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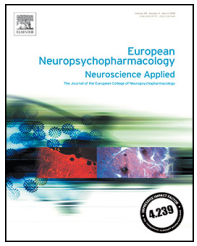

Acute effects of Δ9-tetrahydrocannabinol (THC) on resting state brain function and their modulation by COMT genotype

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Catechol-methyl-transferase (COMT);
Salience

Abstract
Cannabis produces a broad range of acute, dose-dependent psychotropic effects. Only a limited number of neuroimaging studies have mapped these effects by examining the impact of cannabis on resting state brain neurophysiology. Moreover, how genetic variation influences the acute effects of cannabis on resting state brain function is unknown. Here we investigated the acute effects of Δ9-tetrahydrocannabinol (THC), the main psychoactive constituent of cannabis, on resting state brain neurophysiology, and their modulation by catechol-methyltransferase (COMT) Val158Met genotype. Thirty-nine healthy volunteers participated in a pharmacological MRI study, where we applied Arterial Spin Labelling (ASL) to measure perfusion and functional MRI to assess resting state connectivity. THC increased perfusion in bilateral insula, medial superior frontal cortex, and left middle orbital frontal gyrus. This latter brain area showed significantly decreased connectivity with the precuneus after THC administration. THC effects on perfusion in the left insula were significantly related to subjective changes in perception and relaxation. These findings indicate that THC enhances metabolism and thus neural activity in the salience network. Furthermore, results suggest that recruitment of brain areas within this network is involved in the acute effects of THC. Resting state perfusion was modulated by COMT genotype, indicated by a significant interaction effect between drug and genotype on perfusion in the executive network, with increased perfusion after THC in Val/Met heterozygotes only. This finding suggests that prefrontal dopamine levels are involved in the susceptibility to acute effects of cannabis.

1. Introduction

With an estimate between 128 million and 238 million users, cannabis is the most widely used illicit substance in the world (United Nations Office on Drugs and Crime, 2017). It produces a broad range of acute, dose-dependent psychotropic effects, such as 'feeling high', relaxation and euphoria, which are regarded as the main reasons why people use cannabis (Green et al., 2004; Mané et al., 2015). In addition, cannabis has been shown to acutely induce increased heart rate, perceptual alterations and psychotic symptoms (Ashton, 2001; Hall and Degenhardt, 2009; Sherif et al., 2016), and to impair cognitive functions such as learning and memory (Bossong et al., 2014a; Broyd et al., 2016). Finally, epidemiological studies indicate that the use of cannabis increases the risk for developing schizophrenia (Marconi et al., 2016; Moore et al., 2007; Vaucher et al., 2017). These effects are primarily produced through action of Δ9-tetrahydrocannabinol (THC), the main psychoactive ingredient of cannabis, on the cannabinoid 1 (CB1) receptor (Huestis et al., 2001; Ledent, 1999).

In recent years, neuroimaging studies have increasingly demonstrated acute effects of THC on brain function, the vast majority of them involving a cognitive challenge (Bossong et al., 2014b). For example, without affecting performance accuracy, THC caused reductions in activity during encoding of information in temporal and prefrontal areas, but increases in activity during recall, which suggests neural compensation to meet the cognitive demands of a task (Bhattacharyya et al., 2009; Bossong et al., 2012). However, state-of-the-art research on the acute impact of THC on resting state brain function, thereby mapping its psychotropic effects, is limited. An initial series of studies by Mathew and colleagues using Positron Emission Tomography (PET) showed increased perfusion after THC in prefrontal, insular and anterior cingulate regions, which was associated with reported intoxication levels and changed time perception (Mathew et al., 1989, 1992, 1997, 2002). Van Hell et al. (2011a) confirmed these findings by demonstrating increased perfusion measured with Arterial Spin Labelling (ALS), and increased amplitude of fluctuations in resting state functional Magnetic Resonance Imaging (MRI) signal in the frontal cortex and bilateral insula after THC administration (van Hell et al., 2011a). In these studies, altered perfusion is measured as changes in regional cerebral blood flow (CBF), which is directly correlated with the level of local neural activity (Attwell et al., 2010; Dukart et al., 2018). The few studies that investigated acute effects of THC on resting state functional connectivity showed THC-induced connectivity changes within both the sensorimotor and dorsal visual stream networks, including the cerebellum and dorsal frontal cortical regions (Klumpers et al., 2012), and significant reductions in functional connectivity between the ventral striatum and the limbic lobe, prefrontal cortex, striatum and thalamus (Ramaekers et al., 2016). Grimm et al. (2018) did not show any significant effects of oral THC administration on striatal functional connectivity measures.

Importantly, there is marked inter-individual variability in the susceptibility to the effects of cannabis (Atakan et al., 2013). One possibility is that inter-individual differences in the impact of cannabis are explained by genetic variation. In this context, an extensively studied vulnerability gene is catechol-methyl-transferase (COMT), which encodes the main catecholamine degrading enzyme COMT. A functional nucleotide polymorphism (SNP) in the COMT gene (rs4680) results in a valine-to-methionine mutation at position 158 (Val158Met). Variation of the COMT Val158Met is associated with dopamine turnover in the prefrontal cortex. Individuals with the COMT Val/Val allele show increased COMT enzyme activity and, consequently, reduced dopamine levels compared to Met homozygotes (Chen et al., 2004; Tunbridge et al., 2006). Epidemiological studies have demon-

strated the COMT Val158Met genotype as a moderator of the association between cannabis use and the later development of psychotic symptoms or schizophrenia (Caspi et al., 2005; Costas et al., 2011; Estrada et al., 2011; Nieman et al., 2016; Vinkers et al., 2013), although not all studies demonstrated consistent findings (de Sousa et al., 2013; van Winkel, 2011; Zammit et al., 2011). In cannabis users, the COMT Val158Met genotype was shown to moderate executive function, with the Val allele associated with poorer performance (Verdejo-Garcia et al., 2013). Experimental studies on COMT modulation of the impact of cannabis showed stronger responses to cannabis or THC in terms of acute psychotic effects and cognitive impairments in individuals with increased COMT activity, particularly in people with a (genetic risk for a) psychotic disorder (Henquet et al., 2006, 2009). A study by Spronk et al. (2016) in healthy volunteers failed to demonstrate a moderating role of COMT genotype on cannabis-induced effects on reversal learning. Tunbridge et al. (2015) showed that COMT genotype affects the impact of acute THC on working memory performance but not on psychotic experiences in participants vulnerable to paranoia. However, it is unknown how COMT genotype modulates acute THC effects on resting state brain function.

The aim of the present study was to investigate the acute effects of THC on resting state brain neurophysiology, and to examine the impact of COMT genotype on these effects. Thirty-nine healthy volunteers participated in a randomised, placebo-controlled, crossover pharmacological MRI study. Acute effects of THC were assessed on resting state perfusion measured with ASL and on resting state connectivity measured with functional MRI. Consistent with previous neuroimaging studies (Mathew et al., 1997, 2002; van Hell et al., 2011a), we anticipated increased perfusion after THC administration in the insula and frontal cortex. Given the particular role of COMT in prefrontal dopamine degradation (Chen et al., 2004; Tunbridge et al., 2006), we further hypothesised that COMT genotype would modulate perfusion in the executive network, which includes the dorsolateral prefrontal cortex.

2. Experimental procedures

This study is part of the Pharmacological Imaging of the Cannabinoid System (PhICS) project, the design and objectives of which are provided in a methodological paper (van Hell et al., 2011b).

2.1. Subjects

Thirty-nine healthy male right-handed subjects were recruited through flyers, posters and internet advertisements. All subjects used cannabis on an incidental basis, defined as having used cannabis at least four times but at most once a week in the year before inclusion in the study. All subjects were in good physical health as assessed by medical history and physical examination, and were screened for axis I psychiatric disorders using the Dutch version of the Mini International Neuropsychiatric Interview for DSM-IV clinical disorders. Subjects were asked to refrain from cannabis for at least two weeks before the first study day until study completion. Illicit drug use other than cannabis was not allowed within six months prior to inclusion. Compliance was tested by means of a urine sample at the beginning of each test day. For further details on inclusion and exclusion criteria we refer to Van Hell et al. (2011b). All volunteers gave written informed consent before entry into the study. The study was approved by the Independent Ethics Committee of the University Medical Center Utrecht, the Netherlands, in accordance to the Declaration of Helsinki 2008. Subject characteristics are summarised in Table 1. Nineteen of these subjects were also part of the study reported by van Hell et al. (2011a).

Table 1 Subject characteristics ($n = 39$).

Characteristic	Mean ± SD	Range
Age (years)	22.6 ± 4.3	18-40
IQ	105.7 ± 5.2	94-114
Height (cm)	184.7 ± 6.5	175-201
Weight (kg)	76.2 ± 9.8	60-110
BMI (kg/m^2)	22.3 ± 2.02	18.5-27.8
Cannabis use (Occasions / year)	22.5 ± 15.0	4-61
Tobacco smoking (Cigarettes / week)	33.2 ± 55.9	0-203
Alcohol consumption (Units / week)	14.4 ± 9.0	2-40
Coffee consumption (Units / week)	14.8 ± 11.1	0-40
Illicit drug use (Occasions lifetime)	2.5 ± 4.2	0-17

Use of cannabis, tobacco, alcohol and coffee was given for the year before inclusion in the study. Subjects refrained from cannabis for at least two weeks before the first study day until study completion and from alcohol for 48 h before each study day. Caffeine intake and smoking were not allowed from the moment of arrival until the end of a study day. Illicit drug use other than cannabis was at least more than six months before the first study day. All subjects showed negative urine screening at both study days.

2.2. Design and procedure

In a double-blind, randomised, placebo-controlled, crossover pharmacological MRI study, subjects underwent two scanning sessions after administration of placebo and of THC. Study days were scheduled at least two weeks apart to allow for complete clearance of drugs. Two weeks before the first study day, participants were familiarised with the scanner environment using a mock scanner.

On the beginning of each study day, a catheter was placed intravenously in the left arm for the withdrawal of blood samples. Subjects received THC or placebo by means of a Volcano ® vaporizer (Storz-Bickel GmbH, Tuttlingen, Germany) at several time points. The first dose consisted of 6 mg of THC or placebo. To maintain stable levels of subjective effects throughout the experiment, upload dosages of 1 mg were used, with 30 min intervals. Two ASL scans were performed, one before and one after the first

administration of THC or placebo. Resting state fMRI was measured after the second or third upload dose. See Van Hell et al. (2011b) for detailed study procedures.

2.3. Genotyping

Before drug administration, two blood samples were collected for DNA isolation, which was performed according to standard protocols. Genome-wide genotype data were generated using Illumina® HumanOmniExpress (733,202 SNPs). Quality control procedures were performed using PLINK V1.07 (Purcell et al., 2007). COMT Val158Met genotype was obtained for thirty subjects. Nine subjects had a Met/Met, twelve a Val/Met, and nine a Val/Val genotype.

2.4. Drug levels, subjective effects and physiological measurements

Venous blood samples to determine plasma concentrations of THC and its two most important metabolites, 11-OH-THC and 11-nor-9-carboxy-THC, were collected 5 and 27 min after each drug administration and were processed according to Zuurman et al. (2008).

Subjective effects were determined with two sets of visual analogue scales (VAS) (Bond and Lader, 1974; Bowdle et al., 1998), which were performed consecutively at baseline and 5 and 27 min after each drug administration. From these scales, composite scores of 'perception', 'dysphoria', and 'relaxation' were calculated. This is a new approach of reporting different classes of THC effects, as recently validated by Kleinloog et al. (2014). VAS data were corrected for baseline values, averaged over the six time points, and analysed with paired t tests.

Heart rate and respiratory function were monitored continuously during scanning. Heart rate was assessed by measuring the electrocardiogram using four electrodes attached to the subject's chest, and respiratory function was assessed by measuring the expansion of a respiration band around the subject's abdomen. The cardiac and respiratory signals were sampled at a frequency of 500 and 100 Hz, respectively.

2.5. Image acquisition

Image acquisition was performed on a Philips Achieva 3.0 Tesla scanner with a Quasar dual gradient set (Philips Medical Systems, Best, the Netherlands). A high-resolution whole-brain anatomical scan was performed with the following scan parameters: TR 9.4 ms, TE 4.7 ms, flip angle = 8°, FOV 220.8 × 240 × 159.6, matrix 368 × 400 × 113, voxel size 0.6 × 0.6 × 0.6 mm, 266 slices, sagittal orientation. During both the ASL scans and the Blood-Oxygen-Level Dependent (BOLD) fMRI resting state scan, subjects were instructed to lie still and to keep their eyes open.

2.5.1. Arterial spin labelling

Pseudo-continuous labelling was performed by employing a train of radio-frequency (RF) pulses (duration 0.5 ms) with an interpulse pause of 0.5 ms in combination with a balanced gradient scheme (Dai et al., 2008; Wu et al., 2007). The duration of labelling was 1650 ms. The control situation was achieved by adding 180° to the phase of every other RF pulse. ASL imaging was performed in combination with background suppression, which consisted of a saturation pulse immediately before labelling and inversion pulses at 1680 ms and 2830 ms after the saturation pulse. Imaging was performed with single-shot echo planar imaging (EPI) in combination with parallel imaging (SENSE factor 2.5). In total, 17 slices of 7 mm thickness were acquired in ascending fashion with an in-plane resolution of $3 \times 3\,mm^2$. Imaging was performed 1525 ms after labelling stopped. The total scan time for a pair of control and label images was 8 s. For measurement of the magnetisation of arterial blood (M0) and also for segmentation purposes, an inversion recovery sequence was acquired with the same geometry and resolution as the ASL sequence (inversion times: 100–1900 ms with 200 ms intervals, preceded by a saturation pulse at ×1680 ms) (van Osch et al., 2009).

2.5.2. Resting state fMRI

For the BOLD resting-state scan, a single run of 400 volumes was obtained over a period of 4 min using a SENSE-PRESTO scan protocol (Neggers et al., 2008) with the following scan parameters: TR 22.5 ms, TE 33.2 ms, flip angle = 10°, FOV 224 × 256 × 160, matrix 56 × 64 × 40, voxel size 4.0 mm isotropic, acquisition time per volume 0.6075 s, 40 slices, sagittal orientation. A high-contrast volume with a flip angle of 27° (FA27) was scanned for registration purposes.

2.6. Data analysis

2.6.1. Arterial spin labelling

ASL perfusion images were motion-corrected in SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK) prior to subtraction of control images from perfusion-weighted images. The subtraction images were subsequently averaged. Quantitative CBF maps were calculated in ml / 100 ml per min from the ASL images using a formula described in van Osch et al. (2009).

CBF maps were realigned, normalised, interpolated to 2 × 2 × 2 mm voxels, and spatially smoothed (FWHM = 8 mm) in SPM8. All CBF maps were normalised for global CBF, which was calculated for every CBF map by averaging CBF values in the whole brain except cerebrospinal fluid. Pre-administration scans were subtracted from post-administration scans. These difference images were used in paired sample *t* tests in SPM8 to analyse effects of THC on whole-brain perfusion (placebo vs THC). Results were FDR-corrected at cluster-level ($p < 0.05$).

2.6.2. Resting state fMRI

Resting state data were pre-processed and analysed using SPM8. After realignment of functional images, the BOLD signal was corrected for cardiac and respiratory measures using the RETROICOR method (Glover et al., 2000). For each functional image, the cardiac and respiratory phases were calculated. RETROICOR then modelled the relationship between the cardiac and respiratory phases and the BOLD signal, and corrected the BOLD signal accordingly. Further

pre-processing included co-registration with the anatomical scan using the FA27 volume, normalisation into standard MNI space, interpolation to $2 \times 2 \times 2$ mm voxels, and spatial smoothing (FWHM = 8 mm). Finally, band pass filtering was performed which only allowed signals between 0.01 and 0.08 Hz.

Regions that showed significant effects of THC administration on perfusion were used as seed regions in subsequent whole-brain voxel-wise analyses of resting state connectivity. Single subject analyses were performed on data from the placebo and THC session for each seed region. Individual time series were extracted from the particular seed region and from an eroded white matter and cerebral spinal fluid mask (thresholded at an intensity of 0.8 and 0.4, respectively), and used as regressors. For each seed region, effects of THC on whole-brain resting state connectivity were analysed using paired sample *t* tests (placebo vs THC). Results were FDR-corrected at cluster-level ($p < 0.05$).

2.6.3. Brain function vs subjective effects

For every subject and for both the placebo and THC session, mean CBF values or connectivity coefficients were extracted from those regions that showed a significant impact of THC using the Marsbar SPM tool (Brett et al., 2002). Multiple linear regression was performed with brain function in each of those regions as dependent and subjective effects (VAS scores of 'perception', 'relaxation' and 'dysphoria') as independent variables. For all variables, placebo values were subtracted from THC values.

2.6.4. Impact of COMT genotype

The impact of COMT genotype on resting state perfusion was assessed in three functional brain networks, i.e. executive, salience and default mode network. These networks were previously defined by Sridharan et al. (2008), and spheres of 6 mm were placed around the reported coordinates. For every subject and for both the placebo and THC session, mean CBF values were extracted from each network using the Marsbar SPM tool (Brett et al., 2002). To examine genetic modulation of THC effects on resting state perfusion in each of the three functional brain networks, a multivariate approach to repeated measures ANOVA was used with drug (two levels: placebo and THC) as within-subject factor and COMT genotype (three levels: Met/Met, Val/Met and Val/Val) as between-subject factor. Post hoc analyses were performed with paired t tests. The same approach was used to determine the impact of COMT genotype on THC plasma concentrations and subjective effects.

3. Results

3.1. Drug levels and subjective effects

Plasma concentrations of THC and its main metabolites were 84.9 ± 43.5 ng/ml (THC), 3.1 ± 3.5 ng/ml (11-nor-9-carboxy-THC) and 2.8 ± 2.4 ng/ml (11-OH-THC), 5 min after inhalation of 6 mg THC.

Analysis of subjective effects revealed a significant THC-induced increase in VAS score of 'perception', whereas THC significantly reduced 'relaxation' compared to placebo. VAS score of 'dysphoria' showed a trend towards a significant increase after THC administration. Subjective effects are summarised in Table 2. For a more detailed description of drug levels and subjective effects following THC see van Hell et al. (2011b).

3.2. Effects of THC on resting state perfusion

Thirty-three subjects were included in this analysis. Six subjects were excluded due to movement-related errors during scanning or technical malfunction of the scanner. Whole-brain voxel-wise ASL analysis showed significantly increased perfusion after THC administration in both left and right insula / inferior frontal gyrus (653 voxels, $p = 0.003$ and 1043 voxels, $p < 0.001$, respectively), medial superior frontal cortex (555 voxels, $p = 0.005$), and left middle orbital frontal gyrus (333 voxels, $p = 0.026$) (Fig. 1). There were no brain areas showing a significant decrease in perfusion after THC administration.

3.3. Effects of THC on resting state connectivity

The four regions that showed significant effects of THC administration on perfusion were used as seed regions in a subsequent whole-brain voxel-wise analysis of resting state connectivity. Thirty-four subjects were included in this analysis. Five subjects were excluded due to movement-related errors during scanning or technical malfunction of the scanner. The left middle orbital frontal gyrus showed significantly decreased connectivity with the left middle cingulum / precuneus after THC administration (486 voxels, $p = 0.036$) (Fig. 2). The other seed regions did not demonstrate any significant effects of THC on resting state brain connectivity.

3.4. Brain function vs subjective effects

Multiple linear regression was performed to examine the relationship between brain function in those regions that showed a significant impact of THC administration (perfusion in bilateral insula, medial superior frontal cortex and left middle orbital frontal gyrus; connectivity between left middle orbital frontal gyrus and precuneus) and subjective effects (VAS scores of 'perception', 'relaxation' and 'dysphoria'). A near-significant regression equation was found for the left insula ($F(3,29) = 2.66$, $p = 0.067$), with an R^2 of 0.216. Both VAS scores of 'perception' ($\beta = 0.629$, $p = 0.014$) and 'relaxation' ($\beta = 0.540$, $p = 0.020$) were significantly related to perfusion in the left insula.

3.5. Impact of COMT genotype

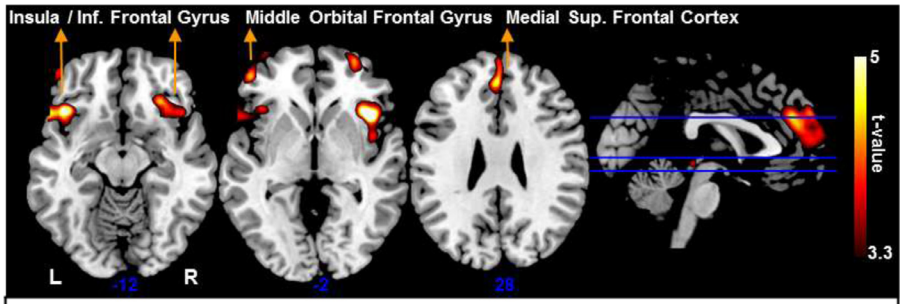
For thirty subjects COMT Val158Met genotype was determined, of which twenty-five had complete ASL data sets. Genetic modulation of THC effects on resting state perfusion was assessed in three functional brain networks

Table 2 Subjective effects of Δ9-tetrahydrocannabinol (THC) ($n = 39$).

Assessment	Mean placebo score (± SD)	Mean THC score (± SD)	p value
VAS Perception	1.5 ± 3.5	17.5 ± 17.7	< 0.001*
VAS Dysphoria	-0.2 ± 1.4	2.1 ± 7.4	0.069
VAS Relaxation	-5.5 ± 7.8	-20.2 ± 13.8	< 0.001*

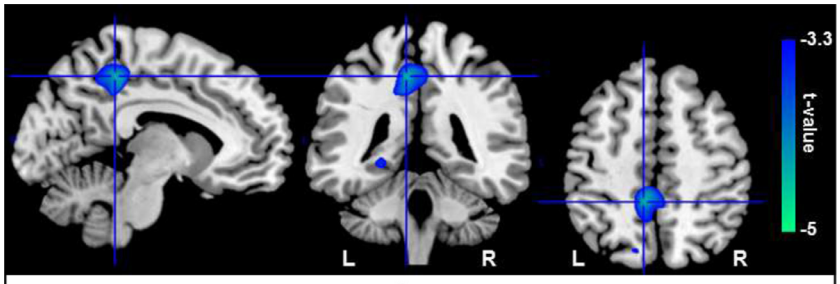
Statistical analysis was performed with paired t tests using baseline corrected values averaged over six time points.

* Significant difference between placebo and THC ($p < 0.05$). VAS, Visual Analogue Scale.



Brain region	BA	mm³	p value	x	y	z	Max T
Insula / Inferior Frontal Gyrus R	47	8344	<0.001	40	20	-2	6.06
Insula / Inferior Frontal Gyrus L	38	5224	0.003	-44	22	-12	5.21
Medial Superior Frontal Cortex	32	4440	0.005	-2	44	28	4.77
Middle Orbital Frontal Gyrus L	46	2664	0.026	-48	48	-2	4.56

Fig. 1 Significant increases in perfusion after THC compared to placebo administration ($n = 33$; FDR-corrected at cluster level; $p < 0.05$). Numbers below slices indicate Montreal Neurological Institute z coordinates. BA, Brodmann Area; L, left; R, right.



Brain region	mm³	p value	x	y	z	Min T
Middle Cingulum / Precuneus L	3888	0.036	-8	-40	50	-4.46

Fig. 2 Significant decreases in resting state connectivity with left middle orbital frontal gyrus after THC compared to placebo administration ($n = 34$; FDR-corrected at cluster level; $p < 0.05$). L, left; R, right.

(Fig. 3). This analysis revealed a significant interaction effect between drug and COMT genotype in the executive network ($F(2,22) = 3.48$, $p = 0.049$), with increased perfusion after THC in individuals with a Val/Met genotype only ($p = 0.006$) (Fig. 4a). The salience network showed significantly increased perfusion after THC irrespective of genotype (drug effect; $F(1,22) = 7.47$, $p = 0.012$) (Fig. 4b). There were no significant drug or interaction effects on resting state perfusion in the default mode network. COMT genotype did not have any significant effects on THC plasma concentrations or subjective effects.

4. Discussion

In the current pharmacological MRI study we examined the acute effects of THC on resting state brain function, and their modulation by COMT genotype. Compared to placebo, THC increased perfusion in bilateral insula, medial superior frontal cortex, and left middle orbital frontal gyrus. This latter brain area showed significantly decreased connectivity with the precuneus after THC administration. THC effects on perfusion in the left insula were significantly related to alterations in subjective measures of perception

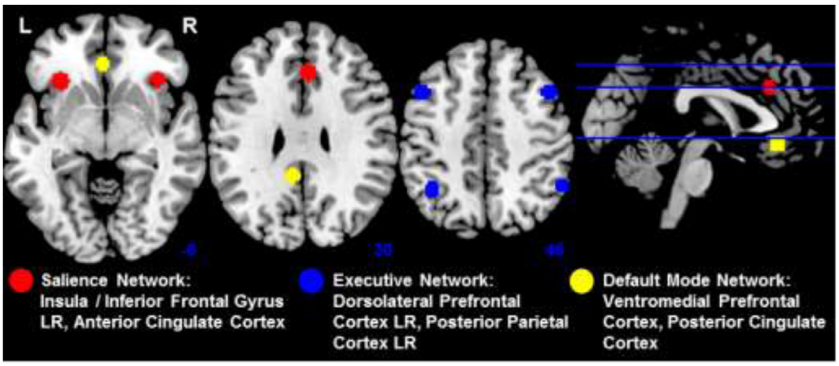


Fig. 3 Functional networks used to examine genetic modulation of THC effects. Networks were defined by placing 6 mm spheres around coordinates previously reported by Sridharan et al. (2008). Numbers below slices indicate Montreal Neurological Institute z coordinates. L, left; R, right.

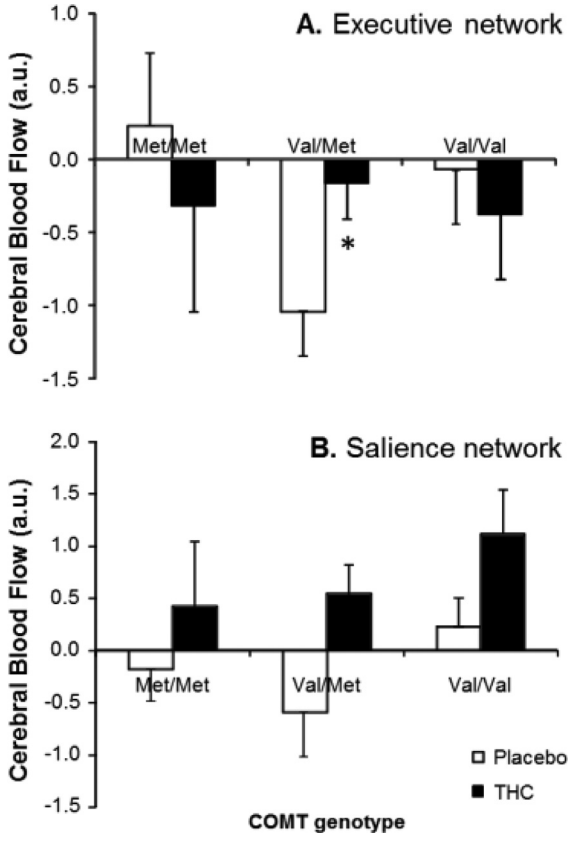


Fig. 4 Genetic modulation of THC effects on resting state perfusion. (A) Significant interaction effect between drug and COMT genotype in the executive network, with increased perfusion after THC in Val/Met heterozygotes only. (B) Significantly increased perfusion in the salience network irrespective of genotype (Met/Met $n = 6$, Val/Met $n = 12$, Val/Val $n = 7$, $p < 0.05$).

and relaxation. Resting state perfusion was modulated by COMT genotype, indicated by a significant interaction effect between drug and genotype on perfusion in the executive network, with increased perfusion after THC in Val/Met heterozygotes only.

Our finding of increased perfusion after THC in bilateral insula and medial superior frontal cortex suggests a THC-induced elevation of neural activity in the salience network. This is a brain system with key nodes in the insular and frontal cortices, which plays a central role in the detection of behaviourally relevant stimuli and the initiation of appropriate responses to this salient input (Craig, 2009; Seeley et al., 2007; Sridharan et al., 2008; Uddin, 2015). In this network, the insula appears to be critically involved in the identification of salient information, as indicated by its fundamental role in behaviour that requires engagement of awareness, such as integration of inner body feelings (interoception), self-recognition, time perception, attention, and performance monitoring (Craig, 2009). Activation of the medial frontal cortex may be responsible for the initiation of subsequent behaviours (Craig, 2009; Uddin, 2015). Thus, elevated perfusion in the salience network after THC administration as shown in the current study most likely reflects a THC-induced increase in awareness and anticipation of salient information. This is consistent with the description of typical THC effects, including perceptual alterations, time distortion, and intensification of ordinary experiences such as eating and listening to music (Ashton, 2001; Hall and Degenhardt, 2009). Furthermore, the reported significant correlations between THC effects on perfusion in the insula and subjective measures of perception and relaxation further suggest that recruitment of brain areas within the salience network is involved in the main acute effects of THC.

THC administration induced a significant reduction in resting state connectivity between the middle orbital frontal gyrus and precuneus. The middle orbital frontal gyrus (also called lateral orbitofrontal cortex) is thought to be particularly involved in the detection, processing and evaluation of non-rewarding or unpleasant information (Kringelbach and Rolls, 2004; Kringelbach, 2005). The precuneus is a key node in the default mode network, which is a brain system that is involved in conscious processes such as mind-wandering, and which is deactivated during performance of demanding cognitive tasks, thereby contributing to successful goal-directed behaviour (Buckner et al., 2008; Raichle et al., 2001). Reduced connectivity between the middle orbital frontal gyrus and precuneus could result in

difficulties in communication between these regions, which may lead to the insufficient integration of negatively valent information in goal-oriented behaviour. Interestingly, this is further supported by studies that showed a significant increase in resting state connectivity between the middle orbital frontal gyrus and important nodes in the default mode network (precuneus and posterior cingulate cortex) in unmedicated patients with major depressive disorder, which was attenuated in patients that received medication (Cheng et al., 2016, 2018).

Our results on the acute effects of THC on resting state brain function are in line with previous neuroimaging findings. Increased perfusion after THC administration in insular and prefrontal regions has previously been reported in smaller samples (Mathew et al., 1997, 2002; van Hell et al., 2011a). Although the perfusion data of the current study are an extension of the data set reported by van Hell et al. (2011a), we did not demonstrate any decreases in perfusion after THC administration in contrast to reduced CBF in the right postcentral gyrus and bilateral occipital gyrus as reported in the original study. Possible explanations for these discrepant results may be found in the larger sample size or the more stringent multiple comparison corrections that were applied in the current study. Our connectivity findings are consistent with those of Klumpers et al. (2012), who also found reduced functional connectivity with the default mode network, in particular between the posterior cingulate cortex and a network of brain regions collectively referred to as the left dorsal visual stream, which is thought to be involved in attentional processes.

In the present study, subjective ratings of 'perception' and 'dysphoria' were increased, whereas those of 'relaxation' were reduced after THC administration. To our best knowledge, this is the first time that the effects of THC on these novel subscales, as recently described by Kleinloog et al. (2014), are reported. Demonstrated acute effects of THC on feeling high and perception ('perception'), psychotic experiences ('dysphoria') and mental aspects of sedation ('relaxation') are consistent with previously reported subjective effects after THC administration (Bossong et al., 2012; Klumpers et al., 2012; van Hell et al., 2011b; Zuurman et al., 2008).

To our best knowledge, we are the first to demonstrate that variation in COMT Val158Met genotype modulated acute THC effects on brain function. This is consistent with previous studies that showed a modulating role of COMT in the effects of cannabis (Caspi et al., 2005; Costas et al., 2011; Estrada et al., 2011; Henquet et al., 2006, 2009; Tunbridge et al., 2015; Vinkers et al., 2013). However, whereas other experimental studies showed strongest acute cannabis effects in Val/Val genotypes (Henquet et al., 2006; Tunbridge et al., 2015), our findings suggest an augmenting effect on activity in the executive network of Val/Met heterozygotes. Possible explanations for these discrepant findings may be found in study population and design. Previous studies examined vulnerable and/or patient groups and investigated the impact of COMT genotype on the acute symptom and/or cognitive effects of cannabis (Henquet et al., 2006; Tunbridge et al., 2015). We demonstrate in the current study that variation in COMT genotype modulates acute THC effects on resting state brain function in healthy volunteers with limited exposure to cannabis or other drugs of abuse. Because it is thought that Val/Met heterozygotes have optimal dopamine levels resulting in maximum prefrontal cortex efficiency (Dickinson and Elvevag, 2009), our findings may suggest that only optimal executive brain function, such as with intermediate COMT activity, is susceptible to the acute effects of THC. This is further supported by a recent resting-state electroencephalography (EEG) study, which showed that brain activity in response to acute nicotine was modulated by COMT genotype, with significant increases in frontal regions in individuals with the Val/Met genotype only (Bowers et al., 2015).

Our results may have implications for understanding the relationship between cannabis use and the development of brain disorders such as schizophrenia or addiction. It is thought that patients inappropriately attribute incentive salience to otherwise relatively neutral environmental cues, which may result in the formation of psychotic symptoms (Heinz, 2002; Kapur, 2003) or the development of addictive behaviour (Berridge, 2012; Flagel et al., 2009). Emerging evidence suggests that reduced engagement of the salience network, and in particular the insula, is a feature of many brain disorders, including schizophrenia and addiction. Insular dysfunction may diminish the capacity of the brain to discriminate between self-generated and external information, thereby contributing to psychotic or addictive behaviour through impaired salience attribution (Whylie and Tregellas, 2010; Droutman et al., 2015; Uddin, 2015). Because we demonstrated increased perfusion after THC administration in the salience network including the bilateral insula, one possibility is that frequent cannabis use may result in desensitisation of the salience network, which may ultimately lead to the development of brain disorders such as schizophrenia or addiction (Hall and Degenhardt, 2009; Marconi et al., 2016; Moore et al., 2007; Vaucher et al., 2017).

This study has several limitations. First, despite the relatively large number of participants for a pharmacological neuroimaging study, sample sizes for examining the modulation of THC effects by COMT genotype were small. Therefore, these particular findings should be interpreted with caution and require replication, as we cannot exclude the possibility that these results are false positives or that additional findings were undetected due to limited statistical power. Second, inclusion of incidental cannabis users, as opposed to non-users, may affect interpretation of results as previous cannabis use may have affected the endocannabinoid system. The choice for incidental cannabis users was based on ethical grounds (van Hell et al., 2011b). Third, although the study was double blind, THC induced behavioural effects that could be identified by most subjects, causing expectancy effects across participants. We tried to limit these effects by randomising the order of drug administration. Finally, we acquired a relatively short resting state fMRI scan of four minutes, whereas a duration of at least eight minutes is commonly used (van den Heuvel et al., 2008).

In conclusion, our findings indicate that THC administration enhances metabolism and thus neural activity in the salience network, and that recruitment of brain areas within this network is involved in the acute effects of THC. THC effects on resting state brain activity were modulated by

COMT genotype, which suggests that prefrontal dopamine levels are involved in the susceptibility to acute effects of cannabis.

Conflict of interest

All authors declare that they have no conflicts of interest.

Contributors

Drs Bossong, van Hell, Schubart, Jager, Boks and Ramsey designed the study and wrote the protocol. Dr Bossong performed the analyses of the data and wrote the first draft of the manuscript. All authors were responsible for the interpretation of data, contributed to critical revision of the manuscript, and approved the final manuscript.

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