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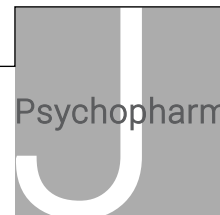
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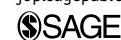
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# Cue-induced striatal activity in frequent cannabis users independently predicts cannabis problem severity three years later

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## Abstract

Cannabis is the most frequently used illicit drug worldwide, but little is known about the mechanisms underlying continued cannabis use. Cue-reactivity (the physical, psychological, behavioural and neural reaction to substance-related cues) might be related to continued cannabis use. In this 3-year prospective neuroimaging study we investigated whether cannabis cue-induced brain activity predicted continued cannabis use and associated problem severity 3 years later. In addition, baseline brain activations were compared between dependent and non-dependent cannabis users at follow-up. Analyses were focussed on brain areas known to be important in cannabis cue-reactivity: anterior cingulate cortex, orbitofrontal cortex, ventral tegmental area, amygdala and striatum. At baseline, 31 treatment-naïve frequent cannabis users performed a cue-reactivity functional magnetic resonance imaging task. Of these participants, 23 completed the 3-year follow-up. None of the cue-induced region of interest activations predicted the amount of cannabis use at follow-up. However, cue-induced activation in the left striatum (putamen) significantly and independently predicted problem severity at follow-up ( $p < 0.001$ ) as assessed with the Cannabis Use Disorder Identification Test. Also, clinically dependent cannabis users at follow-up showed higher baseline activation at trend level in the left striatum compared with non-dependent users. This indicates that neural cue-reactivity in the dorsal striatum is an independent predictor of cannabis use-related problems. Given the relatively small sample size, these results are preliminary and should be replicated in larger samples of cannabis users.

## Keywords

Addiction, cannabis, cannabis use disorder, cue-reactivity, fMRI, striatum

## Introduction

Cannabis is the most frequently used illicit drug (Degenhardt and Hall, 2012), thereby leading to large numbers of individuals with cannabis dependence (Stinson et al., 2006). With the exception of individual differences in genetic vulnerability (Agrawal and Lynskey, 2006), very little is known about why some people develop cannabis use-related problems and cannabis dependence whereas others do not. More insight in the underlying neurobiological mechanisms of cannabis use-related problems and cannabis dependence is important, as it can contribute to development of new prevention and treatment strategies. Cue-reactivity, defined as the physical, psychological, behavioural and neural reactions triggered by exposure to substance-related cues (Chiamulera, 2005), reflects enhanced motivational processes towards substance use and has been associated with increased substance use (Henry et al., 2014). Furthermore, cue-reactivity has been found to predict treatment outcome and relapse in cigarette, alcohol and heroin addiction (Grüsser et al., 2004; Janes et al., 2010; Marissen et al., 2006; Payne et al., 2006), suggesting that it may play a role in the continuation of drug use, including cannabis use and dependence. Much less is known about the role of cannabis cue-reactivity in continued cannabis use and relapse. However, initial studies suggest that both brain and behavioural responses to cannabis cues may predict short-term (i.e. over the course of 6 months or less) levels of cannabis use, associated psychiatric problems and treatment outcome (Cousijn et al., 2011, 2012, 2015; Feldstein Ewing et al., 2013). In this study we

aimed to investigate if baseline cue-induced brain activity in brain regions associated with cue-reactivity predicts the amount of cannabis use and associated problem severity in treatment-naïve frequent cannabis users 3 years later.

In a previous report on the baseline findings of the present study, we found significant activation in the ventral tegmental area (VTA) when viewing cannabis versus neutral stimuli in frequent cannabis users compared with control subjects. These

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frequent cannabis users had used cannabis on average 5 days per week for a minimum of 2 years and did not have a treatment history. Moreover, these frequent cannabis users had varying levels of cannabis use-related problems. Interestingly, activation in the orbitofrontal cortex (OFC), anterior cingulate cortex (ACC) and striatum was only found in those frequent users with high problem severity (Cousijn et al., 2013). These cross-sectional findings suggest that neural cue-reactivity may be an important predictor of cannabis problem severity. To test this hypothesis, we assessed levels of cannabis use and problem severity in the same sample of frequent cannabis users 3 years later. Using a similar approach as in the report on the baseline findings (Cousijn et al., 2013), regions of interest (ROIs) were the OFC, ACC, VTA, striatum and amygdala. All of these regions play a prominent role in substance use disorders, and activity within these areas has been associated with cue-reactivity and reward evaluation in substance-abusing populations (Cousijn et al., 2013; Filbey et al., 2009). Given the cross-sectional association between activity in the OFC, ACC and striatum and cannabis problem severity, we hypothesized that cue-induced activity in these ROIs would specifically predict problem severity: higher activity for cannabis versus neutral images was expected to predict an increase in cannabis use-related problems 3 years later and the development of cannabis dependence. To the best of our knowledge, this study is the first to investigate the causal role of neural cue-reactivity in continued cannabis use and the development of cannabis dependence, using a 3-year follow-up design.

## Experimental procedures

This study was part of a 3-year follow-up study investigating several neurobiological and behavioural factors underlying continued cannabis use (Cousijn et al., 2013, 2014), and was approved by the medical ethics committee of the Academic Medical Centre (AMC) of the University of Amsterdam. All participants signed informed consent prior to participation after the procedure had been fully explained.

### Participants

Of the 31 treatment-naïve frequent cannabis users recruited from the general Dutch population at baseline, 23 (16 male and seven female) frequent cannabis users completed the 3-year follow-up session. The remaining eight subjects did not participate in the follow-up assessment for different reasons: contact lost ( $n=3$ ), refused ( $n=3$ ), not available ( $n=2$ ). At baseline, participants were recruited through advertisements and in cannabis outlets (“coffee shops”). Participants were approached by email and telephone and invited for a follow-up test session at the AMC. Time interval between the two test sessions varied from 35 to 42 months (Mean=39 months,  $SD=2.10$ ). Mean age at follow-up was 24.14 years ( $SD=2.44$ ). Frequent cannabis use was defined at baseline as using cannabis on more than 10 days per month for at least 2 years. Baseline exclusion criteria were: a score higher than 10 on the Alcohol Use Disorder Identification Test (AUDIT) (Saunders et al., 1993), smoking more than 20 cigarettes daily, a positive urine screen for alcohol, amphetamines, benzodiazepines, opioids or cocaine, or using non-cannabinoid drugs on more than 100 occasions (Pope et al., 2001), major medical disorders or a

history of axis I psychiatric disorders. Three of the frequent cannabis users at baseline had quit using cannabis completely for at least 4 months at follow-up. All participants were instructed to abstain from alcohol, cannabis and other drugs 24 h prior to both the baseline assessment and the follow-up assessment. Urine samples were taken during the test session to control for recent illicit substance use. Although urine analysis of  $\Delta^9$ -tetrahydrocannabinol (THC) is not suitable for detection of 24-hour abstinence, it increases accuracy of self-reported substance use (Roese and Jamieson, 1993). At the start of both baseline and follow-up measurements, time of last cannabis use was recorded. The mean time of abstinence was 2.18 days ( $SD=2.77$ ) at baseline and 59 days at follow-up ( $SD=171$ ).

### Questionnaires

At baseline and follow-up, demographic information was collected. In addition, a detailed history of cannabis use was taken (e.g. age of onset of regular use, amount of cannabis use in grams per day and number of joints per day). Cannabis-related problem severity was measured using the Cannabis Use Disorder Identification Test (CUDIT) (Adamson and Sellman, 2003). The CUDIT has good test–retest reliability ( $r=0.85$ ). The severity of nicotine dependence was assessed with The Fagerström Test for Nicotine Dependence (FTND) (Heatherton et al., 1991). Craving was assessed before and after the baseline and follow-up test session with the Marijuana Craving Questionnaire (MCQ) (Heishman et al., 2009) from which session-induced craving was computed. Alcohol use and problem severity was assessed with the AUDIT (Saunders et al., 1993). All questionnaires had satisfactory or good test–retest reliability (Selin, 2003; Singleton et al., 2002; Vink et al., 2005). At follow-up, the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998) was conducted by two experienced psychologists to screen for evidence of the presence of cannabis abuse and dependence and other mental disorders according to DSM-IV. All questionnaires and interviews were conducted at baseline and at follow-up.

### Neural cue-reactivity

As described in detail in Cousijn et al. (2013), to measure cue-induced brain activity, an event-related functional magnetic resonance imaging (fMRI) task was performed during which participants viewed full-colour 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 colour and composition. Target images were photos of animals. Participants were instructed to pay close attention to the images and to press a key on a response box when they saw an animal to ensure maintained attention. Each image was presented for 4 s preceded by a fixation-cross that lasted on average 4 s, and jittered between 2 s and 6 s. Total task time was 11 minutes. The stimuli were presented in the same semi-random order (maximum of three images of the same category in a row) for each participant. The task was only conducted at baseline.

### *fMRI image parameters and data pre-processing*

For detailed information on image parameters and pre-processing the reader is referred to Cousijn et al. (2013). Briefly, images were acquired with a 3T MRI scanner (Philips Intera, Best, The Netherlands) with a phased array SENSE RF eight-channel receiver head coil. BOLD signal during the cue-reactivity task was measured with a T2\* gradient-echo EPI sequence (TR 2.29 s, TE 30 ms, 38 slices, slice thickness 3 mm, interslice gap 0.3 mm, FOV 220×220 mm, in-plane resolution 96×96, flip angle 80°). FEAT (fMRI Expert Analysis Tool) version 4.1, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) was used for data pre-processing. Pre-processed functional data were registered to participants' structural image and transformed to MNI space (Montreal Neurological Institute) using FLIRT (FMRIB's Linear Image Registration Tool). At baseline, 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 using the Talairach Daemon implemented in FSL and the LONI probability atlas (Shattuck et al., 2008) as described by Cousijn et al. (2013). The mean percentage BOLD signal change for cannabis versus neutral stimuli was calculated in FSL 5.0.1 using the Featquery toolbox for all ROIs (ACC, OFC, VTA, striatum and amygdala).

### *Data analyses*

**Demographic variables.** Analyses were conducted using the Statistical Package for the Social Science (IBM SPSS Statistics 20.0). Little's Missing Completely At Random (MCAR) test with all study variables was used to check if participants who did not complete the follow-up assessment were missing at random. Demographic data were analysed using paired sample *t*-tests to check for differences between baseline and follow-up.

**Predictors of weekly cannabis use and problem severity at 3-year follow-up.** First, Pearson's *r* was calculated to check which behavioural variables and ROIs (percentage signal change) correlated with weekly cannabis use (grams) and problem severity at follow-up. Then a hierarchical multiple regression analysis was carried out to determine whether cue-induced brain activation predicted weekly cannabis use and problem severity at 3-year follow-up. We entered the baseline percentage signal change of the ROIs in which activity correlated significantly with total CUDIT scores and weekly cannabis use (in grams) at follow-up in the model. Behavioural variables that correlated significantly with total CUDIT scores and weekly cannabis use were entered in the regression model as covariates, as were the behavioural variables that significantly changed during the 3-year follow-up period.

**Exploratory ROI analyses.** ROIs (percentage signal change) that were found to significantly predict weekly cannabis use and/or problem severity were separately and exploratively analysed to determine the exact location of the significant activation within that ROI. We used FLAME (FMRIB's local analyses of mixed

effects) stages 1 and 2 implicated in FSL. The contrast of interest was cannabis > neutral.

**Activation patterns in dependent and non-dependent users.** Finally, to check for baseline differences in activation patterns between dependent and non-dependent users measured at follow-up, an independent samples *t*-test was performed between dependent and non-dependent cannabis users (determined by the MINI) with cue-induced brain activation at baseline as dependent variable. To adjust for multiple testing we applied a Bonferroni correction corrected for the number of ROIs and outcome variables with a critical *p*-value  $\leq 0.005$ .

## **Results**

### *Demographic variables*

Little's MCAR showed that the frequent cannabis users that did not partake in the follow-up were missing at random ( $\chi^2=80.93$ , d.f.=108,  $p=0.98$ ). A paired sample *t*-test showed that the mean severity of cannabis use-related problems was stable over time, but that mean severity of tobacco dependence, mean alcohol use-related problems and mean lifetime use of other psychotropic substances significantly increased during the 3-year follow-up period (Table 1). An additional independent sample *t*-test using change scores was conducted with all variables listed in Table 1 to check for differences in changes over time between dependent and non-dependent users at follow-up. Except for lifetime cannabis use duration in years ( $p=0.033$ ), no significant differences were found between the two groups.

### *Predictors of weekly cannabis use and problem severity at 3-year follow-up*

Linear correlational analyses showed that there were no significant associations between cannabis cue-induced brain activations at baseline in the ROIs and the amount of cannabis that was used during follow-up. However, the total CUDIT score at follow-up was significantly associated with cue-induced left striatal ( $r=0.604$ ,  $p=0.002$ ) and left VTA activity ( $r=0.462$ ,  $p=0.027$ ) at baseline: higher activity during cannabis versus neutral images was related to more cannabis use-related problems. Regarding behavioural variables, total CUDIT score at follow-up correlated significantly with baseline total CUDIT score ( $r=0.581$ ,  $p=0.004$ ), baseline FTND score ( $r=0.528$ ,  $p=0.010$ ), baseline number of cigarettes per day ( $r=0.466$ ,  $p=0.025$ ) and baseline subjective craving (MCQ post-test-pre-test;  $r=-0.439$ ,  $p=0.036$ ).

Subsequently, the hierarchical multiple regression analysis was performed. Total CUDIT score at baseline was entered first. In the second step, the baseline variables that correlated significantly with the CUDIT score at follow-up (baseline FTND score, number of daily cigarettes, session-induced craving) were entered in the regression model as covariates. In addition, baseline AUDIT scores and lifetime use of other psychotropic substances at baseline were entered as covariates, because these variables changed significantly over time. To investigate the unique variance in cannabis use and cannabis use-related problems (CUDIT scores) at follow-up explained by neural cannabis cue-reactivity, left striatal and left VTA

**Table 1.** Paired sample *t*-test with sample characteristics of frequent cannabis users ( $n=23$ ) at baseline and follow-up.

	Baseline	Follow-up
	Mean (SD)	Mean (SD)
Age	20.9 (2.4)***	24.1 (2.4)***
Estimated verbal IQ (Dutch Reading Test)	104.4 (5.3)	105.7 (4.7)
Years of education	13.8 (2.2)***	16.7 (3.2)***
Current cannabis use days/week	4.7 (1.5)	4.6 (2.6)
Current cannabis use gram/week	2.6 (1.7)	3.1 (3.2)
Lifetime cannabis use duration (years)	2.6 (2.1)***	6.6 (2.9)***
Cannabis use and related problems (CUDIT)	12.7 (6.4)	12.1 (8.6)
Lifetime use other psychotropic substances	6.0 (6.62)*	36.8 (57.15)*
Severity of nicotine dependence (FTND)	2.5 (2.4)***	4.9 (1.8)***
Number of cigarettes per day	6.2 (7.2)	11.7 (8.3)
Duration cigarette smoking (years)	3.1 (3.5)***	7.7 (3.2)***
Session-induced craving (MCQ)	3.7 (11.5)	3.7 (7.5)
Alcohol use and related problems (AUDIT)	6.0 (3.2)*	7.8 (5.0)*

\* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ .

CUDIT: Cannabis Use Disorder Identification Test; FTND: Fagerström Test for Nicotine Dependence; MCQ: Marijuana Craving Questionnaire; AUDIT: Alcohol Use Disorder Identification Test.

An additional independent sample *t*-test using change scores was conducted with all variables listed in Table 1 to check for differences between dependent and non-dependent users at follow-up. Except for lifetime cannabis use duration in years ( $p=0.033$ ), no significant differences were found between the two groups.

activations were entered in the third and final step. Preliminary analyses revealed one outlier with a total CUDIT score at follow-up of more than 3 standard deviations above the mean. This subject was excluded from further analyses. The assumptions of normality, linearity, multicollinearity and homoscedasticity were not violated (maximum standardized residual=1.9, maximum Cook's distance=0.280).

The hierarchical multiple regression analysis showed that activation in the left striatum significantly predicted problem severity ( $p < 0.001$ ), uniquely explaining 13% of the variance in follow-up CUDIT scores after correction for baseline CUDIT score, baseline AUDIT score, baseline FTND score, baseline number of cigarettes per day, baseline craving and baseline lifetime use of other psychotropic substances. VTA activation, on the other hand, did not. Besides striatal activity, total CUDIT score at baseline ( $p < 0.001$ ) was a significant predictor in the final model, as were the number of daily cigarettes at baseline ( $p=0.014$ ) and baseline AUDIT score ( $p=0.003$ ). The final model (see Table 2), consisting of left striatum activity at baseline, total baseline CUDIT score, total baseline AUDIT score and baseline number of daily cigarettes, explained 87% of the variance in cannabis use-related problem severity at follow-up, of which 73% was explained by the control variables and an additional 13% by cue-induced left striatal activation at baseline. The additional ROI analysis showed one significant cluster in the left putamen (Figure 1, MNI coordinates  $x=-24$ ;  $y=4$ ;  $z=6$ ).

### Activation patterns in dependent and non-dependent users

Finally, an additional independent samples *t*-test showed a marginally significant difference ( $p=0.053$ ) in cue-induced striatal activation at baseline between dependent ( $n=12$ ) and non-dependent ( $n=11$ ) cannabis users at follow-up (Table 3):

**Table 2.** Final hierarchical multiple regression analysis for variables associated with cannabis problem severity (CUDIT scores) at 3-year follow-up ( $n=22$ ).

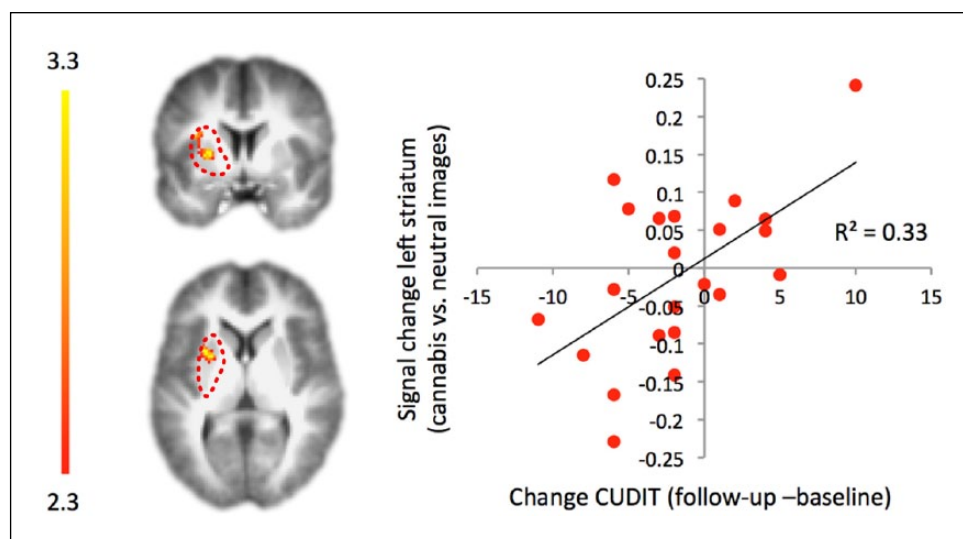
	B	SE B	$\beta$
<b>Step 1:</b> $R^2: 0.62^{***}$			
CUDIT baseline	0.98	0.17	0.79***
<b>Step 2:</b> $\Delta R^2: 0.08^*$			
CUDIT baseline	0.89	0.16	0.72***
Cigarettes daily baseline	0.31	0.14	0.29*
<b>Step 3:</b> $\Delta R^2: 0.06^*$			
CUDIT baseline	0.85	0.15	0.69***
Cigarettes daily baseline	0.33	0.13	0.31*
AUDIT baseline	0.63	0.29	0.25*
<b>Step 4:</b> $\Delta R^2: 0.13^{***}$			
CUDIT baseline	0.67	0.11	0.54***
Cigarettes daily baseline	0.25	0.09	0.23*
AUDIT baseline	0.69	0.20	0.28**
$\Delta$ Striatal activity, Left	29.32	6.50	0.40***

\* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ . Model step 1:  $R^2: 0.62$ , adjusted  $R^2: 0.60^{***}$ ; step 2:  $R^2: 0.70$ , adjusted  $R^2: 0.67^{***}$ ; step 3:  $R^2: 0.77$ , adjusted  $R^2: 0.73^{***}$ ; step 4:  $R^2: 0.89$ , adjusted  $R^2: 0.87^{***}$ . SE: standard error; CUDIT: Cannabis Use Disorder Identification Test;  $\Delta$  Striatum, Left: Percentage signal change in left striatum activity.

dependent cannabis users at follow-up showed higher activity for cannabis versus neutral stimuli at baseline, compared with non-dependent users.

## Discussion

This study examined whether baseline cannabis cue-induced brain activity independently predicted the amount of cannabis



**Figure 1.** Relationship between changes in cannabis use-related problems over 3 years (CUDIT follow-up CUDIT baseline) and striatal cannabis cue-reactivity at baseline (activity cannabis versus neutral images). The scatterplot displays this relation for activity extracted from the entire left striatum. The dotted line indicates the boundaries of the mask that was used. For descriptive purposes, the cluster of significant activation within the left striatum (putamen) is overlaid on a standard MNI brain at  $y=4$  mm and  $z=6$  mm. Thresholded at  $Z>2.3$ , cluster-corrected at  $p<0.05$  for the volume of the left striatum. Right side of the brain is depicted at the right side.

**Table 3.** Independent samples  $t$ -test of all ROIs.

	Non-dependent at FU		Dependent at FU		$t$ -value	$p$
	$n=11$		$n=12$			
	Mean	SD	Mean	SD		
PSC ACC	-0.04	0.12	-0.02	0.09	-0.66	0.515
PSC OFC, R	-0.09	0.18	-0.02	0.13	-1.64	0.116
PSC OFC, L	-0.08	0.18	-0.00	0.11	-1.36	0.187
PSC VTA, R	-0.01	0.17	0.09	0.10	-1.66	0.112
PSC VTA, L	0.05	0.30	0.14	0.22	-0.76	0.459
PSC Striatum, R	-0.04	0.07	-0.02	0.17	-0.36	0.724
PSC Striatum, L	-0.05	0.11	0.03	0.09	-2.05	0.053
PSC Amygdala, R	-0.05	0.13	0.03	0.19	-1.07	0.297
PSC Amygdala, L	-0.00	0.16	0.11	0.15	-1.64	0.116

Note:  $*p\leq 0.05$ ,  $**p\leq 0.01$ ,  $***p\leq 0.001$ .

PSC: percentage signal change; L: left hemisphere; R: right hemisphere; ACC: anterior cingulate cortex; OFC: orbital frontal cortex; VTA: ventral tegmental area.

use and problem severity in frequent cannabis users at 3-year follow-up. For the first time, we showed that neural cannabis cue-reactivity in the left striatum, specifically the putamen, is an independent predictor of cannabis use-related problems 3 years later. However, analyses showed that cue-induced brain activity did not predict the amount of cannabis use at follow-up. In addition, we found that dependent cannabis users at follow-up showed higher left striatal activity at baseline compared with non-dependent cannabis users at trend level.

Interestingly, the correlation between right striatal activity and problem severity did not reach significance. However, given the limited power of the study it is possible that a larger sample

would reveal a bilateral relation between striatal activity and problem severity. Nevertheless, it is possible that involvement of the left putamen in cue-reactivity is stronger than involvement of the right putamen. A study by Wong et al. (2006) has shown a significant correlation between cue-induced craving and change in dopamine receptor occupancy in the left putamen in frequent cocaine users, which is presumably caused by an increased release of intra-synaptic dopamine.

These findings support an important role for the left putamen in the course of frequent cannabis use. As part of the dorsal striatum, the putamen is thought to play an important role in the progression from goal-directed to habitual substance use (Everitt and Robbins, 2005; Sjoerds et al., 2013; Voon et al., 2015). This suggests that habit formation is an important factor in developing problematic cannabis use and dependence. Indeed, in our baseline comparison of cue-reactivity (Cousijn et al., 2013) we found higher striatal activation in frequent cannabis users with high versus low problem severity, whereas no differences in striatal activation were found between frequent cannabis users and controls, suggesting that habit formation is an important factor in problematic cannabis use but not in cannabis use per se. Current treatments of cannabis use disorders are mainly focussed on motivational aspects and contingency management. A study by Feldstein Ewing et al. (2013) indeed found a relation between striatal activity and treatment outcome using motivational interviewing techniques in a 1-month follow-up study in adolescents who had been using cannabis for at least 7 days in the month prior to scanning. However, our results suggest that habit formation/compulsive drug-taking might be an additional target for treatment.

Our results are in line with findings concerning other substances of abuse. Several studies found higher cue-induced striatal activation in heavy alcohol users and that this activity was associated with severity and duration of dependence and amount

of use in alcohol users (Schacht et al., 2013; Sjoerds et al., 2014; Vollstädt-Klein et al., 2010). Moreover, higher activation in the dorsal striatum was associated with obsessive–compulsive craving (Vollstädt-Klein et al., 2010). In addition, Demos et al. (2012) found that striatal activation in response to food images predicted weight gain in first-year college students. These results support the hypothesis generated by Everitt and Robbins (2005) of a shift of learning processes from ventral to dorsal areas of the striatum when substance dependence progresses. Everitt and Robbins (2005) stated that at the early stages of addiction a drug is voluntarily taken because of its rewarding effects, but that this behaviour becomes habitual or compulsive due to loss of control over this behaviour. They hypothesized that this shift from voluntary to compulsive behaviour at the neural level reflects a transition from prefrontal cortical to striatal control and from ventral to dorsal areas of the striatum over drug-taking behaviour. Our results provide preliminary evidence that this hypothesis also applies to cannabis, because cue-induced hyperactivity in the dorsal striatum predicted cannabis problem severity in frequent cannabis users who, at time of scanning, were already using cannabis regularly for at least 2 years. Importantly, cue-induced activation in the other ROIs at baseline did not predict cannabis problem severity at 3-year follow-up. Since the other ROIs are thought to be mainly involved in reward evaluation and motivational aspects of drug-taking behaviour, our results suggest that these processes may be involved in frequent and continued cannabis use, but are less important for the development of cannabis-related problems and dependence.

Baseline alcohol-related problem severity and the number of daily cigarettes also independently predicted cannabis use-related problem severity at 3-year follow-up, suggesting that alcohol, tobacco and cannabis use-related problems and dependence share a common risk factor represented by increased cue-induced activation of the dorsal striatum, probably related to the – already mentioned – shift from goal-directed learning to habit formation.

Interestingly, cannabis problem severity at follow-up correlated positively with VTA activation. However, VTA activation did not explain unique variance as it was no longer a significant predictor when entered in the regression model. Possibly, VTA activation did not differentiate enough between high and low problem severity users at baseline. The VTA projects dopamine into the striatum. Altered dopamine transmission has been reported in *early* stages of cannabis use disorders (Koob and Volkow, 2010). This might explain why VTA activity correlated with, but did not uniquely predict, cannabis problem severity. Indeed, we previously found higher cannabis cue-induced activation in the VTA in frequent cannabis users compared with controls, whereas VTA activation did not differ between cannabis users with high and low problem severity (Cousijn et al., 2013).

When interpreting these findings some limitations have to be taken into account. First, our sample was relatively small, making it difficult to detect subtle effects. The limited sample size could also explain the lack of a bilateral effect, as the relation between right striatal activity at baseline and problem severity at follow-up did not reach significance. Second, since the MINI was used as a screening instrument at baseline and there was no full psychiatric interview, it could not be determined if cue-induced brain activity could predict transition to cannabis dependence. Third, although the participants refrained from cannabis 24 hours prior to

scanning, sub-acute effects of cannabis use could have influenced the results as cannabis can be detected in urine for 24 days after using (Lowe et al., 2009). Fourth, the test–retest and validity of our cannabis history questionnaire was unknown. This could have influenced the results. Finally, not all participants recruited at baseline participated in the follow-up assessment. Although analyses indicated that non-responders were missing at random, a selection bias in the present sample cannot be fully excluded.

In conclusion, cue-induced activation in the left striatum predicted cannabis use-related problem severity in frequent cannabis users at 3-year follow-up, over and beyond behavioural predictors. These findings thereby support the important role of cannabis cue-reactivity in the dorsal striatum (putamen) in the course of cannabis use towards problematic cannabis use and possibly dependence. Since the dorsal striatum is strongly involved in habit formation and compulsive behaviour, treatment of cannabis use disorders should focus on this aspect of addiction. Given the relatively small sample size, these results are preliminary and should be replicated in larger samples of cannabis users.

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