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

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CHAPTER 3

# CUE-INDUCED BRAIN ACTIVITY ASSOCIATED WITH CANNABIS USE & CANNABIS USE-RELATED PROBLEMS

**This chapter based on:**

**Cousijn, J., Wiers, R.W., Ridderinkhof, K.R., Van den Brink, W., Veltman, D.J., Goudriaan, A.E. 2012. Neural responses associated with cue-reactivity in frequent cannabis users. Addiction Biology, in press.**

## ABSTRACT

Cue-reactivity reflects enhanced motivational processing underlying continued substance use and relapse in substance use disorders. Substance use disorders are associated with greater cue-reactivity in the orbitofrontal cortex, anterior cingulate cortex, striatum, ventral tegmental area, and amygdala. Here we examine whether this also holds for heavy cannabis users. Using functional Magnetic Resonance Imaging (fMRI), neural responses to neutral and cannabis related cues were compared between heavy cannabis users (n = 31), sporadic cannabis users (n = 20), and cannabis-naive controls (n = 21). In addition, fMRI findings were correlated with the level of cannabis use, problem severity, and craving. In heavy users compared to sporadic users and controls, cannabis images engendered higher activation than control images in the ventral tegmental area. Activation of the orbitofrontal cortex, anterior cingulate cortex, and striatum was only higher in a subgroup of heavy users with high compared to low problem severity. Activity in the right putamen and right dorsolateral prefrontal cortex correlated negatively with subjective craving in heavy users. Activity was not correlated with level of cannabis use. These findings indicate that cannabis cues activate areas associated with addiction pathology in heavy cannabis users compared to sporadic users and controls. However, among heavy users, cue-reactivity seems to be primarily associated with problem severity, not with amount of cannabis use. These findings imply neural cue-reactivity as a tool in assessing problem severity of cannabis use.

## INTRODUCTION

Cannabis is one of the most frequently used illegal substances worldwide and seven to eight percent of all heavy cannabis users meet DSM-IV criteria for substance dependence (UNODC World Drug Report, 2009; Perkonigg et al., 2008). However, compared to other substances of abuse, relatively little is known about mechanisms underlying continued cannabis use.

In individuals with a substance use disorder (SUD), cue-reactivity (physical and neural reactions triggered by substance-related cues) is thought to reflect motivational processes underlying continued substance-use and relapse (for a review see Carter and Tiffany, 1999). Repeated pairing of substance-use with substance-cues, such as certain contexts, emotional states, and/or substance-related paraphernalia, causes the substance users' brain to become sensitized and conditioned towards these cues (Koob and Volkow, 2010; Leyton, 2007; Robinson and Berridge, 2000). Indeed, substance-related cues tend to grab attention (e.g., Field et al., 2005; Hester et al., 2006; Marissen et al., 2006; Mogg et al., 2005), increase craving (e.g., Goldstein et al., 2009a; Tiffany et al., 2000; Zijlstra et al., 2009), and activate approach tendencies (e.g., Field et al., 2005; Mogg et al., 2005; Wiers et al., 2009b). Further, cue-reactivity has been found to predict treatment outcome and relapse (Janes et al., 2010; Marissen et al., 2006; Payne et al., 2006).

Cue-reactivity is a multi-faceted process and this is reflected in the brain circuits involved: functional Magnetic Resonance Imaging (fMRI) studies showed that exposure to substance-related cues (compared to neutral cues) is related to widespread activations in reward, motivation, cognitive control, and memory circuits in groups with different SUDs compared to healthy controls (e.g., Garavan et al., 2000; Goldstein et al., 2009a; Janes et al., 2010; McClernon et al., 2005; Myrick et al., 2004; Smolka et al., 2006; Zijlstra et al., 2009). Self-reported craving, severity of dependence, and frequency of substance use tend to correlate with cue-induced brain activity, although not consistently over studies (e.g., Goldstein et al., 2009a; Janes et al., 2010; McClernon et al., 2005; Myrick et al., 2004; Smolka et al., 2006; Zijlstra et al., 2009). Among core brain regions associated with cue-reactivity are the orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), striatum, ventral tegmental area (VTA), and amygdala (Everitt and Robbins, 2005; Wilson et al., 2004). Dopaminergic projections from VTA to nucleus accumbens (NAc) and prefrontal areas like ACC and OFC comprise the brain-reward circuit. In early stages of SUD, the reward threshold of dopamine neurons in the VTA changes, resulting in increased firing of these neurons in response to substance related cues (Koob and Volkow, 2010). The NAc connects motivational aspects of salient stimuli with motor actions (Everitt and Robbins, 2005), whereas the ACC is involved in attention, conflict monitoring, and assessing salience of motivational information (Ridderinkhof et al., 2004). The OFC is involved in reward evaluation and together with the ACC and amygdala, the OFC is thought to play a key role in the subjective experience of craving (Koob and Volkow, 2010). The amygdala is thought to attribute emotional salience to cues and initiates approach and avoid actions (Schneider et al., 2001). The dorsal striatum is involved in habit formation as compulsive

substance use progresses (Everitt and Robbins, 2005), which is supported by a recent cue-reactivity study showing increased dorsal striatum activation in heavy-social, but not light-social drinkers (Vollstadt-Klein et al., 2010).

Most studies investigating cue-reactivity in relation to substance use focus on nicotine, alcohol, cocaine, and heroin, not on cannabis. Behavioral studies indicate that cue-reactivity in (heavy) cannabis users is also associated with increased craving (Haughey et al., 2008; Wolfling et al., 2008), attentional bias (Field et al., 2006; Field et al., 2004), and approach tendencies (Cousijn et al., 2011; Field et al., 2006). Only one study investigated cue-induced brain activity related to cannabis use. In heavy cannabis users, Filbey et al. (2009) showed that tactile cannabis cues activated various brain areas previously implicated in cue-reactivity, including OFC, ACC, striatum, VTA, and amygdala. Moreover, problems related to cannabis use correlated positively with activation in the OFC and NAc. These findings suggest similar cue-reactivity mechanisms for cannabis compared to other substances, like nicotine, alcohol, cocaine, and heroin. However, an important limitation of this study is lack of a control group. Moreover, tactile cues were used, which leaves the question whether results hold for cues of other sensory modalities, e.g., visual stimuli.

The main goal of the current fMRI study was to investigate neural mechanisms associated with (visual) cue-reactivity in cannabis users in relation to quantitative levels and problem-severity of cannabis use. Cue-reactivity was compared between heavy cannabis users, sporadic cannabis users, and cannabis naive healthy controls. Within heavy users, a subgroup experiencing high problem severity was compared to a subgroup with low problem severity and relations between cue-induced brain activity, craving, frequency of cannabis use, and duration of cannabis use were explored. Sporadic cannabis users were included because according to the incentive sensitization theory and with support from animal and some human studies, repeated sporadic exposure to addictive substances may suffice to induce sensitization and conditioned responses to substance-related cues (Leyton, 2007; Robinson and Berridge, 2000). We were *a priori* interested in the OFC, ACC, striatum, VTA, and amygdala, core regions associated with cue-reactivity in SUD groups. If sporadic users already show evidence of sensitized and conditioned responses towards cannabis they should show (possibly to a lesser extent) higher activations in similar areas. Within the group of heavy users we expected levels of cannabis use and craving to correlate positively with activity in these brain structures. Since the dorsal striatum is involved in more habitual/compulsive substance use (Everitt and Robbins, 2005), heavy users with a high problem severity were expected to show increased activation in the dorsal striatum compared to heavy users with a low problem severity.

## MATERIALS AND METHODS

The present study was part of a prospective fMRI study investigating the role of different neurocognitive and neuroimaging factors in the course of drug use in heavy cannabis users (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 informed consent prior to participation.

***Participants***

Thirty-three heavy cannabis users, twenty sporadic cannabis users, and twenty-two controls aged 18-25 were recruited through advertisements on the Internet and in cannabis outlets (coffee shops). Participants were left- and right-handed with normal or corrected-to-normal vision (fMRI compatible lenses). Groups were matched for age, gender, education, and estimated intelligence (Nelson, 1984; Schmand et al., 1991), see Table 1. Heavy cannabis use was defined as using cannabis more than 10 days per month for at least two years and not seeking treatment or having a history of treatment for cannabis use. Sporadic cannabis use was defined as having used cannabis 1 to 50 times ever (Pope et al., 2001). The control group had never used cannabis. Drug and alcohol use was controlled for by excluding participants with an Alcohol Use Disorder Identification Test (AUDIT) score higher than 10 ((Saunders et al., 1993), daily smoking more than 20 cigarettes, a positive urine screen for alcohol, amphetamines, benzodiazepines, opioids or cocaine, or using non-cannabinoid drugs on more than 100 occasions (5 participants > 10 occasions, no participant > 25 occasions). Other exclusion criteria were general MRI-contraindications, major medical disorders, or a history of major type I psychiatric disorders, which was assessed with the Mini-International Neuropsychiatric Interview [MINI (Sheehan et al., 1998), Dutch version 5.0.0]. All participants were asked to refrain from alcohol and drug use (except for nicotine and caffeine) 24-hours before testing (average 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, no metabolite quantifications were performed (all heavy cannabis users scored positive for cannabis use, whereas all sporadic cannabis users and controls scored negative). Testing took place in the late afternoon. Participants were financially compensated for their participation.

**Table 1:** Sample characteristics

| | Heavy users | | Sporadic users | | Controls | | Heavy users subgroups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Low-problem | | High-problem | |
| N (% female) | 31 (35) | | 20 (35) | | 21 (36) | | 16 (44) | | 15 (27) | |
| Age | 21.3 (2.3) | | 22.1 (2.5) | | 22.1 (2.4) | | 21.4 (2.4) | | 21.1 (2.3) | |
| Verbal IQ (Dutch Reading Test) | 103.9 (4.9) | | 105.5 (7.5) | | 104.2 (7.2) | | 103.1 (5.2) | | 104.7 (4.6) | |
| Alcohol use and problems (AUDIT) | 6.3 (3.3) | | 5.5 (3.8) | | 4.6 (3.0) | | 7.1 (4.1) | | 5.5 (2.2) | |
| Cigarette smoking (%) | 68** | | 25 | | 9 | | 75 | | 60 | |
| Nicotine dependence (FTND) | 2.8 (2.5)** | | .8 (1.4) | | .3 (.9) | | 3.4 (2.6) | | 2.3 (2.2) | |
| Duration cigarette smoking (year) | 3.7 (3.5)** | | 1.0 (2.0) | | .3 (.9) | | 4.3 (3.5) | | 3.0 (3.7) | |
| Cigarettes per day | 6.8 (7.4)** | | 2.2 (4.4) | | .7 (2.3) | | 8.9 (8.1) | | 4.4 (5.9) | |
| Lifetime cannabis use (# joints) | 1579.0 (1429.8) | | 9.9 (11.4) | | 0 (0) | | 1862.5 (1605.5) | | 1268.3 (1193.2) | |
| Cannabis use and problems (CUDIT) | 12.6 (5.9) | | 0 (0) | | 0 (0) | | 17.1 (4.4)** | | 7.8 (2.1) | |
| Duration heavy cannabis use (year) | 2.5 (1.9) | | -- | | -- | | 2.9 (2.4) | | 2.2 (1.2) | |
| Cannabis use days/week | 5.0 (1.5) | | -- | | -- | | 5.2 (1.4) | | 4.7 (1.6) | |
| Cannabis use gram/week | 3.1 (2.3) | | -- | | -- | | 3.8 (2.7) | | 2.4 (1.4) | |
| | Pre-test | Post-test | Pre-test | Post-test | Pre-test | Post-test | Pre-test | Post-test | Pre-test | Post-test |
| MCQ score | 30.3 (12.8) | 36.8 (14.0)## | 13.1 (2.1) | 13.0 (1.9) | 12.0 (.0) | 12.8 (2.3) | 35.4 (13.9)* | 43.3 (14.5)*# | 24.6 (8.6) | 30.2 (10.5) # |

Means (SD); *$p < .05$ and **$p < .001$ for group and subgroup comparisons; #$p < .05$ and ##$p < .001$ for pre-test post-test comparison; SD, standard deviation; MCQ, Marijuana Craving Questionnaire; AUDIT, Alcohol Use Disorder Identification Test; CUDIT, Cannabis Use Disorder Identification Test. High-problem cannabis use: CUDIT ≥ 12. Low-problem cannabis use: CUDIT < 12.

***Questionnaires***

Problem severity of cannabis use was assessed with the Cannabis Use Disorder Identification test (CUDIT: Adamson and Sellman, 2003). The CUDIT is a screening-instrument for at-risk cannabis use and consists of 10 items on cannabis use-frequencies and severity of use-related problems (Adamson et al., 2010; Adamson and Sellman, 2003). 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 present cannabis and nicotine use was recorded. The short version of the Marijuana Craving Questionnaire (MCQ) was used to assess craving before (pre-test) and after (post-test) the fMRI-session (Heishman et al., 2009).



***Event-related cue-reactivity task***

To measure cue-induced brain activity, an event-related fMRI-task was used during which participants viewed full-color cannabis-related images (*n* = 30), control images (*n* = 30), and target images (*n* = 15). Cannabis images were photos of cannabis, individuals smoking cannabis, and objects for using cannabis. Control images were photos of individuals and objects visually matched to the cannabis images on color and composition. Target images were photos of animals. Participants were asked to pay close attention to the images. To ensure maintained attention, they were instructed to press a key on a response box when they saw an animal. The task was similar to a task previously used to assess the relation between nicotine use, craving, and cue-induced brain activity (McClernon et al., 2005). Each image was presented 4s preceded by a fixation-cross that lasted on average 4s, jittered between 2s and 6s. The cannabis, control, and animal images were presented in the same semi-random order (max three images of the same category in a row) for each participant. Images were projected on a screen viewed through a mirror attached to the MRI head coil. Total task time was 11 minutes. To verify validity of cannabis and control cues, participants performed an image-rating task outside the MRI-scanner, after scanning. All images presented inside the scanner were presented a second time on a computer screen. Craving was assessed after each image with a visual analogue scales ranging from -50 (not at all) to 50 (extremely) asking 'How much do you crave for a joint?'.

***Imaging parameters and data 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 start of each scan-session a T1 structural 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°). During the cue-reactivity task, BOLD signal was measured with a T2* gradient-echo EPI sequence (TR 2.29s, TE 30 ms, 38 slices, slice thickness 3 mm, interslice gap .3 mm, FOV 220 x 220 mm, in-plane resolution 96 x 96, flip angle 80°). Data pre-processing was conducted with FEAT (FMRI Expert Analysis Tool)

version 4.1, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). First, non-brain tissue and skull was removed with BET (Brain Extraction Tool). Images were then slice-time aligned, motion corrected, high-pass filtered in the temporal domain (sigma = 50 s), spatially smoothed with a 5 mm full-with-half-maximum Gaussian kernel, and prewhitened (Woolrich et al., 2001). Next, functional data were registered to the participants' structural image and transformed to MNI space (Montreal Neurological Institute) using FLIRT (FMRIB's Linear Image Registration Tool).



**Table 2** Brain regions activated by cannabis Images versus neutral Images in heavy users, sporadic users, and controls: main effects per group and group comparisons

| Clustersize (voxels) | Brain region | Hemisphere | MNI coordinates | | | $Z_{max}$ |
|---|---|---|---|---|---|---|
| | | | *x* | *y* | *z* | |
| ***Heavy users*** | | | | | | |
| 2546 | Posterior cingulate gyrus/precuneus | L/R | -2 | -34 | 32 | 5.97 [b] |
| 2168 | Medial frontal/orbitofrontal cortex | L/R | -2 | 58 | -12 | 5.11 [b] |
| 914 | Superior frontal gyrus | L | -22 | 46 | 46 | 4.21 [b] |
| 1241 | Lateral occipital/angular gyrus | L | -58 | -66 | 24 | 4.14 [b] |
| 593 | Lateral occipital/angular gyrus | R | 44 | -66 | 42 | 3.89 [b] |
| ***Sporadic users*** | | | | | | |
| 1795 | Posterior cingulate gyrus/precuneus | L/R | -10 | -54 | 28 | 4.42 [b] |
| 1217 | Medial frontal gyrus | L/R | -2 | 66 | 22 | 3.81 [b] |
| 683 | Lateral occipital/angular gyrus | L | -46 | -66 | 42 | 4.28 [b] |
| ***Controls*** | | | | | | |
| 2493 | Posterior cingulate gyrus/precuneus | L/R | -2 | -68 | 30 | 5.57 [b] |
| 1667 | Lateral occipital/angular Gyrus | L | -58 | -60 | 34 | 5.26 [b] |
| 1160 | Dorsolateral prefrontal cortex | L | -34 | 16 | 40 | 4.90 [b] |
| ***Heavy users > sporadic users and controls*** | | | | | | |
| 88 | VTA | L | -16 | -16 | -10 | 3.07 [a] |
| 66 | VTA | R | 12 | -18 | -14 | 3.38 [a] |
| ***Sporadic users and controls > heavy users*** | | | *No significant differences* | | | |

L, left; R, right; MNI, Montreal Neurological Institute; VTA, Ventral Tegmental Area; MNI coordinates of maximum Z-scores are shown for each cluster; [a] Significant at Region of interest level, $Z > 2.3$, cluster-corrected $p < .05$; [b] Significant at whole brain level, $Z > 2.3$, cluster-corrected $p < .05$.

***Statistical analysis***

Demographics and scores on questionnaires were compared between groups with standard univariate analysis of variance procedures. fMRI time-series analysis was carried out with FILM (FMRIB's Improved Linear Model) with local autocorrelation correction. Explanatory variables were created for cannabis images, control images, and target images by convolving timing parameters with a double gamma hemodynamic response function. Higher-level analysis of cannabis-cue vs. neutral-cue contrast-images were conducted using FLAME (FMRIB's local analysis of mixed effects) stages 1 and 2.

*A priori* ROIs implicated in cue-reactivity were the OFC, ACC, striatum, VTA, and amygdala. The Nielsen and Hansen's volume of interest database (Nielsen and Hansen, 2002) was used to obtain anatomical masks of the OFC, ACC, striatum, and amygdala. The VTA mask (x = -20 to 20, y = -10 to 24, z = -6 to -22) was manually drawn on the standard MNI brain in FSL-view with help of the Talairach Daemon implemented in FSL and the LONI probability atlas (Shattuck et al., 2008). Activity within ROI masks was considered significant if $Z > 2.3$, with a cluster probability of $p < .05$ adjusted for the small search volume (Worsley et al., 1992). Activity outside ROI masks was considered significant if $Z > 2.3$, with a whole-brain corrected cluster probability of $p < .05$ (Worsley et al., 1992). Additionally, main effects per group are reported whole-brain corrected, $Z > 2.3$, with a cluster probability of $p < .05$.

Brain activity in heavy users was first compared with sporadic users and controls. Second, activity in sporadic users was compared to controls. Third, within the group of heavy users, users with high and low levels of problem severity were compared using the median split of the CUDIT-score (score = 12), resulting in a group of 16 high-problem (CUDIT scores ≥ 12; mean 17.1) and 15 low-problem heavy users (CUDIT scores < 12; mean 7.8). Fourth, within the group of heavy users, regression analyses were performed to investigate associations between craving, history of cannabis use, nicotine use, and cue-induced brain activity, for which we used post-test MCQ scores, weekly cannabis use (gram), lifetime use (number of joints), duration of heavy use (years), duration of cigarette smoking (years), cigarettes per day, and FTND scores as dependent measures. To further assess possible confounding effects of nicotine use, smoking heavy users were compared with non-smoking heavy users. Finally, within the group of sporadic users, the relation between lifetime cannabis use and brain activity was assessed. For each analysis contrasts were analyzed in both directions (i.e., heavy users > sporadic users and controls and sporadic users and controls > heavy users). Clusters of activation were localized with the Talairach Daemon database implemented in FSL and the LONI probability atlas (Shattuck et al., 2008).

## RESULTS

### *Sample characteristics*

One heavy user and one control were excluded from analyses due to errors in image acquisition. In addition, one heavy user was excluded due to excessive motion (> 3mm). The remaining sample consisted of 31 heavy users, 20 sporadic users, and 21 controls.

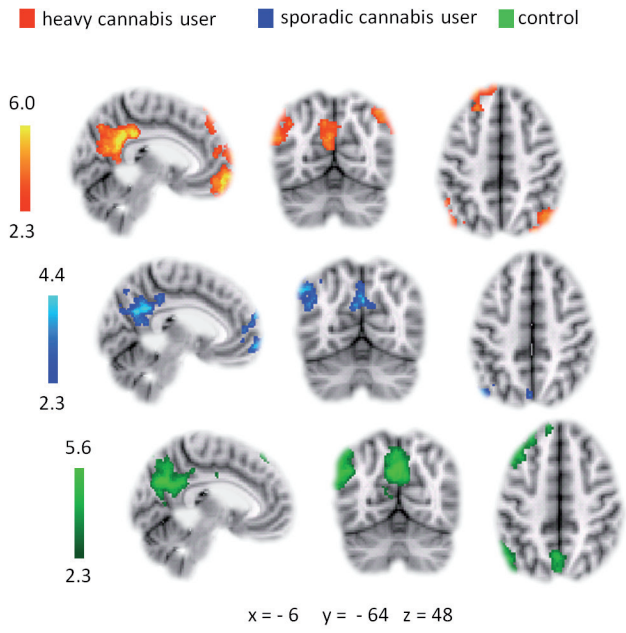
Heavy users, sporadic users, and controls did not significantly differ in age, gender, IQ, and alcohol use/problems. However, heavy cannabis users scored higher on measures of nicotine use [smokers (%), $X^2 = 22.80$, $p < .001$; smoking duration (years), $F_{2,70} = 12.67$, $p < .001$; cigarettes/day, $F_{2,70} = 8.86$, $p < .001$], and nicotine dependence (FTND, $F_{2,70} = 15.06$, $p < .001$; Table 1). Heavy users reported higher post-test craving compared to pre-test craving ($t_{30} = 3.4$, $p = .002$). Image-ratings were analyzed with a mixed ANOVA with group as between subject factor and image type as within subject factor with two levels (cannabis and neutral images). A main effect of image type ($F_{1,70} = 4.96$ , $p = .029$) indicated that participants generally reported more craving after seeing cannabis images. The interaction between image type and group was significant ($F_{2,70} = 20.4$ , $p < .001$). Post-hoc analysis confirmed that only heavy users craved more after seeing cannabis images compared to sporadic users ($t_{49} = 4.44$, $p < .001$) and controls ($t_{51} = 5.28$, $p < .001$). No other post-hoc test was significant.

Within the group of heavy users, high- and low-problem users did not differ in age, gender, verbal IQ, alcohol use, measures of nicotine use and dependence, lifetime cannabis use, duration of heavy cannabis use, cannabis use in days per week, and cannabis use in gram per week. Post-test compared to pre-test craving was higher in both high-problem ($t_{15} = 2.2$, $p = .046$) and low-problem users ($t_{18} = 3.3$, $p = .005$). However, high-problem users reported higher pre-test ($t_{29} = 2.6$, $p = .015$) and post-test ($t_{29} = 2.9$, $p = .008$) craving compared to low-problem users (see Table 1). Rating of the cannabis and control images did not differ between high- and low-problem users.

### *FMRI analysis*

Verifying general task-related activity, main effects per group showed overlapping bilateral activity for cannabis images vs. control images in posterior cingulate gyrus, precuneus, occipital cortex, and angular gyrus. Bilateral clusters in the medial frontal gyrus and OFC were only significant in heavy and sporadic users. The left superior frontal gyrus was only significant in heavy users whereas the left DLPFC was only significant in the control group (Table 2 and Figure 1). Next, ROI analyses comparing heavy users with sporadic users and controls demonstrated significantly higher activity in heavy users in bilateral VTA (Table 2 and Figure 2). Activity in the OFC, ACC, striatum, and amygdala did not differ significantly between heavy users, sporadic users, and controls. No significant differences were observed between sporadic users and controls.

**Figure 1** Activity related to cannabis images vs. neutral images in heavy users, sporadic users and controls. Clusters of significant activation ($Z > 2.3$, cluster-corrected at $p < .05$) are overlaid on a standard MNI brain. Right side of the brain is depicted at right side.




Within the group of heavy cannabis users, high-problem and low-problem subgroups were compared. ROI analysis demonstrated higher activity in high-problem compared to low-problem heavy users in the bilateral OFC, bilateral ACC, bilateral NAc, and right caudate (Table 3 and Figure 3). Activity in the amygdala did not differ between high-problem and low-problem users. Outside ROIs, high-problem users showed higher activity in the left medial frontal gyrus and left temporal pole (Table 3). Low-problem users did not show any significant increases in activity compared to high-problem users.

Within the group of heavy users, ROI analysis demonstrated that craving (MCQ) was inversely correlated with activity in the right putamen (Table 3 and Figure 4). Activity in the OFC, ACC, amygdala, and other parts of the striatum were not correlated with craving. Outside ROIs, activity in the right dorsolateral prefrontal cortex (DLPFC) was inversely correlated with craving. No correlations were found between cue-induced activity and measures of cannabis use [i.e., gram, duration of heavy use (years), total lifetime use (episodes)] and nicotine use and dependence [i.e., duration of cigarette smoking (years), cigarettes per day, and FTND scores]. Additionally, no significant differences were observed between smoking heavy users and non-smoking heavy users. Within the sporadic users, no correlation was found between lifetime cannabis use and brain activity.

**Table 3** Analyses within heavy users: brain regions activated in high-problem vs. low-problem subgroups and brain regions correlating negatively with craving

| Clustersize (voxels) | Brain region | Hemisphere | MNI coordinates | | | $Z_{max}$ |
|---|---|---|---|---|---|---|
| | | | *x* | *y* | *z* | |
| ***High-problem > low-problem*** | | | | | | |
| 541 | Medial frontal gyrus | L | -6 | 64 | 10 | 3.62 [b] |
| 279 | Anterior cingulate cortex | L | -10 | 42 | 24 | 3.45 [ab] |
| | Anterior cingulate cortex | R | 10 | 42 | 20 | 3.75 [ab] |
| 292 | Orbitofrontal cortex | L | -34 | 26 | -14 | 3.73 [ab] |
| | Orbitofrontal cortex | R | 4 | 50 | -12 | 4.01 [ab] |
| 177 | Putamen | L | -28 | -10 | -6 | 3.43 [ab] |
| | Caudate | L | -6 | 12 | 2 | 3.18 [ab] |
| | Caudate | R | 16 | 12 | 6 | 3.67 [ab] |
| | Nucleus accumbens | L | 8 | 8 | -6 | 3.10 [ab] |
| | Nucleus accumbens | R | -4 | 8 | -8 | 3.01 [ab] |
| 497 | Temporal pole | L | -52 | 6 | 26 | 3.67 [b] |
| ***Low-problem > high-problem*** | | | *No significant differences* | | | |
| ***MCQ negative correlations*** | | | | | | |
| 817 | Dorsolateral prefrontal cortex | R | 46 | 10 | 30 | 3.27 [b] |
| 149 | Putamen | R | 30 | 0 | 4 | 3.25 [a] |

L, left; R, right; MNI, Montreal Neurological Institute; High-problem cannabis use: CUDIT ≥ 12; Low-problem cannabis use: CUDIT < 12; MCQ: Marijuana Craving Questionnaire; MNI coordinates and Z-scores of local maxima are shown for each cluster; [a] Significant at Region of interest level, $Z > 2.3$, cluster-corrected $p < .05$; [b] Significant at whole brain level, $Z > 2.3$, cluster-corrected $p < .05$.

## DISCUSSION

The main goal of this fMRI study was to investigate neural mechanisms associated with cue-reactivity in cannabis users in relation to levels of cannabis use, craving, and problem severity. To the best of our knowledge, this is the first study comparing cue-induced brain activity in cannabis users and controls. Analyses were *a priori* focused on the OFC, ACC, striatum, VTA, and amygdala, all core regions associated with cue-reactivity in SUDs (Everitt and Robbins, 2005; Wilson et al., 2004). In line with our hypothesis, viewing cannabis vs. control images was associated with more VTA activation in heavy users compared to sporadic users and controls. However, the OFC, ACC, ventral striatum, and dorsal striatum were only activated in heavy users with high problem severity (CUDIT). Outside *a priori* ROIs in reward/attention/motivational brain circuits, these high-problem

users also showed higher activity in the medial frontal cortex and areas involved in visual processing. These results are generally consistent with an fMRI study in cannabis users that did not include a control group and used tactile stimuli (Filbey et al., 2009) and support similar cue-reactivity mechanisms for cannabis compared to other substances, like nicotine, alcohol, cocaine, and heroin (e.g., Garavan et al., 2000; Goldstein et al., 2009a; Janes et al., 2010; McClernon et al., 2005; Myrick et al., 2004; Smolka et al., 2006; Zijlstra et al., 2009). Craving in heavy users was inversely correlated with cue-induced brain activity in the putamen and DLPFC.

Importantly, cue-induced brain activity in cannabis users seems to be primarily associated with problem severity, not with levels of cannabis consumption. Although heavy users compared to sporadic users and controls showed higher VTA activity, most striking differences were observed between heavy users with high compared to low problem severity. The relative short duration and moderate to heavy levels of cannabis use in this sample suggests that quantitative levels of cannabis might have been too low to detect cue-reactivity differences in the OFC, ACC, striatum, and amygdala. However, neither frequency nor history of cannabis use correlated with cue-reactivity. Furthermore, high- and low-problem subgroups did not differ on quantities and duration of cannabis use. In view of this, average problem severity rather than quantitative cannabis use of the heavy users might have been too low to detect cue-reactivity differences in the OFC, ACC, striatum, and amygdala. As a measure of problem severity, the CUDIT does not distinguish between dependent and non-dependent cannabis use. However, most heavy users in the high-problem subgroup probably were dependent whereas most low-problem users were cannabis abusers [estimated sensitivity and specificity for identifying cannabis dependence of scoring ≥ 12 is 90.8 % and 75.4 %, respectively (personal communication from S. Adamson based on data from Adamson et al., 2010)]. Beside problem severity, craving was also higher in high-problem compared to low-problem users. Craving is thought to play a key role in relapse and most areas showing hyperactivation in high-problem users have been linked to relapse in nicotine and alcohol dependent populations (Grusser et al., 2004; Janes et al., 2010). Together, these findings may suggest cue-reactivity as a tool in the diagnosis and prediction of cannabis dependence. However, prospective studies in (dependent) cannabis users are necessary to confirm this.

In addition, we also identified a brain region in which cue-reactivity was associated with level of use, but not problem severity. Cannabis cues activated the VTA in heavy users compared to sporadic users and controls. However, cue-reactivity in the VTA did not differ between high-problem and low-problem heavy users. Higher VTA activity in heavy users may reflect altered dopamine transmission due to heavy cannabis exposure. Neuro-adaptations within the VTA are thought to arise in early stages of substance abuse and dependence (Koob and Volkow, 2010), which might explain why VTA activity did not differ between high- and low-problem heavy users. Interestingly, neurons in the VTA are also sensitive to cannabis withdrawal (Spiga et al., 2010).

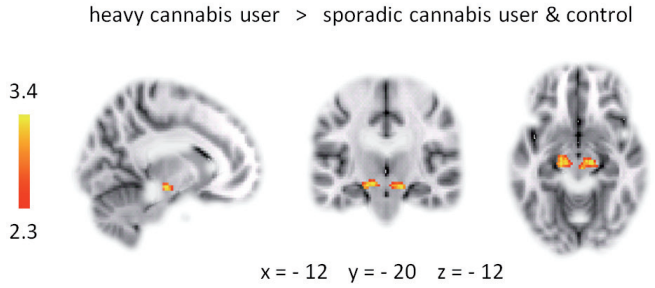



**Figure 2** Clusters in bilateral VTA in which heavy users showed higher activity related to cannabis images vs. neutral images compared to sporadic users and controls. Clusters of significant activation ($Z > 2.3$, cluster-corrected at $p < .05$) are overlaid on a standard MNI brain. Right side of the brain is depicted at right side.

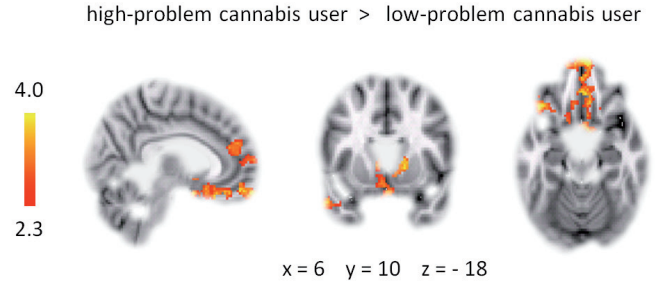



**Figure 3** Clusters in right ACC, bilateral OFC, bilateral NAc, bilateral caudate, and left temporal pole in which high-problem users (CUDIT score ≥ 12) showed higher activity compared to low-problem users (CUDIT score < 12)**.** Clusters of significant activation ($Z > 2.3$, cluster-corrected at $p < .05$) are overlaid on a standard MNI brain. Right side of the brain is depicted at right side.

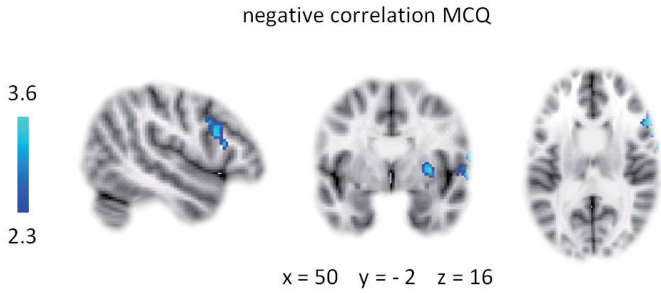



**Figure 4** Cluster in right DLPFC and right putamen ($Z > 2.3$, cluster-corrected at $p < .05$) in which activity in heavy users correlated negatively with craving (MCQ). Clusters of significant activation are overlaid on a standard MNI brain. Right side of the brain is depicted at right side.

Future research is needed to explore the connection between cannabis cue-reactivity and cannabis withdrawal in this region.

According to the incentive sensitization theory repeated sporadic exposure to substances of abuse may suffice to induce sensitized and conditioned responses to substance-related cues (Leyton, 2007; Robinson and Berridge, 2000). However, in the current study cue-induced brain activation did not differ between sporadic users and controls. These results indicate that sensitized and conditioned responses towards cannabis cues in sporadic users are not very likely.

In contrast to our hypotheses, a negative relation between craving and activity in the right putamen and right DLPFC was observed. The DLPFC is involved in self-control and hypoactivation has been linked to poor decision-making in dependent cannabis users (Bolla et al., 2005). A potential explanation for the negative relation between cue-reactivity and putamen activity is currently lacking. Although the findings indicate a relation between craving and cue-reactivity in cannabis users, clearly more research is needed. Another discrepancy is that amygdala activity did not differ between heavy users, sporadic users, and controls and also not between high- and low-problem heavy users. This might be a direct effect of cannabis since THC exposure (the primary psychoactive ingredient in cannabis) is associated with reduced amygdala reactivity (Phan et al., 2008). In the present study, heavy cannabis users were abstinent for at least one day whereas Filbey et al. (2009) found amygdala activation in heavy users after at least three days of abstinence.

Some potential limitations must be taken into account. First, there were more smokers among heavy users and almost all (90 %) heavy users smoked cannabis combined with tobacco [most common in the Netherlands (Bennett, 2008)]. Although nicotine use was not associated with cue-reactivity in heavy users, nicotine possibly increases cannabimetic effects (McPartland et al., 2008) and resemblances between cannabis and nicotine cigarettes might account for part of the group differences. However, strongest differences were observed independently of nicotine use since tobacco smoking did not differ between high- and low-problem heavy users. Including a group of cannabis naive cigarette smokers is recommended in future studies in order to distinguish cannabis from nicotine effects. Second, there were no general appetitive/positive images in the cue-reactivity task, thus it could not be determined if abnormal motivational processing of cannabis cues in heavy users generalizes to other rewarding stimuli. To address this issue and improve understanding of motivational aspects of cannabis cues in relation to general rewarding stimuli, future studies should include general rewarding stimuli. Third, inclusion of both right- and left-handed participants may be a limitation. However we are not aware of fMRI-studies showing confounding effects of hand dominance in cue-reactivity paradigms.

In summary, the current fMRI study is the first to demonstrate that heavy cannabis users exhibit higher activity in reward related brain areas in response to visual cannabis-cues compared to sporadic cannabis users and controls. Reactivity to cannabis images was primarily related to

severity of cannabis-related problems, not to levels of cannabis use: most core regions associated with cue-reactivity were only active in heavy users scoring high for problem-severity, independently of frequency and history of cannabis use. These findings are consistent with previous findings and support a role of cue-reactivity in assessing problem severity of cannabis use.

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