Addiction Biology

HUMAN NEUROIMAGING STUDY

doi:10.1111/adb.12111

Relationship between working-memory network function and substance use: a 3-year longitudinal fMRI study in heavy cannabis users and controls

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ABSTRACT

Deficient executive functions play an important role in the development of addiction. Working-memory may therefore be a powerful predictor of the course of drug use, but chronic substance use may also impair working-memory. The aim of this 3-year longitudinal neuro-imaging study was to investigate the relationship between substance use (e.g. alcohol, cannabis, nicotine, illegal psychotropic drugs) and working-memory network function over time in heavy cannabis users and controls. Forty-nine participants performed an n-back working-memory task at baseline and at 3-year follow-up. At follow-up, there were 22 current heavy cannabis users, 4 abstinent heavy cannabis users and 23 non-cannabis-using controls. Tensor-independent component analysis (Tensor-ICA) was used to investigate individual differences in working-memory network functionality over time. Within the group of cannabis users, cannabis-related problems remained stable, whereas alcohol-related problems, nicotine dependence and illegal psychotropic substance use increased over time. At both measurements, behavioral performance and network functionality during the n-back task did not differ between heavy cannabis users and controls. Although n-back accuracy improved, working-memory network function remained stable over time. Within the group of cannabis users, working-memory network functionality was not associated with substance use. These results suggest that sustained moderate to heavy levels of cannabis, nicotine, alcohol and illegal psychotropic substance use do not change working-memory network functionality. Moreover, baseline network functionality did not predict cannabis use and related problems three years later, warranting longitudinal studies in more chronic or dependent cannabis users.

Keywords Cannabis, cannabis use disorder, fMRI, n-back, working-memory.

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INTRODUCTION

Approximately half of near-daily cannabis users meet DSM-IV criteria for cannabis dependence (Van der Pol *et al*. 2013). Little is known, however, about the neurobiology underlying continued cannabis use and the potential progression toward dependence. Addiction models suggest that the imbalance between strong drug-oriented motivational processes (cue-reactivity, craving) and compromised regulatory executive functions (disinhibition) plays an important role in the trajectory from recreational use toward dependence (Goldstein & Volkow 2002; Wiers *et al*. 2007; Verdejo-Garcia & Bechara 2009).

Working-memory refers to the capacity to retain and manipulate *online* information, and its integrity is required for many cognitive functions (Kane & Engle 2002; Baddeley 2010). Working-memory capacity constrains attentional control and is especially important when behavior is triggered that conflicts with pursued goals (Kane & Engle 2002). This suggests that individuals with working-memory deficits may more easily give in to the urge to use. Indeed, working-memory deficits appear common across different substance use disorder (SUD) populations, including cannabis dependence (Fernandez-Serrano, Perez-Garcia & Verdejo-Garcia 2011). Also, associations between substance use and

motivational processes like craving and cue-reactivity are stronger in individuals with relatively poor working-memory (Grenard *et al.* 2008; Thush *et al.* 2008). Moreover, working-memory deficits have been found to predict the development alcohol dependence (Penick *et al.* 2010) and relapse in smokers (Patterson *et al.* 2010), whereas training of working-memory may be effective in SUD treatment (Bickel *et al.* 2011; Houben, Wiers & Jansen 2011).

We previously showed that stronger connectivity within the working-memory network during an n-back working-memory task was related to an increase in cannabis use 6 months later (Cousijn *et al.* 2013b). Behavioral performance and functionality (in terms of connectivity and activity) of the network did not differ between heavy cannabis users and control. This fronto-parietal network consists of the dorsolateral prefrontal cortex (DLPFC), ventrolateral prefrontal cortex (VLPFC), premotor cortex, lateral/medial parietal cortex, paracingulate gyrus and frontal pole (Wager & Smith 2003; Owen *et al.* 2005). Within the working-memory network, connectivity and activity generally increase with higher working-memory load levels (i.e. Wager & Smith 2003; Owen *et al.* 2005; Cousijn *et al.* 2013b). Working-memory impairments have been linked to hyperactivity of the network (Wager & Smith 2003; Owen *et al.* 2005). Contrasting our earlier findings (Cousijn *et al.* 2013b), chronic cannabis users generally show increased network activity during a working-memory task compared with non-using controls, despite similar task performance (Kanayama *et al.* 2004; Jacobsen *et al.* 2007; Padula, Schweinsburg & Tapert 2007; Jager *et al.* 2010; Schweinsburg *et al.* 2010). Stronger connectivity and activity within the network have therefore been hypothesized to reflect increased effort to perform the task.

Relatively poor working-memory and suboptimal functioning of the associated brain network may be a pre-existing risk factor for cannabis use disorders (Cousijn *et al.* 2013b). The development of SUD may also aversively influence functioning of prefrontal brain areas, resulting in impaired cognitive functioning (Koob & Volkow 2010). Moreover, drug-specific neurotoxic effects may worsen cognitive functioning. Δ9-Tetrahydrocannabinol (THC), the main psychoactive component of cannabis, binds predominately to the endogenous cannabinoid receptor-1 (CB1) (Burns *et al.* 2007). Animal studies have shown neurotoxic effects of chronic THC exposure, resulting in structural and functional changes of brain areas with high CB1 levels like the prefrontal cortex (Jager & Ramsey 2008). Human studies investigating the neurocognitive and potential neurotoxic effects of chronic cannabis exposure show contradicting findings. Both acute and chronic cannabis exposure have been associated with working-memory impairments; however, many studies investigating the association between cannabis exposure and working-memory impairments fail to find a dose–response relationship (i.e. Kanayama *et al.* 2004; Schweinsburg *et al.* 2008; Becker *et al.* 2010; Cousijn *et al.* 2013b). Moreover, cannabis abstinence may restore working-memory capacity (Hanson *et al.* 2010; Schweinsburg *et al.* 2010). Nevertheless, a recent 20-year longitudinal study showed a decline in working-memory throughout adolescence in adolescent heavy cannabis users, suggesting neurotoxic effects of cannabis exposure during adolescent brain development (Meier *et al.* 2012). In summary, it appears that working-memory deficits and functional alterations of the underlying brain network are evident in cannabis-dependent individuals, and that age of onset plays an important role in the potential neurotoxic and neuroplastic consequences of chronic cannabis exposure (Solowij & Battisti 2008; Fernandez-Serrano *et al.* 2011; Meier *et al.* 2012).

The primary goal of the present study was to elucidate the relationship between substance use and working-memory network functionality (connectivity and activity) in heavy cannabis users over time. At baseline and at 3-year follow-up, heavy cannabis users and matched controls performed an n-back working-memory task, during which brain activity was recorded. Additionally, a detailed history of substance use and related problems was obtained at both measurements. Similar to our previous study, tensor-independent component analysis (Tensor-ICA; Beckmann & Smith 2005) was used to identify and investigate functional characteristics of the working-memory network (Wager & Smith 2003; Owen *et al.* 2005). For the first time, it was investigated if exposure to cannabis, alcohol, nicotine and illegal psychotropic substances (i.e. cocaine, heroine, amphetamine, XTC) was associated with deteriorating working-memory network functionality over this 3-year period. We expected poor working-memory network functionality to be a pre-existing risk factor for SUDs rather than a (neurotoxic) consequence of prolonged cannabis use. We therefore did not expect working-memory-related network functionality to change over time in the heavy cannabis users or to correlate with quantitative measures of cannabis use.

In the present sample of heavy cannabis users, we previously observed a predictive relationship between working-memory network functionality and cannabis use 6 months later (Cousijn *et al.* 2013b). The secondary goal of this study, therefore, was to investigate if we could replicate our prior findings: Can working-memory network functionality as identified with ICA at baseline also predict cannabis use three years later?

MATERIALS AND METHODS

The present study was part of a 3-year longitudinal study investigating the effect of neurocognitive processes on the course of cannabis use in heavy cannabis users (Cousijn, Goudriaan & Wiers 2011; Cousijn *et al.* 2012, 2013a,b). In the current report, only participants performing the n-back at baseline and at 3-year follow-up are included in the analyses. The medical ethical 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 were financially compensated for their participation.

Participants

A total of 49 participants performed the n-back working-memory task at baseline and at 3-year follow-up. At baseline, 23 were treatment-naïve heavy cannabis users and 26 were healthy matched non-cannabis-using controls. At 3-year follow-up, four heavy cannabis users discontinued their cannabis use for at least 4 months (abstinence ranged between 120 and 640 days), whereas three controls became heavy cannabis users. For various reasons [1], 9 out of 33 heavy cannabis users at baseline did not participate at follow-up (attrition 27%). Neuro-imaging data at baseline were missing from one heavy cannabis user because of scanning issues. A Little's missing completely at random (MCAR; Little 1988) test with all study variables indicated that these non-responders were missing at random ($\chi^2 = 80.93$, d.f. = 108, $P = 0.98$). At baseline, participants were aged 18–25 years old and recruited through advertisements on the Internet and in cannabis outlets. Groups were matched for age, gender, education, pre-morbid intellectual functioning (Schmand *et al.* 1991) and alcohol use (Saunders *et al.* 1993). All heavy cannabis users used cannabis on more than 10 days/month for at least 1.5 years at baseline. At baseline, drug and alcohol use was controlled for by excluding participants (1) with an alcohol use disorder identification test (AUDIT; Saunders *et al.* 1993) score higher than 10; (2) smoking more than 20 cigarettes daily; (3) with a positive urine screen for alcohol, amphetamines, benzodiazepines, opioids or cocaine; and (4) using non-cannabinoid drugs on more than 100 occasions in their lifetime (5 participants > 10 occasions, no participant > 25 occasions). Other exclusion criteria were general magnetic resonance imaging (MRI)-contraindications and a lifetime prevalence of major physical or psychiatric disorders [assessed with the Mini-International Neuropsychiatric Interview, Dutch version 5.0.0 (Sheehan *et al.* 1998)]. All participants were asked to refrain from using alcohol and drugs 24 hours prior to the test sessions. Although urine analysis of THC metabolites is insensitive to 24-hour abstinence, it increases accuracy of self-reported substance use (Roese & Jamieson 1993). Urine samples were therefore taken to control for recent alcohol and illegal psychotropic substance use. Testing took place in late afternoon.

Questionnaires at baseline and follow-up

Problem severity of cannabis use during the past 6 months was assessed with the cannabis use disorder identification test (CUDIT; Adamson & Sellman 2003). Problem severity of alcohol use during the past 6 months was assessed with the AUDIT (Saunders *et al.* 1993). Scores on the CUDIT and AUDIT can range between 0 and 40, with a cut-off score of 8 for at-risk cannabis or alcohol use. Severity of nicotine-related problems during the last 6 months was measured with the Fagerström test for nicotine dependence (FTND; Heatherton *et al.* 1991). In addition, a query on past and present cannabis and nicotine use was administered, including questions on daily use, lifetime use and duration of use. Severity of depressive symptoms was assessed with the Beck depression inventory (BDI; Beck *et al.* 1961).

n-back task

Participants performed a letter n-back task, during which functional MRI–blood oxygen level dependent (fMRI–BOLD) responses were recorded. The task consisted of alternating blocks with three load levels: 0-back, 1-back and 2-back. During each block, participants viewed a stream of 15 letters with 5 targets. In 0-back blocks, participants were instructed to indicate when the target letter 'X' appeared on the screen. In 1-back blocks, participants had to decide if the letter on the screen was identical to the previous one. In 2-back blocks, targets were those letters identical to the letter presented two trials back. Participants were instructed to press a right response box button for targets and a left button for non-targets. The 0-back blocks provide a letter recognition

[1] The study was originally designed as a prospective neuro-imaging study with a baseline neuro-imaging test session and a 6-month follow-up telephone interview. Additional funding (from the Academic Medical Centre) gave the opportunity to include a neuro-imaging test session after 3 years. At 3-year follow-up, participants were contacted again using the phone number, email and home address information they provided at 6-month follow-up. Contact was lost with two heavy cannabis users at 6-month follow-up and with two additional heavy cannabis users at 3-year follow-up. For various reasons, three heavy cannabis users did not want to participate at 3-year follow-up. A total of two heavy cannabis users wanted to participate but were not available at the time of the data collection. All 26 of the control participants had participated at baseline and 6-month follow-up and were invited (from a total control group pool of 43 individuals) to take, matching their age and gender to the heavy cannabis users group.

baseline, whereas the 1-back and 2-back blocks represent measures of working-memory at low and high load, respectively (Jaeggi *et al.* 2010). Blocks lasted 30 seconds (each stimulus lasted 2 seconds) and inter-block interval was 5 seconds, during which block instructions were repeated. Each load level was repeated four times resulting in a 7-minute task of 12 blocks (Fig. 2c). Block order was the same for each participant. Letters were projected on a screen viewed through a mirror attached to the MRI head coil. Prior to scanning, the participants briefly practiced each block of the task outside the scanner.

Imaging parameters and pre-processing

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. At the start of each scan-session, a T1 structural image was acquired [T1 turbo field echo, repetition time (TR) 9.6 seconds, echo time (TE) 4.6 milliseconds, 182 slices, slice thickness 1.2 mm, field of view (FOV) 256×256 mm, in-plane resolution 1×1 mm, flip angle 8°]. During the n-back task, BOLD signal was measured with a T2* gradient-echo EPI sequence (TR 2.29 seconds, TE 30 milliseconds, 38 slices, slice thickness 3 mm, inter-slice gap 0.3 mm, FOV 220×220 mm, in-plane resolution 2.3×2.3 mm, flip angle 80°, sequential slice acquisition). Data pre-processing was conducted with FEAT (FMRI Expert Analysis Tool version 6.0, part of FMRIB's software library (FSL, http://www.fmrib.ox.ac.uk/fsl). First, non-brain tissue and skull was removed with brain extraction tool (BET). Images were then slice-time aligned, motion-corrected, high-pass filtered in the temporal domain ($\sigma = 50$ seconds), spatially smoothed with a 5 mm full-with-half-maximum Gaussian kernel and pre-whitened (Woolrich *et al.* 2001). Next, functional data were registered to the participants' structural image and transformed to MNI space (Montreal Neurological Institute) using FNIRT (FMRIB's non-linear image registration tool). Inspection of individual motion plots indicated that none of the participants showed excessive motion (>3 mm).

Statistical analyses

Group analyses were focused on the comparison between current heavy cannabis users ($n = 22$, including three controls who became heavy cannabis users) and controls ($n = 23$) at 3-year follow-up. The four abstinent heavy cannabis users were left out of these analyses because of the small sample size. Scores on questionnaires and n-back behavioral performance were compared between groups over time, with repeated measures analysis of variance (rANOVA) in IBM SPSS Statistics for Windows, Version 20.0. (IBM Corp. Armonk, NY, USA). n-back behavioral performance was assessed in terms of median reaction time (RT) of correct responses and accuracy (proportion correct – error responses).

Tensor-ICA (Beckmann & Smith 2005) was used to investigate working-memory network functionality within and between groups over time. Tensor-ICA [version 3.13, implemented in multivariate exploratory linear optimized decomposition into independent components (MELODIC) part of FSL] allowed model-free fMRI analysis of all the n-back data of both test sessions by means of a three-way data decomposition into independent components (ICs), representing signal or artifacts in the data in terms of temporal, spatial and participant-dependent variations (see Beckmann & Smith 2005 for a detailed description of Tensor-ICA and a comparison with other methods). The number of ICs was set to 20, and each IC was finally represented by a normalized time-course and a spatial map of normalized Z-scores reflecting the degree to which each voxel time-course correlated with the overall IC time-course. MELODIC also provides the relative effect size per participant for a given component, thereby providing information regarding the strength of a component per participant at baseline and 3-year follow-up.

Similar to our previous study using only the baseline n-back data (Cousijn *et al.* 2013b), the IC representing the working-memory network was identified by comparing the IC maps with a meta-analysis activation map of 24 normative n-back fMRI studies (Owen *et al.* 2005). ICs with motion artifacts, a mean power above 0.1 Hz, or driven by one participant were excluded from analysis. Overlap between the meta-analysis map and the IC maps (both thresholded at $Z > 2.3$) was calculated in terms of percent significant meta-analysis voxels falling within the thresholded IC map. Talairach Daemon database implemented in FSL and the Laboratory of Neuroimaging probability atlas (Shattuck *et al.* 2008) were used to verify significant cluster locations.

Within the general linear model (GLM) framework implemented in MELODIC, we assessed whether the network responded to task-load. Changes in network function between heavy cannabis users and controls were investigated in multiple steps. First, to assess changes in network response strength between groups, individual network effect size (e.g. degree to which individual data contributed to the overall network) at baseline and follow-up were compared between groups with a rANOVA. Second, individual structural maps of the network were compared to assess voxel-wise spatial changes in connectivity between groups. These structural maps were reconstructed from the group network map with a dual regression approach (Filippini *et al.* 2009), which uses the network time-course in a temporal regression against individual fMRI data. The individual maps were compared using non-parametric permutation testing with 10 000

permutations. The resulting contrast maps were thresholded with a threshold-free cluster enhancement approach (Smith & Nichols 2009), $P < 0.05$ corrected for multiple comparisons. Third, a standard region of interest (ROI) GLM analysis was performed to assess changes in activation amplitude of individual network areas between groups. Per participant, percent BOLD signal change was extracted from each area within the working-memory network for the 1-back > 0-back and 2-back > 0-back contrast at baseline and follow-up with Featquery (implemented in FSL). These were subsequently compared between groups with rANOVAs. Separate masks were made per area per hemisphere based on the working-memory network map thresholded at $Z > 2.3$. Percent BOLD signal change was quantified with Featquery (implemented in FSL).

Within the group of individuals with a history of heavy cannabis use ($n = 26$, including the four abstainers), we investigated the relationship between network function and history of substance use. Separately for baseline and follow-up, Pearson correlations were calculated between individual network strength, network activation amplitude (1-back versus 0-back and 2-back versus 1-back) and measures of cannabis, alcohol, nicotine and other illegal psychotropic substance use exposure. Furthermore, the predictive power of baseline measures of substance use and working-memory-related network function for the prediction of 3-year changes in cannabis use and problem severity was investigated. Similarly, it was also investigated if baseline measures could predict changes in alcohol use and problems (AUDIT). Univariate associations were first assessed by calculating Pearson's correlations with change in cannabis use (g/week follow-up—g/week baseline) and change in problem severity (CUDIT follow-up—CUDIT baseline) as the dependent variables. Second, potential significant predictors were entered in a regression model to assess the unique variance explained by each predictor. In the analyses, we used a Bonferroni correction for multiple comparisons.

RESULTS [2]

Sample characteristics over time

Table 1 shows the changes in sample characteristics between groups over time. Verbal IQ did not significantly differ between groups and did not change over time. Analysis of symptoms of depression (BDI) indicated a main effect of group ($F_{1,43} = 4.10$, $P = 0.049$, $\eta^2 = 0.09$) and an interaction effect between group and time ($F_{1,43} = 4.62$, $P = 0.037$, $\eta^2 = 0.10$). Symptoms of depression were higher in the heavy cannabis users at baseline only ($P_{corr} = 0.006$) because of a reduction in symptoms in the heavy cannabis users group over time ($P_{corr} = 0.016$). Analysis of AUDIT scores indicated a main effect of time ($F_{1,43} = 10.97$, $P = 0.002$, $\eta^2 = 0.20$). Alcohol use and problems did not significantly differ between groups at baseline, but the group difference was marginally significant at follow-up ($P_{corr} = 0.052$) because of an increase in AUDIT scores in the heavy cannabis users ($P_{corr} = 0.003$). Analysis of illegal psychotropic substance use (lifetime episodes) indicated a main effect of time ($F_{1,43} = 7.44$, $P = 0.009$, $\eta^2 = 0.15$), group ($F_{1,43} = 11.42$, $P = 0.002$, $\eta^2 = 0.21$) and an interaction effect between time and group ($F_{1,43} = 6.53$, $P = 0.014$, $\eta^2 = 0.13$). Illegal psychotropic substance use differed significantly between groups at baseline ($P_{corr} < 0.001$) and follow-up ($P_{corr} = 0.005$), and increased in the heavy cannabis users over time ($P_{corr} < 0.001$). Percentage of smokers did not change over time, but there were more cigarette smokers among the heavy cannabis users at baseline ($x^2 = 8.32$, $P_{uncorr} = 0.006$) and follow-up ($x^2 = 11.03$, $P_{uncorr} < 0.001$). Given the low number of smokers in the control group, changes in smoking characteristics were only statistically investigated within the group of smoking heavy cannabis users; paired sample *t*-tests indicated an increase in FTND scores ($t_{11} = 3.56$, $P_{uncorr} = 0.005$) and duration of smoking ($t_{11} = 7.19$, $P_{uncorr} < 0.001$), whereas the average number of cigarettes per day did not change. Regarding cannabis use within the group of heavy cannabis users, average weekly cannabis use, cannabis-related problems (CUDIT) and self-reported abstinence did not significantly change over time, whereas duration of heavy cannabis use ($t_{22} = 7.14$, $P_{uncorr} < 0.001$) and lifetime cannabis use ($t_{22} = 2.44$, $P_{uncorr} = 0.024$) significantly increased. None of the heavy cannabis users sought treatment for their cannabis use during the follow-up period.

n-back behavioral performance over time

Due to a technical error, behavioral n-back data from two heavy cannabis users at follow-up were lost (remaining sample $n = 20$). RTs and accuracy (Fig. 1a and b) were analyzed using rANOVAs with group as between-subject factor and memory-load (0-back, 1-back and 2-back) and time (baseline, follow-up) as within-subject factors. Analysis of RTs and accuracy indicated a main effect of memory-load (RTs: $F_{2,40} = 72.29$, $P < 0.001$, $\eta^2 = 0.78$; accuracy: $F_{2,40} = 38.55$, $P < 0.001$, $\eta^2 = 0.66$) that did not differ between groups (RTs: $F_{2,40} = 2.00$, $P = 0.25$; accuracy: $F_{2,40} = 0.89$, $P = 0.42$). Both at baseline and

[2] All group analyses were performed a second time comparing individuals with a history of heavy cannabis use (including four abstinent heavy cannabis users) to the control group. Results and interpretations were very similar.

Table 1 Sample characteristics.

	Heavy cannabis users, n = *22*		*Abstinent cannabis users*, n = *4*		*Controls*, n = *23*	
	Baseline	*3-year follow-up*	*Baseline*	*3-year follow-up*	*Baseline*	*3-year follow-up*
Gender, male versus female	15:7	15:7	3:1	3:1	14:9	14:9
Age	21.0 (2.3)	24.5 (2.6)	22.4 (3.6)	24.7 (2.9)	22.1 (2.5)	25.3 (2.5)
Pre-morbid IQ (Dutch reading test)	103.7 (6.2)	104.6 (7.3)	108.3 (7.1)	109.8 (4.6)	105.8 (6.2)	103.5 (9.5)
Alcohol use and related problems (AUDIT)	6.0 (3.3)	8.2 (4.7)***	4.8 (1.3)	5.5 (3.8)	4.7 (3.4)	5.8 (3.2)
Beck depression inventory	8.9 (6.8)	5.3 (5.2)***	4.5 (2.7)	3.8 (4.2)	4.7 (6.0)*	4.0 (4.0)
Cigarette smoking, smokers versus non-smokers	13:9	12:10	2:2	2:2	4:19*	2:21**
FTND score	4.2 (1.6)[a]	4.9 (1.9)[a]****	3.0 (0.0)	3.5 (2.1)	3.0 (0.0)	4.5 (2.1)
Duration cigarette smoking (years)	4.7 (2.9)[a]	7.9 (3.3)[a]****	6.0 (1.4)	9.0 (1.4)	3.8 (1.5)	8.5 (2.1)
Cigarettes per day	10.9 (6.4)[a]	12.2 (8.3)[a]	10.9 (6.4)	12.2 (8.3)	7.8 (2.6)	15.0 (14.1)
Lifetime cannabis use episodes	1307.8 (1383.6)	2843.8 (3187.0)***	1531.2 (1191.2)	2022.5 (2114.9)	1.1 (2.0)	2.9 (5.3)***
Cannabis use and related problems (CUDIT)	10.9 (6.9)	12.4 (8.1)	9.3 (1.5)	0.5 (1.0)	0 (0)	0.3 (0.7)
Duration heavy cannabis use (year)	2.4 (2.3)	6.6 (3.0)	1.9 (0.3)	5.0 (1.2)	–	–
First cannabis use (age)	15.3 (2.7)	15.3 (2.7)	15.0 (0.8)	15.0 (0.8)	18.3 (3.1)[b]	18.3 (3.1)[b]
Onset heavy cannabis use (age)	17.9 (3.0)	17.9 (3.0)	19.7 (2.1)	19.7 (2.1)	–	–
Current cannabis use days/week	4.2 (2.3)	4.7 (2.3)	4.0 (0.7)	0.0 (0.0)	–	–
Current cannabis use gram/week	2.3 (1.9)	2.4 (2.3)	1.6 (0.9)	0.0 (0.0)	–	–
DSM-IV cannabis dependence (%)	–	55	–	0	–	0
DSM-IV cannabis abuse (%)	–	55	–	0	–	0
Symptoms DSM-IV cannabis dependence	–	3.1 (2.1)	–	0.0 (0.0)	–	0.0 (0.0)
DSM-IV alcohol dependence (%)	–	5	–	0	–	0
DSM-IV alcohol abuse (%)	–	32	–	0	–	13
Symptoms DSM-IV alcohol dependence	–	0.82 (1.2)	–	0.25 (0.5)	–	0.52 (0.5)
Self-reported abstinence (days)	1.6 (1.5)	3.2 (6.2)	4.8 (5.5)	411.0 (224.3)	–	834.4 (1085.0)[b]
Lifetime use other illegal psychotropic substances	6.0 (6.8)	38.0 (58.2)****	3.0 (3.5)	7.0 (6.8)	0.4 (2.0)**	1.5 (6.2)*

Mean (SD). *$P < 0.05$ and **$P < 0.001$ for group difference between current heavy cannabis users and controls. ***$P < 0.05$ and ****$P < 0.001$ for baseline follow-up comparison within group. [a]Mean of smokers only. [b]Mean of controls who ever used cannabis ($n = 15$). Smoking characteristics were only statistically investigated in current heavy cannabis users. Because of low sample size, no statistical analyses were performed on the abstinent heavy cannabis users as a group. AUDIT = alcohol use disorder identification test; CUDIT = cannabis use disorder identification test; FTND = Fagerström yest for nicotine dependence; SD = standard deviation.

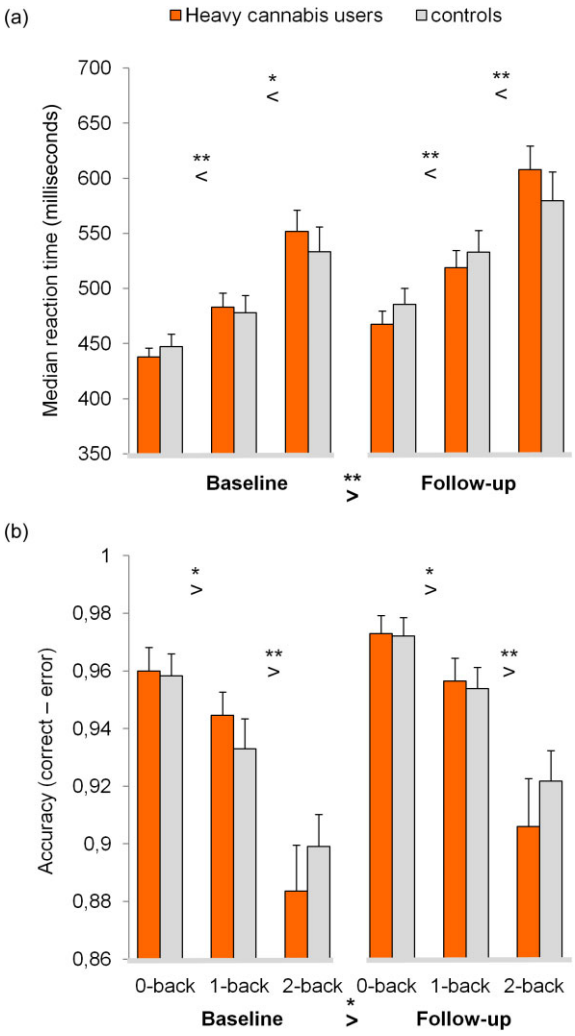

Figure 1 n-back behavioral performance per memory load level over time in heavy cannabis users ($n = 22$) and controls ($n = 23$). (a) 0-back, 1-back and 2-back median group reaction time of correct responses with standard error bars. (b) 0-back, 1-back and 2-back average group accuracy expressed as proportion correct responses, minus errors with standard error bars. *$P < 0.05$, **$P < 0.001$

follow-up, and consistent with normative n-back performance (Owen *et al.* 2005), RTs increased and accuracy decreased with increasing memory-load (1-back versus 0-back and 2-back versus 1-back: RTs $P_{corr} < 0.001$, accuracy $P_{corr} < 0.006$). Moreover, there was a main effect of time (RTs: $F_{1,41} = 16.84$, $P < 0.001$, $\eta^2 = 0.29$; accuracy: $F_{1,41} = 12.88$, $P < 0.001$, $\eta^2 = 0.24$) which did not differ between groups (RTs: $F_{1,41} = 0.11$, $P = 0.74$; accuracy: $F_{1,41} = 0.16$, $P = 0.69$) or memory-load (RTs: $F_{2,40} = 1.08$, $P = 0.35$; accuracy: $F_{2,40} = 0.31$, $P = 0.73$). Although accuracy for each memory-load increased in both groups, RTs also increased over time (0-back: RTs $P_{corr} < 0.001$, accuracy $P_{corr} = 0.018$; 1-back: RTs $P_{corr} < 0.001$, accuracy $P_{corr} = 0.018$; 2-back: RTs $P_{corr} = 0.01$, accuracy $P_{corr} = 0.035$).

Working-memory network selection

The 20 ICs identified by Tensor-ICA explained 87% of the variance in the baseline and follow-up data. Of these components, six were excluded from analysis: three contained motion artifacts and three were driven by a single participant. From the remaining 14 components, the working-memory network was identified. The best-matched component showed 86% overlap with the meta-analysis map provided by Owen and colleagues [second-best component 51%, 90% overlap with working-memory network identified in baseline data (Cousijn *et al.* 2013b)].

Working-memory network functionality over time: group comparison [3]

The working-memory network consisted of the bilateral frontal pole, VLPFC, DLPFC, premotor cortex, paracingulate cortex and inferior parietal cortex (Fig. 2a), and time-course of the network correlated strongly with the modeled n-back time-course ($r = 0.96$, $P < 0.001$; Fig. 2b). GLM analysis showed that network response strength significantly increased with task-load (1-back > 0-back, $Z = 8.52$, $P < 0.001$; 2-back > 1-back; 0-back, $Z = 15.48$, $P < 0.001$). Network strength, however, was not associated with behavioral n-back performance. Network strength did not significantly change over time ($F_{1,43} = 0.26$, $P = 0.62$) or differ between groups ($F_{1,43} = 0.09$, $P = 0.77$), and there was no significant interaction between time and group ($F_{1,43} = 3.55$, $P = 0.07$). Further, dual-regression analysis indicated that there were no significant voxel-wise spatial differences between groups regarding the working-memory network at baseline, at follow-up and over time. Also, ROI GLM analyses indicated that activity amplitude of areas within the network did not significantly change over time, did not differ between groups and showed no interaction between time and group (Table 2). There was a trend, however, toward an interaction between time and group driven by a baseline group difference in the right DLPFC, left premotor cortex and the left/right paracingulate gyrus. A *post hoc* analysis with the other seven ICs that significantly reacted to task-load also did not reveal significant group differences or changes over time.

[3] A standard whole-brain higher-level GLM analysis with FEAT yielded similar results. When looking at brain activations related to the 2-back versus 0-back and 1-back versus 0-back contrasts, there were no main effect of group and time and no interaction effect between group and time ($Z > 2.3$, whole-brain cluster corrected at $P < 0.05$).

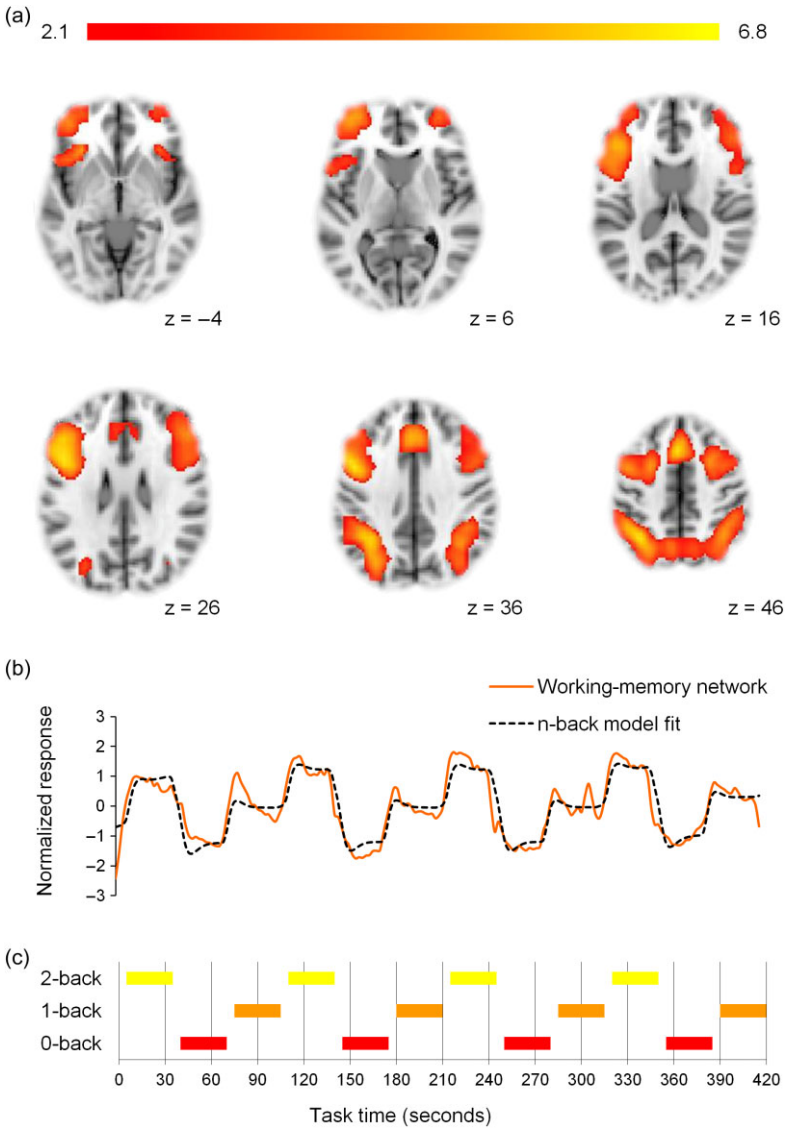

Figure 2 Spatial and temporal characteristics of the working-memory network extracted by tensor-independent component analysis (Tensor-ICA) across groups. (a) Spatial characteristics. Significant clusters are overlaid on a standard MNI (Montreal Neurological Institute) brain. Right side of the brain is depicted at right side. (b) Temporal characteristics. *Y*-axis: normalized response, *X*-axis: time (seconds), orange line: network time-course, black dotted line: task-model time-course. (c) Task-model: order of 0-back, 1-back and 2-back blocks during n-back task, *X*-axis: time (seconds)

Working-memory network functionality: relationship with substance use

Within the group of participants with a history of heavy cannabis use ($n = 26$), separate cross-sectional correlational analyses at baseline and follow-up indicated that network strength and activity amplitude were not significantly correlated with any measure of cannabis (including onset, duration, lifetime use, weekly use and problems), alcohol, nicotine or other illegal psychotropic substance use. Network strength and activity amplitude at follow-up did not differ between dependent ($n = 13$) and non-dependent ($n = 13$) cannabis users. Baseline network strength, activation amplitude and measures of cannabis, alcohol, nicotine or other illegal psychotropic substance use were not significantly associated with change in weekly cannabis use and problem severity over time. Similarly, none of the baseline measures were significantly associated with changes in alcohol use and problems.

DISCUSSION

The aim of this 3-year longitudinal fMRI study was to investigate the relationship between working-memory network functionality and substance use over time in heavy cannabis users and non-cannabis-using controls. Accuracy during the n-back task increased over time, both in heavy cannabis users and in controls, whereas working-memory network functionality did not differ or change between groups. Within the group of individuals with a history of heavy cannabis use, network functionality showed no cross-sectional correlations with measures of substance use and problems (e.g. cannabis, alcohol, nicotine, illegal psychotropic substances) and did

Table 2 Change in working-memory network activation over time: 1-back versus 0-back and 2-back versus 1-back per region in heavy cannabis users and controls.

					Signal change over time: 3-year follow-up—baseline					
	MNI coordinates				*1-back versus 0-back*			*2-back versus 1-back*		
Brain region	x	y	z	Z_{max}	*Cannabis users*	*Controls*	P	*Cannabis users*	*Controls*	P
Working-memory network:	−46	10	32	6.81	−0.10 (0.33)	−0.03 (0.30)	0.44	0.07 (0.27)	−0.08 (0.26)	0.07
Frontal pole, L	−38	54	4	5.25	−0.08 (0.56)	0.02 (0.37)	0.46	−0.03 (0.41)	−0.03 (0.32)	0.99
Frontal pole, R	34	54	4	4.00	−0.15 (0.52)	−0.02 (0.36)	0.35	0.07 (0.33)	−0.05 (0.33)	0.21
Ventrolateral prefrontal, L	−30	26	−8	5.27	−0.12 (0.35)	−0.01 (0.29)	0.23	0.11 (0.28)	−0.06 (0.30)	0.06
Ventrolateral prefrontal, R	34	26	−4	3.86	−0.13 (0.36)	0.02 (0.41)	0.18	−0.02 (0.45)	−0.09 (0.45)	0.61
Dorsolateral prefrontal, L	−46	10	32	6.81	−0.10 (0.36)	−0.03 (0.35)	0.54	0.06 (0.27)	−0.08 (0.35)	0.15
Dorsolateral prefrontal, R	50	18	28	4.73	−0.12 (0.35)	−0.04 (0.39)	0.46	0.09 (0.29)	−0.13 (0.27)	0.01
Premotor, L	30	10	52	5.33	−0.14 (0.45)	−0.09 (0.31)	0.63	0.14 (0.44)	−0.13 (0.33)	0.02
Premotor, R	−26	2	56	5.72	−0.01 (0.31)	−0.08 (0.28)	0.37	0.06 (0.31)	−0.05 (0.24)	0.20
Paracingulate, LR	−4	18	44	6.08	−0.14 (0.37)	−0.09 (0.38)	0.65	0.09 (0.30)	−0.11 (0.35)	0.05
Inferior parietal, L	−34	−54	42	6.40	−0.08 (0.34)	−0.02 (0.33)	0.51	0.09 (0.34)	−0.04 (0.34)	0.18
Inferior parietal, R	38	−54	44	5.16	−0.04 (0.34)	0.00 (0.34)	0.70	0.05 (0.27)	−0.02 (0.27)	0.38

L = left hemisphere; R = right hemisphere; MNI = Montreal Neurological Institute. MNI coordinates of maximum Z-scores are shown for the working-memory network and for each region separately. Values represent average change in percent signal change over time (3-year follow-up – baseline) for all significant voxels within the working-memory network at baseline. *P*-values are shown for the interaction between Group × Time. Critical *P*-value = 0.005 (0.05/11 regions).

not differ between dependent and non-dependent heavy cannabis users. Moreover, individual differences in network response at baseline did not predict changes in weekly cannabis use, cannabis-related problems, three years later, contrasting our previous findings in the same sample showing that working-memory network functionality predicted changes in cannabis use 6 months later (Cousijn *et al.* 2013b). Network functionality also did not predict changes in alcohol-related problems. These findings indicate that working-memory network function in terms of connectivity, location and activity remained stable over time in heavy cannabis users, despite the increases in substance use including cannabis, nicotine, alcohol and other illegal psychotropic substance use, and therefore these findings do not support the presence of neurotoxic or neuroplastic consequences of sustained frequent cannabis use. The present findings also do not support the hypothesis that working-memory network functionality predicts cannabis use and problems over a multiple year period (at least, not in a relatively healthy and relatively stable heavy cannabis users).

Cannabis use disorders tend to increase during adolescence and then decrease again during the transition toward adulthood (Schulenberg *et al.* 2005). However, average weekly cannabis use and cannabis-related problems did not change over the course of 3 years in our heavy-cannabis-using group. Heavy cannabis users and controls were matched on IQ, and because working-memory is related to performance IQ (Kane & Engle 2002), IQ matching may have resulted in a selection of heavy cannabis users with relatively good working-memory. One could therefore question whether the current sample of heavy cannabis users is at high risk for developing a cannabis use disorder. Together, these factors may explain the lack of a 3-year longitudinal association between working-memory network functionality and cannabis use-related problems. The decrease in depression symptoms in the heavy cannabis users further supports this explanation: internalizing disorders like depression and anxiety are strongly associated with cannabis dependence (Van der Pol *et al.* 2013). Moreover, potential participants were excluded if they had any psychiatric disorder, which may have resulted in the selection of relatively healthy cannabis users. Nevertheless, more than half of the heavy cannabis users met DSM-IV criteria for cannabis dependence at follow-up, and working-memory network functionality did not differ between these dependent ($n = 13$) and non-dependent ($n = 13$) cannabis users. To further elucidate the role of working-memory, more longitudinal research is needed, including a large group of chronic or dependent cannabis users. It is thereby recommended to approach ecological validity by including participants with co-morbid psychiatric problems.

Our current findings do not replicate our previous findings that baseline working-memory network functionality predicted cannabis use 6 months later. A total of seven heavy cannabis users dropped out between the 6-month and 3-year follow-up [1]. Although the MCAR

test (using the main study variables) indicated that these participants were missing at random, dropout could still be systematically driven by secondary factors not captured by the present design. Non-random dropout and the decrease in power potentially explain the discrepant findings. Indeed, a *post hoc* analysis excluding the individuals who dropped out showed that baseline network functionality no longer significantly predicted cannabis use at 6-month follow-up ($\beta = 0.18$, $P = 0.21$). Alternatively, one could speculate that the predictors of more short-term cannabis use differ from predictors of long-term cannabis use. Nonetheless, this is the first longitudinal neuro-imaging study in heavy cannabis users, generating new insights and hypotheses to be tested in future studies.

Mean alcohol use-related problems did increase to at-risk levels after 3 years (mean AUDIT score > 8; Saunders *et al.* 1993). Similarly, nicotine use and dependence and illegal psychotropic substance use also increased. Heavy cannabis use often parallels heavy alcohol, nicotine and illegal psychotropic substance use (Swift *et al.* 2012). Within the group with a history of heavy cannabis use, we therefore also investigated the relationship between working-memory network function and alcohol, nicotine and illegal psychotropic substance use. We did not observe a relationship between working-memory-related network functionality and any of the substance use measures. The current results therefore suggest that there is no dose-dependent relationship between working-memory network functionality and moderate to heavy levels of cannabis, nicotine, alcohol and illegal psychotropic substance use in young adults.

Onset of heavy cannabis use during adolescence may be an important contributor to the potential neurotoxic and neuroplastic effects of chronic cannabis exposure (Meier *et al.* 2012). Maturation of the prefrontal cortex is thought to continue into adulthood (Casey, Getz & Galvan 2008). However, we did not observe a relationship between onset of heavy cannabis use and functionality of the working-memory network in the current sample of young adult users. Average age of onset of heavy cannabis use (mean = 17.9) was relatively high, which is in line with the hypothesis that the aversive effects of cannabis exposure are prominent during early and mid-adolescence. Indeed, most studies that report hyperactivity of the working-memory network in heavy cannabis users were performed in mid-adolescent populations (Jacobsen *et al.* 2007; Padula *et al.* 2007; Jager *et al.* 2010; Schweinsburg *et al.* 2010). Neurocognitive decline is most evident in cannabis users with a DSM-IV diagnosis of dependence with an onset before age 18 years old (Meier *et al.* 2012), and onset of heavy cannabis use before the age of 16 years old was found to be related to hyperactivity of the working-memory network in adult cannabis users (Becker *et al.* 2010). The current findings argue against neurotoxic effects of chronic cannabis exposure during late adolescence and thereafter; however, more longitudinal studies are needed, preferably including a measurement before the onset of substance use.

Some potential limitations must be taken into account. Unfortunately, we did not measure cannabis dependence at baseline. We therefore could not investigate the relationship between working-memory network functionality and the transition toward dependence. Also, n-back performance was very high and ceiling effects (90% accuracy for the highest memory-load) may have obscured cannabis-induced working-memory deficits. We recommend including a 3-back level in future research. Moreover, the n-back task is a reliable task to investigate functionality of the working-memory network, but it has poor reliability as a behavioral measure of working-memory (Jaeggi *et al.* 2010). One should therefore also consider including a more reliable working-memory task outside the MRI scanner. Furthermore, gender may moderate cannabis effects on brain function (McQueeny *et al.* 2011). In accordance with estimated gender ratios among cannabis-dependent individuals, we included one-third female participants. Because of the limited number of female heavy cannabis users, we did not investigate gender effects. Finally, the results should be interpreted bearing in mind that all substance use measures were based on self-reports, which may have influenced the results.

In summary, the current 3-year longitudinal fMRI study is the first to demonstrate that functionality of the working-memory network in young adult heavy cannabis users remains at normative levels over time, despite prolonged and increasing substance use. These findings imply that moderate to heavy levels of cannabis, nicotine, alcohol and illegal psychotropic substance use do not seem to impair working-memory network functionality and working-memory performance in young adults. Moreover, baseline network functionality did not predict cannabis use and related problems three years later, warranting longitudinal studies in more chronic or dependent cannabis users.

Acknowledgements

This study was made possible through funding received from the Academic Medical Centre of the University of Amsterdam, a grant from the Netherlands Organization for Scientific Research—Health Research and Development, ZON-Mw Grant No. 31180002 awarded to A.E.G. and W.B., and a VICI grant 453.008.001 awarded to R.W.W. by the Dutch Organization for Scientific Research (Nederlandse Organisatie voor Wetenschappelijk

Onderzoek). Scanning costs were partly funded by a grant from the Amsterdam Brain Imaging Platform to J.C.

Authors Contributions

JC, RWW, AEG and WB were responsible for the study concept and design. JC, CWAMV and LK collected the data. JC performed the data analysis, assisted by CWAMV and LK. All authors assisted with the data interpretation. JC drafted the manuscript. All authors critically reviewed content and approved final version for publication.

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