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TITLE:
Brain Morphology of Cannabis Users with or without Psychosis: A Pilot MRI study

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KEYWORDS:
cannabis-induced psychosis, chronic substance use, magnetic resonance imaging, grey matter, cannabis.

SUMMARY:
This is a 3T magnetic resonance imaging study aiming to investigate grey matter volume differences between cannabis-induced psychosis patients and non-psychotic chronic cannabis users.

ABSTRACT:
Cannabis is the illicit drug most commonly used worldwide, and its consumption can both induce psychiatric symptoms in otherwise healthy subjects and unmask a florid psychotic picture in patients with a prior psychotic risk. Previous studies suggest that chronic and long- term cannabis exposure may exert significant negative effects in brain areas enriched with cannabinoid receptors. However, whether brain alterations determined by cannabis dependency will lead to a clinically significant phenotype or to a psychotic outbreak at some point of an abuser's life remain unclear. The aim of this study was to investigate

morphological brain differences between chronic cannabis users with cannabis-induced psychosis (CIP) and chronic cannabis users without any psychiatric conditions and correlate brain deficits with selective socio-demographic, clinical and psychosocial variables.

3T magnetic resonance imaging (MRI) scans of 10 CIP patients and 12 chronic cannabis users without psychosis were acquired. The type of drug, the frequency, and the duration, as well socio-demographic, clinical and psychosocial parameters of dependency were measured. CIP patients had extensive grey matter (GM) decreases in right superior frontal gyrus, right precentral, right superior temporal gyrus, insula bilaterally, right precuneus, right medial occipital gyrus, right fusiform gyrus, and left hippocampus in comparison to chronic cannabis users without psychosis. Finally, in CIP patients, the results showed a negative correlation between a domain of the Brief Psychiatric Rating Scale (BPRS), BPRS-Activity, and selective GM volumes. Overall, the results suggest that cannabis-induced psychosis is characterized by selective brain reductions that are not present in nonpsychotic cannabis users. Therefore, neuroimaging studies may provide a potential ground for identifying putative biomarkers associated with the risk of developing psychosis in cannabis users.

INTRODUCTION:

According to the European Monitoring Center for Drugs and Drug Addiction, around 96 million (or 29%) of adults (aged 15-64) in the European Union are estimated to have tried illicit drugs, especially cannabis, during their life. When considering the youngest and most vulnerable part of the general population, an estimated 16% of young adults (aged 15-34) used cannabis in the last year, with a male to female ratio of about 2:1[1]. Importantly, cannabis use seems to lead to the development of psychiatric symptoms in healthy subjects, such as mood alterations, increased anxiety, racing thoughts, distorted perceptions, difficulty in thinking and problem solving, ongoing problems with learning and memory, slow reaction time, and loss of control[2]. Such signs and symptoms, though, are normally transient and do not outline a psychiatric condition per se or the need of a treatment. However, cannabis, through its principal psychoactive constituent, named tetrahydrocannabinol (THC), can also induce positive psychotic symptoms including suspiciousness, paranoid delusions, disorders of thought processes, and perceptual alterations[3], as well as negative symptoms similar to those observed in schizophrenia, such as blunted affect, apathy, avolition, lack of spontaneity, lack of interest, passivity, and cognitive deficits (e.g., memory, executive function, abstract ability, decision making, and attention)[3]. Therefore, at the present time, there is evidence that cannabis consumption can both induce transient psychiatric symptoms in otherwise healthy subjects and unmask a florid psychotic picture in patients with a prior psychotic risk[3]. However, whether this relationship is causal, or purely correlational, is still controversial and debated[4]. Indeed, despite epidemiological studies suggesting a relationship between heavy cannabis consumption and risk of psychosis[5], the worldwide increased incidence of cannabis use is not accompanied by an augmented incidence of psychosis[4]. This paradox could be explained by the presence of specific confounding differences between cannabis abusers, with early onset of use, daily assumption of high-potency cannabis, and consumption of synthetic cannabinoids carrying the greatest psychotic risk[3]. Moreover, some genetic factors, such as the presence of specific catechol-O-methyltransferase (COMT) polymorphisms, may also confer an augmented vulnerability to develop psychotic symptoms after cannabis exposure in a small proportion of users[6].

In this regard, human neuroimaging studies attempted to investigate the potential neural mechanisms through which cannabis may lead to psychotic symptoms[7], since preclinical studies previously showed that THC is active within brain areas rich in cannabinoid type 1

receptors (CB1R), including hippocampus, amygdala, striatum, and prefrontal cortex (PFC)[8]. Indeed, experimental THC administration to healthy cannabis users have been shown to attenuate ventrostriatal activation during a learning task and concurrently induce psychotic symptoms[9] as well as altered prefrontal-striatal activation during attentional salience processing[10]. With regard to structural magnetic resonance imaging (MRI) studies, some authors detected significant grey matter (GM) volume reductions in the prefrontal cortex[11–13], the hippocampus[14,15], the amygdala[16] and the putamen[17] in regular cannabis users compared to nonusers while others did not report any significant brain differences between these two groups[18–21] or reported increased GM volumes within the medial temporal, the amygdala, the hippocampus, the posterior cingulate and the cerebellum among adolescents with low cannabis use[22].

Furthermore, few studies explored whether there are any specific brain differences between cannabis users with psychotic symptoms and cannabis users without any psychiatric conditions. One functional MRI study compared healthy subjects who did and did not experience psychotic symptoms after THC consumption and it reported increased activity during a go/no-go task in the right middle temporal gyrus and decreased activity in both parahippocampal and fusiform gyri, which was also associated with greater inhibition errors only in the psychotic group[23]. In contrast, Epstein and Kumra found that both psychotic and nonpsychotic adolescents with cannabis use disorder shared similar brain alterations; specifically, they detected attenuated cortical thinning in the left superior frontal gyrus, the right pars triangularis, the left pars opercularis, the left and right supramarginal gyri, the left and right inferior parietal cortices and the left superior temporal gyrus in both groups[24]. In a previous study, the same authors compared adolescents with early onset schizophrenia (EOS) with (EOS+) and without (EOS-) cannabis use disorder (CUD), adolescents with CUD only and healthy controls[25] . Interestingly, they detected smaller grey matter volumes in the left superior parietal region in both EOS- and CUD groups compared to healthy controls. However, they did not find additive volumetric alterations in adolescents with EOS+ compared to other groups. Finally, a more recent and larger study found a significant total effect from lifetime cannabis consumption to psychotic-life experiences in a sample of adolescents. Interestingly, the authors found an association between psychotic-life experiences and reduced expansion within the uncus of the right hippocampus/parahippocampus[26].

Therefore, these studies, although not all concordant, suggest that cannabis-induced psychosis may be characterized by neurobiological deficits, similar to those detected in pure psychotic disorders. However, whether brain alterations determined by cannabis dependency and highlighted by neuroimaging investigations will lead to a clinically significant phenotype or to a psychotic outbreak at some point of an abuser's life still remains unclear. In this regard, the investigation of brain morphology among psychotic cannabis users in comparison with cannabis users without any psychiatric symptoms could be of paramount importance in order to understand the neurobiological underpinnings of cannabis-induced psychosis. However, to the best of our knowledge, so far no studies have compared cannabis-induced psychotic subjects with healthy cannabis users in terms of brain structural morphology and clinical parameters, such as psychopathology, frequency and duration of dependency, quality of life, personality traits, childbirth complication and childhood abuse. In this context, the aim of this study is to investigate morphological brain differences between chronic cannabis users with substance-induced psychosis (CIP) and chronic cannabis users without any psychiatric conditions and to correlate brain deficits with selective socio-demographic, clinical and psychosocial variables. We hypothesized that CIP patients will show significant

reductions in GM volumes compared to healthy cannabis users as well as possible correlations between GM volumes and socio-demographic, clinical and psychosocial scales.

PROTOCOL:

10 CIP patients and 12 chronic users without psychosis were recruited for this study. All patients were recruited at the psychiatric inward of the University Policlinico Hospital of Milan, Italy, whereas the cannabis users were enrolled in the Milan catchment area. All patients were in stable pharmacological treatment. Either left- or right- handed participants were included. All participants had a habitual cannabis consumption and the type of drug, the frequency and the duration, as well socio-demographic, clinical and psychosocial parameters of dependency were measured. The study was approved by the local ethical committee.

1. Participants

1.1. Use the following inclusion criteria: For patients: age 18-45 years old, DSM-IV diagnosis of Cannabis-induced Psychotic Disorder, heavy cannabis consumption at the time of the study and in the previous 6 months. For healthy controls: age 18-45 years old, no DSM-IV diagnosis, heavy cannabis consumption at the time of the study and in the previous 6 months.

1.2. Use the following exclusion criteria: a diagnosis of mental retardation, any current major medical or neurological illness, a history of traumatic head injury with loss of consciousness, and any other Axis I, including alcohol abuse, or Axis II disorders and pregnancy. Verify that psychotic symptoms do not precede the onset of the cannabis use and do not persist for a substantial period of time after the cessation of acute withdrawal or severe intoxication. Verify that there is no history of recurrent nonsubstance-related episodes.

1.3. To obtain informed consent read the consent form to the participants. Have both the participant and the investigator sign the consent form in duplicate. Store the consent form for records.

1.4. To evaluate the diagnosis of CIP patients, use the Structured Clinical Interview for Diagnosis (SCID-I) of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, text revision (DSM-IV-TR)[27].

1.5. To establish the frequency and the duration of dependency, use the manual for the semistructured clinical interview for children and adolescents SCICA[28].

2. Clinical and psychosocial evaluation

NOTE: Several clinical and psychosocial scales were administered to all the participants.

2.1. To evaluate psychiatric symptoms, use the Brief Psychiatric Rating Scale (BPRS)[29], the Young Mania Rating Scale (YMRS)[30], the Montgomery-Åsberg Depression Rating Scale (MADRS)[31], the Hamilton Depression Rating Scale (HAM-D)[32] and the Hamilton Anxiety Rating Scale (HAM-A)[33].

2.2. To explore the presence of trauma or infection during or immediately after the partum, use the Murray-Lewis Obstetric Complications Scale (MLOCS)[34].

2.3. To assess experiences of neglect or abuse, use the Childhood Experience of Care and Abuse Questionnaire (CECA-Q)[35].

2.4. To estimate the Socio-economic status (SES), use the Socio Economic Status Scale of MacArthur[36].

2.5. Use the Neighbourhood Scale (NS)[37] to assess the specific characteristics of the neighbourhood, in terms of neighbourhood satisfaction (NS-A), sense of security (NS-B), level of degradation (NS-C), willingness on the part of fellow citizens to intervene in adverse situations (NS-D), and degree of acceptance of substances (NS-E).

2.6. Employ the Temperament and Character Inventory (TCI-125) for exploring personality traits[38,39].

2.7. To assess the quality of life and the global functioning use the Manchester Short Assessment of Quality of Life (MANSA)[40] and the Quality of Life Index (QL-index)[41] and the Global Assessment of Functioning (GAF)[27] scales, respectively.

NOTE: All socio-demographic and clinical data are summarized in **Table 1**.

3. Magnetic resonance imaging

3.1. Insert the participant in a supine position on the bed of the 3 Tesla MRI scanner.

3.2. Place a radio frequency (RF) coil over the participant's head.

3.3. Provide earplugs and headphones to block background noise.

3.4. Attach foam pads to immobilize the head.

3.5. Instruct the subject to remain still.

3.6. Run MRI session from the workstation in the control room.

3.6.1. Run a 3-plane gradient echo scan for alignment and localization and perform a shim procedure to generate a homogeneous, constant magnetic field.

3.6.2. Start an echo-planar-imaging protocol for MRI. The acquisition parameters for the acquisition of high-resolution T1-weighted three-dimensional brain scan are already set in the imaging program and should not be changed. The parameters are: repetition time [TR] = 9.8, echo time [TE] = 4.6 ms, in plane voxel size= 0.9375 × 0.9375, matrix= 256 × 256, flip angle = 8°.

3.7. Remove the participant from the MR scanner room. Transfer the MR data to disk and close the session.

NOTE: A total of 185 contiguous 1 mm sagittal slices extending superiorly from the inferior aspect of the cerebellum to encompass most of the brain were selected from a sagittal localizer scan.

4. Pre-processing steps

NOTE: A voxel-based morphometry analysis should be performed using Statistical Parametric Mapping (SPM12) implemented in MATLAB.

4.1. Perform the following pre-processing steps, shown in the **Script_pre-processing** script file, before carrying out group analyses.

4.1.1. Segmentation: Process the structural image to distinguish and separate the white matter tissues, the grey matter tissues and the cerebrospinal fluid into different images. This separation is obtained thanks to the combination of probability maps, elaborated from the general knowledge of tissue distribution combined with model cluster analyses that identifies voxel distributions of specific tissues in the original image. Run the **segment.mat** batch file.

4.1.2. DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra) tools: determine the nonlinear deformations for registering the GM and white matter images of all participants. Run the **create_template.mat** batch file.

4.1.3. Normalization: during the spatial normalization phase, adapt MRI images to an anatomical standard template. This is because every subject has little differences in the form and organization of the brain such as the size and morphologic differences in structures. Run the **normalize_to_MNI.mat** batch file.

4.1.4. Spatial Smoothing: after motion correction, perform an isotropic Gaussian kernel of 6 mm full width at half maximum Gaussian kernel to increase the signal-to-noise ratio and to account for subtle variations in anatomic structures. Run the **normalize_to_MNI.mat** batch file.

4.1.5. Extract the total intracranial volume (ICV) using SPM12: it can be obtained by adding up the density values in GM, white matter, and CSF class images and multiplying by the voxel volumes.

NOTE: Once the pre-processing is completed, it is possible to elaborate the data.

NOTE: Please refer to the SPM manual (https://www.fil.ion.ucl.ac.uk/spm/doc/spm12_manual.pdf) that provides a detailed description of the pre-processing steps employed in this study and the SPM commands to use. Please also refer to the script and Matlab batches included in the supplementary materials with the exact pre-processing steps used for this study.

5. Statistical analyses

5.1. Perform chi-square tests (categorical variables) and two sample t tests (quantitative variables) for exploring differences between the two groups on demographic, clinical and psychosocial scale.

5.2. Perform a one-way Analysis of Variance (ANOVA), in the context of a General Linear Model (GLM)design to compare GM volumes between CIP patients and non-

psychotic cannabis users. Gender and age were used as controlling variables in all the analyses. Run the **one-way ANOVA** batch file.

5.3. Carry out whole-brain regression analyses, only for the CIP group, to explore whether the scores in all the clinical and psychosocial scales employed in this study were significantly correlated with GM volumes changes. Do not use any brain mask but consider all voxels. Run the **Regression analysis** batch file with the clinical scale of interest.

5.4. Convert stereotactic coordinates of the peak maxima of the suprathreshold clusters from the MNI spatial array (www.mni.mcgill.ca) to that of Talairach and Tournoux [42].

NOTE: In all the neuroanatomical analyses, the volumetric differences among subjects were considered by proportional scaling for the total intracranial volume (ICV).

5.4.1. For the ANOVA, set the significance threshold to $p < 0.001$ uncorrected, with a minimum cluster size of k=30, whereas for the multiple regression analyses, a $p < 0.05$ peak Family-Wise Error (pFWE) corrected was considered significant and a minimum cluster size of k=10 was employed. The former threshold was considered due to the small sample size employed in this study and therefore the results emerged from this analysis must be considered as preliminary. The latter threshold is more stringent since the p-value is corrected for multiple comparisons.

NOTE: Please refer to the VBM8 Manual for more details about post-processing steps (http://dbm.neuro.uni-jena.de/vbm8/VBM8-Manual.pdf). Please also refer to the Matlab batches named "one-way ANOVA" and "Regression analysis" included in the supplementary materials with the exact model used for this study. Due to the exploratory nature of this study, a formal sample size calculation would have been of little value and therefore it was not performed.

REPRESENTATIVE RESULTS

Socio-demographic, clinical and psychosocial results

There were no differences in terms of gender (χ^2=0.6, p=0.4), age (t=-0.21; p=0.83), age of onset of dependency (t=-0.79; p=0.44) and educational level (t=1.21; p=0.24) between CIP patients and non-psychotic chronic users. However, some differences between the two groups were observed in one temperament dimension (Harm Avoidance, t=3.71; p=0.001) and one-character dimension (Self-Transcendence, t=2.94; p=0.008) of the TCI where CIP patients showed higher scores compared to chronic cannabis users without psychosis. Finally, non-psychotic cannabis users also showed higher scores compared to CIP patients in one sub-dimension of the Neighborhood Scale (NS-E) (t=-3.55; p=0.002), in the SES total (t=-2.13; p=0.046), in the Quality of Life-Index (t=-8.1; p=0.0001), in the GAF (t=-4.71; p=0.0001) and in one character dimension of the TCI (Self Directedness, t=-3.97; p=0.001)

Specifically, for CIP, the frequency of cannabis dependency was daily for 9 subjects (90%) and several times a week for 1 subject (10%). Instead, the frequency of cannabis dependency in the non-psychotic group was daily for 7 subjects (60%), several times a week for 4 subjects (30%), and multiple times a month for 1 subject (10%). The mean age of onset of dependency was at 18 years old for CIP patients and at 16 years old for the non-psychotic chronic cannabis user group. Although all participants were taking cannabis, some CIP patients (N=6) and non-psychotic chronic users (N=3) also reported previous use of other drugs, including cocaine, LSD and heroin/methadone, but with lower frequency than

cannabis. The frequency of cannabis use did not differ between the two groups (χ^2=1.69, p=0.42). Moreover, no statistical difference in type and frequency of cocaine, heroin/methadone and LSD use was observed between the two groups (cocaine: χ^2=0.06, p=0.79 and χ^2=4.1, p=0.39; heroin/methadone: $\chi^{2=}$1.2, p=0.26 and χ^2=1.2, p=0.26; LSD: χ^2=0.01, p=0.89 and χ^2=2.0, p=0.36). Although we are aware that the presence of poly-consumption in the sample might have negatively affected the generalizability of the findings, it is important to highlight that the use of other drugs was very limited compared to cannabis use. Indeed, in contrast to cannabis use, the consumption of other drugs was lifetime and not occurring during the time of the study. Nonetheless, our results should be taken cautiously and need to be replicated in a more homogeneous sample.

VBM results

VBM analysis showed that CIP patients had extensive GM decreases compared to non-psychotic chronic users in right superior frontal gyrus ((Brodmann area [BA] 10), right precentral (BA 4) , right superior temporal gyrus (BA 22), insula bilaterally (BA13), right precuneus (BA7), right medial occipital gyrus (BA 19), right fusiform gyrus (BA 37) and left hippocampus ($p < 0.001$ uncorrected; **Table 2** and **Figure 1**). No GM differences were observed in non-psychotic chronic users compared to CIP patients.

Correlations between GM regions and clinical scales

In CIP patients, the results showed a negative correlation between a domain of the BPRS, BPRS-Activity, and selective GM volumes within left superior temporal cortex (BA 38, x=-40 y=17 z=-35, z=5.9, cluster size=19) and left cerebellum (x=-12 y=-36 z=-20, z=6.1, cluster size=18). Moreover, the same scale was positively correlated with cuneus bilaterally (BA 18; left: x=-9 y=-90 z=9, z=7.0, cluster size=24; right: x=15 y=-85 z=24, z=7.3, cluster size=13), left inferior occipital gyrus (BA 17; x=-9 y=-88 z=-6, z=7.4, cluster size=34), right inferior parietal lobule (BA 40; x=58 y=-35 z=22, z=6.7, cluster size=33), right superior prefrontal cortex (BA 9; x=3 y=51 z=29, z=6.2, cluster size=23) (all $p<0.05$ pFWE corrected). No differences in any of the other clinical scales were observed between CIP patients and non-psychotic chronic users.

Figure 1. Regions with significant GM difference between Substance-induced psychosis (CIP) patients and non-psychotic chronic cannabis users (p<0.001, uncorrected, k=30).

Table 1. Socio-demographic, clinical and psychosocial variables of the whole sample.

BPRS (Brief Psychiatric Rating Scale); CECA-Q (Childhood Experience of Care and Abuse Questionnaire); CIP (Cannabis-Induced Psychosis); GAF (Global Assessment of Functioning); HAM-A (Hamilton Anxiety Rating Scale); MADRS (Montgomery-Asberg Depression Rating Scale); HAM-D (Hamilton Depression Rating Scale); MANSA (Manchester Short Assessment of Quality of Life); NS-A (Neighbourhood satisfaction); NS-B (Feelings of safety); NS-C (Neighbourhood incivilities); NS-D (Collective efficacy); NS-E (Cannabis acceptance); SD (Standard Deviation); SES (Socio Economic Status); QL-Index (Quality of Life-Index);); TCI (Temperament and Character Inventory); TCI Ns (Novelty Seeking); TCI Ha (Harm Avoidance); TCI Rd (Reward Dependence); TCI P (Persistence); TCI Sd (Self Directedness); TCI Co (Cooperativeness); TCI St (Self Transcendence); YMRS (Young Mania Rating Scale). * NS-A ranges from 0 to 16, where 16 represented extreme satisfaction with the area of residence; NS-B ranges from 0 to 8, where 8 represented a strong feeling of safety; NS-C ranges from 0 to 32, where 32 indicated a high level of incivilities; NS-D ranges from 0 to 12, where 8 represented a high level of collective efficacy amongst neighbours; NS-E ranges from ‘agree strongly’ (score of 4) to ‘disagree strongly’ (score of

0). ** Lower levels of schooling are associated to lower scores while higher levels of schooling are associated to higher scores (ie. Less than 7th grade = 3; Graduate degree= 21). Similarly, Occupations with lower cognitive engagement are associated to lower scores, while occupations requiring more cognitive resources are associated to higher scores (Farm worker= 5; Physician= 45).

Table 2. VBM results. Brain regions showing significant reduced grey matter volumes between the CIP patients and non-psychotic cannabis users (P< 0.001 uncorrected). BA (Brodmann area); CIP (Cannabis-Induced Psychosis); MNI (Montreal Neurological Institute)

DISCUSSION

In the present study, we observed that only the presence of psychotic symptoms discriminated the detection of brain morphological alterations. Indeed, chronic cannabis users with CIP showed decreased GM volumes mainly in the prefronto-temporo-limbic network compared to non-psychotic chronic cannabis users. Moreover, regarding the psychometric questionnaires, correlations between the domain BPRS-Activity and selective GM volumes have been highlighted. Specifically, we observed a negative correlation between this BPRS scale and left superior temporal cortex and left cerebellum together with a positive correlation with the cuneus bilaterally, the left inferior occipital gyrus, the right inferior parietal lobule and the right superior prefrontal cortex. However, we should mention that the lack of a control group of healthy subjects with no cannabis dependency prevented us from exploring if cannabis use caused brain alterations or not.

In general, the results are not surprising since previous MRI studies showed that psychotic disorders, such as schizophrenia, shared similar GM abnormalities, especially in frontal and temporo-limbic regions[29,30]. However, it is still unclear why some chronic cannabis users developed psychotic symptoms while others remained healthy. Indeed, in the sample, we only detected small clinical differences between the two groups and therefore the extensive GM abnormalities observed in the CIP group may not be associated with their specific clinical profile. Specifically, 9 of 10 CIP patients reported a daily cannabis use compared to 7 of 12 in the non-psychotic cannabis users. Moreover, no differences in terms of age, gender, age of onset of cannabis use and educational level were found between the two groups. However, we should consider that this lack of differences could be due to the small sample size that also limited the possibility to statistically analyse and interpret these factors. One hypothesis is that the psychotic process itself is responsible for the decrease in brain volume, regardless cannabis use. Indeed, previous studies showed no GM differences between psychotic patients with and without cannabis consumption, thus finding no clear evidence for cannabis use to be related to GM alterations in first episode psychotic patients[45]. However, cannabis use may have contributed to brain alterations and subsequently induced psychosis only in a subgroup of susceptible cannabis users.

The first hypothesis is in line with those studies showing brain abnormalities in psychotic disorders. Specifically, the results showed that CIP patients compared to nonpsychotic chronic users had extensive GM volume decreases in some brain areas known to be involved in emotional regulation, such as frontotemporal cortices, insula, hippocampus, and fusiform gyrus[46]. Interestingly, disruptions in these structures, especially in prefrontal regions, might explain the mood instability and greater emotional reactivity in adolescents and young adults, as well as impulsive behaviors and substance-seeking[33,34]. Indeed, it has been consistently reported that emotion regulation/processing are associated with recruitment of a set of prefrontal brain regions involved in cognitive control over emotional limbic structures. For

example, greater difficulties in emotional regulation among tobacco smokers have been associated to a weaker connectivity between inferior frontal gyrus and amygdala compared to non-smokers[49]. Therefore, it might be plausible that among CIP patients the development of psychotic symptoms was associated with interfered balance between these structures.

Additionally, we observed that the group of CIP patients showed disruptions in the dorsolateral prefrontal cortex (DLPFC), a key region involved in major cognitive functions, including working memory, executive functions[50] and emotional regulation[51]. Therefore, this finding is not surprising since the DLPFC works together with risk-monitoring regions, such as the insula (a key structure of the salience network recently found to be involved in addiction[52)], which has been also found altered in the group of CIP patients, and anterior cingulate cortex, ultimately suggesting that impairments in distinguishing risky from safe choices may result from a disruption between DLPFC and such risk-monitoring regions[53].

Moreover, CIP patients showed a GM volume decrease in the superior temporal cortex. Interestingly, this result is in line with the evidence reported by a previous multimodal neuroimaging study[54], which employed a larger sample of CIP patients (N=16), the majority of whom overlap with the sample employed in this study that found extensive GM alteration in temporal cortices in CIP patients. Overall such evidence further confirms the key role of the superior temporal cortex in psychosis, since the involvement of this structure has been consistently reported in abilities often found disrupted in psychotic patients, including language processing and theory of mind abilities[39,40]. Also, the results aligned with previous evidence reporting the association between volume reductions of this region and auditory hallucination or thought disorders[41,42] as well as with a previous MRI study suggesting the disruption of this area in substance dependent individuals compared to healthy controls[59].

Finally, from the results emerged a significant GM volume decrease in hippocampus in CIP patients, which is in line with previous evidence showing structural and functional changes in this structure in early psychoses and in at-risk mental state/first-episode psychosis, in comparison to healthy controls[60–63]. Normal hippocampal function is required for a number of mental functions including memory and emotional behaviour[48,49] and it has been proposed that reduced volume in this structure may represent a marker of a negative clinical outcome in patients with a first-episode psychosis[66]. However, in contrast to the results, hippocampal deficits have been also reported in young and adult cannabis users, who have been found to have thinner cortices and reduced volumes in this region[67–70]. Therefore, a clear picture on the role of the hippocampus in substance abuse has still not be attained. Nonetheless, the results point towards the hypothesis that the cortico-limbic system is compromised in the group of CIP patients, as also suggested by a previous MRI study[54] and might explain the emotional elaboration deficits, which has been proposed to be a critical precursor of future psychotic development[55,56], often observed in these patients.

Therefore, it seems reasonable to hypothesize that cannabis-induced psychosis is associated with brain alterations in regions within the prefronto-temporo-limbic network, which may therefore represent a common neurodevelopmental substrate of multiple forms of psychosis. Interestingly, longitudinal studies proposed that some brain disruptions, including smaller orbitofrontal cortex volumes[73], increased fronto-parietal and decreased visual associations regions activation, as well as cognitive deficits, such as poorer executive functions[74], may be present even before the initiation of cannabis dependency. Therefore, it might be that these individuals with underlying brain alterations are more likely to develop psychotic symptoms after cannabis use initiation. In addition, there is evidence reporting that possessing risk

alleles in *AKT1* and *DRD2* genes, which are involved in dopamine signalling, is associated with increased risk of developing psychosis after cannabis use[3]. Therefore, the detection of morphological decreases in CIP patients could reflect an augmented genetic susceptibility to the neurotoxic effect of chronic cannabis use in this group of subjects.

Finally, in the CIP group, the results also showed a negative correlation between a sub-domain of the BPRS, the BPRS-Activity, and selective GM volumes within left superior temporal cortex and left cerebellum. Also, this subscale was positively correlated with cuneus bilaterally, left inferior occipital gyrus, right inferior parietal lobule, and right superior prefrontal cortex. In general, although correlations between clinical symptomatology and GM structures have been extensively reported, especially in schizophrenia[75], the results are still heterogeneous, with a mixed picture of inverse[76], positive[77] or no[78] correlations between selective GM volumes and clinical scales. Notably, the negative correlation observed between BPRS-Activity and superior temporal cortex seems to be in line with previous MRI evidence showing inverse correlations between this structure and positive symptom severity[76], ultimately further suggesting the key role of this structure in the production of psychotic symptoms. Similarly, the positive correlation found between BPRS-Activity and superior prefrontal cortex seems to agree with other MRI studies reporting a similar correlation between negative symptoms and GM volumes with the prefrontal cortex[79].

Therefore, the results overall provide preliminary evidence on the presence of significant association between brain alteration and severity of psychopathology.

The current study suffers from some limitations. First, all psychotic patients were taking pharmacological treatments that could have influenced the results. Second, the lack of a control group formed by healthy subjects not exposed to cannabis does not allow a further comparison with the two groups of cannabis users (psychotic and not). In addition, although the two groups were very similar in terms of number of subjects (10 CIP patients vs 12 chronic cannabis users without psychosis), the small sample size limits the significance of the results achieved and therefore must be considered as preliminary. Further limitations are strictly connected to the nature of the population investigated. Indeed, some patients with CIP (6/10) and a rather small proportion of cannabis users without psychosis (3/12) had a lifetime history of other substance consumption (i.e., cocaine, LSD and heroin/methadone). Moreover, we did not examine the genetic alleles linked to addiction, which could have helped to discriminate the two groups. Nonetheless, the cannabis consumption, although assessed in terms of frequency and volume and duration with a specific tool[20], was not uniform across the two groups. Finally, in this study we did not explore brain activation and we did not assess the neurocognitive state of the sample. Therefore, the lack of these information could have affected the results since previous studies demonstrated the presence of selective brain dysfunctions in patients with schizophrenia with substance abuse in the medial prefrontal cortex, the orbitofrontal cortex and the amygdala as well as a better premorbid neurocognitive profile with a greater long-term decline compared to the same patients without substance abuse[80]. Therefore, further functional MRI studies exploring brain activity coupled with neuropsychological assessments on larger samples and with homogenous consumption habits are needed to confirm our results.

From the results emerged that cannabis-induced psychosis is characterized by GM volume decreases in selective brain structures. Therefore, in view of the crucial and comprehensive role of the endocannabinoid system in the brain, the increasing prevalence of cannabis use, its chronic use during neurodevelopment, as well as the progressively higher THC concentration

in the current market, it seems mandatory to clarify which aspects of cannabis exposure (e.g., age at initiation, quantity, frequency, and duration) determine the greatest risk for the progression towards psychotic-related disorders. However, whether reductions in prefronto-temporo-limbic regions represent a substrate of the psychotic process itself or a direct consequence of cannabis exposure among susceptible subjects remains a complex issue. In this context, the methods employed in the study could be useful to better characterize the neurobiological and clinical features of cannabis-induced psychosis. Finally, longitudinal neuroimaging studies taking into account also potential confounding factors, such as cannabis dose, potency, THC/Cannabidiol ratio, frequency of use, age of onset, familiar history of psychosis, and genetic polymorphisms may provide a potential ground for identifying putative biomarkers which may ultimately help clinicians to detect those cannabis users that are more likely to develop psychosis.

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DISCLOSURES

None.

REFERENCES

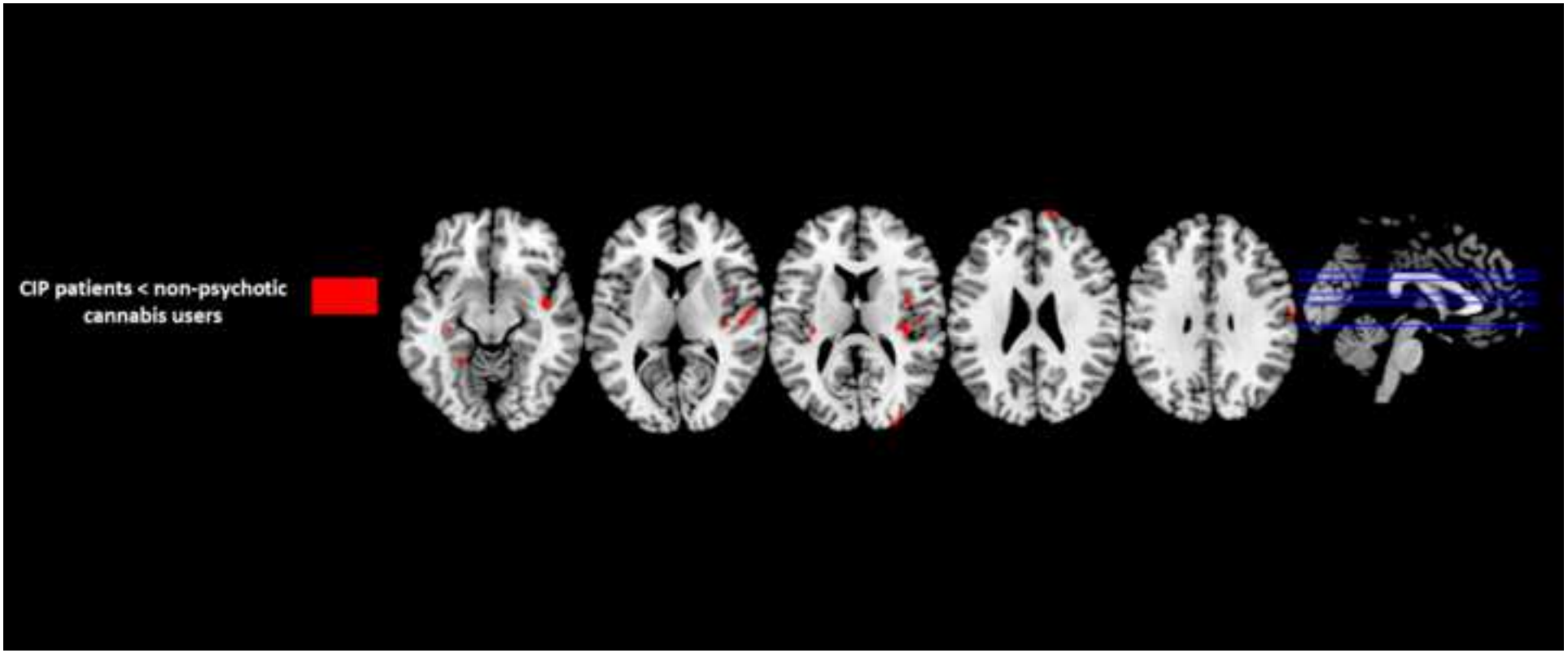
1. European Monitoring Centre for Drugs and Drug Addiction European Drug Report 2019. *European Drug Report 2019: Trends and Developments*. Publications Office of the European Union, Luxembourg. (2019).
2. De Aquino, J.P. et al. The Psychiatric Consequences of Cannabinoids. *Clinical Therapeutics*. **40** (9), 1448-1456 (2018).
3. Murray, R.M. et al. Cannabis-associated psychosis: Neural substrate and clinical impact. *Neuropharmacology*. **124**, 89–104 (2017).
4. Gage, S.H. Cannabis and psychosis: triangulating the evidence. *The Lancet Psychiatry*. **6** (5), 364-365 (2019).
5. Gage, S.H., Hickman, M., Zammit, S. Association between cannabis and psychosis: Epidemiologic evidence. *Biological Psychiatry*. **79** (7), 549-556 (2016).
6. Burns, J.K. Pathways from Cannabis to Psychosis: A Review of the Evidence. *Frontiers in Psychiatry*. **4**, 128 (2013).
7. Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J.A., McGuire, P.K. Neural mechanisms for the cannabinoid modulation of cognition and affect in man: a critical review of neuroimaging studies. *Current Pharmaceutical Design*. **18** (32), 5045–54 (2012).
8. Atkinson, D.L., Abbott, J.K. Cannabinoids and the Brain: The Effects of Endogenous and Exogenous Cannabinoids on Brain Systems and Function. *The Complex Connection Between Cannabis and Schizophrenia*. Academic Press. (2018).
9. Bhattacharyya, S. et al. Modulation of Mediotemporal and Ventrostriatal Function in Humans by Δ9-Tetrahydrocannabinol. *Archives of General Psychiatry*. **66** (4), 442-451 (2009).
10. Bhattacharyya, S. et al. Induction of psychosis by Δ9-tetrahydrocannabinol reflects modulation of prefrontal and striatal function during attentional salience processing. *Archives of General Psychiatry*. **69** (1), 27-36 (2012).
11. Battistella, G. et al. Long-term effects of cannabis on brain structure. *Neuropsychopharmacology*. **39** (9), 2041-2048 (2014).
12. Price, J.S., McQueeny, T., Shollenbarger, S., Browning, E.L., Wieser, J., Lisdahl, K.M. Effects of marijuana use on prefrontal and parietal volumes and cognition in emerging adults. *Psychopharmacology*. **232** (16), 2939-2950 (2015).

13. Filbey, F.M. et al. Long-term effects of marijuana use on the brain. *Proceedings of the National Academy of Sciences of the United States of America*. **111** (47), 16913-16918 (2014).
14. Yücel, M. et al. Regional brain abnormalities associated with long-term heavy cannabis use. *Archives of General Psychiatry*. **65** (6), 694-701 (2008).
15. Lorenzetti, V. et al. Gross morphological brain changes with chronic, heavy cannabis use. *British Journal of Psychiatry*. **206** (1), 77-78 (2015).
16. Schacht, J.P., Hutchison, K.E., Filbey, F.M. Associations between cannabinoid receptor-1 (CNR1) variation and hippocampus and amygdala volumes in heavy cannabis users. *Neuropsychopharmacology*. **37** (11), 2368-2376 (2012).
17. Yip, S.W. et al. Pretreatment measures of brain structure and reward-processing brain function in cannabis dependence: An exploratory study of relationships with abstinence during behavioral treatment. *Drug and Alcohol Dependence*. **140**, 33-41 (2014).
18. Medina, K.L. et al. Prefrontal cortex morphometry in abstinent adolescent marijuana users: Subtle gender effects. *Addiction Biology*. **14** (4), 457-468 (2009).
19. DeLisi, L.E. et al. A preliminary DTI study showing no brain structural change associated with adolescent cannabis use. *Harm Reduction Journal*. **3** (1), 17 (2006).
20. Jager, G. et al. Effects of frequent cannabis use on hippocampal activity during an associative memory task. *European Neuropsychopharmacology*. **17** (4), 289-297 (2007).
21. Tzilos, G.K. et al. Lack of hippocampal volume change in long-term heavy cannabis users. *American Journal on Addictions*. **14** (1), 64-72 (2005).
22. Orr, C. et al. Grey matter volume differences associated with extremely low levels of cannabis use in adolescence. *Journal of Neuroscience*. **39** (10), 1817-1827 (2019).
23. Atakan, Z. et al. Cannabis affects people differently: Inter-subject variation in the psychotogenic effects of Δ9-tetrahydrocannabinol: A functional magnetic resonance imaging study with healthy volunteers. *Psychological Medicine*. **43** (6), 1255-1267 (2013).
24. Epstein, K.A., Kumra, S. Altered cortical maturation in adolescent cannabis users with and without schizophrenia. *Schizophrenia Research*. **162** (1-3), 143-152 (2015).
25. Kumra, S. et al. Parietal lobe volume deficits in adolescents with schizophrenia and adolescents with cannabis use disorders. *Journal of the American Academy of Child and Adolescent Psychiatry*. **51** (2), 171-180 (2012).
26. Yu, T. et al. Cannabis-Associated Psychotic-like Experiences Are Mediated by Developmental Changes in the Parahippocampal Gyrus. *Journal of the American Academy of Child and Adolescent Psychiatry*. **59** (5), 642-649 (2019).
27. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders (4th ed., text rev.)*. Washington. (2000).
28. McConaughy, S.H., Achenbach, T.M. *Manual for the semistructured clinical interview for children and adolescents*. ASEBA (2001).
29. Overall, J.E., Gorham, D.R. The Brief Psychiatric Rating Scale. *Psychological Reports*. **10** (3), 799-812 (1962).
30. Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A. A rating scale for mania: Reliability, validity and sensitivity. *British Journal of Psychiatry*. **133** (5), 429-435 (1978).
31. Montgomery, S.A., Asberg, M. A new depression scale designed to be sensitive to change. *British Journal of Psychiatry*. **134** (4), 382-389 (1979).
32. Hamilton, M. A rating scale for depression. *Journal of Neurology, Neurosurgery, and Psychiatry*. **23** (1), 56 (1960).
33. Hamilton, M. Hamilton Anxiety Rating Scale (HAM-A). *Journal of Medicine*. **61** (4), 81-82 (1959).
34. Lewis, S.W., Murray, R.M. Obstetric complications, neurodevelopmental deviance, and risk of schizophrenia. *Journal of Psychiatric Research*. **21** (4), 413-421 (1987).

35. Bifulco, A., Bernazzani, O., Moran, P.M., Jacobs, C. The childhood experience of care and abuse questionnaire (CECA.Q): Validation in a community series. *British Journal of Clinical Psychology*. **44** (4), 563-581 (2005).
36. Adler, N.E., Stewart, J. The MacArthur Scale of Subjective Social Status. *MacArthur Research Network on SES and Health.* (2007).
37. Hur, M., Nasar, J.L., Chun, B. Neighborhood satisfaction, physical and perceived naturalness and openness. *Journal of Environmental Psychology*. **30** (1), 52-59 (2010).
38. Cloninger, C.R., Svrakic, D.M., Przybeck, T.R. A Psychobiological Model of Temperament and Character. *Archives of General Psychiatry*. **50** (12), 975-990 (1993).
39. Delvecchio, G. et al. Normative data and effects of age and gender on temperament and character dimensions across the lifespan in an Italian population: A cross-sectional validation study. *Journal of Affective Disorders*. **204**, 83-91 (2016).
40. Priebe, S., Huxley, P., Knight, S., Evans, S. Application and results of the Manchester Short Assessment of Quality of Life (MANSA). *International Journal of Social Psychiatry*. **45** (1), 7-12 (1999).
41. Spitzer, W.O. et al. Measuring the quality of life of cancer patients. A concise QL-Index for use by physicians. *Journal of Chronic Diseases*. **34** (12), 585-597 (1981).
42. Talairach, J. Tournoux, P. *Co-planar stereotaxic atlas of the human brain: 3-dimensional proportional system : an approach to cerebral imaging*. Thieme, NY. (1988).
43. Ellison-Wright, I., Glahn, D.C., Laird, A.R., Thelen, S.M., Bullmore, E. The anatomy of first-episode and chronic schizophrenia: An anatomical likelihood estimation meta-analysis. *American Journal of Psychiatry*. **165** (8), 1015-1023 (2008).
44. Fornito, A., Yücel, M., Patti, J., Wood, S.J., Pantelis, C. Mapping grey matter reductions in schizophrenia: An anatomical likelihood estimation analysis of voxel-based morphometry studies. *Schizophrenia Research*. **108** (1-3), 104-113 (2009).
45. Haller, S. et al. Combined grey matter VBV and white matter TBSS analysis in young first episode psychosispatients with and without cannabis consumption. *Brain Topography*. **26** (4), 641-647 (2013).
46. Hou, J. et al. Review on neural correlates of emotion regulation and music: Implications for emotion dysregulation. *Frontiers in Psychology*. **8**, 501 (2017).
47. Martin, R.E., Ochsner, K.N. The Neuroscience of Emotion Regulation Development: Implications for Education. *Current Opinion in Behavioral Sciences*. **10**, 142–148 (2016).
48. Pfeifer, J.H., Allen, N.B. Arrested development? Reconsidering dual-systems models of brain function in adolescence and disorders. *Trends in Cognitive Sciences*. **16** (6), 322–9 (2012).
49. Faulkner, P., Dean, A.C., Ghahremani, D.G., London, E.D. Neural Basis of Smoking-Related Difficulties in Emotion Regulation. *International Journal of Neuropsychopharmacology*. (2020).
50. Dedoncker, J., Brunoni, A.R., Baeken, C., Vanderhasselt, M.-A. A Systematic Review and Meta-Analysis of the Effects of Transcranial Direct Current Stimulation (tDCS) Over the Dorsolateral Prefrontal Cortex in Healthy and Neuropsychiatric Samples: Influence of Stimulation Parameters. *Brain Stimulation*. **9** (4), 501–517 (2016).
51. Sturm, V.E., Haase, C.M., Levenson, R.W. Emotional Dysfunction in Psychopathology and Neuropathology: Neural and Genetic Pathways. *Genomics, Circuits, and Pathways in Clinical Neuropsychiatry*. 345-364 (2016).
52. Koob, G.F., Volkow, N.D. Neurobiology of addiction: a neurocircuitry analysis. *The Lancet Psychiatry*. **3** (8),760-773 (2016).
53. Kohno, M., Morales, A.M., Guttman, Z., London, E.D. A neural network that links brain function, white-matter structure and risky behavior. *NeuroImage*. **149**, 15-22 (2017).
54. Altamura, A.C. et al. Structural and metabolic differentiation between bipolar disorder

with psychosis and substance-induced psychosis: An integrated MRI/PET study. *European Psychiatry*. **41**, 85–94 (2017).
55. Anderson, J.E. et al. An MRI study of temporal lobe abnormalities and negative symptoms in chronic schizophrenia. *Schizophrenia Research*. **58** (2–3), 123–34 (2002).
56. Brüne, M., Brüne-Cohrs, U. Theory of mind--evolution, ontogeny, brain mechanisms and psychopathology. *Neuroscience and Biobehavioral Reviews*. **30** (4), 437–55 (2006).
57. Takahashi, T. et al. Morphologic alterations of the parcellated superior temporal gyrus in schizophrenia spectrum. *Schizophrenia Research*. **83** (2–3), 131–43 (2006).
58. Holinger, D.P. et al. Superior temporal gyrus volume abnormalities and thought disorder in left-handed schizophrenic men. *The American Journal of Psychiatry*. **156** (11), 1730–5 (1999).
59. Ersche, K.D., Jones, P.S., Williams, G.B., Turton, A.J., Robbins, T.W., Bullmore, E.T. Abnormal brain structure implicated in stimulant drug addiction. *Science.* **335** (6068), 601–4 (2012).
60. Baglivo, V. et al. Hippocampal Subfield Volumes in Patients With First-Episode Psychosis. *Schizophrenia Bulletin*. **44** (3), 552–559 (2018).
61. Pruessner, M. et al. Reduced hippocampal volume and hypothalamus–pituitary–adrenal axis function in first episode psychosis: Evidence for sex differences. *NeuroImage: Clinical*. **7**, 195–202 (2015).
62. Walter, A. et al. Hippocampal volume in subjects at high risk of psychosis: A longitudinal MRI study. *Schizophrenia Research*. **142** (1–3), 217–222 (2012).
63. Verma, S. et al. Hippocampal Volumes in First-Episode Psychosis. *The Journal of Neuropsychiatry and Clinical Neurosciences*. **21** (1), 24–29 (2009).
64. Anand, K.S., Dhikav, V. Hippocampus in health and disease: An overview. *Annals of Indian Academy of Neurology*. **15** (4), 239 (2012).
65. Wible, C.G. Hippocampal physiology, structure and function and the neuroscience of schizophrenia: a unified account of declarative memory deficits, working memory deficits and schizophrenic symptoms. *Behavioral Sciences.* **3** (2), 298–315 (2013).
66. Sauras, R. et al. Volumetric and morphological characteristics of the hippocampus are associated with progression to schizophrenia in patients with first-episode psychosis. *European Psychiatry*. **45**, 1–5 (2017).
67. Chye, Y. et al. Alteration to hippocampal volume and shape confined to cannabis dependence: a multi- site study. *Addiction Biology*. **24** (4), 822–834 (2019).
68. Chye, Y. et al. Cannabis-related hippocampal volumetric abnormalities specific to subregions in dependent users. *Psychopharmacology*. **234** (14), 2149–2157 (2017).
69. Burggren, A.C. et al. Subregional Hippocampal Thickness Abnormalities in Older Adults with a History of Heavy Cannabis Use. *Cannabis and Cannabinoid Research*. **3** (1), 242–251 (2018).
70. Schacht, J.P., Hutchison, K.E., Filbey, F.M. Associations between cannabinoid receptor-1 (CNR1) variation and hippocampus and amygdala volumes in heavy cannabis users. *Neuropsychopharmacology*. **37** (11), 2368–76 (2012).
71. Grace, A.A. Gating of information flow within the limbic system and the pathophysiology of schizophrenia. *Brain Research. Brain Research Reviews*. **31** (2–3), 330–41 (2000).
72. Aleman, A., Kahn, R.S. Strange feelings: Do amygdala abnormalities dysregulate the emotional brain in schizophrenia? *Progress in Neurobiology*. **77** (5), 283–298 (2005).
73. Cheetham, A. et al. Orbitofrontal volumes in early adolescence predict initiation of cannabis use: A 4-year longitudinal and prospective study. *Biological Psychiatry*. **71** (8), 684-692 (2012).
74. Tervo-Clemmens, B. et al. Early Cannabis Use and Neurocognitive Risk: A

Prospective Functional Neuroimaging Study. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. **3** (8), 713-725 (2018).
75. Nestor, P.G. et al. Dissociable contributions of MRI volume reductions of superior temporal and fusiform gyri to symptoms and neuropsychology in schizophrenia. *Schizophrenia Research*. **91** (1–3), 103–6 (2007).
76. Nesvåg, R., Saetre, P., Lawyer, G., Jönsson, E.G., Agartz, I. The relationship between symptom severity and regional cortical and grey matter volumes in schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. **33** (3), 482–490 (2009).
77. Lacerda, A.L.T. et al. Morphology of the orbitofrontal cortex in first-episode schizophrenia: relationship with negative symptomatology. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*. **31** (2), 510–6 (2007).
78. Volkow, N.D. et al. Effects of Cannabis Use on Human Behavior, Including Cognition, Motivation, and Psychosis: A Review. *JAMA Psychiatry*. **73** (3), 292–7 (2016).
79. Walton, E. et al. Prefrontal cortical thinning links to negative symptoms in schizophrenia via the ENIGMA consortium. *Psychological Medicine*. **48** (1), 82-94 (2018).
80. Adan, A. et al. Neurobiological underpinnings and modulating factors in schizophrenia spectrum disorders with a comorbid substance use disorder: A systematic review. *Neuroscience and Biobehavioral Reviews*. **75**, 361–377 (2017).



Livello incollato

	CIP patients n=10	Non-psychotic cannabis users n=12	Statistics	p-value
Age, mean (SD)	27 (9.21)	26 (0.89)	t= -0.213	p=0.833
Sex, male/female	2-Aug	1-Nov	χ^2=0.630	p=0.427
Age of onset of cannabis use, mean (SD)	18 (9.69)	16 (1.83)	t= -0.786	p=0.441
Type (N); frequency of other drug use	Cannabis (N=10); Daily (N=9), multiple times a week (N=1).	Cannabis (N=12); Daily (N=7), multiple times a week (N=4), multiple times a month (N=1).	Frequency: χ^2=1.69,	p=0.42
	Cocaine (N=4); multiple times a week (N=2), multiple times a month (N=2).	Cocaine (N=3); multiple times a week (N=1), multiple times a month (N=1), less than one a month (N=1).	Type: χ^2=0.06 Frequency: χ^2=4.1	Type: p=0.79 Frequency: p=0.39
	Heroin/Methadone (N=1); multiple times a week.	No Heroin/Methadone users.	Type: $\chi^{2=}$1.2 Frequency: $\chi^{2=}$1.2	Type: p=0.26 Frequency: p=0.26
	LSD (N=1); less than one a month.	LSD (N=1); multiple times a month.	Type: χ^2=0.01 Frequency: χ^2=2.0	Type: p=0.89 Frequency: p=0.36
Age of onset, mean (SD)	25 (8.46)	-	-	-
BPRS TOT, mean (SD)	43 (9)	20 (3)	t=8.860	p=0.0001
Anxiety-Depression	10 (5)	6 (2)	t=2.629	p=0.016
Anergia	8 (3)	4 (1)	t=3.284	, p=0.004
Thought Disorders	12 (3)	4 (0)	t=9.754	p=0.0001
Activity	6 (2)	3 (0)	t=4.557	p=0.0001
Hostility- Suspiciousness	8 (4)	3 (0)	t=4.053	p=0.001
HAM-D, mean (SD)	11 (6.42)	4 (4.96)	t=3.258	p=0.004
HAM-A, mean (SD)	11 (6.62)	3 (3.93)	t=3.487	p=0.002
MADRS, mean (SD)	14 (7.76)	6 (6.35)	t=2.635	p=0.016

YMRS, mean (SD)	13 (7.92)	0 (1.44)	t=5.378	p=0.0001
CECA-Q, mean (SD)				
CECA-QMA	13 (5.20)	13 (3.89)	t=-0.069	p=0.946
CECA-QMN	19 (5.83)	19 (4.64)	t=-0.284	p=0.779
CECA-QPA	14 (6.44)	14 (5.56)	t=-0.130	p=0.990
CECA-QPN	24 (11.69)	24 (7.12)	t=0.070	p=0.945
Neighbourhood scale*, mean (SD)				
NS- A	9 (1.78)	8 (2.23)	t=0.782	p=0.443
NS- B	6 (2.50)	7 (1.56)	t=-1.070	p=0.298
NS- C	9 (5.87)	10 (7.66)	t=-0.265	p=0.794
NS-D	6 (2.31)	5 (1.53)	t=1.378	p=0.183
NS-E	3 (1.35)	4 (0.29)	t=-3.546	p=0.002
SES** total, mean (SD)	33.6 (12.60)	45.3 (13.05)	t=-2.132	p=0.046
Study	11.3 (4.22)	15.3 (5.93)	t=-1.800	p=0.087
Occupation	22.3 (10.39)	30.0 (8.79)	t=-1.885	p=0.074
QL - Index, mean (SD)	6 (1.65)	10 (0.62)	t=-8.098	p=0.0001
GAF, mean (SD)	58 (15.21)	83 (9.68)	t=-4.715	p=0.0001
MANSA, mean (SD)	54 (14.16)	61 (6.01)	t=-1.250	p=0.226
TCI, mean (SD)				
TCI Ns	59.92 (10.75)	55.95 (12.86)	t=0.173	p=0.864
TCI Ha	55.67 (7.71)	45.61 (5.68)	t=3.708	p=0.001
TCI Rd	48.67 (10.41)	50.49 (9.02)	t=-0.668	p=0.512
TCI P	49.82 (11.49)	39.32 (8.83)	t=2.033	p=0.056
TCI Sd	28.64 (11.85)	49.89 (7.42)	t=-3.969	p=0.001
TCI Co	42.15 (12.21)	49.07 (5.60)	t=-1.430	p=0.168
TCI St	65.56 (12.34)	50.82 (8.16)	t=2.940	p=0.008

Table 2

Gyrus	BA	Laterality	MNI coordinates x y z			Cluster size	z-values	Cohen's *d* effect size
CIP patients < non-psychotic cannabis users								
Superior Frontal	10	Right	13	65	22	38	3.4	-1,26
Precentral	4	Right	59	-5	26	61	3.8	-0,83
Superior Temporal	22	Right	62	-7	3	146	4.2	-0,60
Insula	13	Right	36	-21	13	142	4.1	-0,43
Insula	13	Left	-33	-23	14	32	3.8	-0,46
Precuneus	7	Right	6	-66	50	41	3.7	-0,51
Medial Occipital	19	Right	33	-86	21	80	4	-0,84
Fusiform	37	Left	-25	-47	-8	32	3.7	-0,29
Hippocampus	-	Left	-33	-22	-5	36	3.8	-0,68
Non-psychotic cannabis users < CIP patients								
No suprathreshold clusters								

Table of Materials

Name of Material/Equipment	Company	Catalog Number	Comments/Description
Not applicable	Not applicable	Not applicable	Not applicable

Editorial comments:

1. There are scattered typos throughout the manuscript. Please copy-edit the manuscript.

2. Please provide first names and email addresses for each author.

3. Please provide a citation for each evaluation in step 2.

4. Please provide all user input commands for steps 4 and 5. We need all terminal commands and/or buttons clicks in a GUI needed to perform the experiment: File | Save | etc. You need to answer the question of how every analysis is done so that others can replicate the protocol.

5. Please focus the manuscript on the protocol instead of the results. We are a methods based journal with a focus on increasing the reproducibility of the protocol. The Discussion is especially results focused.

6. Table 1 is not cited in the manuscript.

7. Please provide journal, issue, and page numbers for all references.

RESPONSE: All the changes requested have been now addressed. We have now provided all the scripts employed for the pre- and post-processing analyses. We have attached a .zip file with all the scripts and matlabbatches used for this study. Please let us know what is the best way to include this file in the manuscript.

Reviewers' comments:
Reviewer #4:
Manuscript Summary:
The manuscript provides data of interest in the field of cannabis addiction and dual pathology, especially regarding Magnetic Resonance Imaging measurements. However, the authors should make an in-depth review of this version taking into account my concerns

RESPONSE: We thank the reviewer for appreciating our study.

Major Concerns:

1. Regarding diagnosis, both the frequency and duration of cannabis use by the majority of participants suggest that instead of abuse, the term dependency should be used according to DSM-IV criteria used or cannabis use disorder. It is important to clarify this diagnostic terminology.

RESPONSE: We thank the reviewer for this suggestion. We have now replaced the term "abuse" with the term "dependency" and the term "abusers" with "users" in the whole manuscript.

2. The presence of polyconsumption can mediate the results of both groups, but to a greater extent in that of comorbid psychosis. It is stated that this is in "lower frequency that cannabis", although this statement does not solve the problem posed by the different proportion of patients in each group with said consumption pattern. Authors should consider whether to refer to the main substance of consumption. In fact, polyconsumption is the globally widespread pattern of substance use disorders and dual disorders.

RESPONSE: We thank the reviewer for this suggestion. We have now clarified this aspect in the results section, which now reads as follows:

"*Although we are aware that the presence of poly-consumption in our sample might have negatively affected the generalizability of our findings, it is important to highlight that the use of other drugs was very limited compared to cannabis use. Indeed, in contrast to cannabis use, the consumption of other drugs was lifetime and not occurring during the time of the study. Nonetheless, our results should be taken cautiously and need to be replicated in a more homogeneous sample."* (lines 360-365)

3. The term substance-induced psychosis is used. A greater detail of clinical evaluation is needed to support that psychosis has been induced. If there is no clear evidence that psychosis is primary or secondary in all patients, the use of concomitant / comorbid psychosis would be more correct. Moreover, in patients with "psychosis" the specific diagnoses presented should be detailed.

RESPONSE: We thank the reviewer for this clarification. We used the term "Substance-induced psychosis" following the criteria of DSM-IV that include: a) Prominent hallucinations or delusions; b) there is evidence from the history, physical examination, or laboratory findings that either the symptoms in Criterion "a" developed during, or within a month of, substance intoxication or

withdrawal or medication used is etiologically related to the disturbance; c) the disturbance is not better accounted for by a psychotic disorder that is not substance induced. Evidence that the symptoms are better accounted for by a psychotic disorder that is not substance induced included the following: the symptoms precede the onset of the substance use (or medication use); the symptoms persist for a substantial period of time (e.g., about a month) after the cessation of acute withdrawal or severe intoxication, or are substantially in excess of what would be expected given the type or amount of the substance used or the duration of use; or there is other evidence that suggests the existence of an independent nonsubstance-induced psychotic disorder (e.g., a history of recurrent nonsubstance-related episodes).

We agree with the reviewer that more details about the clinical evaluation is needed, therefore we have now added the following paragraph in the methods section, which now reads as follows:

"*Verify that psychotic symptoms do not precede the onset of the cannabis use and do not persist for a substantial period of time after the cessation of acute withdrawal or severe intoxication. Verify that there is no history of recurrent nonsubstance-related episodes.*" (lines 177-179)

4. A wide clinical evaluation has been carried out, but in the case of the group with psychosis, the specific symptoms should have been considered with e.g. PANNS. The claim that all patients were in stable pharmacological treatment is insufficient. The authors should include this aspect as a limitation. Furthermore, section 2 (Clinical and Psychosocial evaluation) does not detail all measurements made on patients (HAM, YMRS, ...).

RESPONSE: We thank the reviewer. We have now added all the clinical measurements that we have performed in the methods section, which now reads as follows:

"NOTE: Several clinical and psychosocial scales were administered to all the participants.

1.1. To evaluate psychiatric symptoms, use the Brief Psychiatric Rating Scale (BPRS)[29], the Young Mania Rating Scale (YMRS)[30], the Montgomery-Åsberg Depression Rating Scale (MADRS)[31], the Hamilton Depression Rating Scale (HAM-D)[32] and the Hamilton Anxiety Rating Scale (HAM-A)[33].

1.2. To explore the presence of trauma or infection during or immediately after the partum, use the Murray-Lewis Obstetric Complications Scale (MLOCS)[34].

1.3. To assess experiences of neglect or abuse, use the Childhood Experience of Care and Abuse Questionnaire (CECA-Q)[35].

1.4. To estimate the Socio-economic status (SES), use the Socio Economic Status Scale of MacArthur[36].

1.5. Use the Neighbourhood Scale (NS)[37] to assess the specific characteristics of the neighbourhood, in terms of neighbourhood satisfaction (NS-A), sense of security (NS-B), level of degradation (NS-C), willingness on the part of fellow citizens to intervene in adverse situations (NS-D), and degree of acceptance of substances (NS-E).

1.6. Employ the Temperament and Character Inventory (TCI-125) for exploring personality traits[38, 39].

1.7. To assess the quality of life and the global functioning use the Manchester Short Assessment of Quality of Life (MANSA)[40] and the Quality of Life Index (QL-index)[41] and the Global Assessment of Functioning (GAF)[27] scales, respectively.

NOTE: all socio-demographic and clinical data are summarized in Table 1." (lines 195-223)

Finally, as per reviewer's suggestion we also included in the limitations that our patients were taking pharmacological treatments. The new sentence now reads as follows:

"First, all psychotic patients were taking pharmacological treatments that could have influenced the results" (lines 533-534)

5. The inclusion of personality measures is only specified as a descriptive evaluation in Table 1. These, in the same way that we have proceeded with clinical measurements, should be correlated with GM regions. Currently, it is being pointed out that the personality traits of the Cloninger model are endophenotypes of substance use disorder (regardless of the substance) and dual disorders. The paper by Marquez-Arrico et al. (2016, Psychiatry Research, 237, 1-8) provides data in relation to this and should be incorporated into the review.

RESPONSE: We thank the reviewer for allowing us to clarify this aspect. We fully agree with the reviewer of the importance of correlating personality measures with GM regions. Indeed, we carried separate multiple regression analyses considering all the clinical scales used in this study, including TCI. However, the correlations between TCI measures and GM volumes resulted not significant at a $p < 0.05$ pFWE corrected. We therefore add a sentence in the results section to clarify this aspect:

"No differences in any of the other clinical scales were observed between CIP patients and non-psychotic chronic abusers." (lines 385-386)

6. The statistical analyzes must include, together with the "p" values, the effect sizes and power sample. This information is extremely important considering the small total sample and in each group of participants.

RESPONSE: We thank the reviewer for allowing us to include these information. In Table 2, here attached, we have now included the effect size (Cohen's *d)* associated to each brain region. Moreover, since the exploratory nature of this study we did not perform a formal power calculation. However, we specify this aspect in the methods section, which now reads as follows:

" *NOTE: Due to the exploratory nature of this study, a formal sample size calculation would have been of little value and therefore it was not performed."* (lines 328-329)

Table 2. VBM results. Brain regions showing significant reduced grey matter volumes between the CIP patients and non-psychotic abusers (P< 0.001 uncorrected).

Gyrus	BA	Laterality	MNI coordinates x	y	z	Cluster size	z-values	Cohen's *d* effect size
CIP patients < non-psychotic abusers								
Superior Frontal	10	Right	13	65	22	38	3.4	-1,26
Precentral	4	Right	59	-5	26	61	3.8	-0,83
Superior Temporal	22	Right	62	-7	3	146	4.2	-0,60
Insula	13	Right	36	-21	13	142	4.1	-0,43
Insula	13	Left	-33	-23	14	32	3.8	-0,46
Precuneus	7	Right	6	-66	50	41	3.7	-0,51
Medial Occipital	19	Right	33	-86	21	80	4.0	-0,84
Fusiform	37	Left	-25	-47	-8	32	3.7	-0,29
Hippocampus	-	Left	-33	-22	-5	36	3.8	-0,68
Non-psychotic abusers < CIP patients								
No suprathreshold clusters								

BA (Brodmann area); MNI (Montreal Neurological Institute); CIP (Cannabis-Induced Psychosis)

7. The discussion can be reworked with a better integration of the results obtained and the preceding findings. A limitation is not having also made functional neuroimaging records that would probably be more informative than the anatomical ones, as well as not evaluating the neurocognitive state (executive functions that could be affected). In this sense, the review work by Adan et al. (2017, Neuroscience and Biobehavioral Reviews, 75, 361-77) should be included.

RESPONSE: We thank the reviewer for suggesting these integrations. We have now added the lack of functional imaging and neurocognitive state as a limitation as well as the suggested review in the following paragraph:

"Finally, in this study we did not explore brain activation and we did not assess the neurocognitive state of the sample. Therefore, the lack of these information could have affected our results since previous studies demonstrated the presence of selective brain dysfunctions in patients with schizophrenia with substance abuse in the medial prefrontal cortex, the orbitofrontal cortex and the amygdala as well as a better premorbid neurocognitive profile with a greater long-term decline compared to the same patients without substance abuse[80]*. Therefore, further functional MRI studies exploring brain activity coupled with neuropsychological assessments on larger samples and with homogenous consumption habits are needed to confirm our results."* (lines 547-555)

Reviewer #5:
Manuscript Summary:
This article examines brain morphological differences between chronic cannabis use without psychotic symptoms, and chronic cannabis used with psychotic symptoms. In doing so, this manuscript proves to be interesting and may be important to the field. It uses largely excellent, scientifically-sound methodology and statistical analyses.

There are nevertheless a few issues that need to be addressed, mainly surrounding the poor use of language and lack of clarity that ensues because of this, as well as the lack of clarity as to whether brain imaging analyses have controlled for total intracranial volume (TIV), and clarity regarding participants' specific drug use behaviours. However, I believe that all of these issues are perfectly addressable. As such, I recommend that this manuscript be published in the Journal of Visualized Experiments, but only once the below comments have been tended to.

RESPONSE: We thank the reviewer for appreciating our study.

Major Concerns:

1. There are a number of grammatical errors that need addressing. For example, line 1 of the abstract; 'Cannabis is one of the most abused drugs in the world and its consume can both enable and…'. I suggest that these grammatical mistakes be carefully tended to before publication.

RESPONSE: We thank the reviewer for allowing us to correct all the grammatical errors within the manuscript. All authors have carefully re-read the manuscript and all the grammatical errors have been now corrected.

2. Please be very careful regarding your use of important, psychiatry-related words. For example, on line 61 you use 'psychic symptoms' when I think you mean 'psychiatric symptoms' or even 'psychotic symptoms'. Please change this.

RESPONSE: We thank the reviewer for this suggestion. We have now changed the words "psychic symptoms" with "psychiatric symptoms".

3. Lines 80 - 82: You mention genetic factors could also confer risk for cannabis-induced psychosis, but do not go into any detail about this. Please do so, as it currently seems like a 'throw-away' comment.

RESPONSE: We thank the reviewer for the suggestion. Unfortunately, the length of the paper is limited and we could not detail more about genetic risk factors. However, since we consider them relevant in substance-induced psychosis, we have now modified the sentence in the discussion section, which now reads as follows:

"Moreover, some genetic factors, such as the presence of specific catechol-O-methyltransferase (COMT) polymorphisms, may also confer an augmented vulnerability to develop psychotic symptoms after cannabis exposure in a small proportion of users[6]*."* (lines 99-101)

4. Exclusion criteria: Were left-handed participants excluded? Please state.

RESPONSE: We thank the reviewer for allowing to clarify this aspect. No, the handedness was not an exclusion criterion. We highlighted this aspect in the methods section:

"Either left- or right- handed participants were included" (lines 166-167)

5. The 'NOTE:' starting on line 165 should be moved to the results section. Further, groups should be statistically compared on these measures - did the two groups differ in terms of cannabis use, frequency or amount of use, for example? This is important to know.

RESPONSE: We thank the reviewer for this suggestion. We have now move the suggested paragraph in the results section. Moreover, following the reviewer's suggestion, we have now compared the two groups on type and frequency of drug use. Details regarding these analyses have been included in Table 1 and in the results section, which now reads as follows:

"*The frequency of cannabis use did not differ between the two groups ($\chi2=1.69$, $p=0.42$). Moreover, no statistical difference in type and frequency of cocaine, heroin/methadone and LSD use was observed between the two groups (cocaine: $\chi2=0.06$, $p=0.79$ and $\chi2=4.1$, $p=0.39$; heroin/methadone: $\chi2=1.2$, $p=0.26$ and $\chi2=1.2$, $p=0.26$; LSD: $\chi2=0.01$, $p=0.89$ and $\chi2=2.0$, $p=0.36$)."* (lines 355-359)

6. The amount of lifetime use of other drugs should be carefully described in this paragraph. This is because you are referring to 'substance-induced psychosis', instead of cannabis-induced psychosis. You need to explain exactly the lifetime use of all drugs for participants, to rule out

the fact that the SIPs do not have psychosis due to other drug use. This is imperative. Perhaps a table in the results section could suffice.

RESPONSE: We thank the reviewer for pointing out this aspect. Following the reviewer's #1 suggestion we decided to modify the term "Substance-induced psychosis (SIP)" with the term "Cannabis-induced Psychosis (CIP) in the whole manuscript since with the term "Substance-induced psychosis" we meant "Cannabis users with Substance-induced psychosis".

Moreover, we have added all the information you requested in Table 1 and in the text, which now reads as follows:

"NOTE: Specifically, for CIP, the frequency of cannabis dependency was: daily for 9 subjects (90%), several times a week for 1 subject (10%). Instead, the frequency of cannabis dependency in the non-psychotic group was: daily for 7 subjects (60%) and several times a week for 4 subjects (30%) and multiple times a month for 1 subject (10%). The mean age of onset of dependency was at 18 years old for CIP patients and at 16 years old for the non-psychotic chronic cannabis user group. Although all participants were taking cannabis, some CIP patients (N=6) and non-psychotic chronic users (N=3) also reported previous use of other drugs, including cocaine, LSD and heroin/methadone, but with lower frequency than cannabis. The frequency of cannabis use did not differ between the two groups (χ^2=1.69, p=0.42). Moreover, no statistical difference in type and frequency of cocaine, heroin/methadone and LSD use was observed between the two groups (cocaine: χ^2=0.06, p=0.79 and χ^2=4.1, p=0.39; heroin/methadone: $\chi^{2=}$1.2, p=0.26 and χ^2=1.2, p=0.26; LSD: χ^2=0.01, p=0.89 and χ^2=2.0, p=0.36). Although we are aware that the presence of poly-consumption in our sample might have negatively affected the generalizability of our findings, it is important to highlight that the use of other drugs was very limited compared to cannabis use. Indeed, in contrast to cannabis use, the consumption of other drugs was lifetime and not occurring during the time of the study. Nonetheless, our results should be taken cautiously and need to be replicated in a more homogeneous sample." (lines 346-365)

7. Line 205: Unless this is otherwise instructed by the journal itself, having a bullet-point list of the MRI scanning protocol seems rather bizarre, and this should instead be explained (as usual) in a paragraph.

RESPONSE: We thank the reviewer for pointing out this aspect. Unfortunately, the way the methods were written were instructed by the journal and therefore we cannot change the suggested paragraphs.

8. The same goes for the 'Pre-processing steps' starting on line 235 and 'Post-processing steps and statistical analysis' starting on line 271.

RESPONSE: We thank the reviewer for pointing out this aspect. Unfortunately, the way the methods were written were instructed by the journal and therefore we cannot change the suggested paragraphs.

9. Line 286: 'What exactly do you mean by 'Proportional scale all the analyses for the total intracranial volume'? Have you not controlled for TIV in all of your GLMs in SPM? If so, it is imperative to do so. Please show that you have done this for all models, and that you have not run any models without controlling for TIV. As such, point 5.4 (line 286) should not discuss

TIV values, as this variable should be controlled for in all analyses. Failure to do this will seriously undermine your findings.

RESPONSE: We thank the reviewer for allowing us to clarify this aspect. We have indeed controlled for TIV in all the neuroanatomical analyses since this is a common practice in neuroimaging studies. Since JoVE is a methodological journal it instructed us to include all the details regarding the analyses and therefore we also discuss TIV values. However, in order to better clarify that the ICV was used in all the analyses we have now added in the results section the following sentence:

"NOTE: In all the neuroanatomical analyses, the volumetric differences among subjects were considered by proportional scaling for the total intracranial volume (ICV)." (lines 312-313)

10. Please explain why you have chosen the significant and cluster size thresholds that you have. I understand that this is preliminary data, but you need to provide a more scientifically-sound reason as to why you chose specifically 30 voxels and 10 voxels. Perhaps another published study has used these thresholds?

RESPONSE: We thank the reviewer for this observation. However, the cluster size of the analyses was chosen arbitrarily and the reason why we chose different voxel sizes is the following. As we specified in the methods section, since in the group analysis we employed a less stringent threshold of $p<0.001$ uncorrected, we therefore decided to use a higher cluster size in order to exclude brain regions with only few voxels. Instead, for the multiple regression analyses we employed a more stringent threshold of $p<0.05$ pFWE, which is the highest threshold you could use in SPM. However, with this threshold, due to the small sample size, we decided to include a lower cluster size with the final goal of retain some meaningful information. We have therefore applied an arbitrary cluster size threshold based on the presence or absence of a peak-based multiple comparison correction.

11. Line 304: No differences in terms of what? Between the two groups? Be careful as to how you report your results. You should state something like 'There were no group differences in terms of….'

RESPONSE: We thank the reviewer for this suggestion. We have now followed his/her advice and modified the text accordingly.

12. Please put these variables, as well as drug use (see point 6 above) in Table 1. Further, some of these variables do not make sense on their own; occupation for example - why is this denoted by a number? Please ensure that in the table legend, you explain exactly what every variable is, and what a higher or lower number actually means.

RESPONSE: We thank the reviewer for this clarification. We have now included a legend below the Table l where we explain what some of the scales mean.

13. Line 373: You state that no differences between groups were found in terms of age, gender, age of onset and educational level - however this could also be due to the small sample size which should be discussed.

RESPONSE: We thank the reviewer for the suggestion. We have now modified the paragraph by including the small sample size as an explanation for the lack of differences between the groups:

"*Moreover, no differences in terms of age, gender, age of onset of cannabis use and educational level were found between the two groups. However, we should consider that this lack of differences could be due to the small sample size that also limited the possibility to statistically analyse and interpret these factors*." (lines 429-433)

14. Line 376: Which brain areas showed GMV alterations? Are these areas similar to those that you report? This warrants much more discussion please.

RESPONSE: We thank the reviewer for this suggestion. However, in the previous version of the manuscript in line 376 we stated that "*One hypothesis is that the psychotic process itself is responsible for the decrease in brain volume, regardless cannabis use, in line with a previous study, which found no GM differences between psychotic patients with and without cannabis consumption*[17]". However, the study by Haller et al. compared first episode psychotic patients with and without cannabis consumption and showed no GM differences between the two groups, without, though, performing comparisons with non-psychotic healthy controls. We therefore used this study in order to state that in our case it could be that psychotic cannabis users showed GM reductions compared to non-psychotic cannabis users given to the psychotic process itself, and not because of cannabis consumption. In order to clarify this aspect, we have now restructured the paragraph as follows:

"One hypothesis is that the psychotic process itself is responsible for the decrease in brain volume, regardless cannabis use. Indeed, previous studies showed no GM differences between psychotic patients with and without cannabis consumption, thus finding no clear evidence for cannabis use to be related to GM alterations in first episode psychotic patients[45]*."* (lines 436-440)

15. Lines 381 - 391: This is a valid point, but I think that you need to relate this to work that examines the neural mechanisms of difficulties in emotion regulation in substance abusers. While there are not many published studies on this yet, please cite Faulkner et al (2020) who examined this point in tobacco smokers.

RESPONSE: We thank the reviewer for this suggestion. We have now added the suggested reference and modified the paragraph that now reads as follows:

"*Indeed, it has been consistently reported that emotion regulation/processing are associated with recruitment of a set of prefrontal brain regions involved in cognitive control over emotional limbic structures. For example, greater difficulties in emotional regulation among tobacco smokers have been associated to a weaker connectivity between inferior frontal gyrus and amygdala compared to non-smokers*[49]. (lines 454-458)

16. Line 394: 'A region crucial for the maintenance of valid (cognitive) function' is not specific enough - please specify exactly what you mean, which cognitions etc (i.e. the DLPFC is also involved in emotion regulation I believe, which helps your prior argument).

RESPONSE: We thank the reviewer for allowing us to clarify this aspect. We have now better explained the role of DLPFC in cognition in the following paragraph:

"*Additionally, we observed that the group of CIP patients showed disruptions in the dorsolateral prefrontal cortex (DLPFC), a key region involved in major cognitive functions, including working memory, executive functions*[50] *and emotional regulation*[51]. (lines 462-464)

17. Lines 404 - 405: Are the participants in this previously published study different from the current set of participants? Please be clear on this.

RESPONSE: We thank the reviewer for allowing us to clarify this aspect. We have now better specified this point in the discussion section. The new sentence now reads as follows:

"Interestingly, this result is in line with the evidence reported by our previous multimodal neuroimaging study[54]*, which employed a larger sample of CIP patients (N=16), the majority of whom overlap with the sample employed in this study that found extensive GM alteration in temporal cortices in CIP patients*." (lines 472-475)

Minor Concerns:

1. Line 74: Please change to 'However, whether this relationship is causal, or purely correlational, is still controversial and debated'.

2. Line 76: Please change to 'heavy cannabis consumption'

3. Lines 101 - 105: 'Increased activity' during what? Rest? Performance of a task? Please state clearly.

4. Lines 109 - 114: your description of this study is not clear. How many groups are there - 4? EOS+, EOS-, CUD and healthy controls? You do not mention the latter until you describe the results. Further, your sentence beginning 'However' on line 113 needs to be clearer also.

RESPONSE: We thank the reviewer for allowing us to include these changes in our manuscript. All the suggestions have been addressed.

5. Please clearly state your hypotheses towards the end of the introduction.

RESPONSE: we thank the reviewer for this suggestion. We added a paragraph explaining our main hypotheses at the end of the introduction section, which reads as follows:

"*We hypothesized that CIP patients will show significant reductions in GM volumes compared to healthy cannabis abusers as well as possible correlations between GM volumes and socio-demographic, clinical and psychosocial scales*." (lines 156-159)

6. Line 279: Remove the first word of the sentence ('Use').

RESPONSE: we thank the reviewer for this suggestion. We removed the word "Use" in that sentence.

7. Please report your p value on line 304 to three decimal places, to remain consistent.

RESPONSE: We thank the reviewer for raising this issues. We have now modified the manuscript according to the reviewer's suggestion.

8. The quality of the image in Figure 1 is rather poor. However, if the journal is happy with this, or can improve it, then it is fine.

RESPONSE: We thank the reviewer for raising this issues. We have now created the figure in 600 DPI.

9. Line 376: Change to 'regardless of cannabis use'

RESPONSE: We thank the reviewer for raising this issues. We have now modified the manuscript according to the reviewer's suggestion.

10. Line 387: Change to 'substance-seeking'
12. Line 437: Please change to 'there is evidence'

RESPONSE: We thank the reviewer for raising this issues. We have now modified the manuscript according to the reviewer's suggestion.

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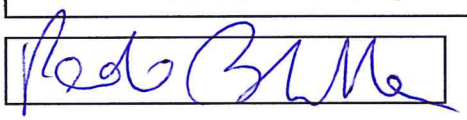
Signature:	
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