

## Review Article

# Is cannabis neurotoxic for the healthy brain? A meta-analytical review of structural brain alterations in non-psychotic users

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**Aims:** Despite growing research in the field of cannabis imaging, mostly in those with a psychotic illness, the possible neurotoxic effects of smoked cannabis on the healthy brain have yet to be fully understood. There appears to be a need to evaluate the existing imaging data on the neuroanatomical effects of cannabis use on non-psychotic populations.

**Methods:** We conducted a meta-analytical review to estimate the putative neurotoxic effect of cannabis in non-psychotic subjects who were using or not using cannabis. We specifically tested the hypothesis that cannabis use can alter grey and white matter in non-psychotic subjects.

**Results:** Our systematic literature search uncovered 14 studies meeting the inclusion criteria for the meta-analysis. The overall database comprised 362 users and 365 non-users. At the level of the individual

studies there is limited and contrasting evidence supporting a cannabis-related alteration on the white and grey matter structures of non-psychotic cannabis users. However, our meta-analysis showed a consistent smaller hippocampus in users as compared to non-users. Heterogeneity across study designs, image acquisition, small sample sizes and limited availability of regions of interest to be included in the meta-analysis may undermine the core findings of this study.

**Conclusions:** Our results suggest that in the healthy brain, chronic and long-term cannabis exposure may exert significant effects in brain areas enriched with cannabinoid receptors, such as the hippocampus, which could be related to a neurotoxic action.

**Key words:** amygdala, cannabis, hippocampus, neuroimaging, psychosis.

OVER THE PAST 2 decades, available imaging techniques have allowed researchers to carefully address the neurobiology of cannabinoids by employing functional or structural methods. One of the first functional magnetic resonance imaging

(fMRI) studies carried out on healthy volunteers with less than 25 times cannabis life-time use directly compared delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD),<sup>1,2</sup> two major compounds of the plant, and found distinct modulatory effects on regional neural responses to fearful faces.<sup>3</sup> Specifically, the authors observed a CBD-induced attenuation of neural responses to intensely fearful faces in the amygdala and cingulate cortex, which was correlated with an electrophysiological response and behavioral evidence for an anxiolytic effect. There

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was also a distinct effect for CBD on the brain connectivity linking these two regions.<sup>4</sup> In subsequent fMRI studies, THC and CBD were found to have opposing effects on striatal activation during verbal recall, on hippocampal activation during response inhibition, on amygdala activation in response to fearful faces, on temporal activation during an auditory task, and on occipital activation during visual processing.<sup>5,6</sup> A further recent experiment in the same study showed that pre-treatment with CBD prevented acute induction of THC-induced psychotic symptoms compared to pre-treatment with placebo, and suggested that THC and CBD can have opposing effects on regionally-specific brain activation, which may underlie their different symptomatic and behavioral effects.<sup>7</sup>

While these studies have allowed the investigation of the neurofunctional circuitries underlying the psychopharmacological properties of cannabinoids, the effect on the healthy brain structure is still highly debatable.<sup>8</sup> A number of individual studies have been published addressing structural abnormalities in cannabis users, but the results are conflicting because of methodological and sampling differences.<sup>8</sup> In particular, the effect of cannabis use on the brain structure may be confounded by the underlying disorders (for a comprehensive review of the effect of cannabis in psychotic patients see Rapp *et al.*<sup>9</sup>). In particular, there is compelling evidence that psychotic disorders can impact brain neuroanatomy independent from cannabis exposure.<sup>10</sup> The final common effects that could be related with cannabis exposure and that can impact brain structures or functioning can broadly be termed as putative 'neurotoxic'. A number of different mechanisms encompassing alterations during the neurogenesis and neuroplasticity processes and dysregulation of the endocannabinoid system may ultimately interplay to cause the neuroanatomical alterations associated with cannabis exposure (for a comprehensive review see Hermann *et al.*<sup>11</sup>). The neuroimaging correlates of cannabis exposure have been recently summarized in systematic reviews (see for example Jager *et al.*,<sup>12</sup> Martin-Santos *et al.*,<sup>13</sup> Lorenzetti *et al.*<sup>8</sup>). However, to our best knowledge, no study has ever addressed in a quantitative fashion the effect of cannabis exposure in non-psychotic users.

We present here a meta-analysis of structural imaging studies in non-psychotic cannabis users. We clearly selected studies enrolling subjects who were without a diagnosis of any psychotic disease accord-

ing with DSM-IV and ICD-10 criteria, and who were using or not using cannabis. The meta-analytic method allowed us to quantify the consistency of individual contrasting studies and to address the associated heterogeneity by controlling for different confounders. We specifically tested the hypothesis that cannabis abuse can exert an action on the healthy brain structures, resulting in volume alteration of grey or white matter in cortical and subcortical brain regions in non-psychotic users.

## METHODS

### Search strategies

A systematic search strategy was used to identify potential relevant studies. Three independent researchers (P.F.-P., M.R. and A.C.) conducted a two-step literature search. First, we carried out a Web of Knowledge search (which includes different databases, such as Medline and Web of Science) to identify putative studies employing structural neuroimaging techniques that had reported data on non-psychotic cannabis users and matched controls. The search was conducted between January and February 2013, and no time-span was specified for date of publication. We employed the following keywords: MRI, DTI, VBM, cannabis, neuroimaging, structural, grey matter, white matter. Three reviewers independently reviewed the database and extracted the data in order to avoid bias or error in the selection of articles and by the extraction of data from studies. Discrepancies in inclusion and exclusion criteria were resolved through discussion and consensus. In a second step the reference lists of the articles included in the review were additionally checked for relevant studies not identified by computerized literature searching. All reports published until February 2013 were included, without any language restriction, though all included papers were in English. To achieve a high standard of reporting we have adopted Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines<sup>14</sup> and the revised Quality of Reporting of Meta-analyses (QUOROM) Statements.<sup>15</sup>

### Inclusion criteria

To qualify for inclusion in the review, studies must have: (i) been an original paper or a short communication and appeared in a peer-reviewed journal;

(ii) recruited cannabis-user subjects without a diagnosis of DSM/ICD psychosis and matched controls; (iii) employed structural imaging techniques (structural magnetic resonance imaging [sMRI] with whole brain automated analyses [VBM] or region of interest on different brain volumes [ROI]); and (iv) reported sufficient data to allow meta-analytical computations. Studies were independently ascertained and checked by the two researchers and inclusion and exclusion criteria were evaluated by consensus.

### Exclusion criteria

We excluded from our review studies that: (i) enrolled subjects with a diagnosis of a psychotic disorder according to DSM or ICD criteria; (ii) included overlapping samples; (iii) were systematic or critical reviews; and (iv) did not report enough data to be included in the meta-analysis. We did not exclude samples that included non-psychotic subjects presenting with incidental comorbidities (both psychiatric and medical) other than psychosis.

### Data extraction

We have appended summary tables of all included structural studies to assist the readers in forming an independent view on the core results. The recorded variables for each article were: imaging technique (sMRI), imaging analysis (whole-brain/ROI), duration of cannabis use, age when the regular use began (as estimated by the individual studies), incidental psychiatric comorbidities other than psychosis, magnetic field strength, proportion of female subjects, mean age of participants, ROI analyzed and principal findings.

### Meta-analyses

The primary outcomes of interest were global/regional volumes for structural alterations in non-psychotic cannabis users (CU+) versus non-users (CU-). Whole brain volume (WBV) was defined as the sum of the volume of all voxels designated as grey matter volume (GMV) and white matter volume (WMV). Intracranial volume (ICV) was defined as the volume resulting from WBV plus cerebrospinal fluid (CSF).<sup>16</sup> We recorded all the ROI investigated from any study. Meta-analyses were conducted when at least three independent studies were available for a preselected ROI (see below). Where there were two or more studies from the same centre, we have carefully

checked putative overlapping samples by directly contacting the authors to verify there was not a significant overlap in the samples. Statistical analysis was carried out using Comprehensive Meta-Analysis Software version 2 (Biostat, Englewood, NJ, USA).<sup>17</sup> This package employs the same computational algorithms used by the Cochrane Collaborators to weight studies. The effect size was calculated for each study included in the meta-analyses. As a measure of effect size, Hedges' *g* was adopted, that is, the difference between the means of the CU+ and CU- groups, divided by the SD and weighted for sample size, in order to correct for bias from small sample sizes.<sup>18</sup> This metric is normally computed by using the square root of the mean square error from the ANOVA testing for differences between the two groups.<sup>18</sup>

We applied both fixed- and random-effects models depending on the observed heterogeneity. Random-effect models are suitable when there is a relevant heterogeneity because they are more conservative than fixed-effect models, and argued to better address heterogeneity between studies and study populations, allowing for greater flexibility in parsing effect size variability.<sup>19</sup> Moreover, they are less influenced by extreme variations in sample size.<sup>20</sup> Heterogeneity among studies was assessed with the *Q* statistic<sup>21</sup> with magnitude of heterogeneity being evaluated with the *I*<sup>2</sup> index.<sup>22</sup> The *Q* statistic was also used to determine between-group differences. To determine whether categorical factors modified the progressive brain changes, subgroup analyses were performed.<sup>21</sup> The influence of continuous moderator variables was tested using meta-regression analyses. The slope of meta-regression ( $\beta$ -coefficient: direct (+) or inverse (-)) of the regression line indicates the strength of a correlation between moderator and outcome. The possibility of publication bias was examined by visually inspecting funnel plots and applying the regression intercept of Egger *et al.*<sup>23</sup> In this way we assessed whether there was a tendency for selective publication of studies based on the nature and direction of their results. In addition, we used the fail-safe procedure<sup>24</sup> to generate a number of unpublished studies that would be needed to move estimates to a non-significant threshold. If a publication bias was found, we also applied Duval and Tweedie's 'trim and fill' procedure, which allowed us to control the meta-analytical estimates for publication biases.<sup>25</sup> To assess the robustness of the results, we performed sensitivity analyses by sequentially removing each study and rerunning the analysis.

## RESULTS

### Database

Fourteen studies published between 2000 and 2013 met the inclusion criteria. The database included 362 non-psychotic cannabis users and 365 non-users (mean age of participants was respectively 25.1 and 23.7 years). The details of the included studies are summarized in Table 1.<sup>26–39</sup>

### ROI analyzed

As stated in the Method section, meta-analyses were performed when at least three studies were available for a given ROI and we performed meta-analyses for the following regions: (i) ICV (Batalla *et al.*,<sup>26</sup> Schacht *et al.*,<sup>27</sup> Cousijn *et al.*,<sup>30</sup> McQueeney *et al.*,<sup>32</sup> Mata *et al.*,<sup>33</sup> Yucel *et al.*,<sup>34</sup> Medina *et al.*,<sup>35,36</sup> Block *et al.*,<sup>39</sup>); (ii) WBV (Zalesky *et al.*,<sup>28</sup> Ashtari *et al.*,<sup>29</sup> Lopez-Larson *et al.*,<sup>31</sup> Mata *et al.*,<sup>33</sup> Yucel *et al.*,<sup>34</sup> Delisi *et al.*,<sup>37</sup> Tzilos *et al.*,<sup>38</sup> Block *et al.*,<sup>39</sup>); (iii) left amygdala (Schacht *et al.*,<sup>27</sup> Ashtari *et al.*,<sup>29</sup> McQueeney *et al.*,<sup>32</sup> Yucel *et al.*,<sup>34</sup>); (iv) right amygdala (Schacht *et al.*,<sup>27</sup> Ashtari *et al.*,<sup>29</sup> McQueeney *et al.*,<sup>32</sup> Yucel *et al.*,<sup>34</sup>); (v) left hippocampus (Schacht *et al.*,<sup>27</sup> Ashtari *et al.*,<sup>29</sup> Yucel *et al.*,<sup>34</sup> Medina *et al.*,<sup>35,36</sup> Tzilos *et al.*,<sup>38</sup>); and (vi) right hippocampus (Schacht *et al.*,<sup>27</sup> Ashtari *et al.*,<sup>29</sup> Yucel *et al.*,<sup>34</sup> Medina *et al.*,<sup>35,36</sup> Tzilos *et al.*,<sup>38</sup>).

### Meta-analysis

#### ICV

No significant differences were observed in ICV between CU+ and CU– (Hedges'  $g = -0.024$ ; 95% confidence interval [CI],  $-0.198$ – $0.150$ ;  $P = 0.785$ ; fixed models applied).

#### WBV

The formal meta-analysis did not uncover significant differences in WBV between CU+ and CU– (Hedges'  $g = 0.087$ ; 95%CI,  $-0.390$ – $0.564$ ;  $P = 0.721$ ; random models applied).

#### Amygdala

There were no side-effects (left: Hedges'  $g = -0.273$ ; 95%CI,  $-0.559$ – $0.013$ ;  $P = 0.061$ ; right: Hedges'  $g = -0.352$ ; 95%CI,  $-0.729$ – $0.026$ ;  $P = 0.068$ ; random models applied). When the amygdaloid was

considered as a whole, we detected significant reductions in CU+ vs CU– (Hedges'  $g = -0.302$ ; 95%CI,  $-0.529$  to  $-0.074$ ;  $P = 0.009$ ; random models applied), but this effect was underlined by significant publication biases (see below).

### Hippocampus

There was a significant grey mater reduction in the whole hippocampus (left and right) in CU+ as compared to CU– (Hedges'  $g = -0.439$ ; 95%CI,  $-0.777$  to  $-0.101$ ;  $P = 0.011$ , random models applied; Fig. 1). However, no significant differences were observed in the right or left sides (left, Hedges'  $g = -0.470$ ; 95%CI,  $-0.970$ – $0.030$ ;  $P = 0.065$ , random models applied; right, Hedges'  $g = -0.412$ ; 95%CI,  $-0.871$ – $0.046$ ;  $P = 0.078$ ; random models applied). These findings were not affected by significant publication biases (see below).

### Test for heterogeneity, publication bias, and sensitivity analysis

According to the criteria set by Higgins and Thompson,<sup>40</sup> heterogeneity in the amygdala meta-analysis was not statistically significant ( $Q = 8.798$ ;  $P = 0.268$ ;  $I^2 = 20.43$ ). As visual inspection of funnel plot revealed possible evidence of publication bias, we performed a quantitative evaluation as measured by the Egger intercept. The analysis revealed significant publication bias ( $P = 0.043$ ). When we applied Duval and Tweedie's trim and fill procedure,<sup>25</sup> the observed differences in the amygdala were no more significant (Hedges'  $g = -0.159$  with a 95%CI of  $-0.331$ – $0.013$ ).

Heterogeneity in the hippocampal volumes was significant and moderate in magnitude ( $Q = 36.33$ ;  $P < 0.001$ ;  $I^2 = 69.73$ ). From a visual inspection of the funnel plot, there was no evidence of publication bias, in agreement with the quantitative evaluation of Egger's intercept ( $P = 0.297$ ). The fail-safe procedure estimated that 59 unpublished studies would be needed to bring the overall meta-analytic estimate of hippocampal volumes to be non-significant. As heterogeneity across hippocampal studies was found to be significant, we performed meta-regressions to address the possible influence of potential moderators.

### Meta-regressions

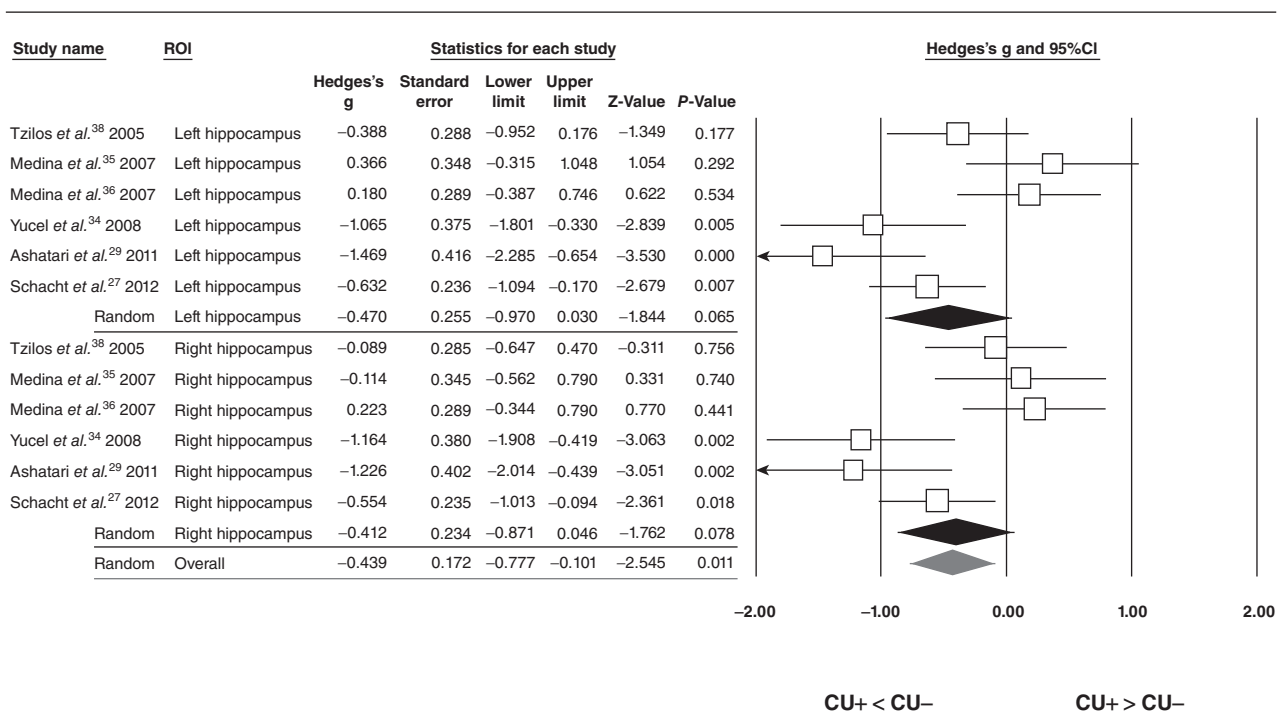
We considered as potential moderators the duration of regular use (years), the percentage of female



**Table 1.** Structural studies in non-psychotic subjects with (CU+) and without (CU-) cannabis use, included in the meta-analysis. The table reports the neuroanatomical findings in the regions of interest analyzed

Author	Year	Imaging technique	Duration of cannabis use (years)	CU+		CU-		Volumes analyzed	Main findings: CU+ vs CU-
				n	Age	n	Age		
Batalla <sup>26</sup>	2013	MRI (VBM)	5.9	29	21	28	22	ICV	No significant differences in ICV between CU+ and CU-.
Schacht <sup>27</sup>	2012	MRI	10.1	37	28	37	27	ICV L Amy R Amy L Hip R Hip	No significant differences in ICV and right amygdala volume. CU+ showed smaller left and right hippocampus and left amygdala than controls.
Zalesky <sup>28</sup>	2012	MRI-DTI	15.6	59	33	33	32	WBV	No significant differences in WBV between CU+ and CU-.
Ashtari <sup>29</sup>	2011	MRI	5.3	14	19	14	18	L Amy R Amy L Hip R Hip WBV	No significant differences in amygdala volumes between CU+ and CU-. Instead, CU+ showed smaller volumes of the right and left hippocampus. WBV was significantly greater in CU+ than in CU-.
Cousijn <sup>30</sup>	2011	MRI	2.5	33	21	42	22	ICV	There was no statistically significant difference between CU+ and CU- in ICV.
Lopez-Larson <sup>31</sup>	2011	MRI	1.7	18	18	18	17	WBV	No significant differences in WBV between CU+ and CU-.
McQueeney <sup>32</sup>	2011	MRI	NA	35	18	47	18	ICV L Amy R Amy	No significant differences in ICV, right and left amygdala volumes, between CU+ and CU-.
Mata <sup>33</sup>	2010	MRI	8.4	30	26	44	26	ICV WBV	No differences in volumetric measures of ICV and WBV, between CU+ and CU-.
Yucel <sup>34</sup>	2008	MRI	19.7	15	40	16	36	ICV L Amy R Amy L Hip R Hip WBV	No significant differences in ICV and WBV. CU+ showed smaller left and right hippocampus and amygdala than CU-.
Medina <sup>35</sup>	2007	MRI	3.4	16	18	16	18	ICV	No significant differences in ICV and hippocampus between CU+ and CU-.
Medina <sup>36</sup>	2007	MRI	NA	26	18	21	17	ICV L Hip R Hip	No significant differences in ICV and hippocampal volume and between CU+ and CU-.
Delisi <sup>37</sup>	2006	MRI-DTI	NA	10	21	10	23	WBV	No significant differences in WBV between CU+ and CU-.
Tzilos <sup>38</sup>	2005	MRI	22.6	22	38	26	29	ICV L Hip R Hip	No significant differences in ICV and hippocampus between CU+ and CU-.
Block <sup>39</sup>	2000	MRI	3.9	18	22	13	23	ICV WBV	No significant differences in ICV and WBV between CU+ and CU-.

DTI, diffusion tensor imaging; ICV, intracranial volume; L Amy, left amygdala; L Hip, left hippocampus; MRI, magnetic resonance imaging; NA, not assessed; R Amy, right amygdala; R Hip, right hippocampus; VBM, voxel-based morphometry; WBV, whole brain volume.



**Figure 1.** Meta-analysis of hippocampal volume in non-psychotic cannabis users (CU+) vs non-users (CU-). Hedges'  $g = -0.439$ ; 95% confidence interval,  $-0.777$  to  $-0.101$ ;  $P = 0.011$ ; random models applied. Negative values indicate lower hippocampal volumes in CU+ as compared to CU-.

subjects and the age of participants, and publication year. There were not enough data to perform other meta-regression analyses, including cumulative intake of cannabis.

No correlation has been found between hippocampal estimates and overall duration of cannabis use expressed in years ( $\beta = -0.014$ ; 95%CI,  $-0.045$ – $0.017$ ;  $P = 0.381$ ). Conversely, we found a significant correlation between hippocampal volume reduction in CU+ versus CU- and the year of publication. Specifically, the most recent articles reported greater inter-group differences of volumes ( $\beta = -0.127$ ; 95%CI,  $-0.231$  to  $-0.023$ ;  $P = 0.017$ ). There were no significant effects with respect to the other moderators tested ( $P > 0.05$ ).

## DISCUSSION

We present here the first quantitative meta-analysis addressing neuroanatomical alterations in non-psychotic cannabis users. In our work, we tested the

whole brain volume, the amygdala and the hippocampus as ROI to address the putative neurotoxic effect of cannabis on the healthy brain. We found no significant differences in whole brain volume between cannabis users and non-users. Conversely, when looking specifically at the hippocampus and amygdala, the meta-analysis found that cannabis users have smaller volumes as compared to non-users. However, amygdala results did not survive after controlling for publication biases. Conversely, the hippocampal reductions were consistent.

The small number of studies included in this analysis limited the number of brain areas to be analyzed. The core finding of our approach was of significant grey matter reductions in the hippocampus of cannabis users as compared to non-users. There was no correlation with the duration of cannabis use. Duration of cannabis use highly varied across the included studies, ranging from 2.5<sup>30</sup> up to 19.7<sup>34</sup> years. We cannot exclude that the overall cumulative effect of smoking cannabis, as indexed by the total amount of

cannabis intake, rather than the length of use, can better reflect putative neurotoxic effects of the substance. There is evidence indicating that, within the group of heavy cannabis users, grey matter volume in the hippocampus is negatively correlated with the intensity of cannabis use and with the severity of dependence,<sup>30</sup> while no associations were related to onset age or duration of cannabis use. Functional imaging studies confirmed that frequency of use critically impacts hippocampal functioning in cannabis users.<sup>41</sup> Unfortunately, in our meta-analysis, the total amount of cannabis use reported in the retrieved studies was not measured consistently enough to perform a meta-regression. For example, this data has been reported as number of 'joints',<sup>26</sup> 'smoking events',<sup>31</sup> 'cones'<sup>34</sup> or simply 'use'<sup>27</sup> per different period of time by different authors.

Our finding of reduced hippocampal volume in non-psychotic cannabis users is interesting. At a developmental level, the hippocampal region is subject to significant neurogenesis (the birth of new neurons) during adolescence.<sup>42</sup> Adolescence is a period during which the brain undergoes dramatic developmental changes. Maturation of the human brain is a complex and comprehensive process, with critical changes occurring at key points throughout development.<sup>43</sup> We had not enough power to test the possible modulating effect of the age at which the regular use started.

The potential neurotoxicity of cannabinoids in the hippocampal region amongst non-psychotic users is important due to the high developmental sensitivity of this region to the effect of cannabis. At a pharmacological level, the hippocampus is particularly enriched with cannabinoid receptors.<sup>44</sup> Furthermore, there is consistent pharmacological evidence on animal studies supporting the notion that delta-9-THC appears particularly neurotoxic to hippocampal neurons.<sup>45–48</sup> At a neuropsychological level, the effect of cannabinoids in the human hippocampus is reflected by significant neurocognitive impairments. In fact, one of the most robust behavioral effects of cannabis is that it impairs memory.<sup>49</sup> It is generally accepted that the hippocampus includes a system of anatomically related structures that are essential for memory functions.<sup>50</sup> Thus, chronic administration of cannabis may decrease hippocampal volume and this may be one of the reasons why memory performance is affected. A number of parameters have a negative impact on the memory, including the age of first use, the average frequency of use, the cumulative lifetime

dose, the average dose per occasion, and the duration of regular use.<sup>51</sup> Although available evidence suggests memory impairments in non-psychotic cannabis users can be reversible,<sup>52</sup> more studies are needed to clarify the correlation between the hippocampal volumetric changes that emerged from our findings and their potential neurocognitive effects.<sup>53</sup> Compensatory functional mechanisms and plasticity of the brain can further complicate the findings described by our meta-analysis and account for preserved memory function even in the presence of long-lasting structural changes.

The association between the age of publication and the size effects found could derive from a broad range of methodological issues. Indeed, we could not exclude sociodemographic differences in the samples recruited in the studies (even if a number of demographic variables have been tested and found to be not responsible for the observed heterogeneity) or qualitative differences in the nature of the smoked cannabis. With respect to this point there is also evidence of a progressive change in the d-9-THC potency of street cannabis over more recent years that should be carefully considered when interpreting our findings (see below).<sup>54</sup>

### Reflection on limitations

In considering the results of this meta-analysis, we have to acknowledge a number of possible limitations. First of all, studies varied broadly in terms of total amount of cannabis use. However, the high inconsistency in reporting the total amount between different studies did not allow quantifying the heterogeneity of this putative moderator. Furthermore, smoked cannabis contains different compounds with opposing effects,<sup>5</sup> which include not only THC but also CBD. Interestingly, a recent whole brain structural imaging study found inverse correlations of bilaterally hippocampal grey matter concentration with the ratio of THC/CBD.<sup>55</sup> In particular, the authors found positive correlations with CBD, pointing to a putative neuroprotective effect of this molecule in the hippocampal region.<sup>55</sup> This result can help to explain the above divergent results in cannabis users with inconsistent findings. Furthermore, the observed inconsistencies may also be the consequence of variations of street cannabis strengths.<sup>54,56</sup> In our investigation, most of the reviewed papers do not adequately consider this fact and more attention should be paid in conducting further studies.

Additionally, apart from THC and CBD, there are other compounds, such as delta-8-THC, tetrahydrocannabivarin and cannabinal, which all have different effects, and their roles have not yet been broadly investigated in the available literature. Furthermore, there may be genetic variations in sensitivity to such effects even amongst non-psychotic users. Comparing results between studies presented in this meta-analysis is also hindered by differences in inclusion criteria and design of the studies. Not all studies used DSM-IV criteria for cannabis dependence or abuse and studies varied in how they set criteria to define their cannabis using and non-using group. Also, a considerable overlap between cannabis and other illicit drug use may have played a confounding role. In particular, some of the included samples presented a significant history of alcohol abuse<sup>30,36,29</sup> or nicotine dependence.<sup>30,29</sup> Moreover, although we carefully excluded the presence of comorbid psychotic diagnoses, some samples were presenting other psychiatric problems, either diagnosed or self-reported. However, the majority of the reviewed samples did not have any comorbid psychopathologies. Of the three samples presenting comorbidities, two samples presented psychopathology in a significant minority of the sample: in Lopez-Larson *et al.*,<sup>31</sup> one participant had past depression and one a history of heavy alcohol use; in Cousijn *et al.*,<sup>30</sup> no participants were reported with a diagnosis of attention deficit hyperactivity disorder. The study by Ashtari *et al.* examined samples with a high proportion of participants with a number of comorbid current and past disorders, including post-traumatic stress disorder ( $n = 2$ ), attention deficit hyperactivity disorder ( $n = 2$ ), and oppositional defiant/conduct disorder ( $n = 4$ ).<sup>29</sup> Finally, it is relevant to acknowledge that, even though a correlation between cannabis use and hippocampal volume reduction has been found, correlation is not causation and our meta-analysis of cross-sectional studies does not allow us to infer a causal role for any of the variables. Though few studies have taken a longitudinal approach whilst investigating the relation between cannabis use and structural abnormalities, a recent study suggests that some structural abnormalities could predate the onset of cannabis use.<sup>57</sup>

## Conclusions

Our results suggest that in the healthy brain, chronic and long-term cannabis exposure may exert signifi-

cant effects in brain areas enriched with cannabinoid receptors, such as the hippocampus, which could be related to a neurotoxic action.

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