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RH=Cannabis-Associated PLEs Mediated by Parahippocampus

Tao Yu, MD, PhD, Tianye Jia, PhD, Liping Zhu, MD, PhD, Sylvane Desrivières, PhD, Christine Macare, PhD, Yan Bi, PhD, Arun L.W. Bokde, PhD, Erin Burke Quinlan, PhD, Andreas Heinz, MD, PhD, Bernd Ittermann, PhD, ChuanXin Liu, PhD, Lei Ji, PhD, Tobias Banaschewski, MD, PhD, Decheng Ren, PhD, Li Du, PhD, Binyin Hou, PhD, Herta Flor, PhD, Vincent Frouin, PhD, Hugh Garavan, PhD, Penny Gowland, PhD, Jean-Luc Martinot, MD, PhD, Marie-Laure Paillère Martinot, MD, PhD, Frauke Nees, PhD, Dimitri Papadopoulos Orfanos, PhD, Qiang Luo, PhD, Congying Chu, PhD, Tomas Paus, MD, PhD, Luise Poustka, MD, Sarah Hohmann, MD, Sabina Millenet, MD, Michael N. Smolka, MD, Nora C. Vetter, PhD, Eva Mennigen, MD, Cai Lei, PhD, Henrik Walter, MD, PhD, Juliane H. Fröhner, MSc, Robert Whelan, PhD, Guang He, MD, PhD, Lin He, PhD, Gunter Schumann, MD, PhD, Gabriel Robert, MD, PhD, and the IMAGEN Consortium

Drs. Yu and Jia are co-first authors of this article.

Editorial
Supplemental Material

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Drs. Yu, Bi, Ji, Ren, Hou, Lei, G. He, and L. He are with Bio-X Institutes, Shanghai Jiao Tong University, Shanghai, China. Drs. Yu, L. He, Robert, Jia, Desrivières, Macare, Quinlan, Chu, and Schumann are with the Medical Research Council - Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK. Dr. Robert is also with the Behavior and Basal Ganglia Unit, Medical University of Rennes, France. Drs. Yu, L. He, Du, and Zhu are with the Shanghai Center for Women and Children's Health, China. Drs. Yu and Lu are with Jining Medical University, Shandong, China. Drs. Jia and Luo are with the Centre for Computational Systems Biology, Fudan University, Shanghai, China. Dr. Bokde is with the Discipline of Psychiatry, School of Medicine and Trinity College Institute of Neuroscience, Trinity College Dublin, College Green, Dublin, Ireland. Drs. Banaschewski, Nees, Flor, Hohmann, and Millenet are with the Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany. Dr. Flor is also with the School of Social Sciences, University of Mannheim, Germany. Drs. Frouin and Orfanos are with NeuroSpin, Commissariat à l'Energie Atomique, CEA, Université Paris-Saclay, Gif-sur-Yvette, France. Dr. Garavan is with the University of Vermont, Burlington. Dr. Gowland is with the Sir Peter Mansfield Imaging Centre School of Physics and Astronomy, University of Nottingham, University Park, Nottingham, UK. Drs. Heinz and Walter are with Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin,

Humboldt-Universität zu Berlin, and Berlin Institute of Health, Campus Charité Mitte, Berlin, Germany. Dr. Ittermann is with Physikalisch-Technische Bundesanstalt (PTB), Braunschweig and Berlin, Berlin, Germany. Drs. J. Martinot and M. Martinot are with Institut National de la Santé et de la Recherche Médicale (INSERM), University Paris Sud, Orsay, France. Dr. Paus is with Bloorview Research Institute, Holland Bloorview Kids Rehabilitation Hospital and the University of Toronto, Ontario, Canada. Drs. Smolka, Vetter, Mennigen, and Fröhner are with Technische Universität Dresden, Germany. Dr. Whelan is with the School of Psychology and Global Brain Health Institute, Trinity College Dublin, Ireland. Dr. Poustka is with the University Medical Centre Göttingen, Göttingen, Germany. Dr. L. He is also with the Baoan Maternal and Child Health Hospital, Jinan University, Shenzhen, China. IMAGEN consortium authors, affiliations, and acknowledgement are listed in the supplementary materials.

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Health; Prof. Dr. Michael Smolka, Technische Universität Dresden; Juliane Fröhner, Technische Universität Dresden; Dr. Maren Struve, Central Institute of Mental Health; Prof. Dr. Steve Williams, King's College London; Dr. Thomas Hübner, Technische Universität Dresden; Uli Bromberg, University of Hamburg; Semiha Aydin, PTB; John Rogers, Delosis; Alexander Romanowski, Charite; Dr. Christine Schmäl, Central Institute of Mental Health; Dirk Schmidt, Technische Universität Dresden; Stephan Ripke, Technische Universität Dresden; Dr. Mercedes Arroyo, Cambridge University; Dr. Florian Schubert, PTB; Dr. Yolanda Pena-Oliver, University of Sussex; Mira Fauth-Bühler, FOM Hochschule für Oekonomie and Management/Central Institute of Mental Health; Xavier Mignon, PERTIMM; Dr. Robert Whelan, Trinity College Dublin; Dr. Claudia Speiser, GABO:milliarium mbH and Co. KG; Tahmine Fadai, University of Hamburg; Prof. Dr. Dai Stephens, University of Sussex; Dr. Andreas Ströhle, Charite; Dr. Marie-Laure Paillere, INSERM; Nicole Strache, Charite; David Theobald, Cambridge University; Sarah Jurk, Technische Universität Dresden; Dr. Helene Vulser, INSERM; Ruben Miranda, INSERM; Dr. Juliana Yacubilin, University of Hamburg; Vincent Frouin, Commissariat à l'Energie Atomique; Alexander Genauck, Charite; Caroline Parchetka, Charite; Isabel Gemmeke, Charite; Johann Kruschwitz, Charite; Katharina WeiB, Charite; Dr. Henrik Walter, Charité Universitätsmedizin Berlin; Jianfeng Feng, Fudan University/Warwick University; Dimitri Papadopoulos, INSERM; Irina Filippi, INSERM; Alex Ing, King's College London; Dr. Barbara Ruggeri, King's College London; Bing Xu, King's College London; Christine Macare, King's College London; Dr. Congying Chu, King’s College London; Eanna Hanratty, King's College London; Dr. Erin Burke Quinlan, King's College London; Dr. Gabriel Robert, King's College London; Prof. Dr. Gunter Schumann, King's College London; Dr. Tao Yu, King's College London; Veronika Ziesch, Technische Universität Dresden; Alicia Stedman, University of Nottingham.

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Correspondence to Gabriel Robert, MD, PhD, Behavior and Basal Ganglia Unit (EA4712), Medical University of Rennes, Avenue du Professeur Léon Bernard, 35000 Rennes, France; e-mail: gabriel.hadrien.robert@gmail.com

Abstract

Objective: Cannabis consumption during adolescence has been reported as a risk-factor for psychotic-like experiences (PLEs) and schizophrenia. However, brain developmental processes associated with cannabis-related PLEs are still ill-described.

Method: 706 adolescents from the general population that were recruited by the IMAGEN consortium had structural MRI scans both at 14 and 19 years-old. We used deformation-based morphometry to map voxel-wise brain changes between the two time points, using the pairwise algorithm in SPM12b. We used an a-priori region of interest (ROI) approach focusing on the hippocampus/parahippocampus to perform voxel-wise linear regressions. Life time cannabis consumption was assessed using the European School Survey Project on Alcohol and other Drugs (ESPAD) and PLEs were assessed with the Comprehensive Assessment Psychotic-like experiences (CAPE). We first tested whether hippocampus/para-hippocampus development was associated with PLEs. Then, we formulated and tested an a-priori simple mediation model where uncus development mediates the association between lifetime cannabis consumption and PLEs.

Results: We found that PLEs was associated with reduced expansion within a specific region of the right hippocampus/para-hippocampus formation, the uncus (p=0.002 at the cluster level, p=0.018 at the peak-level). The partial simple mediation model revealed a significant total effect from lifetime cannabis consumption to PLEs (b=0.069 95CI [0.04-0.1], p=2 x 10^{-16}), as well as a small yet significant, indirect effect of right uncus development (0.004, 95IC [0.0004-0.01], p=0.026).

Conclusion: We show here that the uncus development is involved in the cerebral basis of PLEs in a population-based sample of healthy adolescents.

Key words: cannabis, psychotic-like experiences (PLEs), uncus, paired designed MRI, deformation-based morphometry

Introduction

Psychosis can be perceived as a continuum ranging from non-help-seeking individuals among the general population with psychotic-like experiences (PLEs), to help-seeking people with ultra-high risk of psychosis, to first episode psychosis and patients with chronic psychotic disorder.[1] The median prevalence of PLEs amounts to 5~8% in general population[2] and they usually emerge during late adolescence. PLEs represent a non-clinical psychosis phenotype, but carry an increased risk to develop schizophrenia-spectrum disorder.[3, 4] Another widely known risk factor for schizophrenia development is cannabis consumption which has been identified both in epidemiological surveys and prospective birth cohort studies.[5, 6] Cannabis is suggested to cause the development of schizophrenia because previous studies have shown that cannabis intoxication can induce acute psychosis[7] and the administration of tetrahydrocannabinol is responsible for acute psychotic symptoms in healthy volunteers [8] and the cannabis/psychosis association follows a dose response effect.[9]

On another hand, strong evidence points to hippocampal/parahippocampus, atrophy both in patients with chronic psychotic disorder,[10] first episode patients[11] and ultra-high risk individuals for psychosis.[12] Similarly, some (but not all)[13] investigators reported association of chronic cannabis use with decreased cortical volume, including the hippocampus/parahippocampus,[14] which is a region known to have high cannabis receptor 1 (CB1) density.[15] The uncus, a subregion of the hippocampus/parahippocampus formation, has been associated with both positive

(hallucination) and negative (social withdrawal) psychotic symptoms[4] and might also be involved in cannabis-associated psychotic-like experiences.

Furthermore, animal models of cannabis intoxication also found hippocampal atrophy, suggesting a causal relation between cannabis intake and specific structural brain abnormalities. Thus, hippocampus atrophy seems to be both associated with psychosis (which is strongly related to cannabis intake) and by cannabis intake itself.

While a recent study adopted a longitudinal approach to detect hippocampal atrophy among heavy young adult cannabis users in the hippocampus formation,[16] it did not integrate any information about the PLEs. Thus, the relation between developmental changes in the hippocampal formation during adolescence, cannabis use and PLEs is unclear.

We therefore adopted a paired designed MRI deformation-based morphometry analysis to investigate the association between brain volume development (from 14 years to 19 years) with PLEs and cannabis use in the IMAGEN sample of healthy adolescents. First, we determined whether longitudinal changes of grey matter (GM) within the hippocampus formation were associated with PLEs, using a literature-based region of interest approach. Then we formulated and tested one model where cannabis intake would be directly associated with PLEs, and hippocampus volumetric changes would partially mediate this association.

Method

Subjects: The subjects were selected from the IMAGEN project, a European multi-center neuroimaging genetic study of adolescent participants[17] recruited from eight research centers across Europe. In the present study, we used data from baseline (BL, age 14, n=780) and follow-up (FU, age 19, total n=780), where paired structural MRI images were available (https://imagen-europe.com/). After quality control of both the MRI images and behavior data, 706 subjects were included in the final analyses (see Figure S1, available online). Subjects with psychiatric disorders were excluded from the IMAGEN project at BL because it aims at identifying genetic, cerebral and behavioural markers of mental disorders which appears during adolescence, including addiction. The study was approved by the local ethics committees and adhered to the Declaration of Helsinki. An overview of demographic information for all subjects is shown in Table 1.

Psychotic-like experiences: PLEs in the general population were assessed by the self-report Community Assessment of Psychic Experiences (CAPE)[18] at FU. The questionnaire consists of 42 items covering three symptom dimensions: positive, negative and depressive dimensions. It includes 20 questions for positive symptoms, 14 for negative symptoms, and 8 for depressive symptoms. The subjects were asked to rate the frequency of lifetime PLEs on a 4-point Likert scale of 'Never',

'Sometimes', 'Often' and 'Nearly Always'. The total score was obtained by calculating the mean of the 42 items.

Drugs Use: European School Survey Project on Alcohol and Drugs (ESPAD) was used to measure the frequency of cannabis, alcohol, cigarette and other illicit drugs use in one's lifetime, past 12 months, past 30 days and past week.

Lifetime cannabis use was assessed by the question: "How many times in your life have you used marijuana or hash?" at 19 years (FU). The answer was scored between 0~6 according to their use frequencies: 0 = "never", 1 = "once or twice", 2 = "3-5 times", 3 = "6-9 times", 4 = "10-19 times", 5 = "20-39 times", 6 = "40 times or more". Other drug uses were scored the same way. We also assessed cannabis use at 14 years (BL) using lifetime cannabis use acquired at 14 years. The frequencies of cannabis use at both BL and FU are listed in the supplementary materials (see Table S1, available online).

Images Acquisition: Details of the structural MRI acquisition protocols and quality controls have been provided elsewhere.[17] Briefly, the high-resolution isotropic anatomical MRI images (voxels size=1mm^3) were obtained using a three-dimensional T1-weighted magnetization-prepared gradient-echo sequence (MPRAGE) based on the ADNI protocol (details at http://adni.loni.usc.edu/methods/documents/mri-protocols/).

Quality Control: Details of standard operation procedure for the baseline and follow up structural MRI quality control can be seen in the supplementary materials (Supplement 1, available online).

Baseline MRI data using standard VBM protocol: We used the standard VBM8 protocol for preprocessing the BL structural data (14). Briefly, all images were spatially normalized to the standard MNI T1 MRI template, segmented into GM, WM and cerebrospinal fluid compartments using MNI priors. The segmented images were modulated by the Jacobian determinants in order to compensate for the possible volume changes during the non-linear spatial normalization procedure. Finally, these GM images were smoothing using an 4mm full width at half maximum (FWHM) isotropic Gaussian kernel.

Pairwise analysis of MRI

This followed 5 sequential steps. First, the SPM12b pairwise tool estimates voxel-wise quantitative measures of expansion and volume reduction over time using a within-subject template in the native space. Then, the native space within-subject template were then segmented using the VBM8 toolbox (http://www.neuro.uni-jena.de/vbm/). Here, the segmentation process was data-driven and unsupervised and did not require a priori tissue probability maps information.[19] This approach has been found to be effective to segment pediatric structural MRI.[20] Third, we used DARTEL to derive a between subject study-specific template, to

which the voxel-wise maps of brain development are registered and normalized to. The use of both data-driven segmentation with DARTEL approaches is similar to the Template-o-Matic toolbox to build non-adult templates and has been proven to be efficient to build early adulthood templates.[21]

Given that we focused on the hippocampus/para-hippocampus, we chose to smooth the registered and normalized maps of brain development with a kernel of 4mm FWHM. Full description of the procedure along with the quality control is detailed in the supplementary materials (Supplement 2, available online).

To investigate the association between GM volumetric changes in hippocampus/para-hippocampus and PLEs, we firstly created a mask in these areas using Wake Forest University (WFU) PickAtlas in SPM12b (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). The mean value of grey matter change within the significant cluster was extracted using MarsBar (http://marsbar.sourceforge.net/).

Statistical analysis

Voxel-wise analyses within the hippocampus/para-hippocampus

We ran voxel-wise multiple linear regression models in SPM12b (see Supplement 3, available online): dependent variable was the rate of GM volume changes; independent variable was CAPE total score at FU; covariates included gender, age, site (dummy-coded), as well as TIV at BL following the latest recommendations

about nuisances variables in VBM studies.[22] Similarly, we tested for associations between BL grey matter volume in the hippocampus/parahippocampus formation and CAPE scores, cannabis use at 14 years and 19 years with the same covariates.

All voxel wise statistics were corrected using the family-wise error (FWE) correction for multiple comparisons over the mask in the hippocampus/parahippocampus and considered to be significant for $p<0.05$ after the FWE correction (approximately 32 000 tests were run).

Mediation analyses

The mediation analysis was performed on data from 706 subjects in which all mediation variables and covariates were available: GM volumetric changes in the right uncus, life time cannabis use and PLEs (Figure 1), using the "mediation" R package.[23] Based on literature-based arguments of cannabis-induced PLEs and hippocampal/parahippocampal atrophy, we formulate the partial simple mediation model where cannabis would be associated with PLEs and that association would be mediated by right uncus development. Within that model, the direct effect means the effect of cannabis use on PLEs when the mediating variable (uncus volume change) is not included. The indirect effect means the effect of cannabis use on PLEs through the mediating variable. The total effect equals the sum of direct and indirect effects. The graphical model is displayed on Figure 1. The covariates included in the mediation analyses were the same as in the voxel-wise analyses (i.e. gender, age, site, TIV at BL, lifetime alcohol and psycho-analeptic drugs). The correlation matrix between

psycho-analeptic drugs and psychotic-like experiences were shown at supplementary Table S2, available online. Given the multisite design of the IMAGEN project, we used hierarchical linear modelling to model a one-level random intercept for the variable "site", using "lme4" package in R.[24] The full code is detailed in the supplementary materials (see Supplement 4, available online).

As post-hoc analyses, we sought for an interaction between gender and cannabis consumption on brain development. Moreover, we explored how cannabis consumption were associated with the CAPE subscales scores (see Figure S2, available online).

Results

Demographic Characteristics

The demographic characteristics of subjects at BL and FU are shown in Table 1. Seven hundred and six subjects were included in which paired design MRI image, CAPE and ESPAD scores were available. The average age at BL was 14.41 years, and 18.88 years old at FU. The percentage of cannabis users who had tried cannabis at least once in their lifetime increased from 5.0% at BL to 46.50% at FU. The overall average score of CAPE at FU was 4.60±0.89, and the scores for CAPE subscales are shown in Table 1.

Voxel-wise analyses

We found significant negative correlations between GM volumetric changes and CAPE total score in the right uncus ([18, 0, -32], cluster size=112 voxels, p=0.020 FWE correction for cluster level, and p=0.018 FEW corrected at the peak-level), suggesting that higher CAPE total score is related to a smaller expansion in the right uncus (Figure 2).

Mediation analyses

As illustrated on Figure 1, we found a significant positive total effect of lifetime cannabis intake on PLEs (b=0.069 95CI [0.04-0.1], p=2 x 10^{-16}). We found a positive significant direct effect of cannabis intake on PLEs (b=0.064, 95IC [0.03-0.1], p=2 x 10^{-16}) and an indirect effect of cannabis intake on PLEs mediated by uncus development (0.004, 95IC [0.0004-0.01], p=0.026), suggesting partial mediation. Six percent of the total effect was mediated by uncus development (95IC [0.006-0.17], p=0.03). The graphical model is displayed on Figure 1. While cannabis consumption was negatively associated with development of the uncus (i.e. reduced expansion over time) in the whole sample ($t_{(1,617)}$ = -2.3, p=0.02), we did not find any interaction between gender and cannabis intake on uncus development, with cannabis being associated with reduced expansion of uncus in both genders (see Figure S3, available online). The detailed associations between cannabis intake and cape sub-scores are provided in supplementary material (see Figure S2, available online).

Discussion

We found that a smaller expansion in the right uncus is associated with greater psychotic like experiences. We also found that uncus development partially mediates (6%) the relation between lifetime cannabis use and psychotic like experiences.

The uncus is located at the junction of hippocampus, amygdala and olfactory lobe, and is involved in olfactory hallucination, memory impairments and social withdrawal.[25] Several cross-sectional and longitudinal MRI studies demonstrated structural abnormalities in hippocampus formation or uncus among individuals with ultra-high risk (UHR) for psychosis[12]. Functional MRI studies also noted that UHR individuals showed less activation than controls in the parahippocampal gyrus, medial frontal gyrus during the encoding process of memory task.[26, 27]

Our results are consistent with previous research findings in community samples of adolescents and young adults showing that lifetime cannabis use was significantly correlated with PLEs both in cross-sectional[28] and longitudinal samples.[29] According to our results, a smaller volume increase of the uncus might be one of the pathophysiological mechanism mediating the link between cannabis consumption and psychotic-like experience. This view is supported by multiple evidence: First, CB1 is mainly expressed in the olfactory lobe, hippocampus, parahippocampus and amygdala regions.[30] Second, animal studies have shown that pubertal exposure to cannabis could induced psychotic-like behaviors and hippocampal structures changes such as decreased density and increased glial cell reactivity.[31] Third, functional and structural cross-sectional neuroimaging studies report an association of hippocampal atrophy

hippocampus with long-term cannabis use.[32] We found volumetric changes on the right uncus while previous studies reported on the bilateral side related to schizophrenia.[33] This discrepancy might be explained by the different stages of the continuum hypothesis of psychosis, which might involve different hippocampus alterations. In agreement with this hypothesis, previous results found atrophy only in the right hippocampus in a homogeneous sample of at-risk individuals who did not transition into schizophrenia whereas atrophy within the left hippocampus was significant within the group who transitioned into schizophrenia.[34] This is consistent with our results of smaller expansion of uncus development in the right uncus in cannabis associated psychotic like experiences in healthy adolescents.

The CAPE is a promising tool for the measurement of PLEs in the general population. It was reported as a valid, simple and cost-effective screening tool for detecting individuals at potential risk for psychosis[18]. In addition, the CAPE has a good prognostic reliability since its scores at baseline could predict schizotypic disorders and psychiatric symptoms severity at follow-up in a large longitudinal cohort followed during 7.7 months.[35] In our study, we found the mean total score of CAPE was 4.6 and subscores for positive, negative and depressive symptoms were 1.3, 1.6 and 1.7, respectively, which were similar to other studies in healthy population.[18, 35] For the CAPE assessment, we only calculated the frequency but not distress of each item, since there was highly correlation ($r=0.89$, $p<0.001$) between frequency and distress. Previous results suggest that early cannabis use (i.e. before age 14 years) is associated with psychotic like experiences[36] which we cannot find

here. In contrary to Gastel at al.,[36] only few individuals had cannabis in our sample (see Table S1, available online), which might explain this discrepant result.

Some limitations must be borne in mind when interpreting these results: first, it must be pointed out that our findings conclude to only 6% of the total effect is mediated by uncus development. This first suggests that our results warrant replication. It also suggests that other pathophysiological mechanisms underlie the association between cannabis consumption and PLEs. Previous results showed that cannabis-related psychotic symptoms are associated with abnormal pattern of pre-fronto-striatal connectivity during an odd-ball paradigm.[37] Decreased fractional anisotropy (a measure of white matter integrity) has been found in a diffuse network including the superior and inferior longitudinal fasciculus, which is later associated with the onset of psychosis[38]. Finally, cannabis consumption in ultra high-risk individuals is associated with increased resting state connectivity between the thalamus and sensori-motor regions.[39] Using the IMAGEN cohort, it has been shown that polygenic risk score for schizophrenia moderates the association between cannabis consumption and cortical thickness development in boys.[40] Having these results in mind, further studies might focus on the uncus resting state connectivity pattern, and model genetic vulnerability to schizophrenia, to more extensively apprehend cannabis-related psychotic like experiences in healthy adolescents.[4, 37, 38] Psychotic like experiences at BL (14 years-old) were not assessed, and conclusions about causality cannot be clearly drawn. However, individuals who suffered psychotic diseases at BL through DSM-IV-RT diagnostic criteria were excluded. In addition,

this study only investigated healthy adolescents with PLEs, and only 15%-25% of normal subjects with self-reported PLEs subsequently develop psychotic disorders.[41] Further follow-up studies may establish if changes in uncus volume between 14 and 19 years old will subsequently be associated with onset of psychosis in the same cohort[17]. It should be noted however that individuals with genetic burden for schizophrenia might show higher cannabis consumption and that the causal relations between cannabis intake and onset of schizophrenia is, for a small number reversed.

Other packages are available to adequately segment hippocampus and parahippocampus with a longitudinal design[42] with recently improved hippocampus parcellation.[43] However, these non-voxel wise approaches are highly valuable when the datasets have more than 2 time points with unbalanced time interval between the scans.[42] We took advantage of the 2 time points but well-balanced design of IMAGEN (all scans acquired at 14 and 19 years-old), to increase resolution and to perform voxel-wise analysis. Although we did not have any a-priori hypothesis on the uncus, it is embedded in the parahippocampus gyrus[42] and using a raw parcellation might have increased type 2 errors by meaning the effect across the parahippocampus gyrus. Further studies might focus on the uncus and total hippocampal volume might be a covariate of interest, in addition to TIV, to test for its association with psychotic-like experiences above and beyond the other regions of the hippocampus. Fifth, the voxel-wise analyses did not model the hierarchical design of the IMAGEN project (i.e. multisite). However, the negative association between PLEs and the uncus development in the resulting cluster remained significant while accounting for

the one-level random intercept for "site" ($t_{(1,697)}$ = -3.2, p=0.002) and the two models (i.e. linear and hierarchical) were not statistically different (χ^2=6, p=0.2). Finally, we only controlled for alcohol and psycho-analeptic drug use in the present study and one cannot rule out that a combination of different drug use might affect the association between cannabis and psychotic-like experience.

Despite these limitations, our results show here that the uncus development is involved in the cerebral basis of PLEs and mediates a small, yet significant, proportion of the cannabis-associated PLEs in normal adolescents. When interpreted in line with the cerebral basis of schizophrenia and emerging psychosis, these results are additional arguments for the continuum hypothesis of psychosis from PLEs, to UHR, to first episode psychosis and finally to chronic psychotic disorders.

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TABLE 1. Demographic Characteristics of Subjects at Baseline (BL) and Follow-up (FU)

Characteristics	BL(n=706)		FU(n=706)	
	Mean	**SD**	**Mean**	**SD**
Age in days	5259.68	338.80	6895.50	241.42
CAPE Scores				
Total Score	-	-	4.602	0.890
Positive Symptom	-	-	1.296	0.209
Negative Symptom	-	-	1.595	0.394
Depressive Symptom	-	-	1.712	0.435
	n	**%**	**n**	**%**
Gender				
Male participants	312	44.20	312	44.20
Female participants	394	55.80	394	55.80
Site				
London	104	14.70	104	14.70
Nottingham	130	18.40	130	18.40
Dublin	47	6.70	47	6.70
Berlin	85	12.00	85	12.00
Hamburg	77	10.90	77	10.90
Mannheim	79	11.20	79	11.20
Paris	71	10.10	71	10.10
Dresden	113	16.00	113	16.00
Drugs Use				
Cannabis	34	5.00	328	46.50
Alcohol	548	77.60	691	97.90
Cigarette	202	28.60	474	67.10
Ketamine	0	0.00	22	3.10

LSD	1	0.10	16	2.30
Amphetamine	2	0.30	37	5.20

Note: CAPE=Community Assessment of Psychic Experiences; ESPAD=European School Survey Project on Alcohol and Other Drugs.

FIGURE 1. Reduced Expansion of Uncus Mediates the Association Between Cannabis Consumption and Psychotic-Like Experiences: A Mediation Model

Note: PLE = psychotic-like experiences.

FIGURE 2. Psychotic-Like Experiences are Associated With Reduced Expansion of Right Uncus Between 14 and 19 Years Old.

Note: The significant cluster is displayed on the study specific template using mricroGL®.

Cannabis-Associated Psychotic-Like Experiences Are Mediated by Developmental Changes in the Parahippocampal Gyrus

RH=Cannabis-Associated PLEs Mediated by Parahippocampus

Tao Yu, MD, PhD,[1,2,4,5#] Tianye Jia, PhD,[2, 6#] Liping Zhu, MD, PhD,[4] Sylvane Desrivières, PhD,[2] Christine Macare, PhD,[2] Yan Bi, PhD,[1] Arun L.W. Bokde, PhD,[7] Erin Burke Quinlan, PhD,[2] Andreas Heinz, MD, PhD,[13] Bernd Ittermann, PhD,[14] ChuanXin Liu, PhD,[5] Lei Ji, PhD,[1] Tobias Banaschewski, MD, PhD,[8] Decheng Ren, PhD,[1] Li Du, PhD,[4] Binyin Hou, PhD,[1] Herta Flor, PhD,[8,9] Vincent Frouin, PhD,[10] Hugh Garavan, PhD,[11] Penny Gowland, PhD,[12] Jean-Luc Martinot, MD, PhD,[15] Marie-Laure Paillère Martinot, MD, PhD,[15] Frauke Nees, PhD,[8] Dimitri Papadopoulos Orfanos, PhD,[10] Qiang Luo, PhD,[6] Congying Chu, PhD,[2] Tomas Paus, MD, PhD,[16] Luise Poustka, MD,[19] Sarah Hohmann, MD,[8] Sabina Millenet, MD,[8] Michael N. Smolka, MD,[17] Nora C. Vetter, PhD,[17] Eva Mennigen, MD,[17] Cai Lei, PhD,[1] Henrik Walter, MD, PhD,[13] Juliane H. Fröhner, MSc,[17] Robert Whelan, PhD,[18] Guang He, MD, PhD,[1] Lin He, PhD,[1,4,20] Gunter Schumann, MD, PhD,[2*] Gabriel Robert, MD, PhD,[2, 3*] and the IMAGEN Consortium[‡]

[1] Bio-X Institutes, Shanghai Jiao Tong University, Shanghai, China;
[2] Medical Research Council - Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom;
[3] Behavior and Basal Ganglia Unit, Medical University of Rennes, Rennes, France;
[4] Shanghai Center for Women and Children's Health, Shanghai, China;
[5] Jining Medical University, Shandong, China;
[6] Centre for Computational Systems Biology, Fudan University, Shanghai, China;
[7] Discipline of Psychiatry, School of Medicine and Trinity College Institute of Neuroscience, Trinity College Dublin, College Green, Dublin, Ireland;
[8] Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany;
[9]School of Social Sciences, University of Mannheim, Mannheim, Germany;
[10] NeuroSpin, Commissariat à l'Energie Atomique, CEA, Université Paris-Saclay, Gif-sur-Yvette, France;
[11] University of Vermont, Vermont, USA;
[12] Sir Peter Mansfield Imaging Centre School of Physics and Astronomy, University of Nottingham, University Park, Nottingham, United Kingdom;
[13] Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Campus Charité Mitte, Berlin, Germany;
[14] Physikalisch-Technische Bundesanstalt (PTB), Braunschweig and Berlin, Berlin, Germany;
[15] Institut National de la Santé et de la Recherche Médicale (INSERM), University Paris Sud, Orsay, France.

[16] Bloorview Research Institute, Holland Bloorview Kids Rehabilitation Hospital and University of Toronto, Toronto, Ontario, Canada;
[17] Technische Universität Dresden, Dresden, Germany;
[18] School of Psychology and Global Brain Health Institute, Trinity College Dublin, Ireland;
[19] University Medical Centre Göttingen, Göttingen, Germany;
[20] Baoan Maternal and Child Health Hospital, Jinan University, Shenzhen, China;

‡ IMAGEN consortium authors, affiliations and acknowledgement are listed in the supplementary materials.
# Both authors should be considered co-first authors
* Both authors should be considered co-corresponding authors
Correspondence should be addressed to Gabriel Robert (Email: gabriel.hadrien.robert@gmail.com) or Gunter Schumann (Email: gunter.schumann@kcl.ac.uk)

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The IMAGEN Consortium consists of: Dr. Michael Rapp, Charite; Dr. Eric Artiges, INSERM; Sophia Schneider, University of Hamburg; Christine Bach, Central Institute of Mental Health; Prof. Dr. Tomas Paus, University of Toronto; Alexis Barbot, Commissariat à l'Energie Atomique; Prof. Dr. Gareth Barker, King's College London; Dr. Arun Bokde, Trinity College Dublin; Dr. Nora Vetter, Technische Universität Dresden; Prof. Dr. Christian Büchel, University of Hamburg; Dr. Anna Cattrell, King's College London; Patrick Constant, PERTIMM; Prof. Penny Gowland, University of Nottingham; Dr. Hans Crombag, University of Sussex; Katharina Czech, Charite; Dr. Jeffrey Dalley, Cambridge University; Benjamin Decideur, Commissariat à l'Energie Atomique; Dr. Tade Spranger, Deutsches Referenzzentrum für Ethik / Institut of Science and Ethics; Dr. Tamzin Ripley, University of Sussex; Dr. Nadja Heym, University of Nottingham; Prof. Herta Flor, Central Institute of Mental Health; Dr. Wolfgang Sommer, Central Institute of Mental Health; Birgit Fuchs, GABO: milliarium mbH and Co. KG; Dr. Jürgen Gallinat, Charite; Dr. Hugh Garavan, Trinity College Dublin; Prof. Dr. Rainer Spanagel, Central Institute of Mental Health; Mehri Kaviani, University of Nottingham; Dr. Bert Heinrichs, Deutsches Referenzzentrum für Ethik; Prof. Dr. Andreas Heinz, Charite; Naresh Subramaniam, Cambridge University; Dr. Tianye Jia, King’s College London; Albrecht Ihlenfeld, PTB; James Ireland, Delosis; Dr. Bernd Ittermann, PTB; Dr. Patricia Conrod, King's College London; Prof. Dr. Tobias Banaschewski, Central Institute of Mental Health; Jennifer Jones, Trinity College Dublin; Dr. Arno Klaassen, Scito; Christophe Lalanne, Commissariat à l'Energie Atomique; Dr. Dirk Lanzerath, Deutsches Referenzzentrum für Ethik; Dr. Claire Lawrence, University of Nottingham; Dr. Hervé Lemaitre, INSERM; Dr. Sylvane Desrivieres, King's College London; Catherine Mallik, King's College London; Prof. Dr. Karl Mann, Central Institute of Mental Health; Dr. Adam Mar, Cambridge University; Lourdes Martinez-Medina, King's College London; Prof. Dr. Jean-Luc Martinot, INSERM; Eva Mennigen, Technische Universität Dresden; Dr. Fabiana Mesquita de Carvahlo, King's College London; Yannick Schwartz, Commissariat à l'Energie Atomique; Dr. Ruediger Bruehl, PTB; Kathrin Müller, Technische Universität Dresden; Frauke Nees, Central Institute of Mental Health; Charlotte Nymberg, King's College London; Dr. Mark Lathrop, CNG; Prof. Dr. Trevor Robbins, Cambridge University; Dr. Zdenka Pausova, University of Toronto; Dr. Jani Pentilla, INSERMIERTEE; Dr. Francesca Biondo, King's College London; Dr. Jean-Baptiste Poline, Commissariat à l'Energie Atomique; Dr. Sarah Hohmann, Central

Institute of Mental Health; Dr. Luise Poustka, University Medical Centre Göttingen/Medical University of Vienna; Sabina Millenet, Central Institute of Mental Health; Prof. Dr. Michael Smolka, Technische Universität Dresden; Juliane Fröhner, Technische Universität Dresden; Dr. Maren Struve, Central Institute of Mental Health; Prof. Dr. Steve Williams, King's College London; Dr. Thomas Hübner, Technische Universität Dresden; Uli Bromberg, University of Hamburg; Semiha Aydin, PTB; John Rogers, Delosis; Alexander Romanowski, Charite; Dr. Christine Schmäl, Central Institute of Mental Health; Dirk Schmidt, Technische Universität Dresden; Stephan Ripke, Technische Universität Dresden; Dr. Mercedes Arroyo, Cambridge University; Dr. Florian Schubert, PTB; Dr. Yolanda Pena-Oliver, University of Sussex; Mira Fauth-Bühler, FOM Hochschule für Oekonomie and Management/Central Institute of Mental Health; Xavier Mignon, PERTIMM; Dr. Robert Whelan, Trinity College Dublin; Dr. Claudia Speiser, GABO:milliarium mbH and Co. KG; Tahmine Fadai, University of Hamburg; Prof. Dr. Dai Stephens, University of Sussex; Dr. Andreas Ströhle, Charite; Dr. Marie-Laure Paillere, INSERM; Nicole Strache, Charite; David Theobald, Cambridge University; Sarah Jurk, Technische Universität Dresden; Dr. Helene Vulser, INSERM; Ruben Miranda, INSERM; Dr. Juliana Yacubilin, University of Hamburg; Vincent Frouin, Commissariat à l'Energie Atomique; Alexander Genauck, Charite; Caroline Parchetka, Charite; Isabel Gemmeke, Charite; Johann Kruschwitz, Charite; Katharina WeiB, Charite; Dr. Henrik Walter, Charité Universitätsmedizin Berlin; Jianfeng Feng, Fudan University/Warwick University; Dimitri Papadopoulos, INSERM; Irina Filippi, INSERM; Alex Ing, King's College London; Dr. Barbara Ruggeri, King's College London; Bing Xu, King's College London; Christine Macare, King's College London; Dr. Congying Chu, King’s College London; Eanna Hanratty, King's College London; Dr. Erin Burke Quinlan, King's College London; Dr. Gabriel Robert, King's College London; Prof. Dr. Gunter Schumann, King's College London; Dr. Tao Yu, King's College London; Veronika Ziesch, Technische Universität Dresden; Alicia Stedman, University of Nottingham.

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