SUPPLEMENTARY INFORMATION

The Effects of Acute Cannabis With and Without Cannabidiol on Neural Reward Anticipation in Adults and Adolescents

Skumlien *et al.*

Contents

Supplemental Methods

Table S1. Inclusion and exclusion criteria for all participants.

Inclusion criteria	Exclusion criteria
• Adolescents: aged 16-17 years • Adults: aged 26-29 years • Self-reported cannabis use frequency of 0.5-3 days/week averaged over the past three months • Adults: BMI between 18.5 and 29.9 • Adolescents: BMI between 2nd and 98th percentile • Willing to be cannulated and have four blood samples taken at every acute session • Self-reported ability to consume approximately half a typical joint of cannabis by themselves within 20 minutes • Right-handed	• Females: pregnant or breast-feeding • Adults: had a period of at least three months in which cannabis was consumed at a frequency of at least once per week before the age of 18 • Severe cannabis use disorder (DSM-5 number of symptoms >5) • Illicit drug use of any one specific drug at a frequency of more than twice per month, averaged over the past three months • Currently receiving pharmacological or psychological treatment for a mental health problem (within the last month) • Lifetime psychosis • Lifetime psychosis in any immediate family member • Hypertension (systolic >160; diastolic >100) • Nicotine dependent (Heaviness of Smoking Index >1) • Currently taking any psychotropic medication that will likely affect dependent variables or interact with cannabis • Any physical or mental health condition, any medication, or any treatment that the study doctor considers to be an exclusion • Significant asthma or respiratory problems, severity judged clinically • Self-reported moderate/severe acute unpleasant effects from cannabis which occur often or always • MRI contraindications

Abbreviations: BMI, body mass index; DSM-5, Diagnostic and Statistical Manual of Mental Disorders; MRI, magnetic resonance imaging.

Monetary Incentive Delay Task

Reward anticipation was assessed with the Monetary Incentive Delay (MID) task (1). At the start of each trial, a cue appeared for 500 ms, which signalled whether the participant could win money on that trial (win trials: orange square) or not (neutral trials: blue square). After the cue disappeared there was an anticipation phase for 2-4 s (jittered, blank screen), after which a white circle appeared, which the participant responded to by pressing a button on a button-box. Participants were instructed to respond to the white circle as quickly as possible, even if they could not win any money on that trial. After a response had been made, the participants received feedback indicating whether they were successful on that trial, and whether they won any money. Participants could win £0.50 on win trials and their total winnings were calculated and given to them at the end of the session. Given the short interval between the anticipation and feedback phase (2-4 s), this version of the task was optimised for detecting effects during reward anticipation. The required response time for a hit was calibrated to each participant's performance to obtain a 50% hit rate across all (win and neutral) trials. The initial threshold was set at 300 ms, and was reduced by 16.66 ms (one screen refresh) after each 'hit', down to a minimum of 250 ms, or increased by 16.66 ms after each 'miss', up to a maximum of 400 ms. There were 66 trials in total, of which 38 were neutral trials and 28 were win trials, with a jittered inter-trial interval between 1.2 s and 9.2 s. The task took approximately 10 minutes to complete.

Additional Measures

Instant saliva drugs tests were either Alere DDSV 703 or ALLTEST DSD-867MET/C, which tested for cocaine, Δ^9-tetrahydrocannabinol (THC), opiates, amphetamine, methamphetamine, and benzodiazepines. A Lion Alcometer 500 breathalyser was used to measure blood alcohol

concentration (BAC). Participants with a BAC >0 or positive result for any illicit drug (including THC) were rescheduled. Drug use was assessed with the timeline followback (2) and symptoms of cannabis use disorder were assessed with the Cannabis Use Disorder Identification Test – Revised (CUDIT-R) (3). Participants self-reported gender and self-selected ethnicity from categories provided by the UK Census. Depression was measured with the Beck Depression Inventory (BDI) (4), a 21 item self-report questionnaire assessing depressive symptomatology. Impulsive behaviour was measured with the Short UPPS-P scale (SUPPS-P) (5). The SUPPS-P is a 20-item self-report questionnaire assessing urgency, premeditation, perseverance, sensation seeking, and positive urgency.

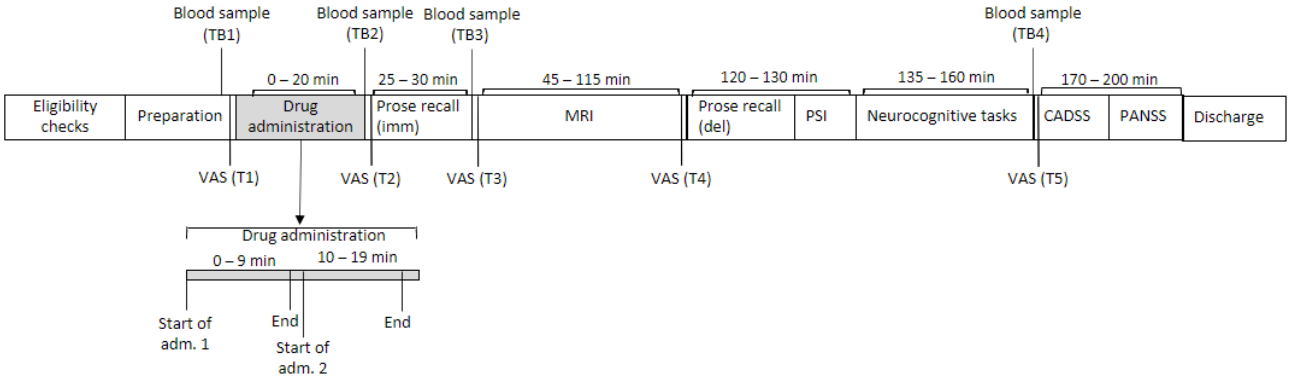

Figure S1. Drug administration session schedule

Abbreviations: Adm, administration; CADSS, Clinician-Administered Dissociative States Scale; del, delayed; imm, immediate; min, minutes; MRI, magnetic resonance imaging; PANSS, Positive and Negative Syndrome scale; PSI, psychotomimetic symptoms inventory; VAS, visual analogue scale.

Time 0 = start of drug inhalation. Administration 1 and 2 = inhalation of first and second balloon filled with cannabis vapour ('THC', 'THC+CBD' or 'PLA'), respectively. Relative to time 0 the time-points correspond as follows: T1 = -5 min, T2/TB2 = +20 min, T3/TB3 = +30 min, T4 = +120 min, and T5/TB4 = +160 min.

Blood Sample Collection and Plasma THC and CBD Quantification

Blood samples (2 x 6ml) were taken from participants via a cannula using BD Vacutainer® Heparin Tubes. Samples were inverted several times and centrifuged at 3000rpm for 10 minutes at 20°C, supernatant plasma was then decanted into four cryovials (1-2ml of plasma per cryovial) and stored at −80°C. Cannabinoids were quantified by isotope-dilution gas chromatography-mass spectrometry (GC/MS) following solvent extraction from plasma, according to the following parameters:

- Standards and reagents: Acetonitrile, water, n-hexane and ethylacetate were of HPLC grade from Fisher Scientific, UK. *N*-*tert*-Butyldimethylsilyl-*N*-methyltrifluoroacetamide with 1% *tert*-Butyldimethylchlorosilane (MTBSTFA) was from Cerilliant via Merck-Sigma, UK.

- THC and CBD were obtained as 0.1 mg/mL or 1 mg/mL methanolic solutions from Cerilliant via Merck-Sigma, UK.

- Extraction solvent: n-hexane-ethyl acetate (9:1).

- Sample preparation: 200 uL of blank, calibration, QC and test samples were spiked with a mixture of deuterium labelled standards and subjected to solvent extractions at neutral and acidic pH and the extracts combined. After evaporation to dryness under nitrogen, samples were derivatized with MTBSTFA and transferred to injection vials for analysis by GC/MS: Agilent 6890 GC/5973 MSD.

- Gas chromatography: carrier gas helium at 1 mL/min; capillary column: Agilent J&W DB-5MSUI 30m x 250µm x 0.25µm; splitless injection at 280°C; temperature program: 150°C to 320°C at 20°C/min and held for 5 min (run time 13.5 min).

- Mass spectrometry: EI 70eV, selected ion monitoring mode: THC (m/z 371, 428), CBD (417, 474). Data acquisition control: Agilent Chemstation v. D.03.
- Data processing: Chemstation files were converted and processed using Agilent Masshunter Quantitative Analysis Version B.07.01. Concentrations were calculated by reference to the standard curves obtained. Concentrations below the lower limit of quantification were replaced with zeros.

MRI Data Acquisition

Participants practised the MID task before going into the scanner, and then performed the task while in the scanner alongside other tasks, which will be reported elsewhere. MRI data were collected with 3.0 T Siemens Verio and Trio scanners (the Verio scanner was decommissioned partway through data collection). T_2* images were acquired using a multiband gradient echo Echo-Planar Imaging (EPI) sequence (repetition time, TR = 1250 ms, echo time, TE = 30 ms, flip angle = 62°). A total of 484 volumes were collected for each participant, with a field-of-view of 192 mm and a matrix size of 64 x 64 mm, yielding an in-plane resolution of 3 x 3 mm. Slice thickness was 3 mm, resulting in 3 mm isotropic voxels. Forty-four slices were collected using interleaved acquisition, and a multi-band acceleration factor of 2. Slice-based acceleration used GRAPPA, also with an acceleration factor of 2. T_1-weighted structural images were acquired using a Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence (TR = 2300 ms, TE = 2.98 ms, flip angle = 9°, parallel imaging acceleration factor = 2), with a spatial resolution of 1 mm isotropic voxels. The acquisition sequences were identical for both scanners.

Power Calculation

The 'CannTeen-Acute' study was powered to detect a Drug by Age-Group interaction on 'feel drug effect', based on Mokrysz et al. (6,7). Given our sample size of n=47, the current study had 80% power to detect a difference between 'THC' and 'PLA' of effect size Cohen's d=.42 and a 2X2 Drug ('THC', 'PLA') by Age-group interaction of effect size Cohen's f = .26 assuming a conservative repeated measures correlation of 0.2.

Table S2. Region of interest coordinates and source

Region	X	Y	Z	Source
Ventral striatum R	12	10	-4	Oldham et al. 2018 (8)
Ventral Striatum L	-10	10	-6	Oldham et al. 2018 (8)
Anterior cingulate cortex R	2	4	40	Skumlien et al. 2022 (9)
Anterior cingulate cortex L	-4	6	42	Skumlien et al. 2022 (9)
Insular cortex R	34	22	-4	Skumlien et al. 2022 (9)

Abbreviations: L, left; R, right.

Supplemental Results

Table S3. MID task behavioural descriptive statistics

	'PLA'	**'THC'**	**'THC+CBD'**
Mean (SD)			
Win % hit	62 (10)	61 (11)	60 (12)
Neutral % hit	40 (8)	37 (8)	39 (8)
Win RT (ms)	252 (31)	256 (36)	253 (33)
Neutral RT (ms)	256 (32)	263 (36)	258 (38)

Abbreviations: MID, Monetary Incentive Delay; ms, milliseconds; RT response time.

Table S4. Full results of Monetary Incentive Delay task behavioural (reaction time) analyses

	F	**df**	**p**
Drug	3.07	2, 46	.06
Trial-Type	32.83	1, 46	<.001
Age-Group	2.12	1, 42	.15
BDI	0.82	1, 42	.37
Cigarette/roll-up tobacco	1.39	1, 42	.25
Scanner	44.85	1, 42	<.001

Abbreviations: BDI, Beck Depression Inventory.

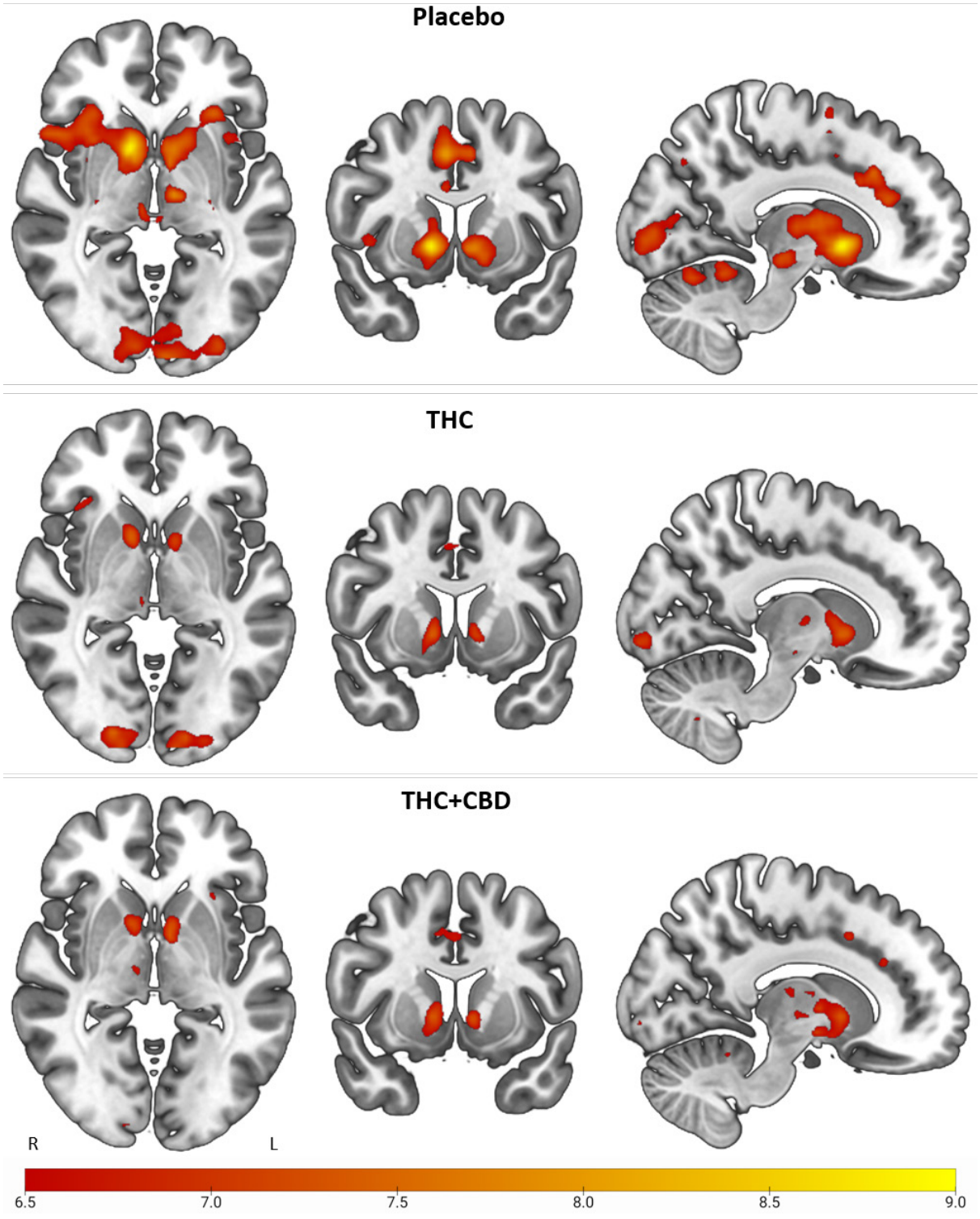

Figure S2. Reward anticipation for each Drug condition

Significant activation for reward anticipation (anticipate win > anticipate neutral) across the full sample of *n*=47 participants. Images are presented in radiological orientation, such that left on the image is the right hemisphere. A higher threshold of Z=6.5 was used for visualisation purposes.

Table S5. Whole-brain one-sample *t*-test results by Drug for reward anticipation on the Monetary Incentive Delay task

	X	Y	Z	K	z
'PLA'					
Pallidum/caudate R	12	8	-2	124 733	8.92
Local maxima					
Insula R	34	22	4		8.48
Cerebellum L	-28	-68	-24		8.20
Thalamus L	-14	-14	4		8.05
Thalamus R	16	-4	12		8.02
Anterior cingulate gyrus R	4	8	44		8.00
'THC'					
Occipital pole R	20	-90	-2	103 312	7.51
Local maxima					
Caudate R	12	10	-2		7.43
Occipital pole L	-16	-92	-4		7.43
Brain stem R	8	-18	-14		7.38
Cerebellum L	-32	-52	-28		7.22
Frontal pole R	18	56	-12	476	4.04
'THC+CBD'					
Caudate L	-8	2	-2	83 819	7.43
Local maxima					
Caudate R	12	8	0		7.37
Paracingulate gyrus R	8	14	42		7.32
Pallidum R	12	-4	-8		7.17
Thalamus R	12	-2	4		7.13
Cerebellum L	-34	-54	-26		7.11
Frontal pole/middle frontal gyrus R	34	42	36	912	5.32
Frontal pole/middle frontal gyrus L	-32	44	28	485	4.61

Abbreviations: L, left; R, right.

Note. X, Y, and Z are coordinates in MNI-space. K refers to number of voxels in the cluster. Peak Z-values are reported for each cluster, and local maxima within clusters, where relevant.

Table S6. Whole-brain paired-sample *t*-test results comparing reward anticipation on the Monetary Incentive Delay task between Drug donditions

	X	Y	Z	K	z
'PLA' > 'THC'					
Occipital fusiform gyrus L	-26	-72	-8	3563	3.85
Local maxima					
Cuneal cortex R	12	-82	22		3.84
Lateral superior occipital cortex R	16	-84	16		3.66
Cerebellum R	8	-66	-24		3.44
Cuneal cortex L	-4	-86	22		3.39
Paracingulate gyrus R	8	26	34	2381	4.41
Local maxima					
Paracingulate gyrus L	-6	26	34		4.22
Insular cortex L	-22	26	-4	1294	3.73
Local maxima					
Accumbens L	-14	14	-6		3.69
Caudate R	10	14	-2		3.66
Frontal operculum L	-36	20	8		3.51
Frontal pole R	22	50	-4	883	3.59
Frontal orbital cortex R	34	34	2	667	3.75
Local maxima					
Insular cortex R	32	20	-2		3.73
Frontal operculum R	50	16	-2		3.48
'PLA' > 'THC+CBD'					
Intracalcarine cortex R	10	-88	8	7700	4.39
Local maxima					
Cerebellum	0	-56	-20		4.12
Lingual gyrus	0	-76	0		3.97
Frontal pole R	22	50	-4	2597	4.56
Caudate R	12	14	-2	1516	4.03
Local maxima					
Insular cortex R	34	24	0		3.91
Frontal operculum	48	16	0		3.64
Frontal orbital cortex R	36	34	2		3.45
Brain stem	0	-30	-2		3.31
Pallidum R	18	2	12		3.25

Superior frontal gyrus R	20	8	72	693	3.45
Local maxima					
Paracingulate gyrus R	8	18	48		3.29
Supplementary motor area R	12	2	58		2.99

Abbreviations: As in Table S5.

Note. X, Y, and Z are coordinates in MNI-space. K refers to number of voxels in the cluster. Peak Z-values are reported for each cluster, and local maxima within clusters, where relevant.

Table S7. Full results of Monetary Incentive Delay task region of interest analyses

	F	**df**	**p**	**η_p^2**
Right ventral striatum				
Drug	4.96	2, 84	.009	.11
Age-Group	10.51	1, 42	.002	.20
Drug*Age-Group	0.80	2, 84	.45	.02
BDI	5.61	1, 42	.02	.12
Cigarette/roll-up tobacco	0.02	1, 42	.91	<.001
Scanner	0.33	1, 42	.57	.01
Drug*BDI	0.86	2, 84	.43	.02
Drug*Cigarette/roll-up tobacco	0.19	2, 84	.83	.004
Drug*Scanner	0.32	2, 84	.73	.01
Left ventral striatum				
Drug	4.18	2, 84	.02	.09
Age-Group	10.94	1, 42	.002	.21
Drug*Age-Group	0.10	2, 84	.91	.002
BDI	1.92	1, 42	.17	.04
Cigarette/roll-up tobacco	0.002	1, 42	.97	<.001
Scanner	0.17	1, 42	.68	.004
Drug*BDI	1.02	2, 84	.37	.02
Drug*Cigarette/roll-up tobacco	0.71	2, 84	.50	.02
Drug*Scanner	0.25	2, 84	.78	.01
Right anterior cingulate cortex				
Drug	0.99	2, 84	.38	.02
Age-Group	4.32	1, 42	.04	.09
Drug*Age-Group	0.07	2, 84	.94	.002
BDI	6.82	1, 42	.01	.14
Cigarette/roll-up tobacco	1.14	1, 42	.29	.03
Scanner	0.20	1, 42	.66	.01
Drug*BDI	0.94	2, 84	.39	.02

Drug*Cigarette/roll-up tobacco	0.53	2, 84	.59	.01
Drug*Scanner	0.26	2, 84	.77	.01
Left anterior cingulate cortex				
Drug	1.35	2, 84	.27	.03
Age-Group	3.81	1, 42	.06	.08
Drug*Age-Group	0.09	2, 84	.92	.002
BDI	5.40	1, 42	.03	.11
Cigarette/roll-up tobacco	1.08	1, 42	.31	.03
Scanner	0.28	1, 42	.60	.01
Drug*BDI	0.62	2, 84	.54	.02
Drug*Cigarette/roll-up tobacco	0.77	2, 84	.47	.02
Drug*Scanner	0.40	2, 84	.67	.01
Right insula				
Drug	6.37	2, 84	.003	.13
Age-Group	6.04	1, 42	.02	.13
Drug*Age-Group	0.003	2, 84	1.00	<.001
BDI	2.49	1, 42	.12	.06
Cigarette/roll-up tobacco	0.05	1, 42	.82	.001
Scanner	5.59	1, 42	.02	.12
Drug*BDI	1.20	2, 84	.31	.03
Drug*Cigarette/roll-up tobacco	0.94	2, 84	.39	.02
Drug*Scanner	0.20	2, 84	.82	.91

Abbreviations: As in Table S4.

Table S8. Results of Monetary Incentive Delay task region of interest analyses without covariates

	F	df	p	η_p^2
Right ventral striatum				
Drug	5.14	2, 90	.008	.10
Age-Group	6.45	1, 45	.02	.13
Drug*Age-Group	1.20	2, 90	.31	.03
Left ventral striatum				
Drug	4.25	2, 90	.02	.09
Age-Group	9.49	1, 45	.004	.17
Drug*Age-Group	0.23	2, 90	.80	.01
Right anterior cingulate cortex				
Drug	1.01	2, 90	.37	.02
Age-Group	1.11	1, 45	.30	.02
Drug*Age-Group	0.09	2, 90	.92	.002
Left anterior cingulate cortex				
Drug	1.38	2, 90	.26	.03
Age-Group	1.08	1, 45	.30	.02
Drug*Age-Group	0.24	2, 90	.78	.01
Right insula				
Drug	6.49	2, 90	.002	.13
Age-Group	3.72	1, 45	.06	.08
Drug*Age-Group	0.08	2, 90	.92	.002

Table S9. Jeffreys-Zellner-Siow Bayes factors in favour of the null hypothesis for each region of interest

	'PLA' vs. 'THC'	**'THC' vs. 'THC+CBD'**	**Adolescents vs. adults ('THC' – 'PLA')**
Ventral striatum R	-	5.99	3.42
Ventral striatum L	-	2.31	3.12
ACC R	3.05	5.56	3.42
ACC L	2.70	6.27	3.39
Insula R	-	5.47	3.33

Abbreviations: ACC, anterior cingulate cortex; L, left; R, right.

Note. Bayes factors are only presented for non-significant Drug and Drug*Age-Group effects in the main analyses.

Table S10. Correlations between 'THC' minus 'PLA' difference scores in regions of interest and cannabis use variables

	Days/week cannabis use	Lifetime days of cannabis use	CUDIT-R
Ventral striatum R	-.139	-.128	.018
Ventral striatum L	-.245	-.088	-.004
ACC R	-.315*	-.168	-.126
ACC L	-.328*	-.140	-.098
Insula R	-.140	-.048	-.109

*Unadjusted $P<.05$

Abbreviations: ACC, anterior cingulate cortex; CUDIT-R, Cannabis Use Disorder Identification Test – Revised; L, left; R, right.

Note. None of the correlations survived multiple comparisons correction with the Benjamin-Hochberg false discovery rate procedure at FDR of 5%.

Supplemental References

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