Received: 15 July 2021 | Revised: 24 September 2021 | Accepted: 29 October 2021

DOI: 10.1111/adb.13113

Addiction Biology SSA WILEY

ORIGINAL ARTICLE

Multimodal MRI data fusion reveals distinct structural, functional and neurochemical correlates of heavy cannabis use

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Abstract

Heavy cannabis use (HCU) is frequently associated with a plethora of cognitive, psychopathological and sensorimotor phenomena. Although HCU is frequent, specific patterns of abnormal brain structure and function underlying HCU in individuals presenting without cannabis-use disorder or other current and life-time major mental disorders are unclear at present. This multimodal magnetic resonance imaging (MRI) study examined resting-state functional MRI (rs-fMRI) and structural MRI (sMRI) data from 24 persons with HCU and 16 controls. Parallel independent component analysis (p-ICA) was used to examine covarying components among grey matter volume (GMV) maps computed from sMRI and intrinsic neural activity (INA), as derived from amplitude of low-frequency fluctuations (ALFF) maps computed from rs-fMRI data. Further, we used JuSpace toolbox for cross-modal correlations between MRI-based modalities with nuclear imaging derived estimates, to examine specific neurotransmitter system changes underlying HCU. We identified two transmodal components, which significantly differed between the HCU and controls (GMV: $p = 0.01$, ALFF $p = 0.03$, respectively). The GMV component comprised predominantly cerebello-temporo-thalamic regions, whereas the INA component included fronto-parietal regions. Across HCU, loading parameters of both components were significantly associated with distinct HCU behavior. Finally, significant associations between GMV and the serotonergic system as well as between INA and the serotonergic, dopaminergic and μ-opioid receptor system were detected. This study provides novel multimodal neuromechanistic insights into HCU suggesting co-altered structure/function-interactions in neural systems subserving cognitive and sensorimotor functions.

KEYWORDS
cannabis, grey matter volume, JuSpace, multimodal data analysis, neurotransmitter, resting state fMRI

1 | INTRODUCTION

According to the United Nations Office on Drugs and Crime, around 192 million of the global population regularly and recreationally (not for medical purposes) use cannabis.[1] Regular and heavy cannabis use (HCU) is a modifiable risk factor for the development of mental illness[2] and a widespread public health issue of mainly young adults.[3–6] According to recent scientific evidence, there is a causal link between cannabis use and long-lasting common mental disorders (e.g., psychotic disorders[6,7] and substance-use disorders).[4–6] In 2010, there were 2 million disability-adjusted life years (DALYs) attributable to cannabis dependence.[3,8] According to recent scientific evidence, there is a causal link between cannabis use and long-lasting common mental disorders (e.g., schizophrenia and other psychotic disorders).[6,7] One of the main hypotheses for psychotic disorders implicates different types of cannabis and a dysregulation (aberrant endogenous signalling) between the two main psychoactive components Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD).[6,7] Furthermore, THC and CBD might differently interfere with the endocannabinoid and other neurotransmitter systems,[9,10] and hence, both substances have different acute-, residual- and long-term effects on affective, cognitive and sensorimotor functioning.[11] On one side, THC can acutely increase positive mood[12] and reduce anxiety at low doses.[13] Further, higher doses of THC might increase the vulnerability of the 5-HT system and lead to depressive, anxiety and psychotomimetic effects (e.g., paranoia, dissociation and depersonalization) as well as impaired memory and attention.[10,14,15] Additionally, long-term effects of cannabis on executive functions are seen in disturbed decision-making, concept formation and planning.[11] On the other side, CBD might decrease the negative effects and perhaps increase the positive effects of THC,[16] leading to antidepressant- and anxiolytic-like effects.[17] The co-localization in the brain and interaction between endocannabinoid and opioid systems play a key role in pain processing, memory, reward and addiction.[18–20] In particular, THC might suppress some opioid signs and symptoms.[20] In turn, CBD may have an "anti-addictive" effect through its action on endocannabinoid, dopaminergic, serotonergic and opioidergic, systems.[21,22]

Although both abnormal brain structure and function has been attributed to HCU, previous neuroimaging studies on HCU showed heterogeneous results mostly prevented through methodological constraints such as unimodal examination of patients with severe comorbid mental disorders. Therefore, it is unclear whether HCU is related to co-altered patterns of brain structure and function, or whether grey matter volume (GMV) and intrinsic neural activity (INA) convey unique and different aspects associated with HCU. Also, it is still unclear how HCU-related structural and functional brain abnormalities are coupled to different neurotransmitter systems. Given the importance of these questions, improving the understanding of the neurobiology underlying HCU is crucial to informing the development of new classes of treatment of both young individuals at high risk for developing a substance-use disorder (SUD).

In order to expand the extant knowledge on network abnormalities spanning across multiple imaging modalities in terms of joint function–structure alterations, which are related to HCU, this study had two major objectives: First, we predicted that there will be a difference in each modality-specific (i.e., brain structure or function) and intermodal (i.e., structure and function) systems comprising cognitive, reward-associated and sensorimotor networks between HCU and a control group without cannabis use. In particular, we chose amplitude of low-frequency fluctuations (ALFF) as primary measures of local intrinsic activity to facilitate comparability with previous research, particularly since several fMRI studies so far have shown abnormal ALFF in both patients with various substance-use disorders and individuals with psychosis.[23–26] Second, acknowledging putative associations between HCU and demographic, clinical and psychopathological variables, we supposed that distinct measures of cannabis use (particularly life-time and current use) will be significantly associated with transmodal components in distinct networks subserving executive control and reward networks.[27–29] Finally, we sought to better understand the relationship between HCU-related brain networks and the underlying molecular features.[30,31] Therefore, we estimated the effects of HCU-related structural and functional brain changes on neurotransmitter systems, including dopaminergic, serotonergic and μ-opioidergic transmission using a novel cross-modal data analysis strategy. Based on previous research,[17,28,32] we expected significant associations between structural/functional networks and serotonergic, dopaminergic and μ-opioid receptor systems. Understanding the molecular architecture underlying HCU might favourably influence the development of future disorder-specific prevention and treatment strategies.

2 | MATERIALS AND METHODS

2.1 | Participants and MRI data

The study was carried out in the Saarland University Hospital Homburg, Germany.[33] A total of 41 participants met eligibility criteria, as outlined below. To reduce potential gender bias,[34,35] in this study, male and right-handed participants aged between 18 and 30 years were considered. We specifically included HCU participants using cannabis and nicotine only. To facilitate comparisons with previous research,[36–38] HCU was defined as cannabis use during at least 10 days/month in the past 24 months and at least 240 days of cannabis use in the past 24 months. Cannabis use criteria for controls was ≤10 joints life-time use and no cannabis use at least 12 months prior to study participation. Current or life-time use of any other illicit substance was an exclusion criterion for all study subjects. Absence of other illicit drugs at the time of testing and MRI was ascertained by qualitative drug-screenings (urine analyses) all study subjects. Participants with a current or life-time mental disorder, as indicated by SCID for DSM-IV-TR interviews, with a history of a neurological disease, significant head trauma or any type of medication were excluded by structured medical history taking. In particular, current or life-time alcohol-use disorder according to DSM-IV-TR was an exclusion criterion. Of note, HCU individuals included in this study did not meet diagnostic criteria for DSM-IV-TR cannabis-use disorder. In addition, the

presence of "attenuated psychosis syndrome," as defined by DSM-5 appendix, was defined as further exclusion criterion.

All HCU participants were evaluated using the Cannabis Use Disorder Identification Test (CUDIT).[39,40] Further rating scales included the Alcohol Use Disorder Identification Test (AUDIT), the Fagerström Test,[41] the German ADHD Self Rating Scale (ADHS-SB)[42] and the Hamilton Depression Rating Scale (HAMD).[43] HCU participants were asked for cannabis abstinence for at least 24 h before clinical assessment and MRI. All HCU consented to these study-specific requirements, and none reported craving or other withdrawal symptoms prior to MRI scanning. The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the ethical review board of the Saarland Medical Association, Saarbrücken, Germany. Written informed consent was obtained from all participants after the procedures of the study had been fully explained.

2.2 | MRI data acquisition

Whole-brain scans were acquired using a 3 T Magnetom Skyra (Siemens, Erlangen, Germany) head MRI system. Structural MRI was acquired via a magnetization-prepared rapid gradient-echo (3D-MPRAGE) sequence with following parameters: TE = 3.29 ms, TR = 1900 ms, TI = 110 ms, flip angle = 9°, FOV = 240 mm, slice plane = axial, voxel size = 0.5 × 0.5 × 0.9 mm^3, distance factor = 50%, number of slices = 192. Afterwards, rs-fMRI was acquired using an echo-planar imaging (EPI) BOLD sequence with parameters as follows: TE = 30 ms, TR = 1800 ms, flip angle = 90°, FOV = 192 mm, slice plane = transversal, voxel size = 3 × 3 × 3 mm^3, distance factor = 25%, number of slices = 32, PAT factor = 2, number of measurements = 230.

2.3 | MRI data analysis

GMV data of 40 (24 HCU, 16 HC; one participant was excluded from the analyses due to head movement >3 mm or 3° during resting state scan) participants from Wolf et al.[33] were considered. Data were analysed via CAT12 (http://www.neuro.uni-jena.de/cat/; last access 06/05/2021; CAT12 version r1109) implemented in SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/; last access 06/05/2021) (see Wolf et al.[33] for details). For processing in CAT12, default parameters, as defined in CAT12, were chosen. GMV data processing comprised spatial normalization, segmentation and smoothing (8-mm Full Width half maximum Gaussian kernel). ALFF was calculated from the resting state data of 41 participants via the Data Processing Assistant for rs-fMRI (DPARSF).[44] Preprocessing comprised removal of the first six scans, slice timing, realignment, coregistration of the T1 image to functional scans, DARTEL[45] based segmentation and normalization (Montreal Neurological Institute [MNI]–space; voxel size 3 × 3 × 3 mm^3) and spatial smoothing with a 6-mm Full Width half maximum Gaussian kernel. Individual whole brain maps of GMV and ALFF were then entered a parallel ICA (pICA)[46,47] using the Fusion ICA Toolbox (FIT; version 2.0e; https://trendscenter.org/software/fit/; last access: 06/11/2021). The number of components for each modality was estimated using the minimum description length (MDL). Five components were identified for each modality. ICASSO[30] was run 20 times to assess the consistency of the components, and the most central run was selected to ensure replicability and stability. Component selection was based on a two-tier approach: First, we used the results of (two-tailed) two sample *t* tests on loading parameters of HCU and HC, as implemented in FIT, to pre-select components of potential interest. For this purpose, in order to ensure that we will not miss subtle but potentially important effects, we chose a liberal threshold of $p < 0.1$. Covariation for nuisance variables, that is, age, was not performed at this stage, nor was correction for multiple comparisons applied. In a second step, loading parameters for these components were extracted and entered into two ANCOVA models—one for GMV loading parameters and one for the loading parameters of the ALFF-component. Both ANCOVA models were adjusted for age. For these analyses, a nominal threshold of $p < 0.05$ was defined, Bonferroni-corrected for multiple comparisons ($p < 0.025$). Anatomical labels and stereotaxic coordinates within significantly differing components were derived from positive clusters above a threshold of $z > 3.5$ by linking the ICA output images to the Talairach Daemon database (http://www.talairach.org/daemon.html; last access: 06/11/2021). Associations between component loadings and using behavior were tested via Spearman correlations and regression models were applied to test how current use in terms of frequency (d/week) and quantity (g/week) of use can be predicted best via lifetime joints and component loadings (all seven possible combinations per measure of current use).

2.4 | MRI-nuclear imaging cross-modal correlations

Components that significantly differed between HCU and controls were used as input for spatial correlation with PET- and SPECT-derived maps in JuSpace (version 1; bugfix for exact *p* value computation manually implemented; https://github.com/juryxy/JuSpace; last visited 06/11/2021).[31] Independent z-score maps based on all 12 PET and SPECT maps implemented in JuSpace (5HT1a_WAY_HC36, 5HT1b_P943_HC22, 5HT2a_ALT_HC19,[48] D1_SCH23390_c11,[49] D2_RACLOPRIDE_c11,[50] DAT_DATSPECT,[51] FDOPA_f18,[52] GABAa_FLUMAZENIL_c11,[51] MU_CARFENTANIL_c11,[53] NAT_MRB_c11,[54] SERT_DASB_HC30,[48] and SERT_MADAM_c11 [https://www.nitrc.org/projects/ki-5htt]) of HCU versus HC were computed using Spearman correlations (based on the Neuromorphometrics atlas; exact *p* values, $N = 10\,000$ permutations; adjusted for spatial autocorrelation).

3 | RESULTS

3.1 | Demographic and psychometric data

For demographic and psychometric details of the two groups, see Table 1. There were no significant false discovery rate (FDR)-corrected differences between the groups (see Table 1).

TABLE 1 Demographics and psychometric scores

	HCU (mean)	SD	Min-max	HC (mean)	SD	Min-max	Statistic	*p*	Effect size[c]
Age (years)	23.13	3.00	19–28	24.81	3.45	18–29	1.6425[a]	0.108	0.26
BDI	6.79	7.82	0–28	2.44	3.12	0–10	274.5[b]	**0.022**	−0.36
HAMD	1.33	1.88	0–8	0.44	0.81	0.2	251[b]	0.070	−0.29
Years of education	14.15	2.93	9–19.5	16.34	2.86	12–20	2.3464[a]	**0.024**	0.36
ADHD-SB total	11.5	5.88	2–23	8.19	8.92	0–26	259.5[b]	0.063	−0.29
Tobacco per year (pack years)	3.33	5.71	0–25.5	1.25	5.00	0–20	301[b]	**<0.001**	−0.53
Joints lifetime	3731.25	4553.59	350–18 000	1.625	2.9	0–10	384[b]	**<0.001**	−0.85
CUDIT total	17.63	8.88	4–36	0	0	0–0	384[b]	**<0.001**	−0.86
Onset age	17.73	3.04	11–25	0	0	0–0	384[b]	**<0.001**	−0.87
Duration use (years)	4.56	3.73	1–14.5	0	0	0–0	384[b]	**<0.001**	−0.87
Current use (d/week)	5.65	1.67	2–7	0	0	0–0	384[b]	**<0.0011**	−0.88
Current use (g/week)	6.06	6.39	0.25–28	0	0	0–0	384[b]	**<0.001**	−0.86

Note: Significant results ($p_{FDR} < 0.05$) in bold font.
Abbreviations: ADHD-SB, attention-deficit/hyperactivity disorder self rating scale; BDI, Beck Depression Inventory; CUDIT, Cannabis Use Disorder Identification Test; HAMD, Hamilton Depression Scale; HC, control group ($n = 16$); HCU, heavy cannabis users ($n = 24$); SD, standard deviation; STAI, State–Trait Anxiety Inventory.
[a]*t* value.
[b]Wilcoxon rank-sum test value.

3.2 | Parallel ICA

In the first step, component pre-selection identified two components, that is, one GMV- ($p = 0.01$) and one ALFF-component ($p = 0.06$). ANCOVA models revealed differences between HCU and HC in both GMV ($F = 6.96$, $df = 1$, $p = 0.01$) and ALFF ($F = 5.00$, $df = 1$, $p = 0.03$) component loadings. The spatial pattern for GMV comprised limbic-, thalamic-, cerebellar-, temporo-parietal- and temporo-occipital structures. For ALFF, the component pattern included occipital-, cerebellar-, frontal- and parietal structures (see Table 2 and Figure 1). The GMV component survived Bonferroni correction ($p < 0.025$), whereas the ALFF component did not. Nevertheless, given that several studies so far consistently highlighted the importance of prefrontal cortical function in substance-use disorders,[55,56] and cannabis-use disorders in particular,[29,57] the ALFF component was also considered for subsequent MRI-nuclear imaging cross-modal correlation analyses.

3.3 | Associations between component loadings and using behavior and prediction of current use

As expected, using behavior was highly intercorrelated (all Spearman's ρ's > 0.7). All loading component-using behavior pairing were significantly correlated (all Spearman's $|\rho| > 0.30$, all p_{FDR}'s < 0.05; generally, INA was stronger correlated than GMV), except lifetime number of joints and GMV, as well as CUDIT and GMV. GMV and INA were not intercorrelated (see Figure 2 and Table S1).

Regression models revealed lifetime use to be the best predictor for current use, whereas amount of current use could be predicted better than frequency of current use (see Table 3 for details). The best performing model (in terms of best adjusted R^2 following Stein's formula[58] and using minimum amount of predictors) predicted amount of current use using number of lifetime joints and GMV component loadings as predictors (adjusted $R^2 = 0.59$, $p < 0.00001$). MRI-nuclear imaging cross-modal correlations between affected receptor systems and pICA components.

Cross-modal correlations revealed associations between GMV and the serotonergic system (5HT1b and 5HT2a) as well as between ALFF and the serotonergic system (5HT1a, 5HT1b, SERT DASB HC30 and SERT MADAM), the dopaminergic system (D2, DAT and FDOPA) and the μ-opioid receptor system (MU; see Figure 3 and Table 4).

4 | DISCUSSION

The present study aimed at investigating interrelationships between GMV, INA and different neurotransmitter systems in HCU. Four main findings emerged: First, the sMRI source identified regions within the cerebello-temporo-thalamic network where HCU showed reduced GMV compared to controls. Second, the rs-fMRI source localized regions within the frontoparietal network where HCU showed reduced ALFF compared to controls. Third, both ALFF and GMV alterations within the respective networks were associated with using-behavior. Fourth, both GMV and INA changes were associated with the estimated serotonergic, dopaminergic and μ-opioid neurotransmitter binding.

4.1 | Alterations of INA and GMV components in HCU

In accordance with our prediction, one structural component significantly differentiated HCU from controls, suggesting that HCU-specific

TABLE 2 Spatial characteristics of identified components of interest

Component Region	Brodmann area	L z-Score/MNI (x, y, z)	R z-Score/MNI (x, y, z)	Volume (cc) L/R
GMV				
Parahippocampal Gyrus	28, 34	6.5 (−23, −6, −20)	6.0 (23, −5, −20)	0.8/1.0
Thalamus	-	5.9 (−8, −18, 11)	5.7 (8, −18, 11)	0.8/0.8
Uncus	-	5.5 (−23, −3, −23)	5.3 (23, −2, −23)	0.4/0.3
Declive	-	4.4 (−21, −65, −18)	5.1 (21, −77, −23)	0.5/1.5
Anterior Cingulate	24, 33	-	4.8 (3, 24, 24)	−/0.5
Cingulate Gyrus	23, 24, 31, 32	4.0 (−2, −32, 33)	4.7 (3, 17, 30)	0.4/0.8
Supramarginal Gyrus	40	-	4.7 (53, −42, 35)	−/0.4
Fusiform Gyrus	19	-	4.6 (24, −80, −21)	−/0.4
INA				
Lingual Gyrus	17, 18	-	6.7 (9, −96, −21)	−/0.3
Declive	-	-	6.2 (15, −90, −27)	−/0.4
Middle Frontal Gyrus	6, 10, 11, 47	6.0 (−33, −6, 60)	-	2.0/−
Superior Frontal Gyrus	6, 8, 9, 10, 11	4.1 (−6, 6, 66)	5.3 (27, 66, −6)	0.4/1.1
Superior Parietal Lobule	7	4.8 (−36, −60, 57)	5.1 (30, −72, 51)	0.3/0.6
Precuneus	7	-	4.6 (24, −72, 51)	−/0.3
Inferior Parietal Lobule	40	4.5 (−45, −48, 57)	-	0.3/−

Note: Voxels with z > 3.5 were coupled with the Talairach Daemon database to provide anatomical labels and were translated into MNI space. For each hemisphere (L = left; R = right), the maximum z-value and MNI coordinate are provided. The volume of voxels in each area is provided in cubic centimetres (cc); the table displays clusters >0.2 cc.

pathology may lie in reduced GMV of cerebello-temporo-thalamic network. This is particularly interesting for a number of reasons: First, cerebellum is a region with a high expression of CBD_1 receptor and might be highly affected by cannabis.[59] Recent sMRI studies also showed reduced cerebellar GMV in HCU.[60,61] Furthermore, according to fMRI studies, cannabis might modulate signalling pathways and functional connectivity (FC) from cerebellum to other brain regions such as thalamus and cortex.[27,62] These findings together with the present study corroborate the crucial role of cerebellar network in cognitive control of HCU. Second, there is a consistent body of literature supporting temporal lobe alterations underlying HCU.[60] Furthermore, according to a longitudinal sMRI study by Koanders et al.,[63] GMV alterations of the medial temporal lobe is considered to be a pre-existing risk factor for later HCU. Third, thalamus is crucial for incentive and reward processing.[57,64] Emerging evidence from fMRI studies (including cue-reactivity fMRI tasks) suggests aberrant thalamus activity in cannabis users.[65] Fourth, we also identified GMV alterations of the cingulate gyrus and anterior cingulate cortex (ACC) in HCU. ACC is crucial for integrating cognitive and emotional processes in support of goal-directed behavior.[66] A recent fMRI study showed that cingulate activation was correlated with self-reported craving in individuals with cannabis use disorder. Interestingly, a recent magnetic resonance spectroscopy (MRS) study[67] showed that significant interaction between dACC glutamate levels and monthly cannabis may predicted the functional connectivity between dACC and nucleus accumbens (NAc). This study was also able to show that cannabis is detrimental to both intrinsic neural connectivity (see also Raymond et al.[68]) and neurochemistry in regions responsible for reward processing.[67] However, no previous study on HCU has found changes in the cingulate cortex.[36,69,70] Taken together, altered structural and functional patterns of temporal regions and basal ganglia may eventually lead to the disturbed reward system and difficulties to interrupt risk-taking behavior as it is known from HCU or other strong drug users.

Furthermore, we chose to investigate neural correlates of HCU with resting-state fMRI, because functional brain alterations underlying HCU might be intrinsic and therefore they may be better examined at rest without the confounders of a non-ecological setting (e.g., scanner and paradigm) and the individual's motivational bias. The identified frontoparietal network comprising frontal gyrus, superior and inferior parietal lobule, and precuneus is crucial for understanding of neuronal mechanisms underlying HCU for three reasons: First, functional abnormalities of the inferior frontal cortex are associated with aberrant response inhibition.[71,72] According to Charboneau et al.,[28] visual cues of cannabis increased craving and activated inferior frontal gyrus in cannabis-dependent adults. Surprisingly, no other fMRI studies could be found that identified aberrant functioning of the middle or superior frontal gyrus as neuronal correlates of HCU. Second, the frontoparietal network includes the orbitofrontal cortex (OFC). The OFC is crucial for cognitive control and emotion regulation and processing, and hence it is highly interconnected with cingulate/medial prefrontal, premotor and parietal cortical areas.[73] Previous studies highlighted the association between structural and functional alterations of the OFC and SUD suggesting a direct substance exposure on the brain.[56,74] Furthermore, Wade et al. showed that larger

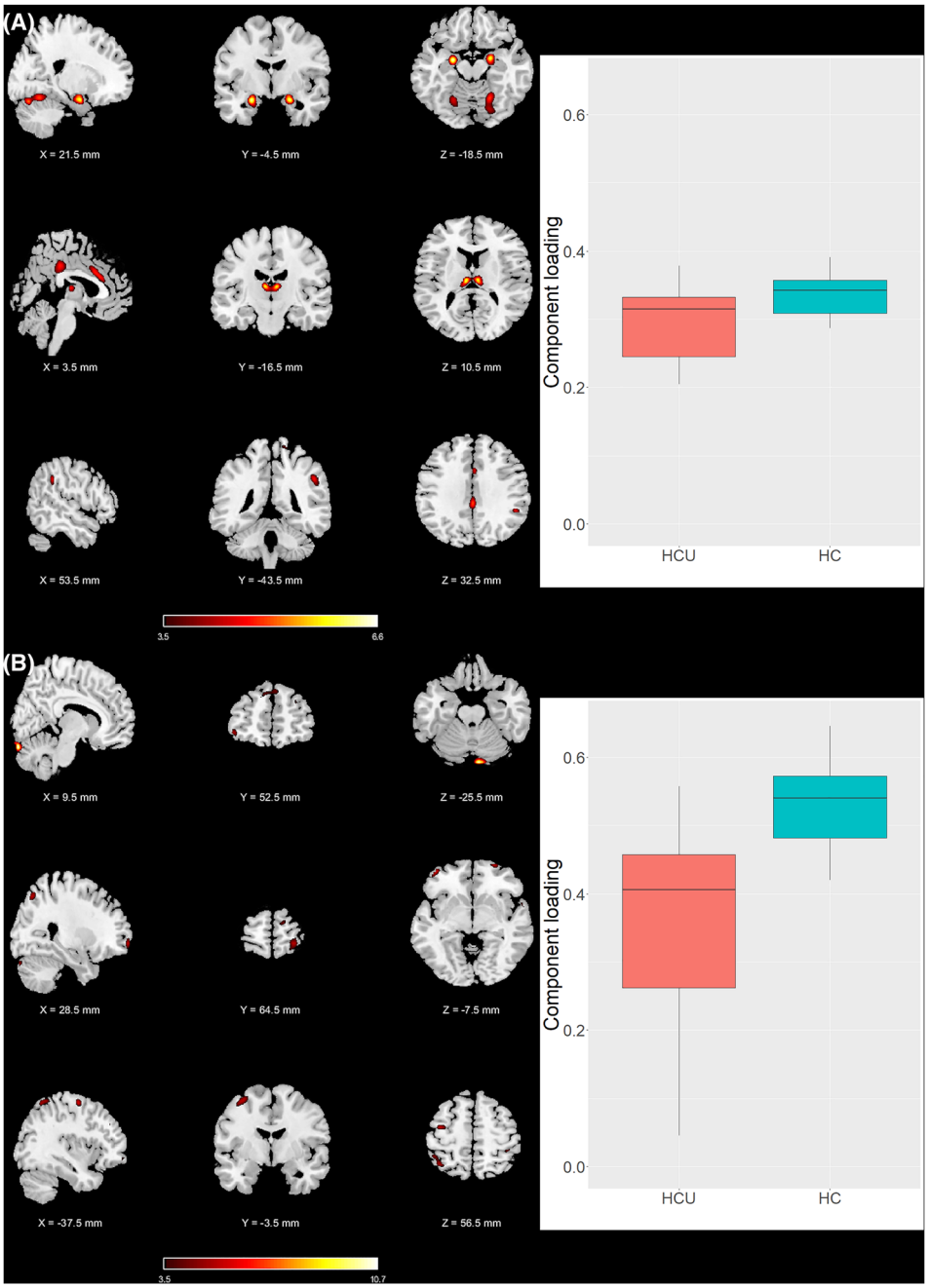

FIGURE 1 Visualization of component patterns and loadings. (A) Left: overlays of the component pattern of GMV onto a brain template (axial slices). Right: boxplot of component loadings by group ($p = 0.01$; ANCOVA, adjusted for age). (B) Left: overlays of the component pattern of INA onto a brain template (axial slices). Right: boxplot of component loadings by group ($p = 0.03$; ANCOVA, adjusted for age). Please note that for the ALFF component, differences between HCU and HC did not survive Bonferroni correction for multiple comparisons (see also main test, Section 3). Colour bars depict z-values. HC, control group; HCU, heavy cannabis use group

OFC (probably due to aberrant maturation) can predict cannabis initiation in adolescents.[75] Interestingly, according to a longitudinal study by Camchong et al.,[76] lower FC between OFC and ACC at baseline predicted higher amounts of cannabis use during the following 18 months in cannabis use disorders. Taken together, aberrant fronto-parietal networks might lead to disturbance of coordinating behavioral control and finally to HCU.

4.2 | INA and GMV components underlying specific HCU-related behavior

Recent evidence suggests that long-term cannabis can cause different psychopathological, psychomotor and cognitive symptoms in both healthy individuals and psychiatric patients.[33] However, little is known about the exact effects of immediate and chronic dose-dependent effects of cannabis on the structure and function of brain networks.[77] Especially when HCU or patients with manifest SUD are studied, the question of the immediate and chronic dose-dependent effects (smoking topography) on brain networks is particularly relevant for methodological reasons.[78] What is further important is the extent to which the levels of cannabis exposure modulate brain structure and function, because differences between HCU or SUD and controls might depended on the levels of THC relative to CBD.[79] This also depends on the current use (in grams) of cannabis. The present study found that current cannabis use is associated with GMV and INA alterations that predominantly include cerebello-temporo-thalamic network. These findings corroborate previous evidence from sMRI

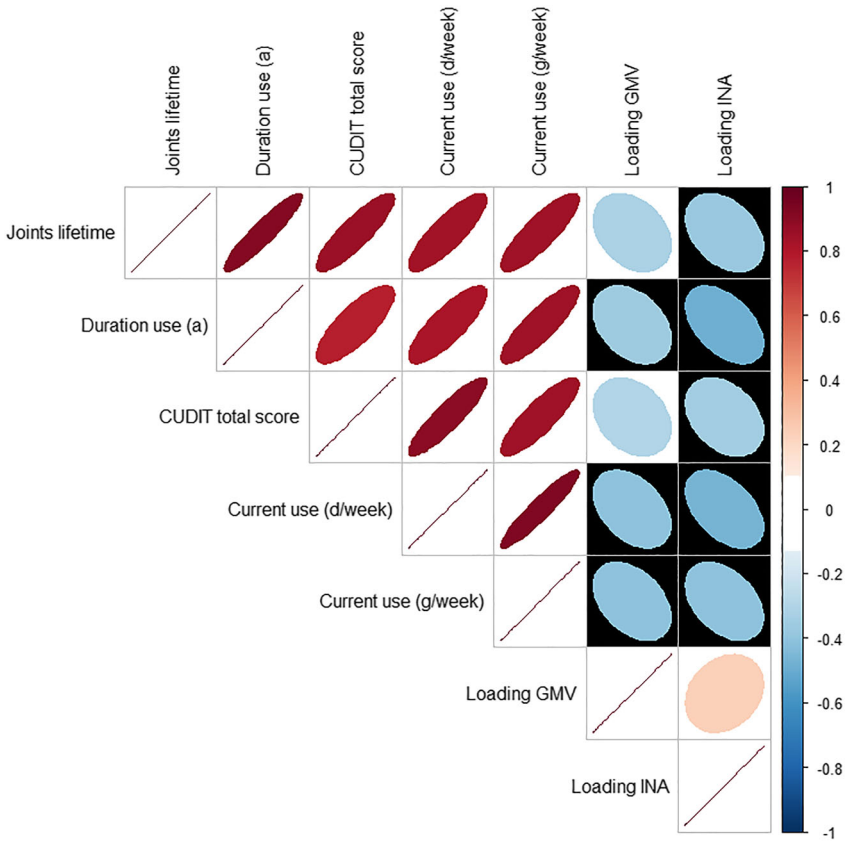

FIGURE 2 Correlograms between component loadings and using behavior. The shape of the ellipse represents the extent of the correlation between two variables, more circular when two variables are uncorrelated. The slope of the longest axis of the ellipse indicates the direction of the correlation, with a positive slope indicating a positive correlation. Black background highlights correlations surviving $p < 0.05$ FDR-correction. Note that for display purposes, no background-highlighting was done for using behavior intercorrelations. Colour bar depicts Spearman's ρ

TABLE 3 Prediction of current use via lifetime use and/or component loadings

Measure of current use Predictors	Multiple R^2	Adjusted R^2 (Stein)	*p* model
D/week			
Joints lifetime	0.28	0.22	**0.0004548**
GMV	0.19	0.12	**0.005556**
ALFF	0.1	0.03	0.04827
Joints lifetime, GMV	0.39	0.3	**0.000107**
Joints lifetime, ALFF	0.36	0.27	**0.0002785**
GMV, ALFF	0.25	0.15	**0.00468**
Joints lifetime, GMV, ALFF	0.45	0.33	**0.00007825**
G/week			
Joints lifetime	0.57	0.53	**<0.00001**
GMV	0.17	0.11	**0.007711**
ALFF	0.04	−0.04	0.2155
Joints lifetime, GMV	0.64	0.59	**<0.00001**
Joints lifetime, ALFF	0.59	0.53	**<0.00001**
GMV, ALFF	0.19	0.08	**0.01878**
Joints lifetime, GMV, ALFF	0.66	0.59	**<0.00001**

Note: Significant results ($p_{FDR} < 0.05$) in bold font. Adjusted R^2 was calculated using Stein's formula, see Stevens.[58]

and fMRI studies in individuals with HCU. Previous sMRI study on chronic HCU identified GMV decrease in frontal, temporal and left occipital gyrus when compared with healthy controls.[60] Interestingly, Cousinjn et al.[36] found that in HCU, GMV of the amygdala and hippocampus are negatively correlated with the amount of cannabis use. Similar results were identified by Koenders et al.[63] who found a negative correlation between cannabis dose (in grams) and regional GMV of the left hippocampus, amygdala and superior temporal gyrus. In a longitudinal MRI study, Koenders et al.[80] found that cannabis dose (in grams) did not affect hippocampal volumes in HCU. Interestingly, a

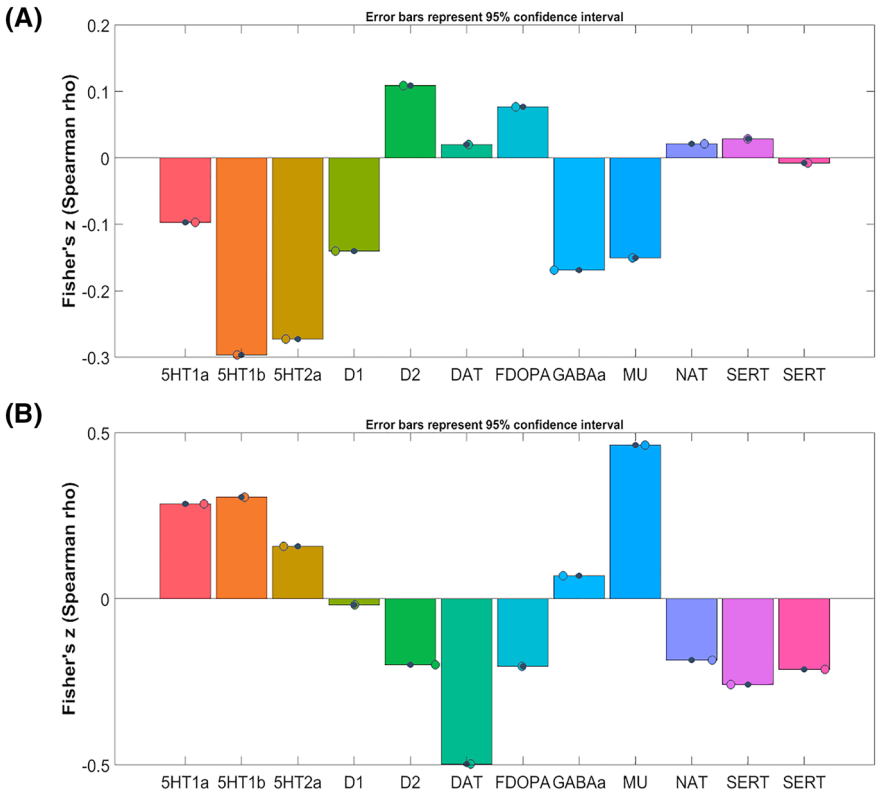

FIGURE 3 Barplots of cross-modal correlations between receptor systems and pICA components. (A) Correlations between GMV and receptor systems (for *p* values, see Table 4). (B) Correlations between INA and receptor systems (for *p* values, see Table 4). The two SERT-labelled bars each are from left to right SERT DASB HC30 and SERT MADAM

previous multi-site-mega-analysis showed that in individuals with cannabis dependence OFC volume was associated with higher monthly cannabis dosage.[81] Furthermore, this study highlighted the distinction between cannabis use and dependence based on subregions of OFC.[81] However, there are also contradictory results that suggest cannabis use has no effect on structural brain morphology.[82] Previous fMRI studies in HCU showed a relationship between chronic cannabis use and aberrant rsFC in the default mode network (DMN), posterior cingulate cortex, cerebellum and supramarginal gyrus.[34] Cannabis exposure was also associated with attenuation of the negative correlation between the striatum and the fusiform gyrus.[55] A combined MRS and fMRI study showed THC-dose-dependent increase of striatal glutamate concentrations and dopamine as indicated by reduced FC between NAc and cortical regions.[83] A more recent combined MRS and fMRI study showed that FC between the dorsal ACC and right NAc was predicted by the interaction between dorsal ACC glutamate levels and monthly cannabis use.[67] Contradictory to the present study, Kuhns et al.[84] showed that cue-induced ventral tegmental area (VTA) but not dorsal ACC activity was positively correlated with grams per week of cannabis. Furthermore, another fMRI studies did not find any correlation between brain activity level of cannabis use.[85]

4.3 | Association between GMV-INA components and neurotransmitter activity maps

Surprisingly, neuroimaging studies on cannabis, serotonergic system and INA/GMV in humans have not been published yet. Therefore, the discussion of the above mentioned findings can only be annexed to animal studies. First, we found an association between HCU-related GMV alterations within the cerebello-temporo-thalamic network and $5HT_{1b}$ and $5HT_{2a}$ activity maps. Accordingly, Bambico et al.[86] found that THC might enhance tonic 5-HT_{1A} receptor activity in the rat hippocampus and suggested that endocannabinoid enhancers might possess antidepressant effects at low doses. Second, there was also an association between HCU-related frontoparietal INA alterations and $5HT_{1a}$ and $5HT_{1b}$ activity maps. This finding corroborate previous findings from animal studies that showed cannabis-induced uncoupling of brain rsFC in the raphe nuclei[87] and vulnerability of the 5-HT system and anxiety symptom.[10] Another animal study by Vinals et al. found that 5-HT_{2A} and CB_1 receptors interact in the mice brain and mediate the memory impairment induced by THC.[88] Interestingly, Galindo et al. found 5-HT_{2A} and CB_1 heteromer expression to be significantly increased in cannabis users. Further, schizophrenia (SZ) is also characterized by serotonergic and endocannabinoid systems dysregulation.[89] A recent study by Guinart et al.[89] showed that 5-HT_{2A} and CB_1 heteromer expression is modulated by cannabis in SZ patients. Further, clozapine (an antipsychotic drug) treatment in SZ patients modulated the 5-HT_{2A} and CB_1 heteromer expression, but cannabis use (SZ patients with cannabis) prevented these alterations.[89] Third, the association between HCU-related frontoparietal INA alterations and dopaminergic system supports the previous evidence that cannabinoids/endocannabinoids directly interact with the dopaminergic system (e.g., stimulating mesolimbic dopamine[32]).[90] The interaction between cannabis products and the human reward system is also clinically relevant. Furthermore, the present findings are noteworthy as they could open up a new avenue into a potential interaction between HCU-associated neural changes

TABLE 4 Associations between pICA components and neurotransmitter activity maps

Component PET map	Mean Fisher's z (Spearman's rho)	*p* (parametric)
GMV		
5HT1a	−0.097	0.295
5HT1b	−0.297	**0.002**
5HT2a	−0.273	**0.004**
D1	−0.140	0.133
D2	0.109	0.242
DAT	0.020	0.829
FDOPA	0.077	0.409
GABAa	−0.169	0.071
MU	−0.150	0.107
NAT	0.021	0.820
SERT DASB HC30	0.029	0.757
SERT MADAM	−0.008	0.934
INA		
5HT1a	0.286	**0.002**
5HT1b	0.305	**0.001**
5HT2a	0.157	0.092
D1	−0.018	0.844
D2	−0.199	**0.033**
DAT	−0.498	**<0.001**
FDOPA	−0.204	**0.029**
GABAa	0.069	0.458
MU	0.463	**<0.001**
NAT	−0.185	0.047
SERT DASB HC30	−0.258	**0.006**
SERT MADAM	−0.213	**0.023**

Note: Significant results ($p_{FDR} < 0.05$) in bold font.

and SZ pathophysiology, a disorder, for which HCU is a well-known major environmental risk factor. In this regard, our results are consistent with a recent study by Chase et al. which combined activation likelihood estimate (ALE) meta-analysis with meta-analytic functional connectivity modelling.[91] The authors reported interconnections between aberrant ventral striatal activation and sensorimotor regions, particularly pre-supplementary area, midbrain and cerebellum in SZ. That meta-analysis, together with findings of this study, supports the notion of a neurobiological link between HCU-related changes and alterations of the reward system, as observed in SZ. Of note, our findings in HCU are in line with recent work by Chen et al.,[30] which found that higher densities of dopaminergic and serotonergic transporters as well as elevated dopamine-synthesis-capacity are related to intrinsic connectivity patterns of networks associated with theory-of-mind, as well as social cognitive and affective processing. Importantly, these networks allowed the prediction of cognitive impairment in SZ patients. Similar mechanisms could possibly also account for cognitive impairment that has been frequently reported in HCU.[92] Overall, better understanding and targeting of the interaction between HCU-related neuronal abnormalities and cognitive as well as reward systems might help to develop novel treatments of SUD.

Eventually, the interaction between HCU-related structural and functional brain alterations and opioid system is important, especially from a clinical perspective, because patients with SUD very often use both cannabis and opioids. This may reflect system levels that have been previously highlighted as both risk and protection factors in cannabis/cocaine/opioid use disorders.[93] Taking the above mentioned results into account together with the fact that opioid analgesics such as fentanyl, tramadol, and methadone are serotonin transporter inhibitors (e.g., 5-HT), it becomes evident that endocannabinoid, serotonin and opioid neurotransmitter systems interact with each other. Interestingly, a recent systematic review showed that cannabis use does not compromise the therapeutic outcome of patients with opioid dependence.[94] In addition, cannabis products are discussed as substitutes or savings for opioids in pain management.[20] Eventually, recent studies showed that allosteric negative modulators of CB_1 receptors and drugs targeting CB_2 receptors are promising agents for the treatment of SUD (for review see Manzanares et al.[95]), because they might modulate the addictive properties of several drug classes.[90] Therefore, we strongly acknowledge future multimodal neuroimaging studies that will prove the advantages (CBD) and disadvantages (THC) of cannabis in SUD. In the case of CBD, this is feasible since approved medication is already available.

4.4 | Strengths and limitations

Strengths of this study include the examination of joint function–structure alterations, JuSpace toolbox allowing for examination of a variety of neurotransmitter systems and clinically well-characterized sample of male participants with HCU presenting without major mental disorders and without current or a history of attenuated psychotic symptoms. Nevertheless, this study has several potential limitations. First, we used cross-sectional data. Second, we used a relatively modest sample size, which may have missed more subtle effects at the structural and functional systems level. Third, we examined an exclusively male population. In this particular case, we chose to investigate male HCU only because certain brain functions are differently represented and modulated in left- and right-handed males or females.[96] Further, we also sought to control for cerebral asymmetries which may putatively induce noise in neuroimaging findings.[97] This said, we acknowledge that the present findings may apply to right-handed individuals only and that future research will need to address the impact of handedness and laterality in HCU in more detail. Fourth, the exclusion of individuals with "attenuated psychosis syndrome" as defined by DSM-5 appendix might be considered as potential selection bias. We acknowledge that this could be indeed the case given the frequent co-occurrence of HCU and attenuated psychotic symptoms (APS). However, in this study, we explicitly renounced the inclusion of individuals presenting with APS in order to exclude potential bias for any other comorbid mental disorder or risk behavior except HCU.

Nevertheless, given the clinical relevance of APS in HCU, future MRI studies will need to address the impact of such comorbidity in more detail, preferably within a longitudinal framework. Keeping the above mentioned limitations in mind, this study provides a first multilevel neuromechanistic understanding of HCU, particularly with respect to aberrant cerebello-temporo-thalamic network and related neurotransmitter systems, and a first starting point for future research that has to elucidate neurobiological underpinnings of HCU in more detail.

4.5 | Concluding remarks

The present findings add to the growing evidence that HCU are characterized by both structural and functional disruptions of networks as well as neurotransmitter systems underlying cognitive control, goal-directed behavior and the reward system. In conjunction with findings from recent MRI studies, structural and functional alterations within the above mentioned networks and their interaction with multiple neurotransmitter systems enhance our understanding why HCU persist to use cannabis despite negative consequences of such behavior.

ACKNOWLEDGEMENTS

We thank all our study participants for their understanding and willingness to participate in this study. Open Access funding enabled and organized by Projekt DEAL. WOA Institution: ZENTRALINSTITUT FUR SEELISCHE GESUNDHEIT Blended DEAL: Projekt DEAL.

FUNDING INFORMATION

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

RCW, FW, NDW and WR were responsible for the study concept and design. RCW, FW, MW and WR contributed to the acquisition of clinical and neuroimaging data. MMS and RCW performed the neuroimaging and statistical analyses. MMS, NDW, VDC, FS and KMK assisted with data analysis and interpretation of findings. DH and RCW drafted the first version of the manuscript. RCW, KMK, VDC and FS and DH provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

ETHICS STATEMENT

The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All participants gave written informed consent as approved by the ethical review board of the Saarland Medical Association, Saarbrücken, Germany.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Hirjak D, Schmitgen MM, Werler F, et al. Multimodal MRI data fusion reveals distinct structural, functional and neurochemical correlates of heavy cannabis use. *Addiction Biology*. 2022;27(2):e13113. doi:10.1111/adb.13113