Schizophrenia and comorbid cannabis use disorders: Brain structure, function and the effect of antipsychotic medications
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Chapter 5.4

Comparing the effect of clozapine and risperidone on cue reactivity in male patients with schizophrenia and a cannabis use disorder: a randomized fMRI study

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Submitted for publication
Abstract

Objective  Cannabis abuse and dependence are highly comorbid in patients with schizophrenia and are associated with poor outcome. Clozapine has been put forward as the first choice antipsychotic in this patient group. However, little is known about the mechanisms underlying the assumed superiority of clozapine.

Methods  A total of 38 patients with schizophrenia (30 cannabis users) and 20 healthy comparison subjects were included between April 2009 and June 2012. Patients were randomised to antipsychotic treatment with clozapine or risperidone. At baseline and after 4 weeks of medication, brain response to cannabis related, positive and neutral images was measured using functional MRI, and subjective craving was assessed using self-report questionnaires. Neural correlates of cue reactivity were assessed in the amygdala, ventral striatum, insula, thalamus, orbitofrontal cortex and anterior cingulate cortex.

Results  At baseline, patients with comorbid cannabis use disorder showed higher subjective craving and greater activation in response to cannabis-related images compared to patients without cannabis use disorder and healthy controls in most regions of interest. Clozapine treated patients reported a greater reduction in craving and showed a larger decrease in amygdala activation during cannabis related images compared to risperidone treated patients. In addition, significant correlations were found between subjective craving and thalamus and insula activation during cannabis related images.

Conclusion  These findings provide evidence that clozapine is superior to risperidone in decreasing subjective craving and cue reactivity for cannabis related images probably due to a differential effect of clozapine on regions associated with dopaminergic neurotransmission.

Key words: clozapine; risperidone; craving; imaging; schizophrenia; cannabis
Introduction

Schizophrenia is highly comorbid with cannabis abuse or dependence (Machielsen et al. 2010, Regier et al. 1990) and cannabis use disorders (CUD) are frequently associated with poor outcome in these patients (Dixon 1999, Linszen et al. 1994, Moore et al. 2007, Regier et al. 1990, Zammit et al. 2008). Discontinuation of cannabis use may improve outcome, and is therefore an important target in the treatment of schizophrenia (Grech et al. 2005). Craving, the psychological urge to take a drug, is considered a key feature of substance abuse, contributing to its continuation and to relapse after a period of abstinence (Franken 2003, Robinson and Berridge 1993). Reducing craving could therefore help schizophrenia patients to stop cannabis use, thereby improving outcome. Clozapine has been put forward as the antipsychotic medication of first choice in the treatment of patients with schizophrenia and comorbid substance use disorder (Brunette et al. 2011, Green et al. 2008, Green et al. 2003, Kim et al. 2010, Machielsen et al. 2012, Machielsen and de Haan 2009). However, the mechanisms underlying clozapine’s superiority are insufficiently known.

The consumption of illicit drugs results in dopamine release to a greater extent than natural rewards. Repeated drug use leads to increased salience of drug-related cues. Furthermore, chronic substance abuse is associated with a reduction of postsynaptic dopamine receptors in the mesocorticolimbic system coupled with a decreased sensitivity for natural rewards (Volkow et al. 2004). This hypodopaminergic state is associated with increased substance abuse (de Haan et al. 2006).

The superior effect of clozapine in diminishing craving in schizophrenia patients may thus reflect differential affinity to dopamine receptors. Clozapine and risperidone show a maximal difference in their affinity to dopamine D₂ receptors (Kuroki et al. 2008, Seeman 2002, Tauscher et al. 2004). Clozapine has a lower D₂ occupancy rate, a higher dissociation rate and a higher D₃/D₂ receptor occupancy ratio than risperidone, which may enhance responses to natural rewards while decreasing responses to drug rewards in clozapine treated patients.

The current study was designed to (a) demonstrate differences in regional brain activity related to cannabis-related cues in patients with cannabis use disorder (CUD) compared to patients without cannabis use disorder (NCUD) and healthy controls (HC), and (b) investigate differences between clozapine and risperidone regarding subjective cannabis craving and associated regional brain activity. We hypothesized that CUD patients show more craving and greater regional brain activation in areas previously implicated in substance-related cue reactivity (ventral striatum, amygdala, insula, anterior cingulate cortex, thalamus and orbitofrontal cortex (Chase et al. 2011, Kuhn and Gallinat 2011, Naqvi and Bechara 2010)) than NCUD patients and HCs; and that CUD patients treated with clozapine compared to risperidone would show decreased craving, a decreased response to cannabis related images (cue reactivity) and an increased response to positive non-cannabis related images. We also hypothesized that subjective craving would be associated with regional brain activation following cannabis-related images.
Methods
We conducted an open-label randomized controlled trial investigating the difference between clozapine and risperidone on subjective cannabis craving and brain activity following visual cannabis-related and non-cannabis related cues during fMRI scanning.

The medical ethics committee of the Academic Medical Centre of the University of Amsterdam approved the study.

Participants
Schizophrenia patients were recruited from inpatient and outpatient treatment settings of the Early Psychosis Department of the Academic Medical Centre between April 2009 and June 2012. Inclusion criteria were male gender, age 18-30, DSM-IV diagnosis of schizophrenia, schizophreniform or schizoaffective disorder (for reasons of brevity this will be referred to as schizophrenia). Exclusion criteria were previous unsuccessful treatment with or a contraindication for the use of risperidone or clozapine, using depot antipsychotic medication three months prior to inclusion, treatment with psychotropic medication other than biperiden or benzodiazepines, and the presence of non-removable metal objects as a contraindication for fMRI scanning.

Healthy controls were recruited through advertisements on schools and sport facilities. HC had a negative history of lifetime neurologic or psychiatric diseases, including substance use disorders. HC had used cannabis for a maximum of 50 times lifetime, last time at least 1 year prior to inclusion, and were matched for age and education. After complete description of the study, written informed consent was obtained.

Study design
Patients were randomly allocated to receive either clozapine or risperidone. Patients started with a standard dose titration scheme in the evening after the first fMRI assessment directed at a dose of 3.5mg risperidone or 350mg clozapine. In patients who already used antipsychotic medication, that medication was tapered down in the week before the first assessment. Concomitant psychotropic medication was restricted to benzodiazepines and biperiden. In case of lack of antipsychotic efficacy or dose-related side effects, the dose was adjusted as clinically applicable. During the study patients received supportive treatment-as-usual.

Assessments (functional MRI and questionnaires) took place at baseline before the first dose of study medication, and at the end of four weeks of treatment. HC were assessed once.

All participants were asked to refrain from alcohol and drugs 24hr before testing, and patients did not smoke cigarettes at least 2hr before testing. Patients were instructed to refrain from cannabis at least 3 days before testing, as was confirmed by urine samples taken 3 days prior to scanning and the day of the scan. In addition, urine screens for amphetamines, benzodiazepines, opioids and cocaine were performed prior to all
assessments. During the second assessment blood samples were taken to test compliance to the study medication.

**Assessment of diagnosis and severity of symptoms**

DSM-IV diagnoses were established using diagnostic interviews based on the Comprehensive Assessment of Symptoms and History (CASH)(Andreasen et al 1992) together with interviews with parents. The Composite International Diagnostic Interview (CIDI)(Robins et al 1988) was used to assess presence of (lifetime) substance use disorders. Craving during the past seven days and current craving were assessed with the cannabis version of the Obsessive Compulsive Drug Use Scale (OCDUS)(Dekker et al 2012a) and the short version of the Marijuana Craving Questionnaire (MCQ)(Heishman et al 2009). The Positive and Negative Symptoms Scale (PANSS) was used to rate symptom severity(Kay et al 1987).

**Cue-reactivity task**

During scanning, participants viewed cannabis-related (n=30), neutral (n=30), positive (n=30) and target images (n=15). Cannabis images were photos of cannabis, individuals smoking cannabis and cannabis use related paraphernalia previously used by Cousijn et al.(2012). Positive and neutral images were selected from the International Affective Picture System (IAPS) database(Lang et al 2008). These groups of images were visually matched on color and composition. To control for sustained attention, participants were asked to pay close attention to all images and to press a key on a response box when an animal (target image) was displayed. Each image was presented for 4s and preceded by a fixation-cross for 1s in five blocks consisting of 6 images and a target picture. Between blocks, a low-level baseline (fixation-cross) was presented for 15s. Two different versions were used in a random order for each participant. Total task duration was 10 minutes.

**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. In the first scanning session, a T1 structural image was acquired (TR=9.6seconds, TE=4.6ms, voxel size=1x1x1.2mm, 182 slices, flip-angle=8°). During the cue-reactivity task, blood oxygen level dependent signal was measured with a T2* gradient-echo echo-planar imaging (EPI) sequence (TR=2.3seconds, TE=28ms, voxel size=2.3x2.3x3mm, 35 slices, flip angle=80°). Data pre-processing was conducted with SPM8 (Statistical Parametric Mapping; Wellcome Trust Centre for Neuroimaging, London, UK). Images were manually reoriented, slice-time corrected, realigned and unwarped, co-registered with the T1-scan, segmented, normalized to MNI space and spatially smoothed with an 8mm full-width-half-maximum Gaussian kernel.
**Statistical analysis**

Demographics were compared between groups with standard univariate analysis of variance (ANOVA) procedures and chi-squared tests. Differences between first and second measurements of the PANSS, OCDUS and MCQ were compared for the CUD patients between medication groups using repeated-measures ANOVAs.

FMRI time-series analyses were carried out using SPM8. Imaging data for each subject were analyzed in the context of the General Linear Model. At single-subject level, contrast images for cannabis and positive images versus both neutral images and baseline were computed for each session and entered into second-level (group) analyses. Low-level baseline was used because neutral images could have an emotional value in some patients.

Given their role in cue reactivity in previous studies, we *a priori* selected the following ROIs: ventral striatum, amygdala, insula, anterior cingulate cortex, thalamus and orbitofrontal cortex (Chase *et al* 2011, Kuhn and Gallinat 2011, Naqvi and Bechara 2010). ROIs were defined using the WFU PickAtlas Tool v2.4 that incorporates the automatic anatomical labeling (AAL) atlas (Tzourio-Mazoyer *et al* 2002). Within specific ROIs small volume Family Wise Error (FWE) corrections for multiple comparisons around the peak activation were performed across the search volume of 6mm (for ventral striatum, insula, thalamus and amygdala) and 14mm (for anterior cingulate cortex and orbitofrontal cortex) (Friston *et al* 1996, Worsley *et al* 1996). To account for the number of ROIs we used a Bonferroni correction adjusted for correlations between our ROIs (http://www.quantiativeskills.com/sisa/calculations/bonfer.htm) (Li *et al* 2012a), resulting in a corrected alpha of .018. Differences between CUD patients, NCUD patients and HCs were assessed using one-way ANOVA’s, differences between clozapine and risperidone were compared over sessions using two-way (Group x Time) ANOVA’s. To assess the relation between subjective craving and cue reactivity in CUD patients, linear regression analyses between craving scores and brain response to cannabis vs. neutral images and cannabis images vs. baseline were performed.

**Results**

**Baseline characteristics**

A total of 67 participants (46 patients and 21 HC) signed informed consent. Of these, 2 patients withdrew their informed consents, 4 patients were too anxious to be scanned, 2 scans were of poor quality due to scanner errors (1 patient, 1 HC) and follow up data could not be obtained from 1 patient. Therefore, in the analysis we included a total of 38 patients (30 CUD) and 20 HC. Twenty patients were randomized to risperidone (16 CUD) and 18 to clozapine (14 CUD). For demographics see table 1.

**Treatment compliance and study completion**

Mean medication dosage of at second assessment was 3.8mg (SD=0.92) for risperidone and 302mg (SD=78.1) for clozapine. Two patients using risperidone stopped taking medication
2 days prior to the second assessment. For all other patients compliance could be confirmed. Eight patients (5 risperidone, 3 clozapine) were scanned after 2-3 weeks instead of 4 weeks treatment with study medication, because their clinicians decided to stop study medication. In case of side effects dose titration schemes were adjusted so that those patients were scanned later. As a consequence, mean treatment duration with study medication was 28.4 days. No significant difference in treatment duration was found between risperidone (mean=27.0 days) and clozapine (mean=29.9 days t=1.42, df=36, p=.16). Oxazepam was used by 11 patients (5 risperidone, 6 clozapine) and biperiden by 1 patient (treated with risperidone) during the study. Eighteen (of 30) patients continued cannabis abuse during the study (10 risperidone, 8 clozapine).

Table 1. Sociodemographic- and clinical characteristics of patients and healthy controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CUD risperidone (n=16)</th>
<th>CUD clozapine (n=14)</th>
<th>NCUD using risperidone (n=4)</th>
<th>NCUD using clozapine (n=4)</th>
<th>HC (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
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<td>22.2</td>
<td>23.3</td>
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<td>23.0</td>
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<tr>
<td>SD</td>
<td>3.39</td>
<td>2.58</td>
<td>3.30</td>
<td>2.83</td>
<td>3.4</td>
</tr>
<tr>
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<td>N%</td>
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<td>N%</td>
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<td>N%</td>
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<tr>
<td>Education: highest achieved level b</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
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<td>9</td>
<td>8</td>
<td>3</td>
<td>3</td>
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<tr>
<td></td>
<td>56.3</td>
<td>57.1</td>
<td>75.0</td>
<td>75.0</td>
<td>60.0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>4</td>
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<tr>
<td></td>
<td>18.8</td>
<td>35.7</td>
<td>25.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>1</td>
<td>7.1</td>
<td>1</td>
<td>4</td>
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<tr>
<td></td>
<td>25.0</td>
<td>7.1</td>
<td>25.0</td>
<td>20.0</td>
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<td>Diagnosis</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Schizophrenia, paranoid</td>
<td>7</td>
<td>43.8</td>
<td>2</td>
<td>3</td>
<td>75.0</td>
</tr>
<tr>
<td>Schizophrenia, undifferentiated</td>
<td>5</td>
<td>31.3</td>
<td>2</td>
<td>1</td>
<td>25.0</td>
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<tr>
<td>Schizophrenia, disorganized</td>
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<td>1</td>
<td>7.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Schizoaffective disorder</td>
<td>3</td>
<td>18.8</td>
<td>2</td>
<td>1</td>
<td>25.0</td>
</tr>
<tr>
<td>Schizophreniform Disorder</td>
<td>1</td>
<td>6.3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Smoking a</td>
<td>13</td>
<td>81.3</td>
<td>3</td>
<td>1</td>
<td>25.0</td>
</tr>
<tr>
<td>Alcohol use disorder a</td>
<td>4</td>
<td>25.0</td>
<td>3</td>
<td>1</td>
<td>21.4</td>
</tr>
<tr>
<td>Cocaine use disorder a</td>
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<td>6.3</td>
<td>2</td>
<td>0</td>
<td>14.3</td>
</tr>
<tr>
<td>Ecstasy use disorder a</td>
<td>1</td>
<td>6.3</td>
<td>2</td>
<td>0</td>
<td>14.3</td>
</tr>
<tr>
<td>Admission in the hospital</td>
<td>13</td>
<td>81.3</td>
<td>3</td>
<td>3</td>
<td>75.0</td>
</tr>
<tr>
<td>Continued cannabis use</td>
<td>10</td>
<td>62.5</td>
<td>8</td>
<td>57.1</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CUD Cannabis use disorder, NCUD no cannabis use disorder, HC healthy control

a: significant differences between CUD patients and NCUD patients/HC, no significant differences between medication groups
b: 1, Lower secondary professional education/intermediate vocational education; 2, higher general secondary education/higher vocational education; 3, pre-university education/university
Clinical changes during treatment

After 4 weeks of treatment, scores on all questionnaires were lower in both treatment conditions (table 2). A significant interaction effect of assessment by medication was found for current craving as measured with the MCQ \[F(1,28)=6.0, \ p=.02, \text{ partial eta squared}=0.18\] and PANSS negative symptoms \[F(1,28)=6.14, \ p=.02, \text{ partial eta squared}=1.18\] with larger decreases between baseline and follow-up in the clozapine compared to the risperidone group. A trend in the same direction was found for craving as measured with the OCDUS \[F(1,28)=3.96, \ p=.06, \text{ partial eta squared}=.12\]. Post-hoc analyses showed that after correcting for change in PANSS negative symptoms, the change in MCQ scores was still significantly different between the treatment conditions \[F(1,28)=5.7, \ p=.03, \text{ partial eta squared}=1.17\].

Table 2. Severity of psychopathology on all patients and scores on craving questionnaires for patients with schizophrenia and a comorbid cannabis use disorder (CUD) using clozapine or risperidone

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Clozapine (n=14)</th>
<th>Risperidone (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st assessment</td>
<td>2nd assessment</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>OCDUS total score</td>
<td>20.4</td>
<td>8.6</td>
</tr>
<tr>
<td>MCQ total score</td>
<td>29.5</td>
<td>16.3</td>
</tr>
<tr>
<td>PANSS Positive items</td>
<td>14.5</td>
<td>5.2</td>
</tr>
<tr>
<td>PANSS Negative items</td>
<td>12.7</td>
<td>6.1</td>
</tr>
<tr>
<td>PANSS General items</td>
<td>29.1</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Abbreviations: OCDUS Obsessive Compulsive Drug Use Scale, MCQ Marijuana Craving Questionnaire, PANSS Positive And Negative Symptoms Scale

a: Differences between risperidone and clozapine were compared with a repeated measures analysis of variance (ANOVA)

fMRI analysis

Main effects

CUD patients (n=30) showed more activation during cannabis vs. neutral images in comparison to healthy controls (n=20) during the first fMRI session in the right amygdala (peak voxel: x,y,z=34,2,-24 T=3.90 pFWE=.004), left thalamus (x,y,z=4,-22,6 T=3.99 pFWE=.003) and right thalamus (x,y,z=2,-22,6 T=3.99 pFWE=.003). CUD patients also showed more activation in the right amygdala for positive vs. neutral images compared to healthy controls (x,y,z=28,0,-28 T=3.09 pFWE=.023). Compared to NCUD patients (n=8), CUD patients (n=30) showed trendwise higher activations for cannabis vs. neutral images in the right amygdala (x,y,z=34,2,-26 T=2.87 pFWE=.041), left thalamus (x,y,z=-6,-20,-2 T=2.78 pFWE=.049) and right thalamus (x,y,z=12,-20,-2 T=2.79 pFWE=.048) and during positive vs. neutral images in the left amygdala (x,y,z=-22,-8,-12 T=3.27 pFWE=.009) and right amygdala (x,y,z=28,0,-28 T=2.85 pFWE=.027).
**Differences between medication conditions**

Significant group x time interactions were found. Compared to risperidone treated CUD patients (n=16), clozapine treated patients (n=14) showed a decreased activation in the right amygdala (x,y,z=36,4,-30 T=3.94 pFWE=.006) between first and second scan in response to cannabis vs. neutral images (figure 1a), whereas risperidone treated patients showed an increased activation in the right (x,y,z=40,10,-6 T=3.34 pFWE=.011) and left insula (x,y,z=-34,22,0 T=3.45 pFWE=.012) during cannabis images vs. baseline (figure 1b). No differences were found between medication conditions for positive vs. neutral images or vs. baseline.

**Figure 1**

![Figure 1a](image1.png) ![Figure 1b](image2.png)

**a.** Differences between medication groups (n=38) for cannabis images vs. neutral images. The clozapine group showed a larger decrease in activation in the right amygdala.

**b.** Differences between medication groups (n=38) for cannabis images vs. low level baseline. The risperidone group showed a larger increase in activation in the left insula. Colour bar represents corresponding T values.

**Relationship between regional brain activation and subjective craving**

No significant associations between changes in craving (MCQ scores) and changes in brain activation were found. In CUD patients, a positive correlation was found between current craving and response to cannabis vs. neutral images in the thalamus (x,y,z=-10,-10,8 T=3.48 pFWE=.010) (figure 2a) and between current craving and activation in the left insula (x,y,z=-26,26,8 T=3.87 pFWE=.008) (figure 2b) in response to cannabis images vs. baseline during the first assessment.
Figure 2

a. Correlation between MCQ-scores and thalamus activation for cannabis vs. neutral images (R² linear = 0.327)
b. Correlation between scores on the marijuana craving questionnaire (MCQ) and insula activation for cannabis images vs. low level baseline. (R² linear = 0.348)

Discussion

The current study is the first randomised controlled trial studying differences between clozapine and risperidone treatment in regional brain responses associated with cue reactivity and subjective craving for cannabis in patients with schizophrenia. The key finding is that compared to risperidone treatment, clozapine treatment was associated with a larger decrease in brain response to cannabis related images and that brain activation in response to cannabis related images was correlated with subjective craving.

Subjective measures

After 2-4 weeks treatment, clozapine treated patients showed larger decreases on negative symptoms and MCQ craving scores. Superior effects of clozapine on negative symptoms and subjective craving have previously been described, but not consistently (Brunette et al 2011, Green et al 2003, Machielsen et al 2012). Craving, substance abuse and negative symptoms, including diminished motivation and dysphoria, have been associated with higher occupancy rates of the dopamine D2 receptor (de Haan et al 2003, de Haan et al 2006, Mizrahi et al 2009). Therefore, clozapine’s lower affinity to dopamine D2 receptors may be related to our finding of a larger decrease in negative symptoms and subjective craving. The ‘self-medication theory’ suggests that decreased negative symptoms may result in decreased craving (Khantzian 1997), however the decrease in subjective craving remained significant after correction for the decrease in negative symptoms.

Regional brain activity

In the current study, the main regions involved in cue reactivity to cannabis related images in CUD patients were the right amygdala and bilateral thalamus. Contrary to our expectations, CUD patients showed increased activation of the amygdala during positive images compared to NCUD patients and HCs. This effect was observed during both
assessments and irrespective of medication condition, and its interpretation is speculative. Previous studies have reported a decreased response to positive images in patients with a substance use disorder, indicating the presence of “reward deficiency” for natural rewards (Garavan et al. 2000, Wexler et al. 2001, Zijlstra et al. 2009), although not consistently (Heinz et al. 2007). However, our CUD patients differed from those in previous studies in several aspects, including duration of the substance use disorder, comorbid symptoms and medication, so that accounting for differential findings is not straightforward.

In line with our hypothesis, we found that treatment with clozapine is associated with a decreased brain response to cannabis vs. neutral images in the right amygdala compared to treatment with risperidone. The amygdala is implicated in cue reactivity and receives extensive dopaminergic projections (Chase et al. 2011, Kuhn and Gallinat 2011). Non-significant findings (possibly caused by insufficient power) in the same direction in the left amygdala were found. Because no differences were found between medication groups in response to positive images, the effects of clozapine may be specific for drug related stimuli. Furthermore, compared to clozapine treatment, treatment with risperidone was associated with an increased insula response to cannabis images vs. baseline. Insula and thalamus activation during presentation of cannabis related stimuli was positively correlated with subjective craving. While the insula is not usually a region of interest in cue reactivity studies, it is considered of key importance in addiction due to its involvement in the conscious cue-induced urge to use drugs (Naqvi and Bechara 2010). Likewise, the thalamus has been implicated in cue reactivity (Kuhn and Gallinat 2011). Associations between cue reactivity and subjective craving have been reported in two meta-analyses with regard to the right amygdala, left middle frontal gyrus (Chase et al. 2011), ACC, and striatum (Kuhn and Gallinat 2011), underlining the complexity of both conscious and subconscious processes involved in craving.

Strengths and limitations
Some potential limitations must be taken into account. First, we intended to include more NCUD patients to exclude the possibility that imaging findings in CUD patients were a consequence of disease or medication effects not of the presence of cannabis use disorder. Since time and financial constraints precluded recruitment of additional NCUD patients, the analyses involving NCUD patients are underpowered. However, because the task effect was associated with subjective craving in CUD patients, it is unlikely that the findings are unrelated to the presence of a cannabis use disorder. Second, due to feasibility and ethical considerations this was an open label study over a relatively short period of time in which dosage of medication could be adjusted in case of side effects or lack of efficacy. This may have influenced our results. However, the presence of significant differences between the medications even after a short treatment period with relatively low doses of study medication underlines the important differential effects of clozapine and risperidone. Future studies should provide follow-up information on the frequency of cannabis use in patients treated with clozapine. The open label design of the study is also not a very likely
explanation for the differences in brain activation since cue reactivity is a largely unconscious process. Third, because patients did not use any substance of abuse or cigarettes before scanning, effects of acute withdrawal cannot be ruled out. However, randomisation prevents systematic differences between medication groups. Fourth, 18 of 30 CUD patients continued using cannabis during the four weeks of medication use. Exploratory analysis revealed no differences between patients who continued using cannabis and those who discontinued. Fifth, almost all CUD patients also smoked cigarettes, and antipsychotic medication may affect smoking behaviour (de Haan et al 2006). We cannot rule out the possibility that cannabis related images also induced craving for cigarettes. However, because smoking behaviour was similar in both medication groups it is unlikely that craving for cigarettes differentially influenced the results. Future research should address the important issue whether clozapine may decrease craving for nicotine as well as for cannabis.

Despite these limitations, results from our study have important clinical and research implications, since we found a larger decrease in subjective craving for cannabis and associated brain response to cannabis related images after only 4 weeks of clozapine treatment compared to risperidone treatment in patients with schizophrenia and a comorbid cannabis use disorder.