TITLE

Differential effect of cannabis use and antipsychotic medication on extracellular free-water in the brain of individuals with early psychosis and controls

Running title: Cannabis, Antipsychotics and Brain Free-Water

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ABSTRACT

Inflammatory changes have been widely reported in psychosis. Cannabis use has been consistently related to increased risk of psychosis, earlier onset, higher rates of relapse and poorer treatment response. However, it is unclear how cannabis use interacts with brain inflammatory changes in psychosis.

In this cross-sectional study we used diffusion imaging to measure extracellular free water in the brain (FW), a measure that has been associated with inflammation, in 62 individuals with recent onset psychosis (ROP) and 38 controls, with and without cannabis use. Past cannabis use was associated with lower FW in controls, and conversely, to elevated FW in ROP. This group x past cannabis use interaction was found significant in average GM (p=0.049), and in cortical regions, including the temporal lobe expanding to parietal regions (TFCE p-FWE<0.05). Within ROP, antipsychotic exposure was related to lower FW in gray matter and white matter only in the non-cannabis users, with no significant association in cannabis users (p interaction in WM = 0.005, in GM = 0.073).

Our results demonstrate a differential effect of cannabis use on FW, a surrogate marker of neuroinflammatory processes and suggest that past cannabis use may influence the effects of antipsychotic medication on the brain. However, given the cross-sectional design and moderate sample size, causal interpretations are limited, and further longitudinal studies are warranted.

INTRODUCTION

Schizophrenia is a disabling disease (1) characterized by core symptoms such as delusions, hallucinations and disorganization manifesting in adolescence and early adulthood (2). Although it is considered a highly heritable disorder (3), several environmental risk factors are thought to interact with this genetic load and modify the risk of presenting the illness phenotype (4). Cannabis use, one of the most well-documented risk factors, is notably prevalent during the onset of the disorder, with consumption rates ranging from 30 to 50% (5). However, its role in different symptom domains and outcomes of psychosis is complex and not fully understood. On the one hand, there is compelling evidence supporting cannabis use as a risk factor not only for developing the illness (6–9), but also for earlier onset (10), higher relapse rate (11), and worse outcome (12). Conversely, past cannabis use in individuals with schizophrenia has been associated with better cognitive functioning compared to non-users (13–17), while the inverse association has been consistently observed in the general population (18).

In an effort to integrate these findings, a hypothesis has been proposed suggesting that cannabis use may act as a negative risk factor for illness onset and outcome in those with high genetic loading for schizophrenia, and may additionally be a proximal causal factor in initiating psychosis in those individuals with intermediate to low genetic risk, leading to a psychotic phenotype with a better cognitive profile (13). Supporting this hypothesis, genetic studies have reported a variety of gene-environment interactions between cannabis use and risk for psychosis in genes associated with the dopaminergic pathway, the endocannabinoid system, and inflammation (19–21).

The association between psychosis and inflammation is well-documented (22) and has been consistently observed in the early phases of the disease (23,24). Interestingly, the primary compounds of cannabis sativa, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD),

have exhibited differential effects on inflammation in animal models, where THC appear to suppress immune response while CBD could enhance it (25–27) . In this regard, only a few studies have focused on the effects of cannabis use on inflammatory markers in psychosis, showing mild decreases of peripheral inflammatory markers in psychotic patients using cannabis in comparison to non-users (28). Thus, how cannabis may interact with inflammatory processes in psychosis remains poorly understood.

Another confounding factor in psychotic patients is exposure to antipsychotic drugs, which are known to modulate the immune response. These drugs have been extensively reported to decrease inflammatory cytokines and inflammatory enzymes such as cyclooxygenase and microglia activation (29). Notably, there is no evidence linking antipsychotic exposure to gliosis, a proliferation of astrocytes in the brain, often but not exclusively related to neuronal damage (30,31). The anti-inflammatory effects of antipsychotics have been proposed as either a mediator of their therapeutic effects, a potential confounding factor in the study of neuroinflammation in psychosis or both (32,33). Interestingly cannabis use in psychosis has been reported to interact with antipsychotic treatment by decreasing treatment response both at the clinical level (34), and at the neurobehavioral level in animal models (35). However, little is known about how this interaction is revealed in terms of inflammatory processes in the brain related to psychosis.

Neuroimaging techniques, such as diffusion tensor imaging (DTI), measure the diffusion pattern of water molecules within the brain providing non-invasive insights into brain tissue properties. By using a bi-compartmental model in DTI, extracellular free water (FW) can be defined as water molecules exhibiting low diffusion restriction or isotropic diffusion, which can be computed as a fraction for each voxel in an MRI brain scan (36). Physiologically, FW is found in cerebrospinal fluid or the surroundings of brain parenchyma, but it may also accumulate in certain pathological conditions, such as vasogenic edema, typically associated

with inflammation (37). Furthermore, elevated FW in gray matter (GM) has been correlated with decreased glutathione brain levels (38). Glutathione is an antioxidant peptide known to be reduced in inflammatory processes (39,40) and during maternal immune activation in animal models of psychosis (39,41). Other work has highlighted significant relationships between elevated white matter FW and peripheral cytokines, including IL-6 and TNF-alpha in patients with schizophrenia (42). Similarly, Wu and colleagues (43) found elevated FW in a subgroup of patients with elevated peripheral proinflammatory cytokines. Accordingly, FW has been interpreted as a surrogate measure of neuroinflammation (44).

Elevated FW both in GM and in WM has been consistently reported during the early stage of psychosis (36,38,45–47) and, to a lesser extent, in established schizophrenia (48,49). To clarify the neurobiological significance of elevated FW in psychosis and its distinction between state and trait characteristics, a few studies have conducted longitudinal measurements of FW. These studies have revealed a decrease in FW over time in a subgroup of first-episode psychosis (46), while other subgroups exhibit no significant change (50). Additionally, we have recently shown that elevated FW in GM of first-episode psychosis predicts clinical improvement at 12 months follow-up (51).

Nevertheless, the influence of relevant clinical variables in psychosis, such as antipsychotic exposure and cannabis use on FW in the brain, remains largely unknown. The association between antipsychotic exposure and FW has yielded inconsistent findings in psychosis, with a few studies indicating a significant correlation (50), while others report no association (46,47). These mixed findings might be attributed to the influence of cannabis use or other confounding factors.

Despite the potential role of cannabinoids in modulating the immune system, the effects of cannabis use on FW remain unexplored. Our primary objective is to determine the relationship

between cannabis use and FW as well as interactions with antipsychotic exposure. Based on previous evidence, we hypothesized that cannabis use would have a different impact on FW levels of patients with first episode of psychosis and healthy controls. Additionally, we posited that cannabis use in patients will modulate the effect of known confounding factors, such as antipsychotic treatment, on FW.

METHODS

Subjects:

Subjects diagnosed with a recent onset psychosis (ROP) and healthy controls, both with and without cannabis use and aged between 18 and 35 years old, were recruited for the present study. The ROP group included subjects with a first episode of psychosis as well as individuals affected by schizophrenia within the five first years since illness onset. Exclusion criteria included a history of treatment with electro-convulsive therapy, present substance use disorder other than cannabis or nicotine, and any medical condition that could contraindicate MRI scan. Participants from the ROP group were recruited from the local early psychosis intervention program and local community mental health services. Healthy controls were recruited through local advertisements. The confirmation of any past or present diagnosis of severe mental disorder, or any present mental disorder through interview with the MINI (52), were additional exclusion criteria for the control group. Groups were mean-matched on age, sex, and educational level. Sample size was based on previous reports on FW and psychosis (38). The study protocol was approved by the local ethics committee, and all subjects gave written informed consent before any study procedure.

Assessment:

As part of a comprehensive assessment, participants underwent two study visits including a clinical interview and an MRI scan. During the clinical interview, duration of illness and duration of untreated psychosis (DUP) were measured as the time since psychotic threshold symptoms emerged to the date of the study visit or the date in which antipsychotic medication was started, respectively. Socio-demographic status was assessed through the Hollingshead-Redlich Scale (53), appropriate diagnoses were confirmed or discarded using the MINI interview, BMI was measured and registered, psychotic symptoms were assessed using the

Positive and Negative Syndrome Scale (54), negative symptoms were measured using the BNSS (55,56), general functioning was evaluated using the DAS-WHO (57), and a history of antipsychotic intake was collected and completed with clinical records. Doses were transformed to chlorpromazine miliequivalents using R package chlopromazineR (https://docs.ropensci.org/chlorpromazineR/) to then calculate the cumulative exposure, which was log transformed before entering the regression models. Information regarding cannabis use was provided by past and present substance use history registered through an in-house structured interview, and biological matrices collected during the study visit. Cannabis use was qualitatively determined in urine, and quantitatively determined in blood and hair as described elsewhere(58,59). While different sources of information regarding cannabis use showed a non-uniform pattern, as a strategy of variable reduction, we implemented a cluster analysis to group subjects according to their past cannabis use profile. To this purpose, past cannabis use frequency, average past cannabis use units per week, and years of use were used to construct a Gower's distance matrix, which was then entered in a clustering model using partition around medoids (10.32614/CRAN.package.parallelpam). This method was chosen because it may be fed with dissimilarity matrix, and it is more robust than the traditional k-means method (60) . Average silhouette width, a parameter that measures the fit of an individual to a specific cluster, was used to visualize and select the appropriate number of clusters in the model, which were used as the variable to describe past cannabis use in further analysis. As for recent cannabis use, we decided to use the self-reported frequency of use during the last month and, in separate analysis, quantitative THC levels in hair if available, as it summarizes cannabis use during the past three months. For this purpose, we used an abbreviated form of Psychiatric Research Interview for Substance and Mental Disorders (PRISM) (61). Information regarding composition or potency of the cannabis used by subjects (e.g., THC/CBD ratio) was not available for this sample.

Participants cognition was briefly evaluated using the vocabulary, and the letter-number subtests of the WAIS-III, the Spain-Complutense Verbal Learning Test (TAVEC) , and the Stroop test.

Neuroimaging acquisition and preprocessing:

MRI images were acquired using Philips Ingenia 3 Tesla scan with a 32 channel head coil, including, among others, the following acquisition sequences 1) structural T1 weighted with FOV= 240x240x180, multishot acquisition with final voxel size = 0.68x0.68x1mm, TR=9.8ms, TE=3.5ms, flip angle=8º, scan duration = 4' and 17'', 2) DWI sequence with FoV= 230x230x135.3, acquisition voxel size=2.05x2.05x2.05mm, multishell single shot, TE= 104ms, TR=9300ms, 68 directions including 2 b0 volumes, 34 volumes with b=1000, 34 volumes with b=2500, flip angle=90º. Structural images were segmented and reconstructed into a surface mesh using the default CAT12 segmentation procedure (https://neuro-jena.github.io/cat/). Diffusion Weighted images were initially preprocessed using DTIPrep (62,63) for quality control, motion effects and Eddy current distortion correction, and denoising. Those volumes showing artifacts were discarded at this step. Then, top-up procedure in FSL (64) using anterior-posterior and posterior-anterior acquisition volumes was implemented to correct for phase encoding distortion effects. Next, a bi-compartmental model in DIPY(65)), was used to calculate free-water fraction and free-water elimination DTI scalar measures in each voxel. Voxels with FW values higher than 0.7 were considered to be predominantly CSF and removed from all scalar images.

Analysis of average values:

To align structural and diffusion images, b0 images were initially coregistered to structural images in cat12 using normalized mutual information cost function. Then, this transformation was applied to FW images. Average FW values were extracted by masking the FW images

with the respective gray matter and white matter masks previously generated by cat12 and using a probabilistic threshold of 0.8. These values were entered in the following linear models.

First, to test the differential effect of cannabis in psychosis and controls, average FW values for GM and WM were entered in separate linear regression models as the dependent variables. Group (ROP or control), past cannabis use, and recent cannabis use were introduced as the main variables of interest. Interaction terms between group and past or recent cannabis use were also entered in the initial model. As BMI and premorbid education level showed significant between-group differences, and they have been associated with inflammation in psychosis (66,67) ,these variables were additionally introduced as covariates in the initial model. To limit the number of covariates in the model, the three variables describing education, i.e. years of education, educational level of the participant and parental educational level, were reduced to one single principal component using PCA analysis. Additionally, FW values were correlated with cognitive assessment most relevant scores to discard a primary effect of cognition (see supplementary material table S7 for details).

Second, to test the differential effect of cannabis use on FW and its interaction with antipsychotic exposure, average FW values in WM or GM were entered as the dependent variable in separate linear models only in the group of ROP. Past and recent cannabis use, cumulative exposure to antipsychotic drugs and its interaction with both past and present cannabis use, were entered as the main variables of interest. As in the previous model, BMI and premorbid education level were also entered. To control for the effect of time since illness onset and DUP, these two variables were also introduced as covariates in the initial models.

For all linear models, a backward stepwise elimination procedure was implemented based on the best AIC parameter in R (MASS package, https://www.stats.ox.ac.uk/pub/MASS4/). Additionally, those variables with *p* value higher than 0.1 were also removed unless involved

in a significant interaction. The surviving measures in the final model were reported and selected for voxel-wise analyses implemented in GM surface or WM tracts as appropriate.

Voxel-wise analysis:

To analyse voxel-wise free-water in gray matter, the coregistered FW images were resampled onto a common cortical surface mesh previously generated for each participant by cat12 and smoothed using a 15mm FWHM. These surface meshes were entered in a general linear model, where those variables surviving the previous average FW in GM stepwise regression models, were entered here as independent variables. The results were corrected for multiple-voxel comparisons using Threshold-Free Cluster Enhancement (TFCE) and p-FWE < 0.05.

To analyze the regional effect in WM of those variables surviving the previous stepwise regression models, a voxel-wise analysis was performed using TBSS in FSL as reported elsewhere (46). Briefly, using the public available ENIGMA scripts (https://enigma.ini.usc.edu/protocols/dti-protocols/ ; RRID:SCR_014649) , FA images were registered to the standard ENIGMA space, and then projected onto a common white matter skeleton template. This registration and projection matrix was then used to project FW images onto the white matter skeleton. This standard TBSS pipeline in ENIGMA space was applied with one adaptation: FA images were slightly eroded and masked using DIPY tools (using "median_otsu" command and parameters: median radius = 2, numpass = 1, autocrop=FALSE, dialte = 1, https://dipy.org; RRID:SCR_000029), instead of the standard TBSS eroding process. Then, the FW skeletonized images were entered in a general linear model, with those variables surviving the stepwise regression model as variables of interest. Permutation methods (45) were applied, and results were corrected using TFCE and p-FWE < 0.05.

RESULTS

Sixty-four participants with ROP and 38 healthy controls participated in the study. Two subjects were discarded due to unusable images. Among the ROP group, 15 individuals fulfilled the criteria for schizophrenia, while the remaining 47 were diagnosed with first-episode psychosis (FEP) within the first year since illness onset, including brief psychotic episode (n=3), schizophreniform disorder (n=9), bipolar disorder with psychotic symptoms (n=7) or psychosis not otherwise specified (n=28).

Clinical features for ROP and differences in sociodemographic characteristics between ROP and healthy controls (HC) were examined either by t-student or Chi-square test and are fully described in Table 1. ROP subjects did not differ from HC in age or sex. However, ROP showed significantly higher BMI, lower educational level, fewer years of education and smaller proportion of both own and parental grad studies. Quantification of THC in hair was unavailable for three control participants and six individuals with ROP. Therefore, we decided to present the results using self-reported recent cannabis use for the entire sample in the main manuscript, and additionally, we replicated the analysis using quantitative THC measurements for the subset of participants with this data available (see Supplementary Table S1). The clinical characteristics of the sample categorized into three groups: first-episode psychosis, established schizophrenia, and controls is available in Supplementary Table S2.

Cluster analysis for the variables describing past cannabis use returned a two-cluster solution, with "frequent past cannabis use, more than one unit per week" serving as the categorizing variable. See supplementary material and Supplementary Figure S1a for details about cluster analysis and resulting cluster characteristics, and Supplementary Figure S1b for a correlation

plot of all cannabis use related variables. While there were no significant differences in rates of past cannabis use between ROP and controls, rates of current cannabis use in ROP were significantly lower than those of controls. FW in WM or GM did not significantly correlate with the score of PANSS positive subscale, PANSS negative subscale, PANSS total, CGI, BNSS, WHO-DAS or PAS.

Table 1 here

The effect of group and cannabis use

The variables that survived the final model to test the effect of group and cannabis use on average FW in GM included a significant effect of group (B=1.562, 95% CI [0.654,2.470], p<0.001) and past cannabis use (B=1.520, 955 CI [0.319, 2.721], p=0.013), a trend towards a significant effect of frequency of recent cannabis use (B=0.025, 95% CI [-0.003, 0.053], p=0.074), and a significant effect of the interaction between group and past cannabis use (B=-1.732, 95% CI [-3.184, -0.279], p=0.019). The coefficients and model fit measures of the initial and final regression models are shown in table 2. Average FW in GM by group and past-cannabis use are plotted in figure 1. Figure 2 and table S3 in supplemental material show clusters with significant effect on FW in GM surface for contrasts ROP higher than controls (A), all participants non-users higher than users (B), and the interaction effect of group x past cannabis use (C).

Table 2 here

Figure 1 here

Figure 2 here

The same analysis using quantitative THC in hair instead of self-reported frequency of recent cannabis use returned the same variables (see supplementary material table S4). Plots showing correlation between both THC in hair and self-reported frequency of recent cannabis use against raw and model-predicted FW are shown in supplementary material figure S1c. The model to predict average FW in WM did not result in any significant effect of group or cannabis use.

The effect of cannabis use and antipsychotic exposure in ROP

Individuals with ROP with and without past cannabis use did not differ in baseline clinical characteristics except for years of education and parental educational level (see details in supplementary material table S5). Regarding FW in GM, the variables that survived the final model to test the effect of cannabis use and antipsychotic exposure within the group of psychosis included a non-significant effect of past cannabis use and group (FEP vs SCH) (B=1.940, 95% CI [-0.060, 3.945], p=0.058), a significant effect of antipsychotic exposure (B=0.871, 95% CI [0.190, 1.553], p=0.013), DUI (B=-0.001, 95% CI [-0.002, -0.001], p=0.014) and BMI (B=-0.090, 95% CI [-0.176, -0.005], p=0.040) (see table 3A), and a trend towards a significant effect of the interaction between past cannabis use and antipsychotic exposure (B=-0.499, 95% CI [-1.044, 0.047], p=0.073). Figure 3A plots the effect of the interaction term between past cannabis use and antipsychotic exposure on FW GM, showing decreasing FW along with increasing antipsychotic exposure only in the non-cannabis past user group. When using quantitative THC in hair instead of self-reported frequency of cannabis

use, the final step returned a similar model except for the removal of BMI and the addition of a significant effect of THC in hair and its interaction with antipsychotic exposure, in the direction of higher FW in GM along THC in hair, specifically in those with low antipsychotic exposure. (see supplementary material table S6A and figure S1d). No significant correlations were found between FW in GM or WM and any of the cognitive assessment outputs (see supplementary material table S7).

Table 3 here

Figure 3 here

The voxel-wise analysis of FW in cortical surface with the variables surviving the final model within the group of patients, found no significant clusters when testing the effect of antipsychotic exposure, past cannabis use, DUI or BMI. The effect of the interaction between past cannabis use and antipsychotic exposure within ROP individuals resulted in significant clusters representing larger decrease of FW as a function of antipsychotic exposure in ROP without past cannabis use in comparison to ROP with past cannabis use, in right temporal, occipital, parietal areas and prefrontal cortex (see figure S2 in supplemental material).

Regarding FW in WM, the variables that survived the final model to test the effect of cannabis and antipsychotic exposure within the group of psychosis included a significant effect of past cannabis use (B=8.348, 95% CI [2.670, 14.025], p=0.005), antipsychotic exposure (B= 0.834, 95% CI [0.189, 1.478], p=0.012), and the interaction term between both (B=-0.749, 95% CI [-1.270, -0.230], p=0.005, see table 3). As shown in table 3B and figure 3B, antipsychotic

exposure was associated with a decrease in FW in WM only in the non-cannabis user individuals with ROP. When using quantitative THC in hair, the model included these same terms (see supplementary table S6B).

Figure 4 shows the voxel-wise representation of the interaction between past-cannabis use and antipsychotic exposure, with clusters showing white matter skeletonized tracts where decrease in FW as a function of antipsychotic exposure in past non-users was significantly larger compared to past cannabis users. These clusters included the bilateral superior and inferior longitudinal fasiculum, corpus callosum, internal capsule and corona radiata.

Figure 4 here

DISCUSSION

Our study not only corroborates an increase of FW in GM in early phases of psychosis compared with healthy controls,but additionally reports new findings of a differential association of cannabis use on FW in individuals with ROP in comparison to controls, with past cannabis use associated with lower FW in controls and, conversely, to higher FW in ROP. This different association was significant in average GM volume, and specifically, in the left inferior temporal and fusiform gyrus. Importantly, antipsychotic exposure in ROP was related to lower FW only in the non-cannabis users, whereas this association was not present in past-cannabis users.

Our findings are consistent with previous reports showing elevated FW in average GM, in ROP (49,50). This elevation in FW has been associated with immune activation in animal models (39,41), decreased glutathione in patients with first-episode schizophrenia (38), and abnormal peripheral cytokines levels in schizophrenia (42,43). Thus, FW elevation has been suggested as a proxy of neuroinflammatory changes (42). In our sample, elevated FW was found specifically in several GM regions overlapping those of previous reports from our group in different samples (38,46), including the temporal lobe, inferior parietal cortex, precuneus, and occipital areas, supporting the consistency of these findings. Interestingly, the temporal lobe, where we observed the largest elevation of FW in our ROP sample, and specifically the anterior hippocampus, has been consistently involved in psychosis (68), and recently reported as a putative epicentre of pathology in psychosis which subsequently extends to other brain regions (69). Thus, FW elevation could translate early neuroinflammatory responses in psychosis in temporal regions spreading to a lesser extent to other regions of the brain (see figure 2).

We did not find significant between-group differences in average FW in WM, which contrasts with the findings of a recent meta-analysis examining FW in psychosis (47) . Regarding ROP populations, two previous studies reported higher FW in WM in FEP compared to healthy controls (45,49) while another study showed only a trend toward elevation (38). In a distinct cohort of subjects, we have previously found increased FW in WM only in those FEP classified in the schizophrenia spectrum but not in FEP with affective psychosis (46). Our sample of ROP includes non-affective and affective psychosis, which could contribute to the differences with previous reports. We provide additional description related to specific diagnostic categories in supplementary data figure S3. Additionally, we used a multishell MRI acquisition protocol, whereas most of the previous reports have used single shell acquisitions. This technical distinction may account for the discrepancies between our findings and previous reports.

Since cannabis is a highly prevalent environmental risk factor in psychosis, our aim was to explore its association with FW in the brain during the early phase of psychosis. We found that past cannabis use was associated with lower FW in controls, and conversely, with higher FW in the group of ROP, suggesting a differential inflammatory brain response to cannabis exposure in psychosis in comparison to the general population. Additionally, regarding recent cannabis use, our results show an association with increased FW in GM irrespective of group, which trend to significance when using measures of self-reported use during the last month and reached significance when using quantitative THC in hair samples. These findings suggests that recent cannabis use is related to an acute inflammatory response in all subjects, followed by a delayed and protracted anti-inflammatory activity, which does not occur in ROP. However, further longitudinal studies are required to elucidate this hypothesis.

Another plausible explanation is that cannabis use may be linked to various aspects of the

421 immune response leading to an immunological dysfunction especially during adolescence, that
422 would represent an underlying susceptibility to psychosis (21,70). In other words, this suggests
423 that it is not psychosis that modifies the impact of cannabis use on neuroinflammation, but
424 rather that cannabis use in certain individuals may be associated with abnormal immune
425 response and increase the risk for psychosis. Although the most important body of literature
426 shows anti-inflammatory properties of cannabis compounds in general population (71,72) a
427 recent in vivo PET imaging study conducted in non-psychotic individuals showed increased
428 neuroimmune activation of the glia among those who were cannabis-users (73). These mixed
429 findings suggest that the effect of cannabis on inflammation may be complex, inhibiting the
430 most relevant inflammatory pathways but activating immune pathways in the glia. In clinical
431 samples of psychosis, suitable evidence for the link between cannabis use and inflammatory
432 processes is not yet clear. Studies of individuals with chronic schizophrenia report an increase
433 of lymphocyte count in those with comorbid cannabis use (74) and a further increase in
434 inflammatory markers following cannabis cessation (75,76). These previous reports are in line
435 with our results showing elevated FW in GM related to past cannabis use in ROP, even though
436 many of these individuals decreased their cannabis use in the last months. However, other
437 studies show a trend to decreased inflammatory cytokines in psychotic patients using cannabis
438 (28) and some fail to find a correlation (77). Evidence in early psychosis populations is scarce,
439 however, one small sample size study showed decreased IL-1 and IL2 among FEP who used
440 cannabis (78), and a recent and larger cross-sectional study comparing HC and FEP did not find
441 a direct relationship between cannabis use and inflammatory markers (79). Our study, in
442 contrast to others that used peripheral inflammatory markers, uses FW as a surrogate for in-
443 brain inflammation. Peripheral and in-brain inflammation might not completely overlap (22,80),
444 which could explain the differences in results. The heterogeneity in methodology for
445 measuring cannabis use as well as the diverse cannabis use pattern including route of

administration (81) are also concerning in order to stablish solid conclusions in these regards.

Alternatively, differences in the impact of cannabis on brain inflammation observed between control subjects and patients with first-episode of psychosis may be influenced by distinct premorbid characteristics of those psychotic patients using cannabis. This alternative hypothesis offers a different interpretation of our results based on previous rationale proposed by Yücel et al (13) and suggests that the different effect of cannabis use in FW between ROP and controls may be in fact a consequence of different premorbid characteristics of those FEP with past cannabis use, rather than a direct effect of exogenous cannabinoids on inflammatory processes in all FEP. According to this hypothesis, our results would suggest that the subset of ROP with cannabis use would represent a population with even higher neuroinflammatory processes, which would co-occur but not interact with cannabis use. Given the fact that no baseline differences in the score of the premorbid adjustment scale were found between ROP with and without cannabis use, our study does not seem to support this hypothesis.

Notably, the majority of our ROP sample had been exposed to antipsychotic drugs, and thus, one could hypothesize that antipsychotic exposure, and not psychosis illness, would be underlying these differences. However, we found that, within the group of ROP, the association between antipsychotic exposure on FW varies depending on past cannabis use. Individuals with no cannabis use exhibited lower FW along with antipsychotic exposure, whereas individuals with past cannabis use showed no difference or even higer FW. This was true for both gray matter and white matter (see figure 3). To put it differently, exposure to antipsychotic drugs was associated to decreased FW when there was no cannabis use, whereas this antipsychotic–related effect was not present in those individuals with past cannabis use. Interestingly, when measuring recent cannabis exposure through quantification of THC in biological matrices (Table S6 and figure S1d in supplementary material), the interaction with antipsychotic

exposure was significant for both past and present cannabis use in GM. This suggests that cannabis in ROP, contrary to promoting an anti-inflammatory effect as observed in controls, may be associated with changes in the anti-inflammatory properties of antipsychotics in the brain. We have previously demonstrated an association between elevated FW and treatment response in first-episode psychosis (51) suggesting that some level of FW elevation during the early phases of psychosis may be associated with a higher likelihood of anti-inflammatory response to treatment (which typically includes antipsychotics). This is in agreement with our results showing antipsychotic exposure inversely correlated to FW in non-cannabis users. The absence of this association within ROP individuals with cannabis use might suggest that cannabis is is influencing the anti-psychotic response. These findings align with previous evidence showing hampering effects of cannabis on antipsychotic response (34,82) and might contribute to elucidate why cannabis use in psychosis is related to increased relapse rates (83). The potential mechanisms by which cannabis could alter antipsychotic function may involve alteration of antipsychotic metabolism (84), modification of its transportation across the blood-brain barrier (35), altering the functionality of targeted receptors of medications (85), or, as suggested by our results, an interference of anti-inflammatory effect of antipsychotic drugs. A schematic conceptual model summarizing these hypothesized interactions is presented in Figure 5.

Figure 5 here

Our findings support the notion that lowering inflammation is part of the mechanisms related to antipsychotic response and not merely a confounding factor by aligning with previous research both in periphery and brain tissue (86–89).

To the best of our knowledge, our study is the first to shed light on the relationship between the influence of cannabis on the efficacy of antipsychotic drugs and neuroinflammation in psychosis. Our findings offer an alternative interpretation concerning the interference of cannabis on antipsychotic response, suggesting that cannabis may be associated with changes in the beneficial effect of antipsychotic on inflammatory processes in psychosis.

Our research counts with several strengths such as a relatively large sample size specifically including subjects with ROP and the use of a single multi-shell MRI system. Additionally, we conducted a thorough exploration of cannabis use and its temporal pattern.

The findings should be interpreted within the scope of study limitations. Firstly, the cross-sectional design of the study limits the ability to draw causal inferences or determine temporal sequence between cannabis use and FW alterations. Because exposure, imaging, clinical and treatment variables were assessed at the same time, directionality cannot be presumed and potentially cannabis exposure could precede, follow, or share common determinants with FW alterations. Longitudinal studies with repeated imaging and detailed substance use could clarify potential directionality suggested by our research.

Additionally, our sample included patients with recent-onset psychosis (ROP) who did not have a definitive diagnosis, as most cases were pending longitudinal assessment. Therefore, there is a potential heterogeneity regarding clinical features associated with affective psychosis. Moreover, while the sample size is reasonable for the scope of this study, statistical power for detecting interaction effects and subgroup differences may be limited, particularly due to the heterogeneity within the ROP group. Secondly the interpretation of our results hinges on the assumption of the increase in FW as a surrogate measure of neuroinflammation. Although FW increase in brain parenchyma is common in other pathological processes like edema and degeneration, FW imaging alteration has been previously related to presence of inflammatory

metabolites such as glutathione (38) and could reasonably be interpreted as an approximation to neuroinflammation. Thirdly, despite including a comprehensive evaluation of cannabis use, composition and potency of the substance were not examined. This could represent a potential limitation, given that THC/CBD ratio has been postulated as a relevant determinant of cannabis impact on psychosis course (90,91) and a differential effect of both components in brain function has been proposed (92). Lastly, the reliance on self-reported cannabis use is another limitation. However, we were able to replicate the findings by biological measure in a subset of participants using THC hair tests, which adds validity to our results.

Our study represents, to the best of our knowledge, the first work to approach the relevance of cannabis in psychosis using FW as an in-brain inflammatory proxy. The finding of higher FW in patients with cannabis use, not observed in control subjects suggests an association between cannabis use and alterations in inflammatory system in early psychosis. Moreover, the potential association of cannabis use with an alteration of FW reduction mediated by antipsychotic treatment provides a novel insight underlying poorer outcome in psychotic users. These findings support the hypothesis of a potential impact of exposure to cannabis on the inflammatory processes already proposed in psychosis and highlight the need of further research on the interplay of these variables, particularly focusing on the endocannabinoid system. Future investigations of FW in early stages of psychosis that incorporate additional inflammatory biomarkers and a comprehensive evaluation of cannabis use will offer a clearer understanding of the environmental impact of cannabis in psychosis pathophysiology.

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AUTHOR CONTRIBUTIONS

LMS and DB contributed to the main design of the study, elaboration of the protocol, submitting the project to the local ethical board, recruitment of the sample, clinical assessments, analysis of the results and writing of the manuscript. MBC, PR, EVE and JGQ contributed to the design of the study, collection, processing and analysis of the biological samples, elaboration of the results and writing of the manuscript. AM, AT and AT contributed to the recruitment of the sample, MRI acquisition and neuroimaging preprocessing. TAL and CSC contributed to the analysis of the results and writing of the manuscript.

DATA AVAILABILITY

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

559 All methods were performed in accordance with relevant guidelines and regulations. The
560 study was approved by the Clinical Research Ethics Committee of Hospital del Mar
561 (reference number 2018/7942/I). Written informed consent was obtained from all participants
562 prior to inclusion in the study.

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564 CONFLICT OF INTEREST

565 The authors declare no conflict of interest.

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FIGURE AND TABLE LEGENDS

Table1:

Socio-demographic and clinical characteristics of recent onset psychosis and healthy controls. *frequent use = more than once per week.*

Table2:

Final regression model for Free-Water in Gray Matter. The upper half part of the table shows initial full model to predict average free-water in gray matter. Group (Recent onset psychosis vs. controls), as well as past and present cannabis use, and the interaction between them, were introduced as the main variables of interest. Body mass index (BMI) and education level were introduced as covariates for its between-group differences and the potential association with neuroanatomical characteristics. The lower half of the table shows final full model after step-wise backwards modeling. The surviving variables and its respective coefficients, together with the final model fit measures are displayed.

Figure 1:

Boxplot showing average free-water values in gray matter in ROP and controls, categorized by past cannabis use. The left side of the plot shows values for the control group, displayed from left to right: with past cannabis users (red boxplot), non-users (green boxplot), and a combined group of both past users and non-user controls (gray boxplot),. The right side of the plot presents values for the ROP group, also showing, from left to right, past cannabis users (red boxplot), non-users (green boxplot), and the combined group (gray boxplot).

Figure 2:

Clusters of free-water in gray matter surface by different variables of interest. A) Clusters showing higher free-water in gray matter surface in ROP in comparison to healthy controls (ROP > HC). B) Higher free-water in gray matter surface in past cannabis users in comparison to non-users (Past Cann > No Cann), C) The effect of cannabis use in ROP in comparison to the effect of cannabis use in controls (interaction effect = ($ROP_{Past\ Cann} - ROP_{No\ Cann}$) > ($HC_{Past\ Cann} - HC_{No\ Cann}$)).

Table 3:

Regression models for predictors of Free-Water in Gray matter and White Matter within the group of psychosis. The upper half part of the table shows initial full model within the group of recent onset psychosis, to predict average free-water in in gray matter (left) and white matter (right). Past cannabis use, cumulative exposure to antipsychotic in log transformed miliequivalents of chlorpromazine log transformed (CPZ), Type of disorder (First-episode pscyhosis [FEP] or chronic schizophrenia [SCH]), and the interaction between them, were introduced as the main variables of interest. Duration of untreated psychosis (DUP), duration of illness (DUI), body mass index (BMI), and cognitive reserve (CR) were introduced as covariates for its between-group differences and the potential association with neuroanatomical characteristics. The lower half of the table shows the respective final full models after step-wise backwards modeling. The surviving variables and its respective coefficients, together with the final model fit measures are displayed.

Figure 3:

Scatter plot and regression lines of free-water in gray matter and white mater as a function of past cannabis use and antipsychotic exposure. A) free-water in gray matter as a function of exposure to antipsychotic drugs and categorized by low or frequent past cannabis use. B) free-water in white matter as a function of antipsychotic exposure and categorized by low or

frequent past cannabis use. Free-water values decrease with increasing antipsychotic exposure only in ROP with low or non past cannabis use, whereas ROP with past cannabis use show no decrease or even an increase with increasing antipsychotic exposure.

Figure 4:

Clusters in skeletonized white matter tracts showing a significant effect of the interaction between antipsychotic exposure and past cannabis use. The clusters show white matter tracts where the interaction effect involving a decrease in FW with increasing antipsychotic exposure occurs only in ROP with low or non past cannabis use, whereas ROP with past cannabis use show the opposite effect as shown in figure 3.Tract-Based spatial statistics in FSL, multiple comparisons corrected by TFCE, p-FWE<0.05.

Figure 5:

Conceptual model illustrating the potential interference of cannabis use on antipsychotic-related reduction of extracellular free-water (FW) in GM and WM in patients with recent-onset psychosis. Antipsychotic exposure is associated with decreased FW only in non-users whereas in cannabis users this effect is null or opposite.

Table 1: Socio-demographic and clinical characteristics of recent onset psychosis and healthy controls.

	Healthy Controls N = 38	Recent Onset Psychosis N = 62	Statistic (t-student or Chi-square)	p value
Age, mean years ± sd	24.97 ± 4.51	24.89 ± 4.81	0.091	0,928
Sex, % male	53%	63%	0.647	0.421
Score PANSS Positive, mean ± sd	NA	12.87 ± 5.54	-	-
Score PANSS Negative, mean ± sd	NA	16.38 ± 6.54	-	-

Score PANSS Total, mean ± sd	NA	61.78 ± 16.21	-	-
Score WHO-DAS, mean ± sd	1.39 ± 3.24	9.77 ± 8.38	-7.060	<0.001
Current cannabis use, frequent use *	61 %	23 %	12.971	<0.001
Past cannabis use, frequent use *	63%	60 %	0.018	0.893
Body mass index, mean ± sd	22.67 ± 3.09	24.55 ± 5.34	-2.226	0.028
Years of education, mean ± sd	16.18 ± 3.68	13.02 ± 3.65	4.192	<0.001
Educational level (grad studies)	58%	19%	13.924	<0.001
Maximum parental educational level (grad studies)	63%	27%	11.006	<0.001

* frequent use = more than once per week

Table 2: Final regression model for Free-Water in Gray Matter

Initial model, regression estimates

	Estimate (B)	**Estimate (B) 95 % CI**	**t value**	***p***
Past cannabis use	1.241	-0.052 to 2.534	1,919	0.058
Group	1.668	0,508 to 2.828	2.876	0.005
Frequency of recent cannabis use	0.015	-0,026 to 0.056	0.745	0.459
BMI	-0.019	-0.096 to 0.059	-0.486	0.628
Education level	0.180	-0.119 to 0.479	1.204	0.232
Past cannabis x Group	-1.556	-3.118 to 0.007	-1.991	0.049
Group x frequency of recent cannabis use	0.022	-0,045 to 0.079	0.789	0.432

Initial model, model fit measures

Residual standard error	Multiple R-squared	Adjusted R-squared	F statistic	DF	*p*
1.697	0.150	0.085	2.310	7, 92	0.032

Final model, regression estimates

	Estimate (B)	**Estimate (B) 95 % CI**	**t value**	***p***
Past cannabis use	1.520	0.319 to 2.721	2.532	0.013 *
Group	1.562	0.654 to 2.470	3.443	<0.001 *
Frequency of recent cannabis use	0.025	-0.003 to 0.053	1.808	0.074 .
Past cannabis x Group	-1.732	-3.184 to -0.279	-2.384	0.019 *

Final model, model fit measures

Residual standard error	Multiple R-squared	Adjusted R-squared	F statistic	DF	*p*

1.694	0.126	0.089	3.410	4, 95	0.012

The upper half part of the table shows initial full model to predict average free-water in gray matter. Group (Recent onset psychosis vs. controls), as well as past and present cannabis use, and the interaction between them, were introduced as the main variables of interest. Body mass index (BMI) and education level were introduced as covariates for its between-group differences and the potential association with neuroanatomical characteristics. The lower half of the table shows final full model after step-wise backwards modeling. The surviving variables and its respective coefficients, together with the final model fit measures are displayed.

Table 3: Regression models for predictors of Free-Water in Gray matter and White Matter within the group of psychosis

A. Gray matter, Initial model, regression estimates

Variables	Estimate (B)	t value	*p*
Past cannabis use	3.974	1.275	0.208
CPZ	0.746	2.080	0.043
FEP vs SCH	-1.580	-0.239	0.0812
Frequency of recent cannabis use	0.223	1.496	0.141
DUP	0.001	0.584	0.562
DUI	-0.001	-1.637	0.108
BMI	-0.062	-1.343	0.186
CR	0.176	0.959	0.342
Past cannabis use x CPZ	-0.427	-1.490	0.142
ROP vs SCH x CPZ	0.297	0.511	0.612
Frequency of cannabis use x CPZ	-0.018	-1.300	0.200

Initial model, model fit measures

Residual standard error	Multiple R-squared	Adjusted R-squared	F statistic	DF	*p*
1.622	0.272	111	1.691	11, 50	0.103

Final model, regression estimates

Variables included	Estimate (B)	Estimate (B) 95 % CI	t value	*p*
Past cannabis use	4.738	-1.190 to 10.667	1.599	0.116
CPZ	0.871	0.190 to 1.553	2.557	0.013 *
Past cannabis x CPZ	-0.499	-1.044 to 0.047	-1.828	0.073 .
FEP vs SCH	1.930	-0.060 to 3.945	1.940	0.058 .
DUI	-0.001	-0.002 to <-0.001	-2.544	0.014 *
BMI	-0.090	-0.176 to -0.005	-2.106	0.040 *

Final model, model fit measures

B. White matter, Initial mo

Variables
Past cannabis use
CPZ
ROP vs SCH
Frequency of recent cannabis use
DUP
DUI
BMI
CR
Past cannabis use x CPZ
ROP vs SCH x CPZ
Frequency of cannabis use x CPZ

Initial model, model fit

Residual standard error	Multiple R-squared	Adjuste R-squar
1.753	0.223	0.033

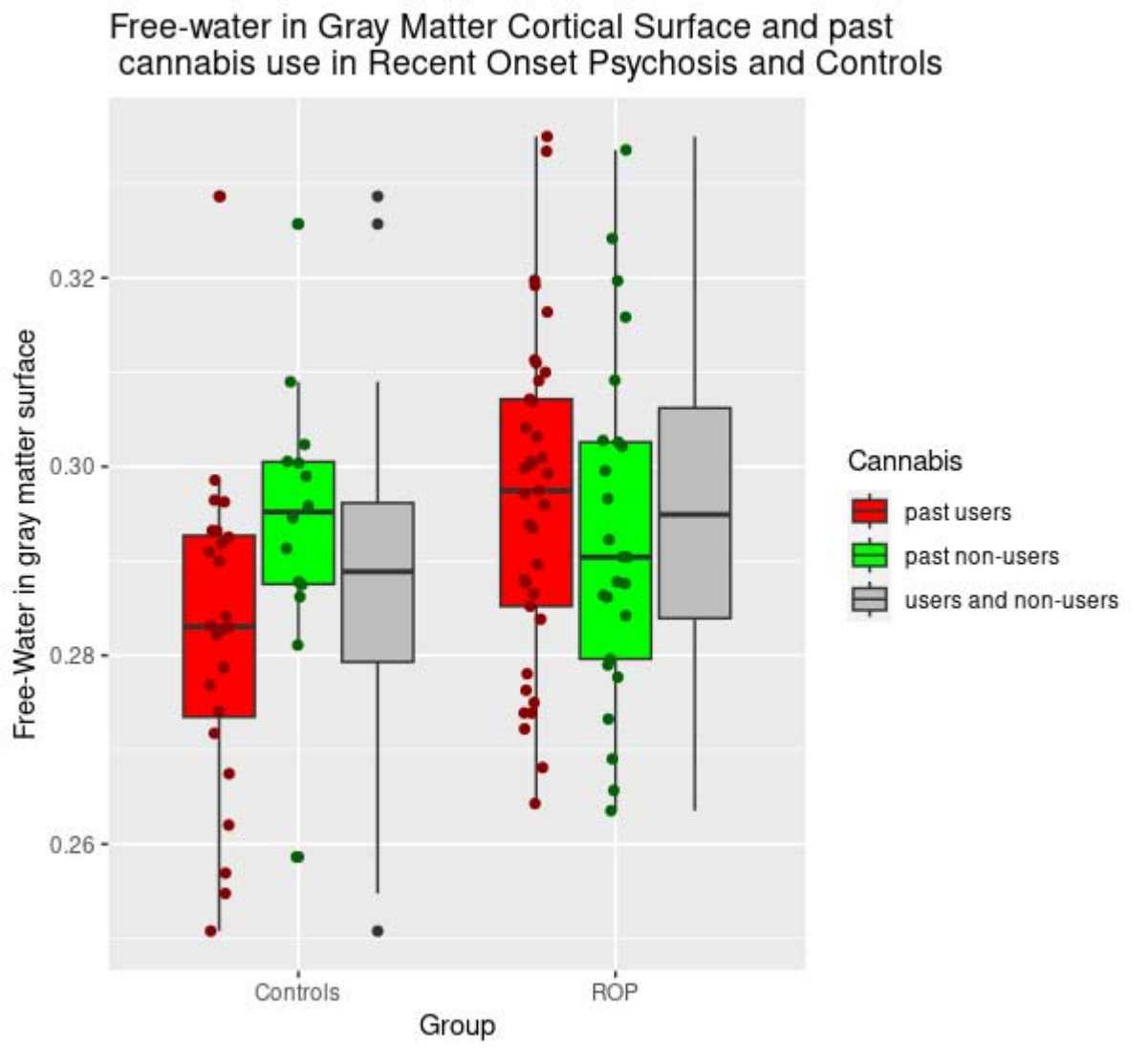
Final model, regression

Variables included	Estima (B)
Past cannabis use	8.348
CPZ	0.834
Past cannabis x CPZ	-0.749

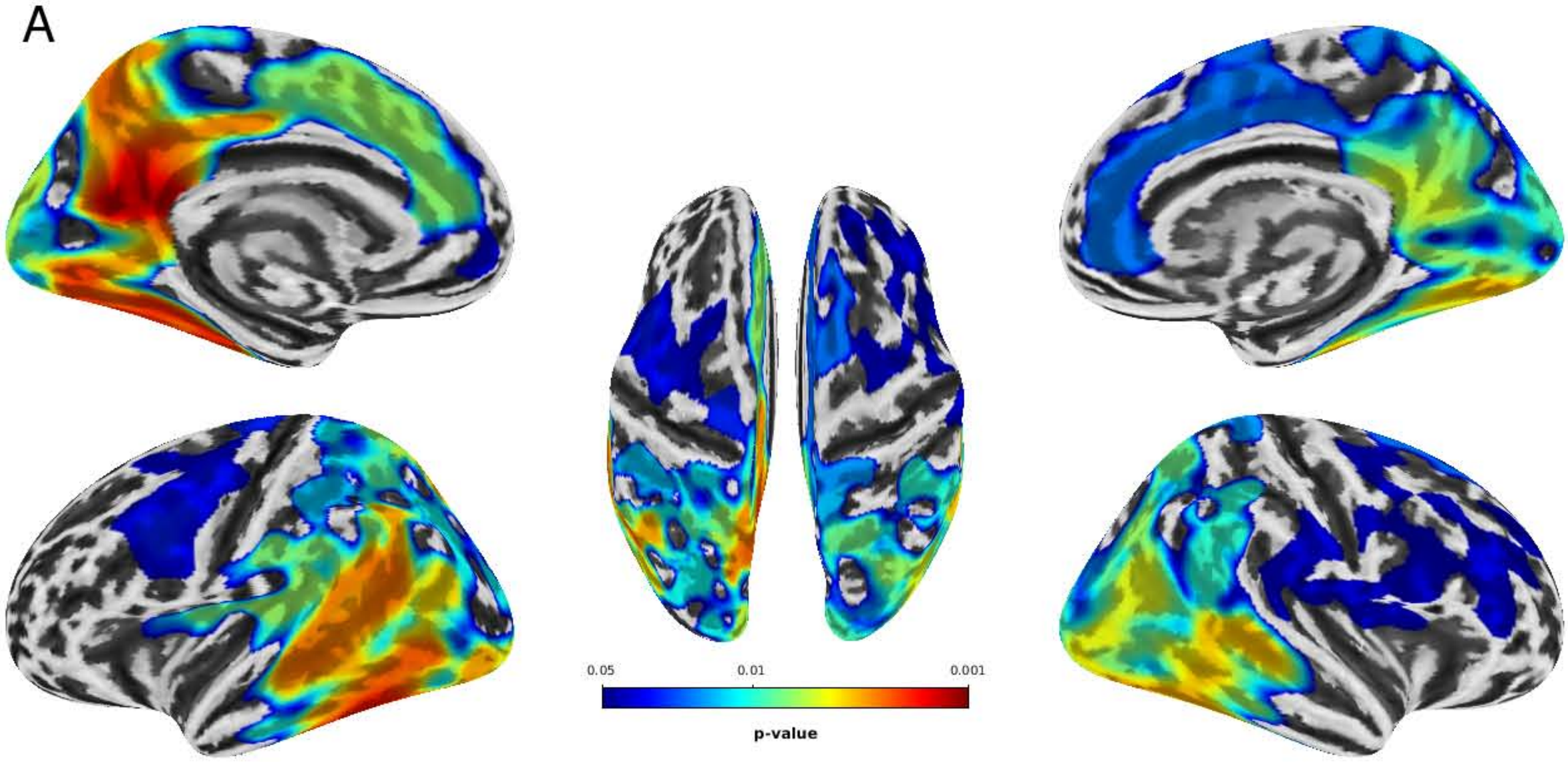
Final model, model fit

Residual standard error	Multiple R-squared	Adjusted R-squared	F statistic	DF	*p*	Residual standard error	Multiple R-squared	Adjuste R-squar
1.621	0.202	0.115	2.324	6, 55	0.045 *	1.702	0.113	0.088

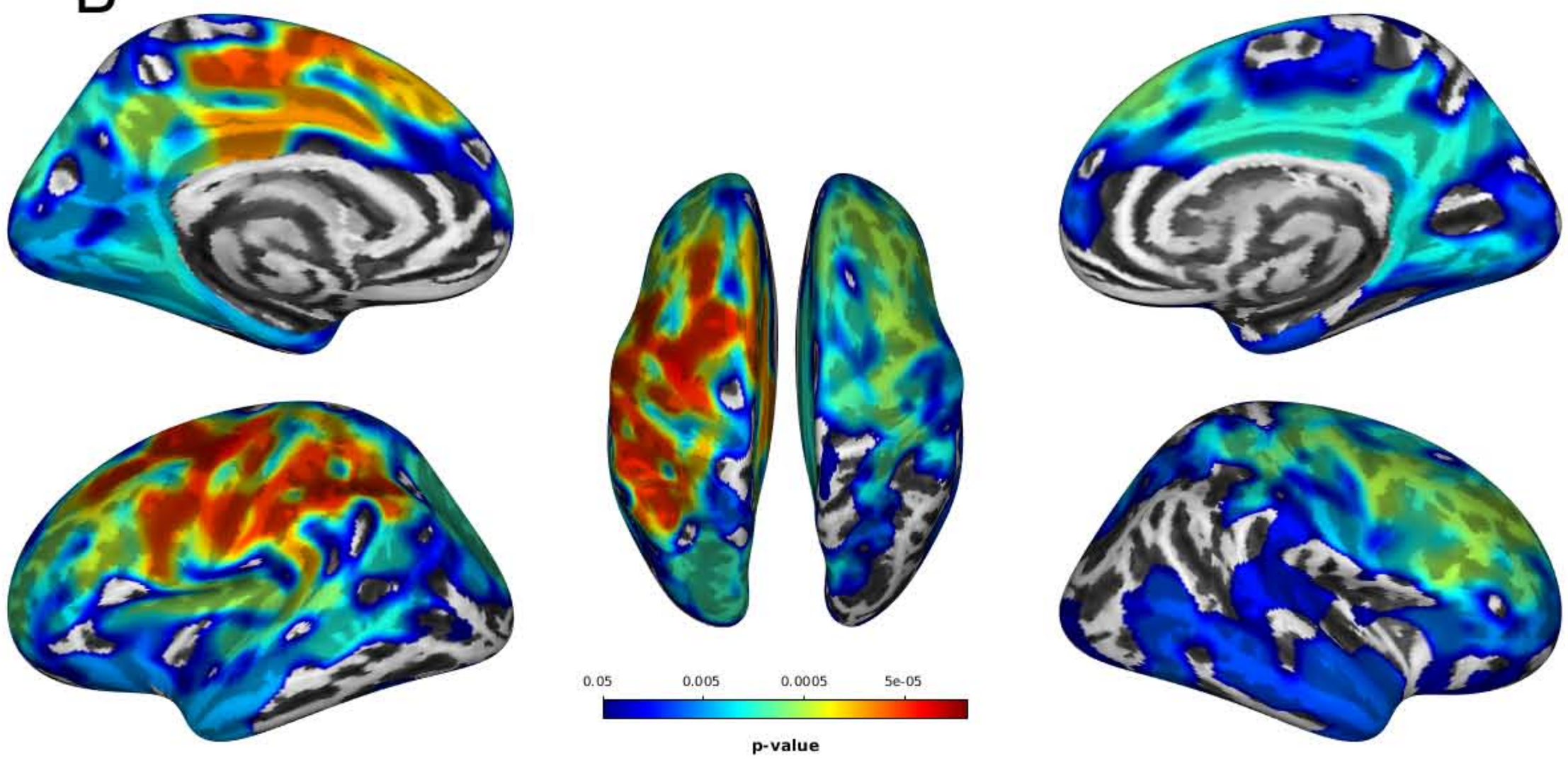
The upper half part of the table shows initial full model within the group of recent onset psychosis, to predict average free-water in in gray matter (left) and white matter (right). Past cannabis use, cumulative exposure to antipsychotic in log transformed miliequivalents of chlorpromazine log transformed (CPZ), Type of disorder (First-episode pscyhosis [FEP] or chronic schizophrenia [SCH]), and the interaction between them, were introduced as the main variables of interest. Duration of untreated psychosis (DUP), duration of illness (DUI), body mass index (BMI), and cognitive reserve (CR) were introduced as covariates for its between-group differences and the potential association with neuroanatomical characteristics. The lower half of the table shows the respective final full models after step-wise backwards modeling. The surviving variables and its respective coefficients, together with the final model fit measures are displayed.


Free-water in Gray Matter Cortical Surface and past cannabis use in Recent Onset Psychosis and Controls
Free-Water in gray matter surface
0.32
0.30
0.28
0.26
Controls
ROP
Group
Cannabis
past users
past non-users
users and non-users

A


0.05
0.01
0.001
p-value

B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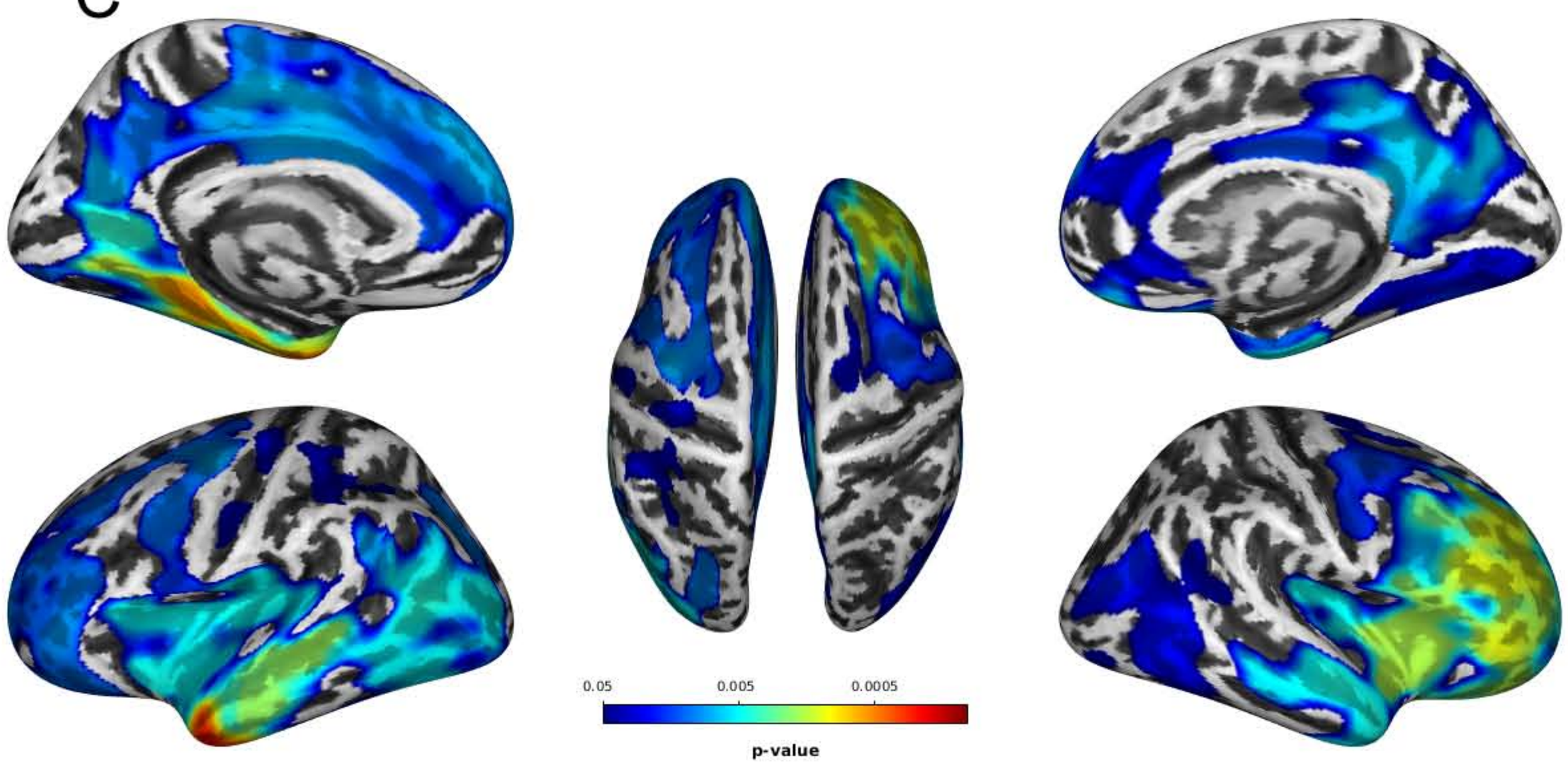
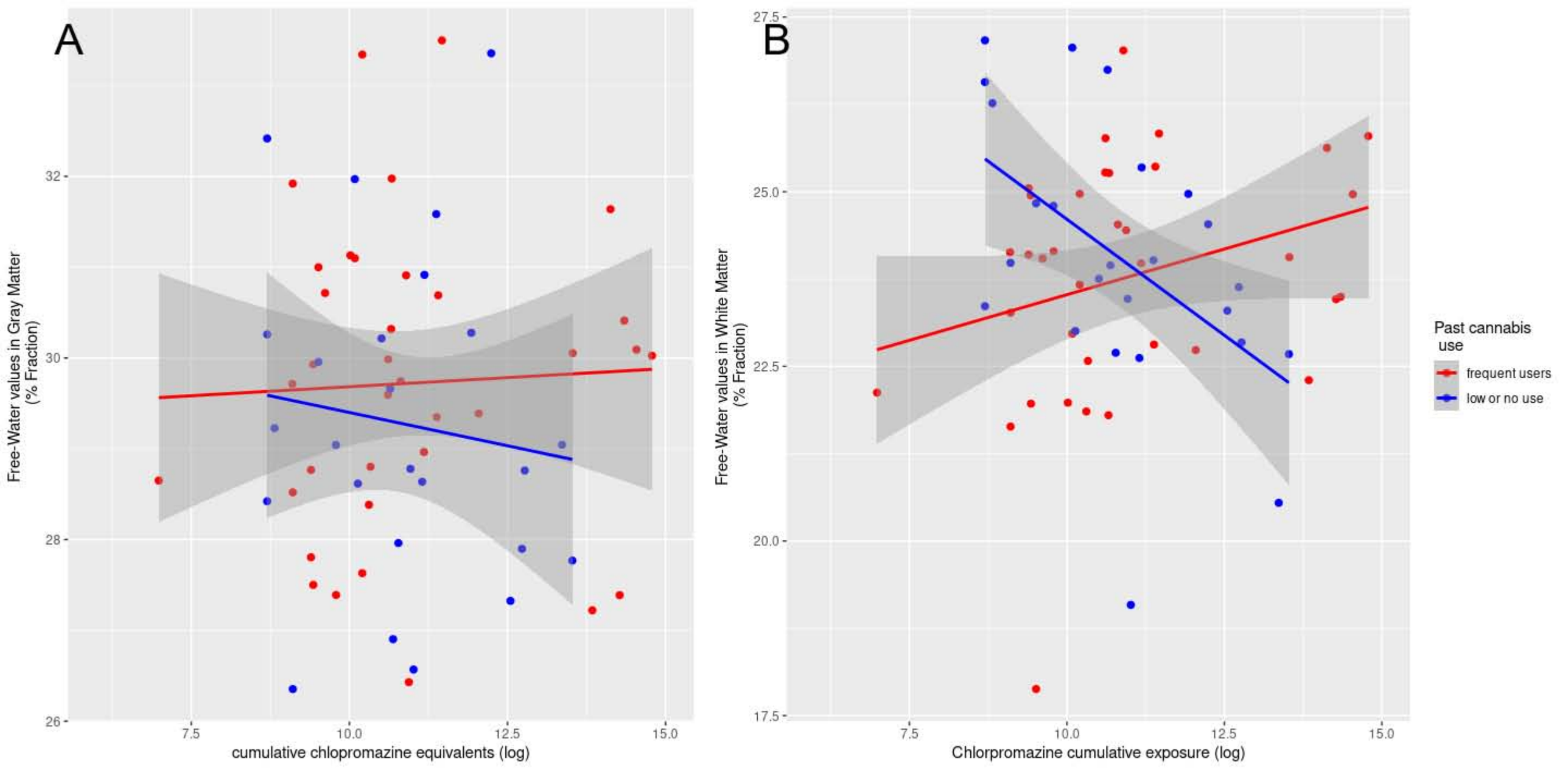
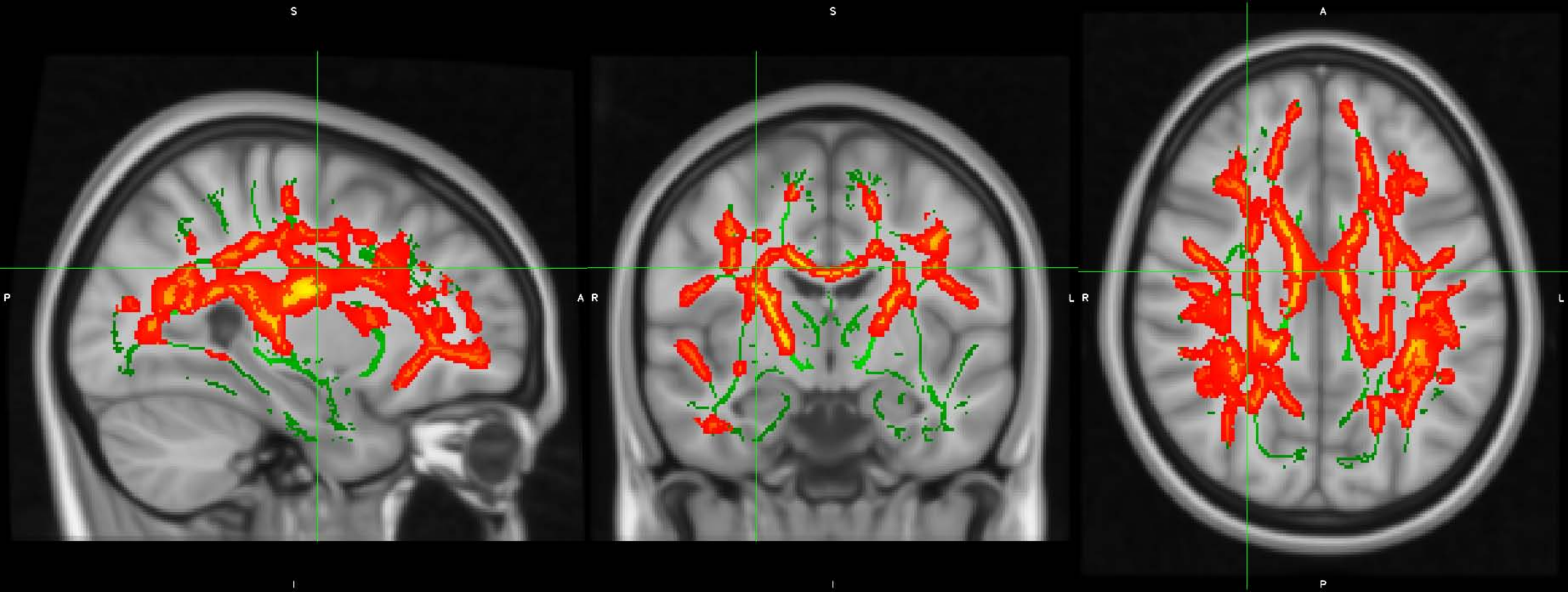
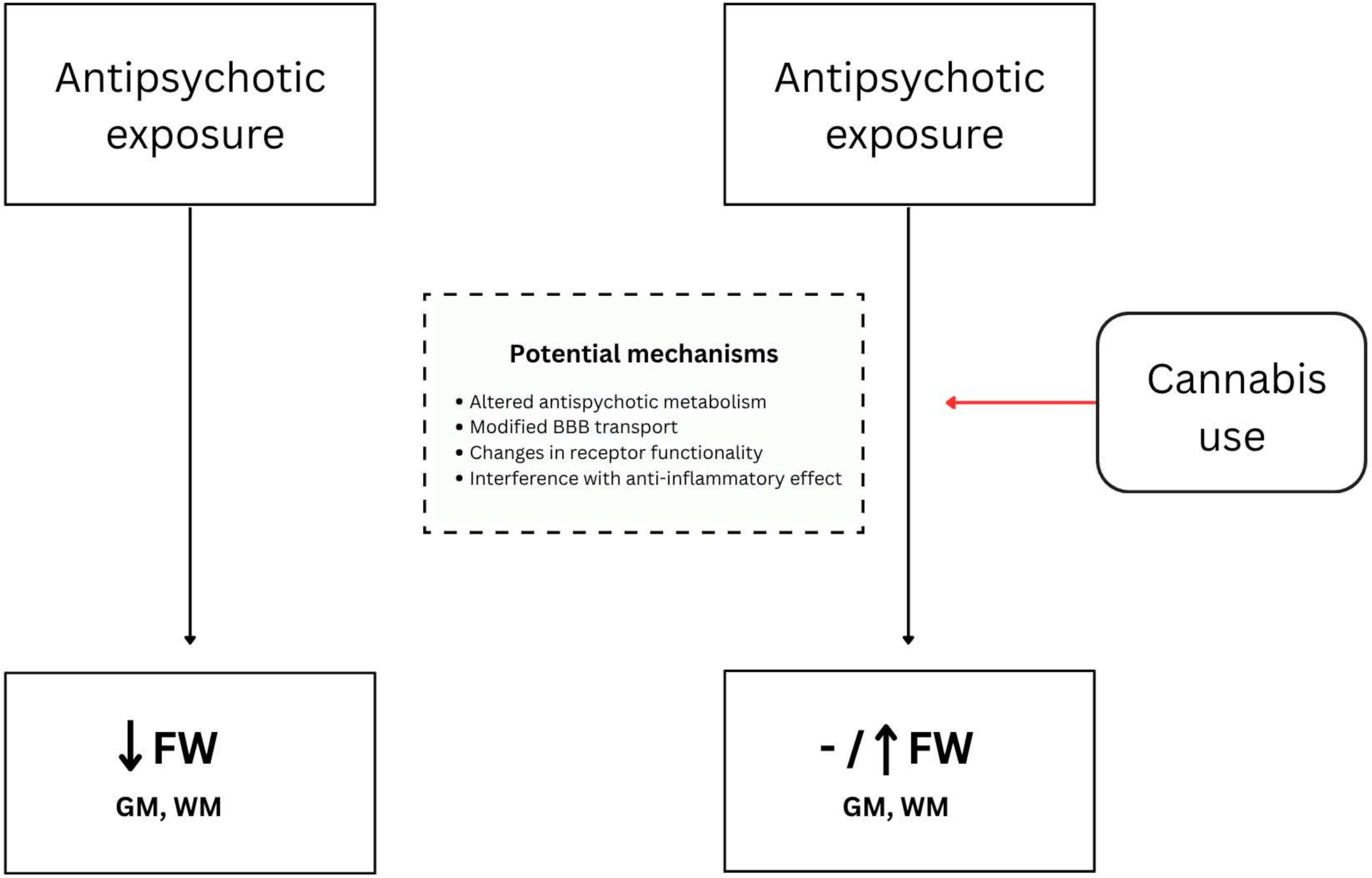
0.005
0.0005
5e-05
p-value

C


0.05
0.005
0.0005
p-value


A
B
Free-Water values in Gray Matter
(% Fraction)
Free-Water values in White Matter
(% Fraction)
cumulative chlopromazine equivalents (log)
Chlorpromazine cumulative exposure (log)
Past cannabis use
frequent users
low or no use
26
28
30
32
17.5
20.0
22.5
25.0
27.5
7.5
10.0
12.5
15.0


S
P
A R
I
S
L R
I
A
L
P


Antipsychotic exposure
Antipsychotic exposure
Potential mechanisms
• Altered antispychotic metabolism
• Modified BBB transport
• Changes in receptor functionality
• Interference with anti-inflammatory effect
Cannabis use
↓ FW
GM, WM
- / ↑ FW
GM, WM