given the reduced sample size (n=10 in placebo, n=19 controls), suggests that our results are unlikely to be driven by cannabis use (mean ± SD in non-using [all] controls= 8.41 ± 1.27; and non-using placebo patients= 7.61 ± 0.87). For the CBD vs placebo pairwise contrast, including mean-centred current cannabis use (yes/no) in Quade's nonparametric (due to inhomogeneity of variances) ANCOVA showed no significant group effect after accounting for cannabis use ($F_{[dfh/dfe]}(1,31)= 1.28$, $p=.27$), which is consistent with the CBD vs placebo pairwise result in the main text.

SUPPLEMENTARY DISCUSSION

Preclinical models show that the hippocampus is a key part of the circuitry mediating anxiety-related behaviours[20] and evidence suggests that both the hippocampus and anxiety may be targeted by CBD and other cannabinoids.[21] In humans, CBD attenuates perfusion and/or activation of mediotemporal and limbic regions in healthy people[22,23] and those with anxiety disorders.[24] CBD also has anxiolytic effects[25,26] in people with social anxiety disorder[11,27] and in healthy people subjected to simulated public speaking.[26,28–30] In our previous work based on the same patient sample as the present study, we showed that 7-day CBD treatment partially attenuated the cortisol and anxiety response to stress in CHR individuals.[31] We also previously showed that a single dose of CBD was sufficient to alter brain activation in CHR patients during fear-processing and in a direction suggestive of normalisation,[32] although in further preliminary analyses, CBD did not 'restore' the aberrant relationship between stress-induced cortisol response and mediotemporal activation seen in the CHR-placebo group vs controls.[33] In view of the above, we cannot rule out the possibility that anxiety-related effects may explain or contribute to some of the findings reported in the present study. Future studies relating CBD-induced changes in brain chemistry and/or function with changes in symptoms (including anxiety) would help to disentangle these mechanisms and better characterise relationships with clinical effects.

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