Supplementary Online Content

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eMethods. Effect of Δ9-tetrahydrocannabinol on attentional salience processing

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

Design

The effects of Δ9-THC and CBD were examined in 15 healthy, occasional cannabis user male volunteers (26.67±5.7 years) over three sessions in a double-blind, placebo-controlled, within-subject design, with counterbalanced order of drug administration using an established protocol.[1] Participants were scanned three times, with at least one-month inter-scan interval. All subjects provided written informed consent to participate. The study was approved by the local research ethics committee and the investigators had a license to use Δ9-THC and CBD for research.

Subjects

Fifteen healthy right-handed Caucasian males with English as the first language, mean age of 26.67 (±5.7) years and a mean IQ of 98.67 (±7.0) as measured using the NART[2] who had only occasionally been exposed to cannabis (<15 times in their life), completed the study. Nine subjects had used cannabis <5 times in lifetime and 6 subjects had used 5-14 times in lifetime. They had minimal exposure to other illicit drugs: 3 subjects had experimented with amphetamines, 1 of them had also experimented with hallucinogenic mushrooms; 2 other subjects had experimented with hallucinogenic mushrooms, 1 of whom had also experimented with ecstasy; 4 other subjects had experimented with only ecstasy and 1 subject had experimented

only with cocaine. Experimentation typically involved use of the drug on <3 occasions lifetime. Seven of the 15 subjects were current tobacco smokers. The mean number of cigarettes smoked in a day was 2.92 (SD-4.9) (range 0-15/ day). Only 2 of them smoked >10 cigarettes/day. They had not used cannabis in the 3 months before the first scan and were requested to abstain from illicit drugs for the duration of the study. Subjects were required to abstain from alcohol and caffeine for 24 h and 12 h before each session, respectively and also to avoid smoking on the morning of the study. Subjects had no history of neurological or psychiatric illness or substance abuse/ dependence. They were asked about history of contact with general practitioner or referral to a Neurologist or Psychiatrist and diagnosis of neurological or psychiatric illness to confirm this. All subjects gave written informed consent.

Procedure

All subjects had a urine drug screen for amphetamines, benzodiazepines, cocaine, opiates, and Δ9-THC before each session. This was negative for all sessions in all the subjects. One hour prior to scanning subjects were given identical gelatine capsules of either 10mg Δ9-THC or 600mg CBD (THC-Pharm, Frankfurt, Germany) or placebo (flour). A dose of 10 mg of Δ9-THC was used, as previous work[3-5] suggests that this dose can induce detectable effects on psychomotor performance without causing marked psychotic or anxiety reactions that may have affected the ability of participants to complete the imaging component of the study. The dose of

CBD was chosen based on its ability to have significant behavioural effects. A dose of 600 mg has previously been found to significantly reduce subtle psychotic-like experiences following ketamine infusion in healthy volunteers and be safely tolerated,[6] and a comparable dose had been shown to affect neural activity in the only published previous neuroimaging study.[7] Illicit substance use was assessed using the Structured Clinical Interview and Addiction Severity Index.[8] Psychopathological ratings were conducted at the time of drug administration and then at 1, 2 and 3 hours post-administration using the Visual Analogue Mood scale (VAMS),[9] the State-Trait anxiety Inventory (STAI),[10] the Analogue Intoxication scale (AIS)[11] and the Positive and Negative syndrome scale (PANSS).[12] Heart rate and blood pressure were measured at the same time points and heart rate was also monitored continuously via a finger-tip sensor throughout the online (inside fMRI scanner) session. Venous blood samples were taken from an I.V. line in the non-dominant arm of each participant at the time of drug administration and then at 1, 2 and 3 hours post-administration to monitor the levels of Δ9-THC and CBD. Whole blood drug levels were measured by Tricho-Tech (Cardiff, UK). MR images were acquired between 1 and 2 hours after administration of the drug as pilot work had indicated that plasma levels of Δ9-THC had reached a plateau approximately 1 hour following ingestion. Subjects performed a simple visual oddball detection task inside the MRI scanner.

Cognitive task: The Visual Oddball detection paradigm is described in detail elsewhere.[13,14] A series of arrows were presented on the right or left side of a screen. The arrows lasted for 600 msec and were followed by a blank screen for an average of 1.2 sec [jittered between 1 sec and 1.4 sec, amounting to a total mean inter-trial interval (ITI) of 1.8 sec]. The 'Standard' stimuli, presented on 160 trials, were horizontal arrows pointing either to the right or left with equal probability. Participants were instructed to press the right or left button that corresponded to the arrow direction, using their right or left thumb on a gamepad. 'Oddball' stimuli, consisting of arrows pointing either to the right or left at a 23⁰ angle, were presented for 600 msec in 24 trials, pseudo-randomly interspersed among the 'Standard' trials. Participants were instructed to press the right or left button according to the arrow direction, as with the 'Standard' stimuli. All the 'Oddball' stimuli were at least 3 repetition times (TR) apart from each other, to allow adequate separation of the associated hemodynamic response. Contrast of the 'Oddball' and 'Standard' stimuli allowed us to assess the neural response to rareness/ deviance (corresponding to stimulus salience), without the potentially confounding effect of other dimensions of stimulus salience, such as targetness, emotional valence and motivational valence (rewarding or non-rewarding).[15] This design also controlled for brain activation related to the motor response. We were thus able to measure the correlates of pure attention allocation to a rare infrequent stimulus.[13,14]

Image acquisition: Images were acquired on a 1.5 Tesla (GE) system. Two hundred and eight T2*-weighted images were acquired with TE 40 ms, TR=1.8 sec, flip angle 90° in 16 axial planes (7mm thick with an inter-slice gap of 0.7mm), parallel to the AC-PC line, with an in-plane voxel size of 3.75 x 3.75 mm for the fMRI data. A high-resolution inversion recovery image dataset was also acquired to facilitate anatomical localization of activation.

Data Analysis: Data from the fMRI tasks were analysed using XBAMv3.4 (http://www.brainmap.it/). Images were realigned and smoothed with an 8mm (FWHM) Gaussian filter. The experimental design was convolved with 2 gamma variate functions (peaking at 4 and 8 seconds) to model the blood oxygen level dependent (BOLD) response with a maximum in this range. Following least squares fitting of the convolved model to the time series at each voxel, the sum of squares ratio (SSQ- ratio of model component to residual sum of squares) was determined for the experimental contrast of interest, i.e. "Oddball – Standard" stimuli. The 'Standard' stimuli were the implicit baseline against which the 'Oddball' stimuli were modelled. The significance of the estimated SSQ values at each voxel was determined using permutation testing.[16] SSQ ratio maps for each individual were transformed into standard Talairach stereotactic space[17] and group activation maps computed for each drug by determining the median SSQ ratio at each voxel. Inter-condition contrasts were studied using nonparametric-repeated measures ANOVA,[18] with a voxel-wise threshold of $p=0.05$ and the cluster-wise threshold set such that the total number of false positive clusters per brain volume was <1: the p

value at which the latter occurred is quoted. The principal advantages of cluster-level testing are that it confers greater sensitivity by incorporating information from more than one voxel in the test statistic and also substantially reduces the search volume or number of tests required for a whole-brain analysis, thereby mitigating the multiple comparisons problem.

For each drug condition (Δ9-THC, CBD and placebo), we contrasted the active task condition ('Oddball' stimuli) against the implicit baseline ('Standard' stimuli) condition. For the event-related analysis of the visual oddball detection task, this involved contrasting the 'Oddball' trials against the 'Standard' trials for each drug treatment, to control for sensorimotor processing. We then examined the effects of Δ9-THC and CBD during visual oddball salience processing in the whole brain by comparing the brain activation maps obtained as described in the previous step for each drug condition separately to the activation map for the placebo condition during the visual oddball detection task. Finally, in keeping with our hypothesis that Δ9-THC and CBD would have opposite effects on regional brain activation, we identified areas within the whole brain where the effects of Δ9-THC and CBD were in the opposite direction relative to the placebo condition.

Measures of task performance, symptom ratings and physiological data, were normally distributed and were analysed using repeated-measures ANOVAs used to compare drug conditions. When significant differences were found, the Tukey test for pair-wise comparisons was applied. The effects of between-drug differences in

symptom levels on activation were examined by correlating measures of activation with the change in the rating from baseline to the mean of those at 1 and 2 hours. We predicted that such a relationship would exist between the effects of Δ9-THC in the striatum and the psychotic symptoms induced by it, based on our previous work and because of the central role played by the striatum in salience attribution[19,20] and in the generation of psychotic symptoms.[21] We also predicted a relationship between the effects of Δ9-THC and performance in the oddball detection task based on previous literature that relates aberrant salience attribution to striatal activation.[22,23] Post-hoc, we tested whether the relationship between the psychotic symptoms induced by Δ9-THC and neural activation was specific to the striatum, by examining the correlation with its effects in the medial temporal and inferior frontal cortex.

eReferences

1. Bhattacharyya S, Fusar-Poli P, Borgwardt S, Martin-Santos R, Nosarti C, O'Carroll C, Allen P, Seal ML, Fletcher PC, Crippa JA, Giampietro V, Mechelli A, Atakan Z, McGuire P. Modulation of mediotemporal and ventrostriatal function in humans by Delta9-tetrahydrocannabinol: a neural basis for the effects of Cannabis sativa on learning and psychosis. *Arch Gen Psychiatry.* 2009;66(4):442-451.

2. Nelson HE. National Adult Reading Test (NART): Test Manual. Windsor: NFER- Nelson; 1982.

3. Chesher GB, Bird KD, Jackson DM, Perrignon A, Starmer GA. The effects of orally administered delta 9-tetrahydrocannabinol in man on mood and performance measures: a dose-response study. *Pharmacol Biochem Behav.* 1990;35(4):861-864.

4. Koethe D, Gerth CW, Neatby MA, Haensel A, Thies M, Schneider U, Emrich HM, Klosterkotter J, Schultze-Lutter F, Leweke FM. Disturbances of visual information processing in early states of psychosis and experimental delta-9-tetrahydrocannabinol altered states of consciousness. *Schizophr Res.* 2006;88(1-3):142-150.

5. Leweke FM, Schneider U, Thies M, Munte TF, Emrich HM. Effects of synthetic delta9-tetrahydrocannabinol on binocular depth inversion of natural and artificial objects in man. *Psychopharmacology (Berl).* 1999;142(3):230-235.

6. Zuardi AW, Crippa JA, Hallak JE, Moreira FA, Guimaraes FS. Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug. *Braz J Med Biol Res.* 2006;39(4):421-429.

7. Crippa JA, Zuardi AW, Garrido GE, Wichert-Ana L, Guarnieri R, Ferrari L, Azevedo-Marques PM, Hallak JE, McGuire PK, Filho Busatto G. Effects of cannabidiol (CBD) on regional cerebral blood flow. *Neuropsychopharmacology.* 2004;29(2):417-426.

8. McLellan AT, Kushner H, Metzger D, Peters R, Smith I, Grissom G, Pettinati H, Argeriou M. The Fifth Edition of the Addiction Severity Index. *J Subst Abuse Treat.* 1992;9(3):199-213.

9. Folstein MF, Luria R. Reliability, validity, and clinical application of the Visual Analogue Mood Scale. *Psychol Med.* 1973;3(4):479-486.

10. Spielberger C. *Manual of the State-Trait Anxiety Inventory*. Palo Alto, CA.: Consulting Psychologists Press, Inc; 1983.

11. Mathew RJ, Wilson WH, Humphreys DF, Lowe JV, Wiethe KE. Regional cerebral blood flow after marijuana smoking. *J Cereb Blood Flow Metab.* 1992;12(5):750-758.

12. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull.* 1987;13(2):261-276.

13. Rubia K, Smith AB, Brammer MJ, Taylor E. Temporal lobe dysfunction in medication-naive boys with attention-deficit/hyperactivity disorder during attention allocation and its relation to response variability. *Biol Psychiatry.* 2007;62(9):999-1006.

14. Rubia K, Hyde Z, Halari R, Giampietro V, Smith A. Effects of age and sex on developmental neural networks of visual-spatial attention allocation. *Neuroimage.*51(2):817-827.

15. Bunzeck N, Duzel E. Absolute coding of stimulus novelty in the human substantia nigra/VTA. *Neuron.* 2006;51(3):369-379.

16. Bullmore E, Long C, Suckling J, Fadili J, Calvert G, Zelaya F. Colored noise and computational inference in neurophysiological (fMRI) time series analysis: resampling methods in time and wavelet domains. *Hum Brain Mapp.* 2001;12:61-78.

17. Talairach J, Tournoux P. Co-planar stereotaxic atlas of the human brain. New York: Thieme Medical; 1988.

18. Brammer MJ, Bullmore ET, Simmons A, Williams SC, Grasby PM, Howard RJ, Woodruff PW, Rabe-Hesketh S. Generic brain activation mapping in functional magnetic resonance imaging: a nonparametric approach. *Magn Reson Imaging.* 1997;15(7):763-770.

19. Zink CF, Pagnoni G, Chappelow J, Martin-Skurski M, Berns GS. Human striatal activation reflects degree of stimulus saliency. *Neuroimage.* 2006;29(3):977-983.

20. Zink CF, Pagnoni G, Martin ME, Dhamala M, Berns GS. Human striatal response to salient nonrewarding stimuli. *J Neurosci.* 2003;23(22):8092-8097.

21. Guillin O, Abi-Dargham A, Laruelle M. Neurobiology of dopamine in schizophrenia. *Int Rev Neurobiol.* 2007;78:1-39.

22. Jensen J, Willeit M, Zipursky RB, Savina I, Smith AJ, Menon M, Crawley AP, Kapur S. The formation of abnormal associations in schizophrenia: neural and behavioral evidence. *Neuropsychopharmacology.* 2008;33(3):473-479.

23. Murray GK, Corlett PR, Clark L, Pessiglione M, Blackwell AD, Honey G, Jones PB, Bullmore ET, Robbins TW, Fletcher PC. Substantia nigra/ventral tegmental reward prediction error disruption in psychosis. *Mol Psychiatry.* 2008;13(3):239, 267-276.
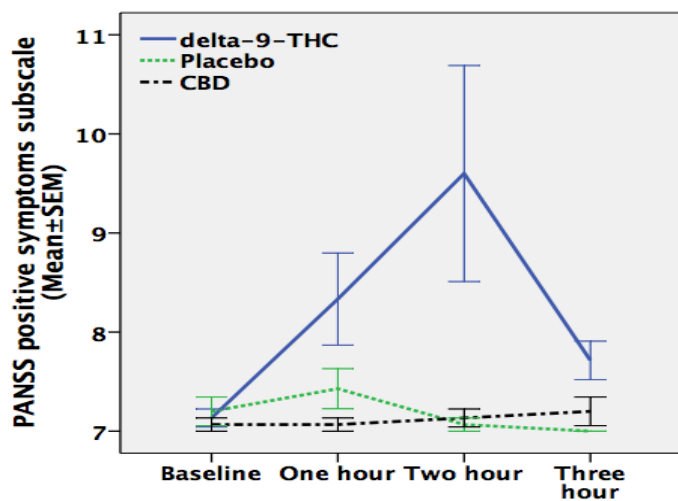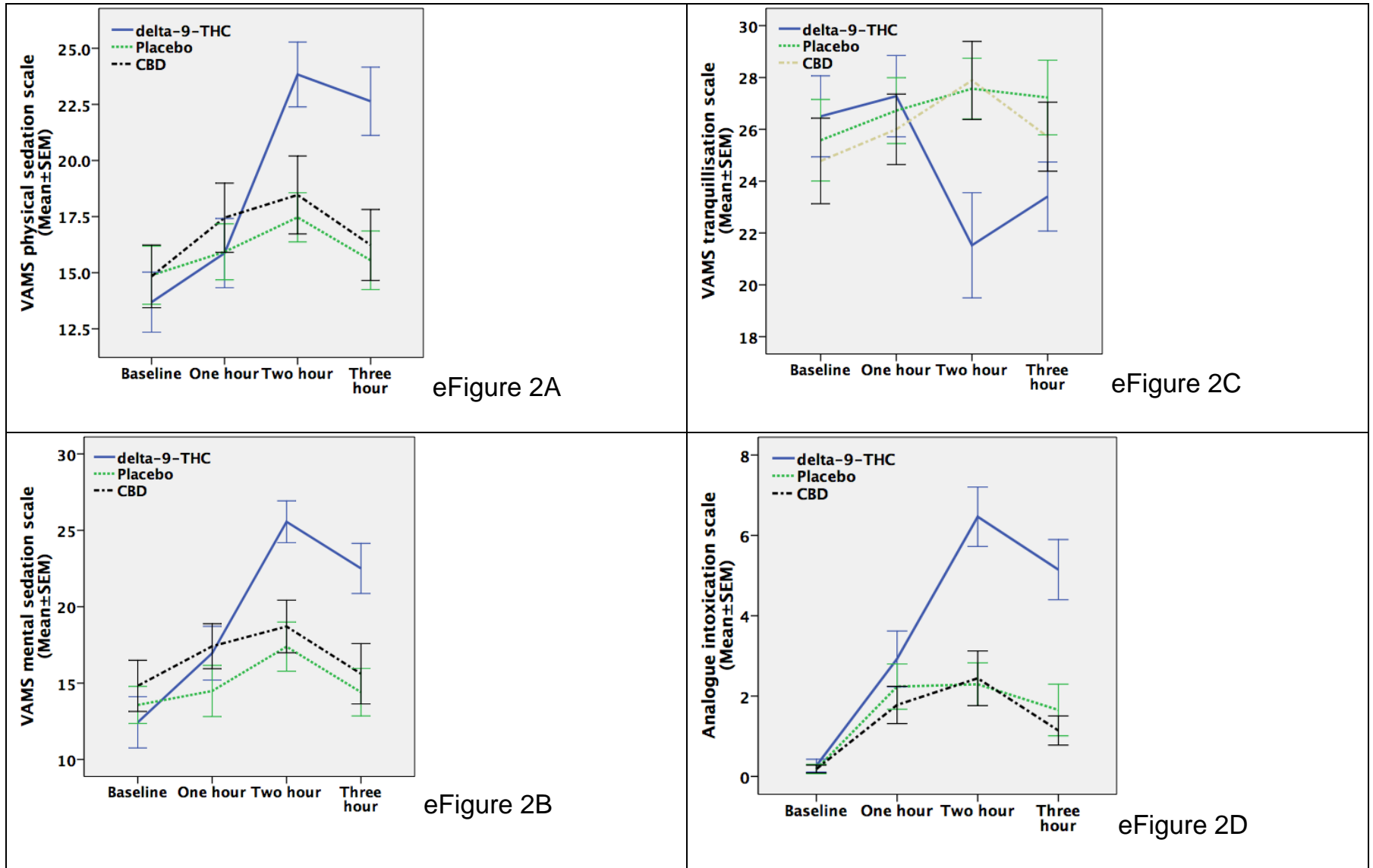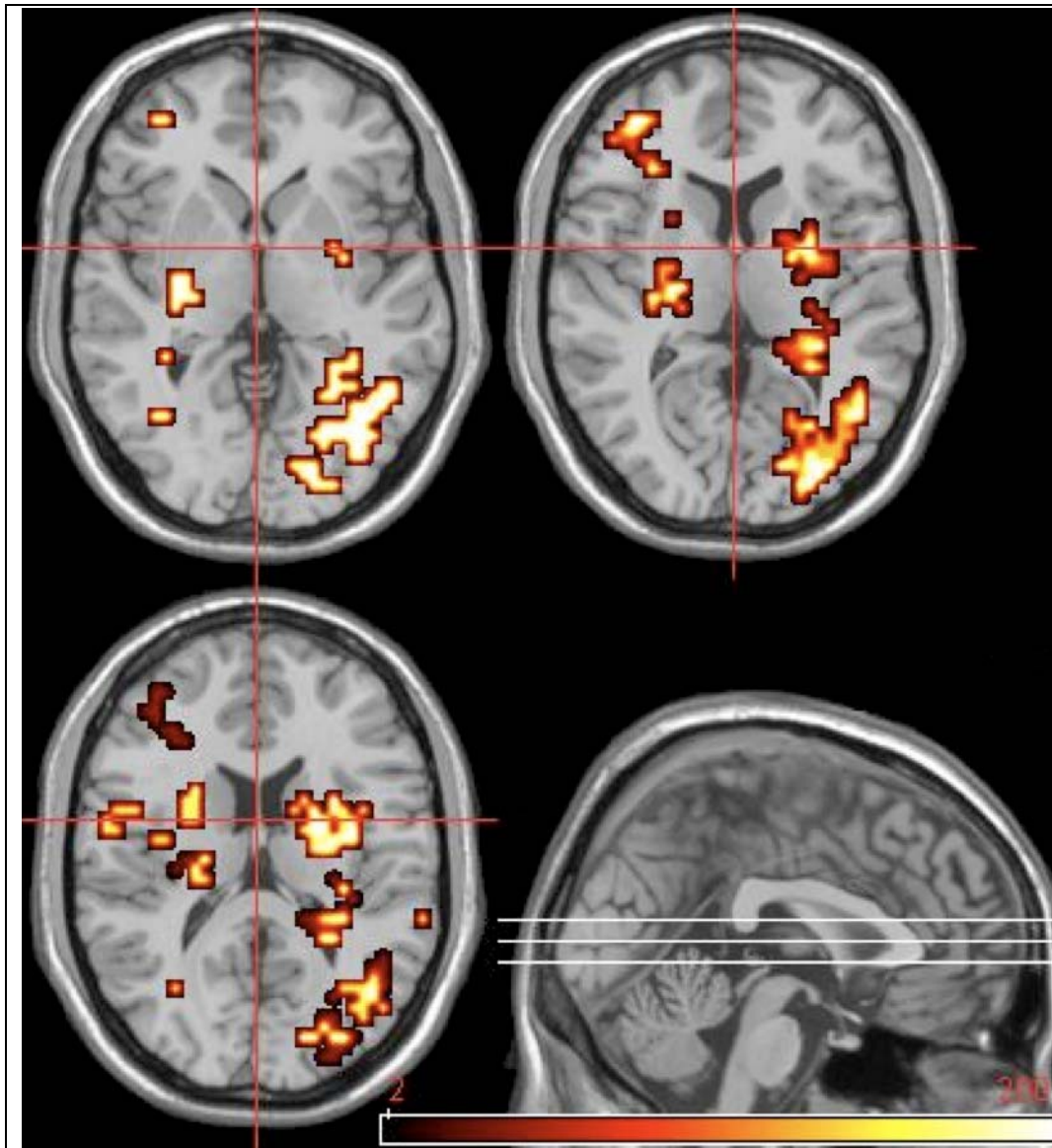
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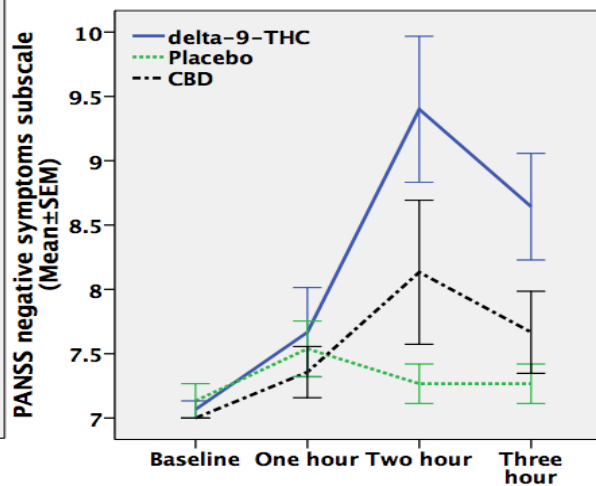
eFigure 1B

eFigure 1: Change in positive (1A) and negative (1B) psychotic symptoms [y-axis; Positive and Negative Symptoms Scale (PANSS)] under the Δ9-THC, placebo and CBD conditions over time (x-axis) [Δ9-THC vs placebo: p<0.05 for positive as well negative symptoms; CBD vs placebo: p=NS for both groups of psychotic symptoms].

eFigure 2: Change in physical sedation (2A), mental sedation (2B), tranquillization (2C) subscales [y-axis; Visual Analogue Mood Scale (VAMS)] and Analogue intoxication scale (AIS) (2D) under the Δ9-THC, placebo and CBD conditions over time (x-axis) [Δ9-THC vs placebo: $p < 0.05$ for all VAMS scales and AIS; CBD vs placebo: p=NS for all comparisons].

eFigure 3: Brain regions activated by the visual oddball salience processing task independent of drug condition: the caudate (-14,22,4; 18,4,20) and putamen (-22,4,9; 25,0,9); the left inferior (-51,4,31), middle (-32,52,-2) and medial prefrontal cortices (-14,33,26); the parahippocampal gyrus (-32,-37,-7; 29,-41,-7), hippocampus (-29,-11,-13; 29,-37,4) and amygdala (25,-7,-13); as well as the insula (43,-26,20; -32,-4,15), thalamus (-18,-11,9; 11,-7,15) and cerebellum (36,-41,-24; 22,-63-35; 29,-56,-40), and the right inferior parietal lobule (43,-48,48) and inferior temporal gyrus (51,-48,-13). The left side of the brain is shown on the left side of the image.