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A joint approach: brain structure & function in heavy cannabis users & their relationship with future use

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CHAPTER 2

BRAIN STRUCTURE ALTERATIONS ASSOCIATED WITH CANNABIS USE & CANNABIS USE-RELATED PROBLEMS

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ABSTRACT

Cannabis abuse is related to impairments in a broad range of cognitive functions. However, studies on cannabis abuse in relation to brain structure are sparse and results are inconsistent, probably due to differences in imaging methodology, severity of cannabis abuse, and use of other substances. The goal of the current MRI study was to investigate brain morphology related to current and lifetime severity of cannabis use and dependence in heavy cannabis users without intensive use of other illicit drugs. Voxel-based morphometry was used to assess differences in regional grey and white matter volume between 33 heavy cannabis users and 42 matched controls. Within heavy cannabis users, grey and white matter volume was correlated with measures of cannabis use and dependence. Analyses were focused a priori on the orbitofrontal cortex, anterior cingulate cortex, striatum, amygdala, hippocampus, and cerebellum, regions implicated in substance dependence and/or with high cannabinoid receptor-1 concentrations. Regional grey matter volume in the anterior cerebellum was larger in heavy cannabis users. Within the group of heavy cannabis users, grey matter volume in the amygdala and hippocampus correlated negatively with the amount of cannabis use or dependence. No associations were found between white matter volume and measures of cannabis use or dependence. These findings indicate that associations between heavy cannabis use and altered brain structure are complex. Differential patterns of structural changes for various cannabis use levels imply that alterations in brain structure are associated with specific characteristics of cannabis use and dependence.

INTRODUCTION

Awareness of the addictive properties of cannabis and the treatment need for cannabis dependence has greatly increased over the past decades (UNODC World Drug Report, 2009). Compared to other substances of abuse, relatively little is known about the (neuro)pathology related to cannabis use disorders. An important question is to what extent different levels of cannabis use and dependence affect brain structure and function (Martin-Santos et al., 2010). Studies on acute and chronic effects of cannabis showed that cannabis use is related to impairments in a broad range of cognitive functions [e.g., learning and memory (Nestor et al., 2008; Pope et al., 2001; Pope et al., 1997; Pope and Yurgelun-Todd, 1996; Solowij et al., 2002), attention (Ehrenreich et al., 1999; Solowij et al., 1995; Solowij et al., 2002), decision-making (Bolla et al., 2005; Whitlow et al., 2004), and cognitive flexibility (Pope and Yurgelun-Todd, 1996)]. However, studies on the relation between cannabis use and brain structure are sparse and results are inconsistent (Lorenzetti et al., 2010).

The main psychoactive component of cannabis, Δ9-tetrahydrocannabinol (THC; Gaoni and Mechoulam, 1971), binds to the endogenous Cannabinoid Receptor-1 (CB1). CB1-receptors are abundant throughout the brain, especially in the cerebellum, prefrontal cortex, cingulate cortex, striatum, amygdala, and hippocampus (Burns et al., 2007). Animal studies have shown neurotoxicity and structural changes in brain areas with high CB1-receptor concentrations after (chronic) exposure to relatively high THC concentrations (Downer et al., 2001; Heath et al., 1980; Lawston et al., 2000; Scallet et al., 1987). Additionally, and irrespective of potential drug-specific neurotoxic effects, the development of addictive behaviors is thought to involve neuroplasticity in the ventral striatum and orbitofrontal cortex (OFC), and eventually other parts of the prefrontal cortex, anterior cingulate cortex (ACC), amygdala and dorsal striatum as the addiction progresses (Koob and Volkow, 2010). Some of these neuroadaptations might already be evident before the start of drug use, thereby predisposing individuals to developing a substance use disorder (Chambers et al., 2003). All these findings suggest that if cannabis use affects human brain morphology, changes could be predominantly expected in the OFC, ACC, striatum, amygdala, and hippocampus, i.e., areas with both an important role in the development of addictive behaviors as well as high CB1-receptor concentrations. Further, findings of impaired cerebellar-mediated associative learning in chronic cannabis users (Skosnik et al., 2008) and high CB1-receptor concentrations in the cerebellum suggest this area might also be affected.

Some human structural Magnetic Resonance Imaging (sMRI) studies showed significant volume reduction of the (para-)hippocampus, amygdala, and cerebellum in adult heavy cannabis users compared to healthy controls, but evidence for alterations in the OFC, ACC, and striatum is lacking (Demirakca et al., 2010; Matochik et al., 2005, Solowij et al., 2011; Yucel et al., 2008; for a review see Lorenzetti et al., 2010). Within heavy cannabis users, hippocampal volume was found to be inversely correlated with cumulative cannabis exposure (Ashtari et al., 2011; Yucel et al.,

2008), psychotic symptoms (Yucel et al., 2008), and THC concentrations in hair (Demirakca et al., 2011). Cerebellar white matter volume was found to be inversely correlated with duration of cannabis use (Solowij et al., 2011). Other sMRI studies did not find a relationship between cannabis use and brain morphology (Block et al., 2000; Jager et al., 2007; Medina et al., 2007b; Tzilos et al., 2005). However, in adolescent cannabis users a significant increase in cerebellar grey matter volume (Medina et al., 2010) and a positive association between severity of dependence and increased left hippocampal volume (Medina et al., 2007a) have been reported. A recent review suggested that inconsistencies between these studies might stem from methodological differences and heterogeneity with regard to cannabis use characteristics like duration, frequency, and cumulative lifetime use (Lorenzetti et al., 2010). Additionally, these discrepancies might be explained, at least in part, by differences in the use of other substances. Also, age and gender differences between studies might account for differential results since both age (Medina et al., 2007a) and gender (Medina et al., 2009) possibly moderate cannabis effects on brain morphology. Structural alterations are most consistently reported in groups with high levels of lifetime cannabis use and dependence. However, the relation between specific cannabis use levels or patterns and morphological changes remains unclear and needs further investigation.

In addition to sMRI studies some Diffusion Tensor Imaging (DTI) studies indicate that heavy cannabis use is related to white matter (WM) changes. DTI quantifies magnitude, directionality, and coherence of water diffusion in WM fibers and thereby provides information about WM integrity and connectivity in the brain (Conturo et al., 1999). DTI studies in both adolescent and adult cannabis users have found reduced WM integrity in the hippocampus (Yucel et al., 2010) and in connecting WM tracts from and to the prefrontal cortex (Arnone et al., 2008; Ashtari et al., 2009; Jacobus et al., 2009). However, two other DTI studies reported no differences between heavy adolescent cannabis users and controls (Delisi et al., 2006; Gruber and Yurgelun-Todd, 2005). These inconsistencies might be explained by a lack of power due to small group sizes (10-15 participants) and by differences in alcohol use in the cannabis users that were studied.

In sum, conflicting results have been reported regarding the effect of cannabis use on brain morphology. Structural abnormalities in the hippocampus have been observed most consistently (Demirakca et al., 2011; Matochik et al., 2005; Yucel et al., 2008; Yucel et al., 2010). However, discrepancies between studies to date, possibly associated with individual characteristics of cannabis use and related problems, preclude strong conclusions about the relation between cannabis use and brain morphology. The goal of the current sMRI study was to investigate structural brain alterations in relation to levels of cannabis use and severity of dependence in a large sample, taking into account the use of addictive substances other than cannabis. Voxel-Based Morphometry (VBM) was performed to assess differences in grey and white matter volume between 33 heavy cannabis users and 42 healthy matched controls. Within heavy cannabis users we investigated the relation between regional grey and white matter volume and individual

differences in cannabis use and severity of dependence. *A priori* regions of interest in which we expected grey and white matter alteration in heavy cannabis users compared to controls were the OFC, ACC, striatum, amygdala, hippocampus, and cerebellum. Within the group of heavy cannabis users we expected greater volume changes in those individuals with higher levels of cannabis use and severe dependence.

MATERIALS AND METHODS

The present study was part of a prospective functional Magnetic Resonance Imaging (fMRI) study with the aim to investigate the (predictive) role of different neurocognitive and neuroimaging factors in heavy cannabis users at risk for the development of cannabis dependence (Cousijn et al., 2011). The medical ethics committee of the Academic Medical Centre of the University of Amsterdam approved the study and all participants signed an informed consent prior to participation.

Participants

A total of 33 heavy cannabis users and 43 controls in the age range of 18-25 were recruited through advertisements on the Internet and in cannabis outlets (known as 'coffee shops' in the Netherlands). However, one control participant was excluded from all analyses due to abnormal brain morphology (extremely large left ventricles). The remaining sample consisted of 33 heavy users and 42 controls. Groups were matched for age, gender, education, and estimated intelligence (Nelson, 1984; Schmand et al., 1991). Table 1 presents the sample characteristics. For inclusion, heavy cannabis use was defined as using cannabis on 10 or more days during the last month, at least on 240 days during the last two years, without seeking treatment or having a treatment history for problematic cannabis use. Participants in the control group had smoked less than 50 cannabis joints during their lifetime and did not use during the last year (5 controls reported lifetime use of more than 10 joints lifetime). Drug and alcohol use was controlled by excluding participants with an Alcohol Use Disorder Identification Test (AUDIT) score higher than 10 (Saunders et al., 1993), smoking more than 20 cigarettes a day, a positive urine screen for alcohol, amphetamines, benzodiazepines, opioids or cocaine, or having used any other type of non-cannabinoid illicit drug during the last year or on more than 100 occasions lifetime (5 participants > 10 occasions, no participant > 25 occasions). Other exclusion criteria were general MRI contraindications (e.g., head injuries, metal implants), major medical disorders, and a history of major DSM-IV Axis I psychiatric disorders (American Psychiatry Association, 1994), which was assessed using the Mini-International Neuropsychiatric Interview [MINI (Sheehan et al., 1998), Dutch version 5.0.0]. Additionally, severity of depressive and ADHD symptoms was assessed with the Beck Depression Inventory (BDI; Beck et al., 1961) and a self-report ADHD rating scale (Kooij et

al., 2005), respectively. All participants were asked to refrain from alcohol and drug use (except for nicotine and caffeine) 24 hours prior to the test session (self-reported abstinence of cannabis use in heavy users was 1.8 days, SD = 2.3). Although urine analysis of THC metabolites is insensitive to 24 hour abstinence, it increases accuracy of self-reported substance use (Roese and Jamieson, 1993). The urine samples were taken to control for recent illicit substance use. Testing took place in late afternoon and beginning of the evening. Participants were financially compensated for their participation.

Cannabis and nicotine use

Severity of cannabis dependence was measured with the Cannabis Use Disorder Identification test (CUDIT: Adamson and Sellman, 2003). The CUDIT is a screening questionnaire used for at-risk cannabis use and consists of 10 items on cannabis use frequency, symptoms of dependence, and cannabis use related problems (Adamson et al., 2010; Adamson and Sellman, 2003). Possible

Table 1 Sample Characteristics

	Heavy cannabis users	Controls
N (% female)	33 (36)	42 (38)
Age	21.3 (2.4)	21.9 (2.4)
Verbal IQ (Dutch Reading Test)	104.2 (5.4)	104.9 (7.3)
Beck Depression Inventory	7.4 (5.7)	4.3 (5.0)*
ADHD self-rating scale	5.5 (4.4)	2.9 (3.0)*
Alcohol use related problems (AUDIT)	6.2 (3.3)	4.9 (3.4)
Cigarette smoking (%)	70	17**
Nicotine dependence (FTND)	2.8 (2.4)	.5 (1.2)**
Duration cigarette smoking (year)	3.7 (3.6)	.6 (1.5)**
Cigarettes per day	7.0 (7.2)	1.4 (3.5)**
Cannabis use lifetime (# joints)	1579.5 (1425.0)	4.7 (9.2)
Cannabis use related problems (CUDIT)	12.4 (5.7)	0 (0)
Onset regular cannabis use	18.8 (2.3)	--
Duration heavy cannabis use (year)	2.5 (1.9)	--
Current cannabis use days/week	4.9 (1.5)	--
Current cannabis use grams/week	3.0 (2.2)	--

Means (SD). *$p < .05$ and **$p < .001$ for group comparison. SD: standard deviation. AUDIT: Alcohol Use Disorder Identification Test. FTND: Fagerström Test for Nicotine Dependence. CUDIT: Cannabis Use Disorder Identification Test.

scores on the CUDIT ranged from 0 to 40 with a suggested cut-off for cannabis abuse of 8. Severity of nicotine dependence was measured with the Fagerström Test for Nicotine Dependence (FTND; Heatherton et al., 1991). In addition, a detailed history on past and current cannabis and nicotine use was recorded, including questions on recent and lifetime cannabis use.

Imaging parameters and data preprocessing

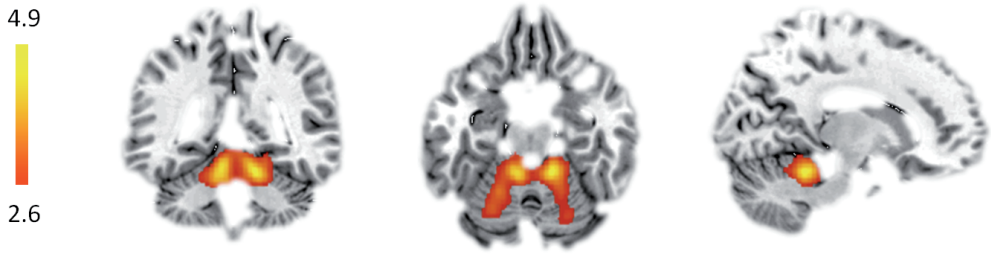
A 3T MRI scanner (Philips Intera, Best, The Netherlands) with a phased array SENSE RF eight-channel receiver head coil was used for image acquisition. For each participant, a T1 structural MRI image was acquired (T1 turbo field echo, TR 9.6 s, TE 4.6 ms, 182 slices, slice thickness 1.2 mm, FOV 256 x 256 mm, in-plane resolution 256 x 256 mm, flip angle 8°). The MRI images were visually inspected for motion artifacts. Data preprocessing was conducted with Statistical Parametric Mapping 8 software (SPM8; http://www.fil.ion.ucl.ac.uk/spm) and the VBM8-toolbox (http://dbm.neuro.uni-jena.de/vbm.html) with default settings. The VBM8-toolbox is an extension of the unified segmentation model (Ashburner and Friston, 2005) in which structural images are bias corrected, segmented into white matter, grey matter, and cerebrospinal fluid, and registered combined within the same model. VBM was used because it facilitates (compared to volumetric approaches) investigation of structural differences in areas with poorly defined structural landmarks (e.g., prefrontal areas) and allows explorative analysis of structural differences. Moreover, the VBM8 toolbox extends the unified segmentation model with a Maximum A Posterior (MAP) technique (Rajapakse et al., 1997) and Partial Volume Estimation (PVE) to account for partial volume effects (Tohka et al., 2004), which results in more subtle segmentation of subcortical areas (Gaser, 2009). The images were bias corrected to remove MRI inhomogeneities and noise with a spatially Adaptive Nonlocal Means (SANLM) filter (Manjon et al., 2010). To further improve signal-to-noise ratio, a spatial constraint (incorporated in the segmentation procedure) was applied based on a classical Markov Random Field (MRF) model (Cuadra et al., 2005). Registration to standard MNI-space consisted of a linear affine transformation and a nonlinear deformation using high-dimensional DARTEL normalization (Ashburner, 2007). This procedure uses the DARTEL template of 550 healthy control participants (defined by default settings VBM8). The normalized segmented images were modulated by applying a nonlinear deformation, which allows for comparing absolute amounts of tissue corrected for individual differences in brain size. To check the quality of the normalization procedure, the normalized unsegmented images were visually inspected. Covariance between normalized segmented images was calculated to check homogeneity of variance and to identify potential outliers. Finally, the segmented images were spatially smoothed with an 8 mm full-width-half-maximum Gaussian kernel.

Statistical analysis

Demographics and scores on questionnaires were compared between groups with standard t-tests for independent samples. Within the group of heavy cannabis users, Pearson correlations were calculated to assess the relation between measures of cannabis use and nicotine use, alcohol use, depression symptoms, and ADHD symptoms. Statistical analyses of the structural MRI images were conducted in SPM8.

A priori ROIs were the OFC, ACC, striatum, amygdala, hippocampus, and cerebellum. The Nielsen and Hansen's volume of interest online database (Nielsen and Hansen, 2002) was used to obtain anatomical masks of these areas. Structural differences within ROI masks were considered significant if $p < .005$, with an FWE-corrected cluster probability of $p < .05$ adjusted for the small search volume (Worsley et al., 1996). Structural differences outside ROI masks were considered significant if $p < .001$, with a whole brain FWE-corrected cluster probability of $p < .05$.

First, grey and white matter volumes were compared between heavy cannabis users and controls. Gender possibly affects regional volume differences in cannabis users (Medina et al., 2009). To take into account and examine moderating gender effects, an ANCOVA was conducted to compare the groups. To control for the effects of age and estimated intelligence, these variables were added as additional covariates. Second, within the group of heavy users, multiple regression analyses were performed to investigate the association between grey and white matter volumes as dependent variables and current weekly cannabis use (gram), lifetime use (number of joints), onset of regular cannabis use (years), duration of heavy cannabis use (years), and CUDIT scores as independent variables. For each independent variable a separate regression analysis was performed for grey and white matter. To assess possible effects of nicotine use, alcohol use, ADHD, and depression, additional regression analyses were performed with duration of cigarette smoking (years), cigarettes per day, FTND scores, AUDIT scores, ADHD self-rating scores, and BDI scores as independent measures. Age, gender, and estimated intelligence were added as additional covariates in these analyses. To avoid possible edge effects between grey and white matter, all voxels with GM values of < .1 were excluded (absolute threshold masking). Locations of structural differences were verified with the LONI probability atlas (Shattuck et al., 2008). For descriptive purposes, proportional regional volume within each ROI was extracted per participant using the MarsBaR region of interest toolbox (http://marsbar.sourceforge.net/).



x = 14 y = - 43 z = - 21

Figure 1 Larger grey matter volume in anterior cerebellum in heavy cannabis users compared to controls. Cluster of significant volume differences (p < .005, FWE cluster-corrected at p < .05 adjusted for region of interest volume) is overlaid on a standard MNI brain. Right side of the brain is depicted at right side.

RESULTS

Sample characteristics

Heavy cannabis users and controls did not differ significantly in age (t_{73} = 1.02, p = .31), gender (X^2 = .02, p = .88), IQ (t_{73} = .49, p = .63), and alcohol use/problems (t_{73} = 1.61, p = .11). However, heavy cannabis users scored higher on measures of nicotine use [smokers (%), X^2 = 21.65, p < .001; smoking duration (years), t_{73} = 5.06, p < .001; cigarettes/day, t_{73} = 4.39, p < .001], nicotine dependence (FTND, t_{73} = 5.63, p < .001), depression symptoms (t_{73} = 2.54, p = .013), and ADHD symptoms (t_{73} = 3.02, p = .003; Table 1). Within the group of heavy cannabis users, the different measures of cannabis use and dependence were not significantly correlated with measures of alcohol use (R < .16, p > .38), depression (R < .25, p > .16), and ADHD (R < .18, p > .31), but the different measures of nicotine use and dependence correlated positively with duration of heavy cannabis use (R > .40, p < .023).

VBM analysis

Comparing heavy cannabis users with controls, ROI analyses showed that regional grey matter volume in both the left and right anterior lobes of the cerebellum was significantly larger in heavy cannabis users compared to controls (Table 2 and Figure 1), whereas grey matter volume in the OFC, ACC, striatum, amygdala, and hippocampus did not significantly differ between heavy users and controls (Table 3). Gender did not significantly interact with grey matter differences between groups.

Within the group of heavy cannabis users, ROI analysis showed that grey matter volume in the right amygdala was inversely correlated with severity of cannabis dependence (R peak = .65). Additionally, grey matter volume in the bilateral hippocampus was inversely correlated with

weekly cannabis use (*R* peak = .55; see Figure 2 and Table 2). No significant associations were found between grey matter volume and onset of regular cannabis use, duration of heavy cannabis use, lifetime cannabis use, nicotine use and dependence, alcohol use and problems, ADHD, and depression White matter volume did not differ between heavy users and controls (Table 3) and there was no significant interaction between group and gender. Within the group of heavy cannabis users, no associations were found between white matter volume and measures of cannabis/nicotine/alcohol use and problems, ADHD, and depression.

Table 2 Regional grey matter differences in heavy cannabis users vs. controls and brain regions correlating negatively with level of dependence and quantitative cannabis use.

Brain region	MNI coordinates			t_{max}	Clustersize (voxels)
	x	*y*	*z*		
Heavy cannabis users > Controls					
Medial cerebellum, L & R	-8	-43	-20	5.15 [ab]	4252
Negative correlations CUDIT					
Amygdala, R	24	0	-24	4.62 [a]	266
Negative correlations weekly use (gram)					
Hippocampus, L	-32	-36	-11	4.38 [a]	361
Hippocampus, R	30	-27	-18	3.95 [a]	345

L, left hemisphere; R, right hemisphere; MNI, Montreal Neurological Institute; CUDIT, Cannabis Use Disorder Identification Test; MNI coordinates of maximum *t*-scores are shown for each cluster; [a] Significant at regions of interest level, $p < .005$, FWE cluster-corrected $p < .05$. [b] Significant at whole brain level, $p < .001$, FWE cluster-corrected $p < .05$.

DISCUSSION

The goal of this VBM-sMRI study was to investigate regional differences in grey and white matter in relation to levels of cannabis use and dependence in heavy cannabis users. We were *a priori* interested in the OFC, ACC, striatum, amygdala, hippocampus, and cerebellum, areas with high CB1-receptor concentrations and/or an important role in the neuropathology of addiction (Burns et al., 2007; Koob and Volkow, 2010). Grey matter volume in the anterior cerebellum was larger in heavy cannabis users compared to controls. White matter volume did not differ between groups. Within the group of heavy cannabis users, reduced grey matter volume strongly correlated with individual differences in cannabis use and dependence: current weekly cannabis use (gram) was associated with reduced hippocampal volume and level of cannabis dependence (total CUDIT score) was associated with reduced amygdala volume. No associations were found in the ACC and

striatum. This is the first study in *adult* cannabis users indicating that heavy cannabis use is associated with larger grey matter volume in the cerebellum. Moreover, we show that different aspects of cannabis use and dependence in young adults are associated with differential structural patterns in the brain.

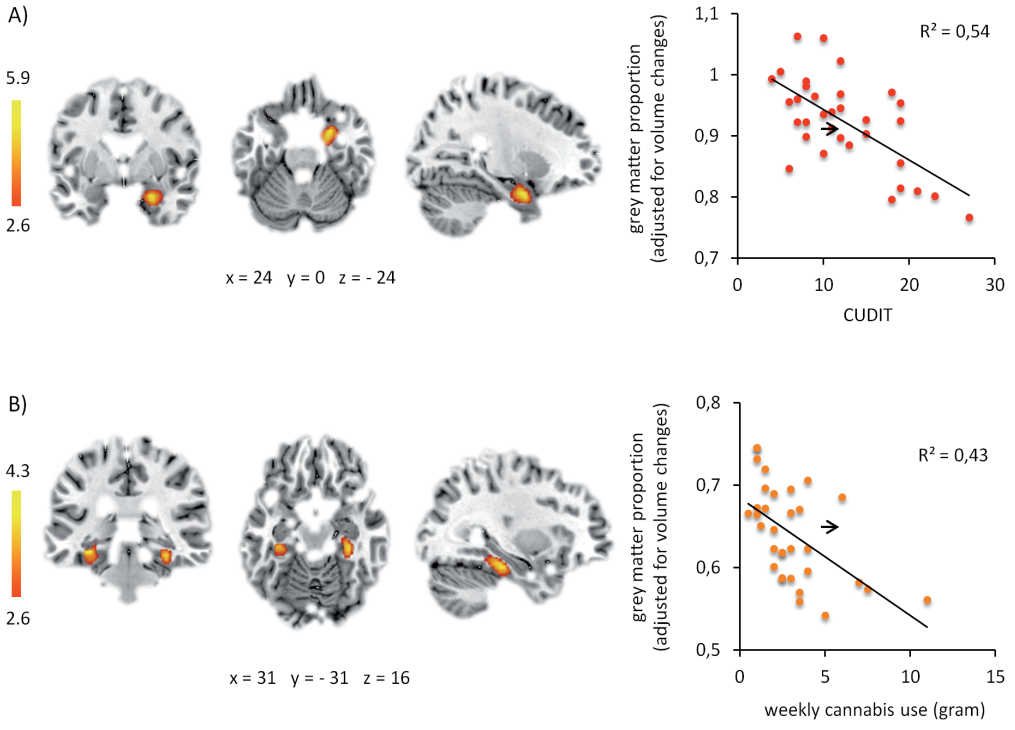
The increase of cerebellar volume in heavy cannabis users compared to controls is consistent with a study in four weeks abstinent *adolescent* cannabis users (Medina et al., 2010). Although the heavy cannabis users in the present study were not abstinent, the findings from Medina et al. after four weeks abstinence suggest that abnormal cerebellar morphology may be of a relatively persistent nature. Medina et al. additionally reported decreased executive functioning in association with larger cerebellar volume, suggesting increased cerebellar volume as an indicator of diminished cognitive functions. Changes in cerebellar volume linked to various psychopathologies predominantly comprise volume *reductions*: psychosis (Berge et al., 2011), ADHD (Seidman et al., 2011), depression (Peng et al., 2010), and addiction (Sim et al., 2007; and in heavy cannabis users: Solowij et al., 2011). Medina et al. suggested that the increase in cerebellar volume might be a developmental effect; before the cerebellum fully matures around the age of 18, cannabis might inhibit neuronal pruning thereby blocking cerebellar volume reductions during late adolescence. In addition, increases in cerebellar volume may be a cannabis specific pharmacological effect. Alternatively, an increase in cerebellar volume may represent an underlying (familial) risk factor for the development of a substance abuse disorder (Hill et al., 2007). Clearly, more research is needed to clarify causal processes and functional implications of these structural alterations in the cerebellum.

Table 3 Total Intracranial Volume and Grey and White matter Volume per Region of Interest in Heavy Cannabis Users and Controls

	Heavy cannabis users		Controls	
Total intracranial volume (SD)	1496.17 (53.87)		1498.64 (59.64)	
	grey matter	*white matter*	*grey matter*	*white matter*
Orbitofrontal cortex, mean (SD)	54.1 (3.8)	51.7 (3.3)	53.6 (3.7)	53.1 (4.7)
Anterior cingulate cortex, mean (SD)	31.5 (4.3)	18.5 (1.6)	30.9 (5.0)	18.8 (1.5)
Striatum, mean (SD)	28.2 (2.6)	36.2 (3.4)	27.2 (1.8)	36.4 (3.1)
Amygdala, mean (SD)	2.5 (.1)	.6 (.1)	2.4 (.2)	.7 (.1)
Hippocampus, mean (SD)	19.0 (.8)	8.9 (.5)	18.6 (1.0)	9.1 (.8)
Cerebellum, mean (SD)	64.5 (5.8)*	20.0 (2.7)	61.4 (4.1)	20.2 (3.8)

Volumes are the sum of the left and right hemisphere in ml. Total intracranial volume = (grey matter + white matter + CSF). Mean proportional volume per region. * Larger cerebellar grey matter volume in heavy users compared to controls, $p = .007$.

Figure 2 Relation between grey matter volume in Regions of Interest and individual characteristics of cannabis use and dependence in heavy cannabis users. (A) Right amygdala volume correlated negatively with severity of cannabis dependence (CUDIT score). (B) Bilateral hippocampus volume correlated negatively with weekly cannabis use (gram). Clusters of significant volume differences ($p < .005$, FWE cluster-corrected at $p < .05$ adjusted for region of interest volume) are overlaid on a standard MNI brain. Right side of the brain is depicted at right side. Graphs show correlation of proportional volume in peak voxel with the dependent variable of interest.



Additionally, the present findings imply different roles for the hippocampus and amygdala during the course of cannabis use towards dependence. Altered morphology and functioning of the hippocampus has been linked to reduced memory performance in heavy cannabis users (Solowij and Battisti, 2008). This might be directly related to absolute THC concentrations in the brain since volume reduction of the hippocampus was found to correlate with THC concentrations from hair analysis (Demirakca et al., 2011), and with current weekly cannabis use in the present study. Also, THC administration in rats has been found to impair memory through binding to CB1-receptor specifically in the hippocampus (Wise et al., 2009). Alterations in the hippocampus might occur relatively early in time as a more direct effect of consuming high amounts of cannabis, which

might explain why structural changes in the hippocampus are reported most consistently. Additionally, direct effects of cannabis on the hippocampus might eventually cumulate over time since loss of hippocampal volume has been found to correlate with lifetime cannabis exposure in a group of long-term (>10 years) daily cannabis users (Yucel et al., 2008). The amygdala is thought to play an important role in craving (Filbey et al., 2009). Supported by the inverse relation between amygdala volume and CUDIT scores in this study, neuroadaptations in the amygdala probably occur when cannabis use becomes a conditioned response and part of a dependence syndrome (Koob and Volkow, 2010).

Findings from the present study also imply that heavy cannabis use is not necessarily related to structural changes in the forebrain and striatum. Changes in grey matter may only be present in those individuals that consume large quantities of cannabis almost daily with symptoms of dependence. This might explain discrepancies between previous studies (Ashtari et al., 2011; Block et al., 2000; Demirakca et al., 2011; Jager et al., 2007; Matochik et al., 2005; Medina et al., 2007b; Medina et al., 2007c; Tzilos et al., 2005; Yucel et al., 2008) and is in line with the idea that moderate cannabis use has a limited effect on frontal or striatal grey matter whereas changes appear to be most likely in long-term heavy and often dependent cannabis users (Lorenzetti et al., 2010). Given the relatively young age and short duration of cannabis use in this sample, more research is needed to clarify the relation between regional grey matter volume and more severe dependent or chronic cannabis use.

An important question arising from these results is to what extent the observed structural differences are actually caused by heavy cannabis use. The associations between specific characteristics of cannabis use and volume reductions in the hippocampus and amygdala suggest a linear relation between volume reduction and severity of cannabis use and dependence. Then again, it could be that alterations in brain structure only occur when a certain threshold of cannabis abuse is reached. Alternatively, the observed structural differences might be pre-existent, causing individuals to be more prone to develop certain psychopathologies such as cannabis dependence. Abnormal morphology of the hippocampus, amygdala, and/or cerebellum has been associated (not always consistently) with numerous disorders that constitute a risk factor for cannabis use and dependence, like ADHD (Sasayama et al., 2010), depression (Frodl et al., 2010), anxiety (Hayano et al., 2009), psychosis (Fusar-Poli et al., 2011), and obsessive compulsive disorder (Kwon et al., 2003). As in the current study, excluding participants with a history of psychiatric illness cannot rule out potential effects of sub-clinical symptoms. Although severity of ADHD and depression symptoms was not associated with regional brain volume and measures of cannabis use and dependence, psychotic and anxiety symptoms were not taken into account. Longitudinal within-subject studies are needed to establish the causal relation between (heavy/dependent) cannabis use and grey matter alterations.

Some potential limitations must be taken into account. First, there were more smokers among heavy cannabis users, nicotine use correlated with duration of heavy cannabis use, and almost all cannabis users smoked cannabis combined with tobacco [the most common form of cannabis use in the Netherlands (Bennett, 2008)]. However, since the heavy cannabis users were relatively light smokers and neither nicotine use nor dependence was associated with structural alterations in heavy cannabis users, it is unlikely that nicotine use accounts for the observed effects. Including a group of cannabis naive cigarette smokers is recommended in future studies in order to distinguish cannabis from nicotine effects. Second, although alcohol use and dependence did not differ between groups [based on the AUDIT (Saunders et al., 1993)], comorbid alcohol and cannabis use may still be a potential confound. In post hoc analyses, the AUDIT score was added as additional covariate in the group analysis and both the AUDIT and FTND scores were added in the within heavy users analyses. Both the group difference in the cerebellum and the results in the amygdala and hippocampus remained unaffected.

In summary, the present VBM-sMRI study showed that grey matter volume in the anterior cerebellum was larger in heavy cannabis users compared to controls. Additional reductions of grey matter volume were only observed in those heavy cannabis users with very high levels of weekly cannabis use (hippocampus) and dependence (amygdala). These findings indicate that associations between heavy cannabis use and altered brain structure are complex: differential cannabis use and dependence patterns may lead to differential patterns of structural change.

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