

# Cannabis induced increase in striatal glutamate associated with loss of functional corticostriatal connectivity



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## Abstract

Cannabis is the most commonly used illicit drug and is known to alter state of consciousness and impair neurocognitive function. However, the mechanisms underlying these effects have yet to be fully elucidated. Rodent studies suggest that  $\Delta^9$ -tetrahydrocannabinol (THC) activates dopaminergic neurons in the limbic system, subsequently enhancing dopamine, which is implicated in the rewarding effects of cannabis. Additional evidence suggests that THC may act indirectly on dopamine firing by modulating GABA and glutamate release. This double-blind, placebo-controlled study assessed the acute influence of two doses of THC on brain kinetics of glutamate, GABA, and dopamine, in relation to behavioral outcomes, by using magnetic resonance spectroscopy and functional magnetic resonance imaging. Twenty occasional cannabis users received acute doses of cannabis (300  $\mu$ g/kg THC) and placebo, in one of two dose regimes (full dose and divided dose), during two separate testing days. Administration of THC increased striatal glutamate concentrations, and dopamine as indicated by a reduction in functional connectivity (FC) between the nucleus accumbens (NAc) and cortical areas. Alterations in glutamate and FC were dose dependent and evident in the full dose group where THC serum concentrations exceeded 2 ng/ml at T-max. Average glutamate changes correlated

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strongly with FC alterations. Additionally, THC induced changes in FC correlated with feelings of subjective high and decreased performance on an attention task. Taken together, this suggests that THC elicits subjective and cognitive alterations via increased striatal dopaminergic activity and loss of corticostriatal connectivity, which is associated with an increase in striatal glutamate.

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## 1. Introduction

Cannabis is the most commonly used illicit drug in the world. About 4% of the global population have used cannabis and 10% of those develop daily use patterns (WHO, 2016). However, with changing legalization and a growing interest in therapeutic utility, there is an increasing trend of use (EMCDDA, 2016; UN, 2016).

The acute effects of cannabis on subjective and behavioral state are well known. Studies have shown that cannabis induces an increase in feelings of euphoria, altered perception, and relaxation (Green et al., 2003; Iversen, 2003; Johns, 2001), and a dose-related decrease in performance on motor, memory, learning, and attentional tasks (Hall and Solowij, 1998; Lundqvist, 2005; Ramaekers et al., 2004) when serum delta-9-tetrahydrocannabinol (THC) concentration levels surpass 2 ng/ml (Ramaekers et al., 2006). Nonetheless, the mechanisms underlying these acute effects have yet to be fully elucidated. THC, the main psychoactive component of cannabis, acts on cannabinoid (CB1) receptors distributed in motor and cognitive regions (Glass et al., 1997; Parsons and Hurd, 2015). These receptors can be found in moderate to high densities in the cerebellum, hippocampus, frontal cortex, posterior cingulate, and the striatum (Burns et al., 2007; Glass et al., 1997). Previous pharmacological neuroimaging studies investigating acute effects of  $\Delta^9$ -THC on brain activation have found increased regional cerebral blood flow (rCBF) in frontal, limbic, paralimbic, and cerebellar regions (Gonzalez, 2007). These alterations have been found to correlate with subjective levels of intoxication (Mathew et al., 1997, 1992) and with task performance (Fusar-Poli et al., 2009; O'Leary et al., 2003). However, it is likely that these acute functional and behavioral changes induced by cannabis are accompanied by changes in neurochemistry (Prescot et al., 2013). As the regions of functional change under the influence of cannabis involve those implicated with other substance use disorders, like dopamine reward pathways (Quickfall and Crockford, 2006), it is suggested that alterations in dopaminergic function may be associated with some of the behavioral effects of THC (Bloomfield et al., 2016).

Rodent studies assessing the acute actions of THC suggest that THC leads to a selective increase of activity in dopaminergic neurons in the ventral tegmental area (VTA), dose-dependently increasing dopamine baseline neuron firing rates (French, 1997; French et al., 1997). These dopamine neurons in the VTA target both neurons located in the nucleus accumbens (NAc), forming the mesolimbic dopamine pathway, as well as in the frontal cortex (Lupica et al., 2004). Accordingly, THC has been shown to increase dopamine in striatal areas, the NAc, and pre-frontal cortex (Diana et al., 1998; Fadda et al., 2006; Ng Cheong Ton

et al., 1988; Pistis et al., 2002b). Dopaminergic modulation in these brain areas is implicated in the euphoric and rewarding effects of cannabis and other drugs of abuse (Gessa et al., 1998; Ng Cheong Ton et al., 1988), as well as the disruption on cognitive (Diana et al., 1998) and motor (Navarro et al., 1993) function seen when under the influence of cannabis.

However, it has been suggested that THC may not act directly on dopamine firing, but instead acts indirectly by inhibiting glutamate release onto gamma-aminobutyric acid (GABA) neurons in the VTA and NAc (Colizzi et al., 2016; Pertwee, 2008; Schlicker and Kathmann, 2001). Accordingly, consistent evidence from animal models suggests that THC disrupts glutamate signaling by depressing glutamate synaptic transmission (Colizzi et al., 2016) in a dose- and brain region- dependent manner (Galanopoulos et al., 2011). In humans, only a few studies have investigated the effects of cannabis use on glutamate signaling in the brain. These studies have found reduced levels of glutamate-related metabolites in the basal ganglia (Chang et al., 2006; Muetzel et al., 2013) and anterior cingulate cortex (Prescot et al., 2011, 2013) in chronic cannabis users during abstinence. As these brain areas have been implicated in contributing to the behavioral effects of cannabis, these studies suggest that cannabis use affects cortical glutamate levels, and that these neurotransmitter changes mediate the behavioral effects. However, the acute effects of THC on glutamate signaling in the human brain and its interplay with GABA and dopamine transmission have yet to be assessed.

The present study was therefore designed to assess acute influences of two different dose regimens of cannabis on brain kinetics of glutamate, GABA, and dopamine in the limbic system during the absorption and elimination phase of THC. One group ( $N=10$ ) received a full dose of 300  $\mu\text{g}/\text{kg}$  THC that was expected to induce subjective high and affect cognitive performance. A second ( $N=10$ ) group received 3 successive doses of 100  $\mu\text{g}/\text{kg}$ , separated in time, that were expected to produce low THC serum concentrations (i.e. around 2 ng/ml) with little behavioral interference (Ramaekers et al., 2006). Participants in both groups also received placebo during a separate testing day. Ultra-High Field (7T) proton magnetic resonance imaging ( $^1\text{H}$  MRS), a non-invasive imaging technique that allows in vivo measurement of neurometabolites and neurotransmitters, was used to assess glutamate and GABA levels in the striatum and anterior cingulate cortex. Resting state functional magnetic resonance imaging (fMRI) was measured to determine functional connectivity between the regions of interest (ROI) in the NAc and remote cortical areas, as an indirect measure of dopaminergic stimulation (Ramaekers et al., 2013, 2016a). It was expected that when serum THC concentration levels at  $T_{\text{max}}$  were above 2 ng/ml, cannabis would alter glutamate and GABA levels, and would lead to dopaminergic

stimulation, reducing functional corticostriatal connectivity (Ramaekers et al., 2013, 2016a). It was further expected that these functional changes would correlate with changes in subjective high and task performance in a dose-dependent manner.

## 2. Experimental procedures

### 2.1. Participants

In total, 20 healthy, occasional cannabis users (male  $N=12$ , female  $N=8$ ) completed both treatment conditions. On average, participants had been occasional users of cannabis for 4.85 years (min-max: 2-11), using a mean frequency (SE) of 5.33 (0.758) times a month. For further details see Supplement and table S1.

This study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and amended in Fortaleza (Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and was approved by the Academic Hospital and University's Medical Ethics committee. All participants were fully informed of all procedures, possible adverse reactions, legal rights and responsibilities, expected benefits, and their right for voluntary termination without consequences.

### 2.2. Design, doses, and administration

This study was conducted according to a double-blind, placebo-controlled, mixed cross-over design. Occasional cannabis users were randomized to one of two groups. In the full dose group ( $N=10$ ) participants received 300  $\mu\text{g}/\text{kg}$  THC (Bedrobinol; 13.5% THC) vapor in one full dose. In the divided dose group ( $N=10$ ) participants received 300  $\mu\text{g}/\text{kg}$  THC vapor divided over 3 successive doses of 100  $\mu\text{g}/\text{kg}$ , separated by 30 min. Participants also received the relative placebo (0% THC) on a separate day, separated by a minimum wash-out period of 7 days to avoid cross-condition contamination. Treatment orders and doses were randomly assigned to subjects according to a balanced, block design. The dosage of cannabis was tailored to individual participants in order to reach 300  $\mu\text{g}/\text{kg}$  bodyweight THC, which has previously been found to be well tolerated by subjects with an average experience of cannabis use (Ramaekers et al., 2016b; Theunissen et al., 2012). For an overview of the testing day schedule, see Table S2.

### 2.3. Procedures

Upon arrival, a baseline blood sample was taken and baseline vital signs (blood pressure and heart rate) were measured. After measurements, the participant was placed in the MRI scanner, where after localizer scans and a T1 structural scan were taken, they inhaled the placebo or cannabis vapor. Throughout the intoxication and elimination phase (approximately a 1.5 h time window) functional connectivity and metabolic changes were assessed three separate times at regular intervals using resting state fMRI and magnetic resonance spectroscopy (MRS) respectively. Participants were also required to perform an attention task, complete a subjective high questionnaire, and give 5 blood samples at fixed intervals during and after scanning. Participants stayed under supervision until the researcher deemed they were fit to go home. For details, please see Supplement.

### 2.4. Proton spectra

Single-voxel proton magnetic resonance spectroscopy (MRS) measurements were performed on a MAGNETOM 7T MR scanner (Siemens Healthineers, Erlangen, Germany) with a whole-body gradient set (SC72; maximum amplitude, 70 mT/m; maximum slew rate, 200 T/m/s) and using an single-channel transmit/32-channel receive head coil (Nova Medical, Wilmington, MA, USA). Spectroscopic voxels of interest were placed by a trained operator at the Anterior Cingulate Cortex (ACC) (voxel size =  $25 \times 20 \times 17 \text{ mm}^3$ ) and the right striatum (voxel size =  $20 \times 20 \times 20 \text{ mm}^3$ ). Spectra were acquired with stimulated echo acquisition mode (STEAM) (Frahm et al., 1989) sequence using the following parameters: TE = 6.0 ms, TM = 10.0 ms, TR = 5.0 s, NA = 64, flip angle =  $90^\circ$ , RF bandwidth = 4.69 kHz, RF centred at 2.4 ppm, receive bandwidth = 4.0 kHz, vector size = 2048, 16-step phase cycling, acquisition time = 5:20 min. Outcome measures for MRS were concentration ratios of glutamate and GABA to total Creatine (Creatine + Phospho-Creatine). For further information and fit quality measures see Supplement.

### 2.5. Resting state fMRI

During resting state, 258 whole-brain EPI volumes were acquired (TR = 1400 ms; TE = 21 ms; field of view (FOV) = 198 mm; flip angle =  $60^\circ$ ; oblique acquisition orientation; interleaved slice acquisition; 72 slices; slice thickness = 1.5 mm; voxel size =  $1.5 \times 1.5 \times 1.5 \text{ mm}$ ). During resting state scans, participants were shown a black cross on a white background, and were instructed to focus on the cross while attempting to clear their mind, and lay as still as possible.

Resting state image preprocessing were conducted using SPM8 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, Institute of Neurology, University College London) Pre-processing steps included: motion correction (registered to the first image with second degree B-spline interpolation), coregistration (linking of functional to anatomical scans), and spatial normalization (the mean EPI image of each session was matched to SPM8's EPI template in Montreal Neurological Institute [MNI] space) where after the parameters were applied to all images of that session. During normalization voxel size was  $1.5 \times 1.5 \times 1.5 \text{ mm}$ . Finally, the data were smoothed at 3 mm Gaussian kernel.

### 2.6. Functional connectivity

Functional connectivity data were produced with the MATLAB toolbox DPARSF (Chao-Gan and Yu-Feng, 2010). Linear trends of time courses were removed followed by low band-pass filtered (0.01-0.08 Hz) of the preprocessed data to remove 'noise' attributable to physiological parameters. Nuisance covariates (motion parameters, white matter signal, CSF signal) were also removed. Two spheres (4 mm radius) were created that were located (in MNI space) in the left ( $-9, 9, -9$ ) and right ( $9, 9, -9$ ) NAC. Seed locations were in agreement with previous work validating structural and functional subdivisions of the NAC (Di Martino et al., 2008; Kelly et al., 2009). Average time courses were obtained for each sphere separately and correlational analysis was performed voxel wise to generate functional connectivity maps for each sphere. The correlation coefficient map was converted into z maps by Fisher's r-to-z transform to improve normality (Chao-Gan and Yu-Feng, 2010; Rosner, 2006). This is in accordance with previous studies investigating drug induced changes in functional connectivity, utilizing such changes as an indirect measure of dopaminergic stimulation, as dopamine levels cannot be directly assessed by MR methods (Ramaekers et al., 2013, 2016a).

## 2.7. Psychomotor vigilance task

The psychomotor vigilance task is a sustained-attention, reaction-time task that measures the speed with which participants respond to a visual stimulus (Dinges and Powell, 1985). The participant is instructed to press a button as soon as the stimulus appears (red circle). The outcome measure of this task was number of attentional lapses (reaction time > 500 ms). For more information see Supplement.

## 2.8. Subjective high

Participants rated their subjective high on visual analogue scales (10 cm) on four consecutive time points after treatment administration. Participants had to indicate how high they felt at that moment, compared with the most high they have ever felt (0 = not high at all; 10 = extremely high).

## 2.9. Pharmacokinetic measures

Blood samples (8 mL) to determine cannabinoid concentrations (THC and metabolites OH-THC and THC-COOH) were taken at baseline, 10, 30, 50, and 70 minutes post administration. Blood samples were centrifuged and serum was frozen at  $-20^{\circ}\text{C}$  until analyses for pharmacokinetic assessments. Cannabinoid concentrations were determined using a validated and proficiency test approved forensic routine method consisting of an automated solid-phase extraction and gas chromatography with tandem mass spectrometric detection with a limit of quantification of 0.3 ng/ml or less.

## 2.10. Statistical analysis

### 2.10.1. Subjective and cognitive effects

Statistical analysis of subjective high and task performance were conducted in IBM SPSS Statistics 24 using a mixed model analysis consisting of the within-subject factors Treatment (THC and placebo) and Time after smoking (3 levels), and the between-subject factor of Dose (full and divided dose). Due to main effect of Treatment or Treatment x Dose, a second analysis was performed for each dose, with treatment and time as within-subject factors. The alpha criterion level of significance was set at  $p = .05$ . Due to a violation of the assumption of normality, the data for the number of lapses were log transformed.

### 2.10.2. Metabolite concentrations

Due to violations of the assumption of normality, separate Friedman's non-parametric tests were used to assess metabolite concentration per time point for each dose.

### 2.10.3. fMRI data

Functional connectivity data (i.e. correlation coefficient maps for each individual in each treatment condition at each time point) were analyzed in 2 GLM models in SPM 12. In the first GLM, data entered a full factorial model with treatment (THC and placebo) and time point (3 levels) as within-subject factors and dose (full and divided dose) as a between-subject factor. Due to main effect of dose, a second GLM was performed for each dose, with treatment and time point as within-subject factors. From this model main effects of treatment were identified for the full dose group, but not the divided dose group.

### 2.10.4. Correlation analysis

Voxel wise correlation analysis between FC data and absolute average change scores of striatal glutamate, subjective high, and number of attention lapses were conducted. Individual treatment maps (placebo > THC) were entered into one-sample *t*-tests in SPM, with the absolute average change scores of striatal glutamate, subjective high, and number of attention lapses. Maps were corrected for multiple comparisons at the cluster level using the family wise error correction (FWE). Average mean voxel activation of SPM identified clusters were put into SPSS and Pearson's correlations were performed to get correlation strengths.

## 3. Results

### 3.1. THC concentrations in serum

Mean (SE) concentrations of THC, THCOH, and THCCOH in serum are given in Table 1.

### 3.2. Subjective and cognitive effects

Mixed model analysis showed a significant main effect of Treatment [ $F(1,17) = 30.491$ ,  $P < .0001$ ] and Treatment x Dose [ $F(1,17) = 9.169$ ,  $P = .008$ ] on subjective high and a main effect of Treatment [ $F(1,16) = 7.278$ ,  $P = .016$ ] on number of attentional lapses. Further analysis revealed that ratings of subjective high were significantly increased by THC in the full dose group [ $F(1,8) = 50.330$ ,  $P = .000$ ], but not in the divided dose group ( $P > .1$ ). THC administration also increased the number of attentional lapses during the psychomotor vigilance test in the full dose group [ $F(1,7) = 10.061$ ,  $P = .016$ ], but not in the divided dose group ( $P > .1$ ). There were no effects of THC on the other outcomes of the psychomotor vigilance test. Mean scores of variables significantly affected by THC are shown in Fig. 1.

### 3.3. Metabolite concentrations

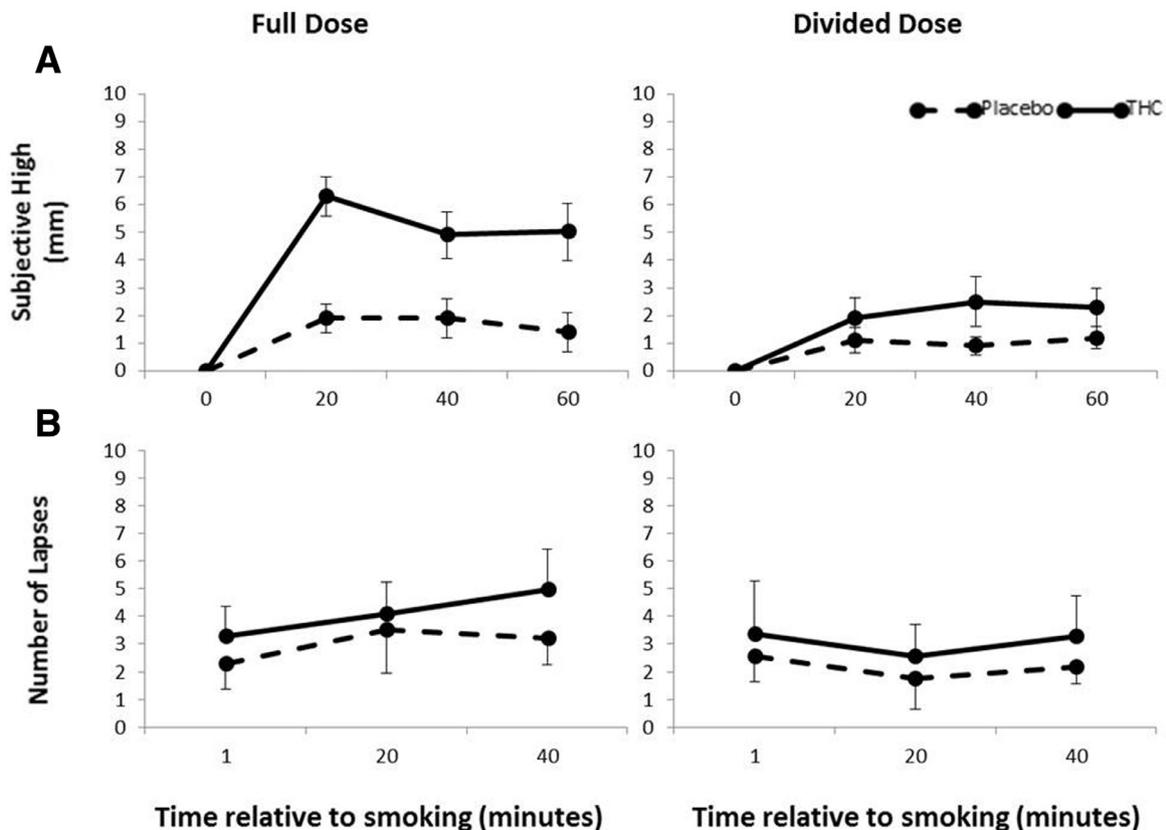
Separate Friedman's non-parametric tests showed that, relative to placebo, THC significantly increased glutamate/PCr + Cr (Glutamate) concentrations in the right striatum at both 30 minutes [ $\chi^2(1) = 9.800$ ,  $P = .002$ ] and 50 minutes [ $\chi^2(1) = 8.000$ ,  $P = .005$ ] post administration. Separate analysis per group showed that this increase was found in the full dose group at both 30 minutes [ $\chi^2(1) = 6.400$ ,  $P = .011$ ] and 50 minutes [ $\chi^2(1) = 5.444$ ,  $P = .020$ ], but not the divided dose group (Fig. 2). There were no effects of THC on GABA/PCr + Cr (GABA) in the striatum, or on GABA or Glutamate in the ACC, after either dose. Mean scores of metabolites unaffected by THC are shown in Table S3.

### 3.4. Functional connectivity

Fig. 3 shows significant FC data in each treatment condition for the left NAc seed averaged over time. GLM revealed a main effect of Dose and a Dose x Treatment interaction (Table S4). Further GLM contrasts showed that relative to placebo, cannabis reduced functional connectivity with NAc

**Table 1** Time course for mean (S.E.) concentrations of THC and its metabolites in serum (ng/ml) following smoking two doses of THC (group 1 who received one full dose of 300  $\mu$ g/kg THC and group 2 who received 3 successive doses of 100  $\mu$ g/kg), as assessed by gas chromatography-mass spectrometry (GC-MS).

Time relative to smoking	Serum (GC-MS)					
	Group 1			Group 2		
	THC	THC-OH	THC-COOH	THC	THC-OH	THC-COOH
0	0.05 (0.05)	0.03 (0.03)	0.80 (0.33)	.019 (0.02)	.07 (0.05)	1.85 (0.70)
6	7.03 (1.83)	1.97 (0.43)	9.43 (2.30)	1.66 (0.49)	.63 (0.18)	3.14 (0.96)
28	2.26 (0.63)	0.91 (0.29)	7.38 (2.00)	2.82 (0.44)	.97 (0.10)	4.72 (1.06)
50	1.87(0.52)	0.89 (0.27)	7.52 (2.01)	2.95 (0.62)	.89 (0.21)	4.86 (0.10)
67	2.52(0.71)	1.07 (0.26)	9.64 (2.16)	2.18 (0.35)	.80 (0.16)	6.82 (1.50)



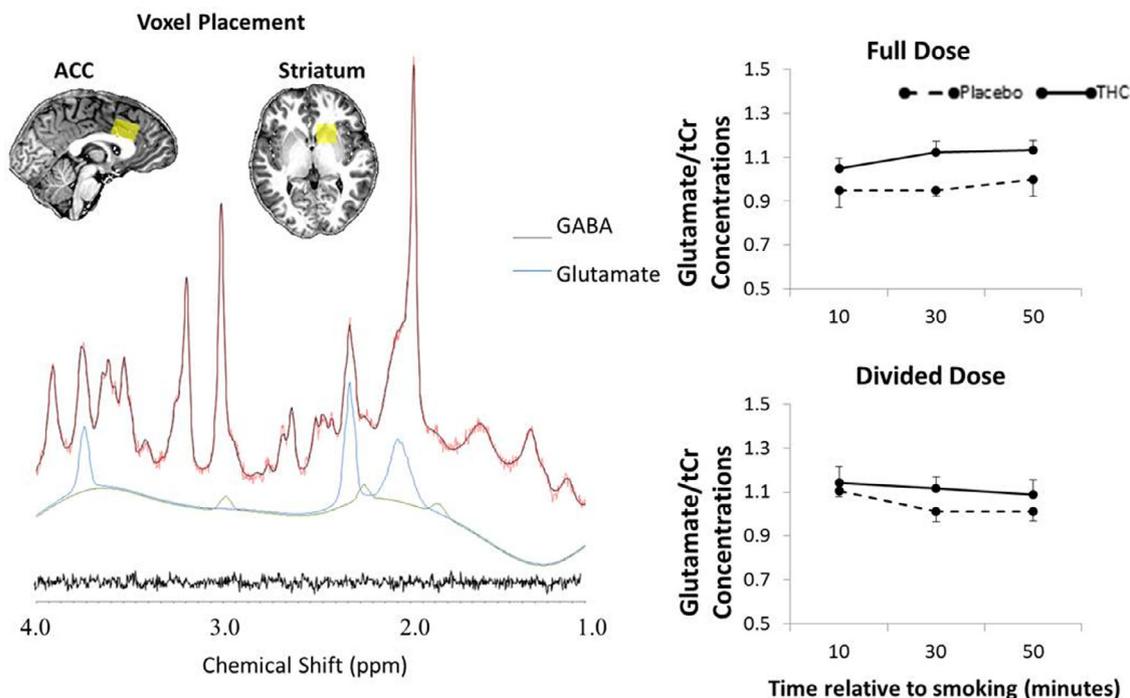
**Fig. 1** Subjective and cognitive effects. A. Group 1 and Group 2 mean (SE) subjective high for both treatments (THC vs placebo) as a function of time relative to smoking (0, 20, 30, and 60 minutes). B. Group 1 and Group 2 mean (SE) number of lapses (RT > 500 ms) for both treatments (THC vs placebo) as a function of time relative to smoking (1, 20, and 40 min).

seeds in both hemispheres in the full dose group, whereas no change was found in the divided dose group. Reductions in functional connectivity were prominent in broad areas of the frontal, temporal, parietal, and occipital lobes, a pattern typical of dopamine increase. However, GLM contrasts did not reveal any positive changes (THC > placebo) in functional connectivity. Table S5 shows the decrements in functional connectivity for the THC-placebo contrast in the full dose group. No significant differences were seen between the left and right NAC seed, so only left NAC seed

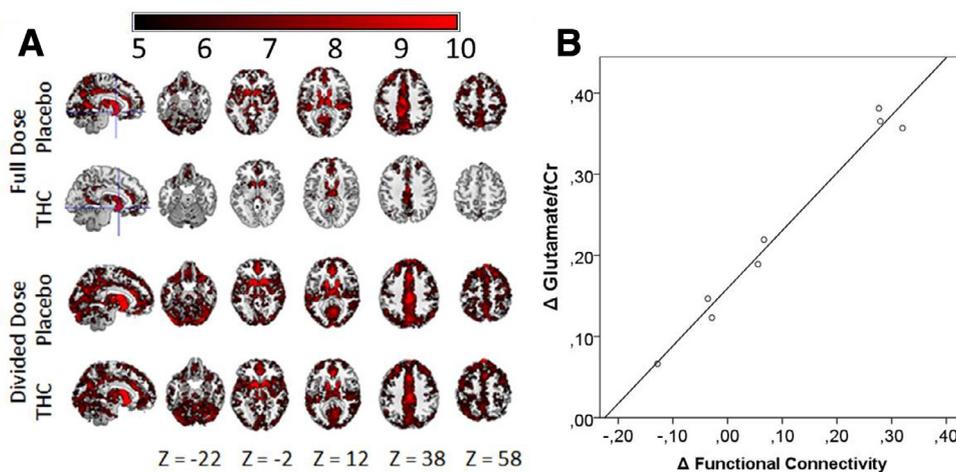
results are shown. There were no main effects of Time or Time x Treatment.

### 3.5. Correlation of subjective and cognitive effects, metabolite and THC concentrations, and functional connectivity

Voxel wise correlation analysis was conducted to evaluate the association between Pla > THC functional



**Fig. 2** A. Example LC-Model fitted  $^1\text{H}$ -MRS data recorded from one participant. The black line spectra corresponds to the phased  $^1\text{H}$ -MRS data with the LC-Model fits overlaid (red). The residual spectra (raw data minus the LC-Model fit) are displayed below the spectrum. B. Group 1 and Group 2 Mean (SE) Glu/tCr concentration levels in the striatum for both treatments (THC vs placebo) as a function of time relative to smoking (10, 30, and 50 min). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 3** A. NAcc-related functional connectivity in the left hemispheres. Shown are thresholded Z-score maps of functional connectivity for each condition. The cross-hair indicates the seed ROI position. B. Example of correlation strength between absolute averaged striatal glutamate change concentration and change in functional connectivity between the left nucleus accumbens and the precentral gyrus ( $r=0.989$ ). Other areas showed correlations of similar strength.

connectivity contrasts and changes in subjective high, attentional lapses, and glutamate changes in the striatum (Table S6). Correlation analysis revealed strong, positive correlations between glutamate changes in the striatum and functional connectivity between the left NAc and 6 cortical brain areas ( $r > 0.975$ ;  $P < .0001$ ) (Fig. 3; Table S6). Further analysis revealed a negative correlation between subjective high change scores ( $r = -0.987$ ,  $P < .0001$ ) and functional connectivity in the left NAc, and a positive correlation ( $r = 0.857$ ,  $P = .006$ ) between attentional lapse

change scores and functional connectivity in the left NAc. Glutamate changes were not significantly correlated with changes in subjective high and attentional lapses.

#### 4. Discussion

The present study demonstrates the first attempt to assess acute influences of two different doses of cannabis on brain kinetics of glutamate, GABA, and dopamine, in

relation to behavioral outcomes. Using a multimodal brain imaging approach, we showed significant neurometabolic and functional connectivity alterations during the absorption and elimination phase of THC in occasional cannabis users. These alterations were associated with changes in cognitive performance. Furthermore, these alterations were only evidenced when peak THC concentration levels were above 2 ng/ml, suggesting a dose and concentration dependency.

MRS showed that, compared to placebo, THC increased glutamate concentrations in the striatum in the full dose group. These findings are compatible with previous human studies which have found reductions of glutamate-related metabolite concentrations in chronic cannabis users in the basal ganglia and dorsal striatum (Chang et al., 2006). Specifically, the decrease found during post-acute studies could be postulated to be a result of increased glutamate activity during the acute phase (Sampedro et al., 2017) or an excessive down-regulation of glutamate signaling in chronic users (Colizzi et al., 2016). Further support stems from rodent studies, which have found that use or exposure to THC disrupts glutamate signaling in the striatum, although the direction of the effect is mixed (Brown et al., 2003; Galanopoulos et al., 2011; Sano et al., 2008).

MRS also showed that, compared to placebo, THC did not alter glutamate concentrations in the ACC. This is in accordance with a previous study by Sung et al. (2013) who did not find glutamate + glutamine alterations in cannabis users compared to healthy controls. However, previous research has also found reductions of glutamate-related metabolite concentrations in adolescent cannabis users in the ACC (Prescot et al., 2011; Prescot et al., 2013). In addition, Prescot et al. (2013) found significantly lower ACC GABA levels in adolescent cannabis users compared to non-using controls. This is in contrast to our study, which did not find any effects of THC on GABA in either the ACC or striatum. The absence of THC induced GABA changes could be due to inherent quantification challenges when assessing GABA concentration levels, arising from low brain concentration levels and metabolite signal overlap (Puts and Edden, 2012; Shungu et al., 2016). However in the present study, quantification precision of GABA/tCr, as estimated by Cramér-Rao Lower Bounds, overall surpassed the estimated signal change requirement, suggesting that null findings are not due to an inability to detect changes. Instead, the disagreement with studies could be due to the population under examination. Specifically, Prescot et al. (2013) assessed GABA levels in adolescents with a mean age of 17.9, whereas our study assessed individuals with a mean age of 22.5. Although animal studies have suggested that acute THC administration increases GABA levels in the prefrontal cortex (Pistis et al., 2002a) it has been suggested that these effects may be more pronounced in adolescence due to ongoing development (Silveri et al., 2013; Sneider et al., 2013). Finally, the finding that THC did not alter GABA concentrations in the striatum is in accordance with a suggestion that GABAergic neurons in the NAc are less sensitive to THC than glutamatergic neurons (Sano et al., 2008).

In the full-dose group, THC decreased synchronicity between BOLD responses in the NAc and other areas of the brain. In particular, THC decreased functional connectivity between the NAc and areas of the frontal cortex (e.g.,

Brodman area 6) and temporal cortex (e.g., Brodmann area 21 and 48), suggesting that THC directly affects the limbic reward circuit and decreases FC of brain areas in the circuit (Ramaekers et al., 2013, 2016a), a marker of elevated dopamine. In accordance with this, previous studies have shown that acute THC induces dopamine release in the striatum (Bossong et al., 2015, 2009). The NAc receives its dopaminergic input from the VTA that is under inhibitory control of GABA interneurons on which presynaptic CB1 receptors are located. Stimulation of CB1 receptors by THC disinhibits the VTA which in turn increases dopamine levels in the NAc (Lupica et al., 2004).

THC induced increments in striatal dopamine correlated strongly with THC induced increments in striatal glutamate. Specifically, as treatment changes in FC between the NAc and areas in the frontal and parietal lobe increased, changes in striatal glutamate concentration increased. Previous evidence suggests that dopaminergic innervation of the NAc increases inhibitory GABAergic neurotransmission to the ventral pallidum, which leads to a reduction of GABAergic, inhibitory tone to the thalamus (Pierce and Kumaresan, 2006). This process of disinhibition increases glutamatergic signaling from the thalamus to frontal cortex areas and subsequently to the NAc (Pierce and Kumaresan, 2006), leading to increased striatal levels of glutamate as observed in the present study. In theory, increased stimulatory glutamatergic input to the NAc will add to the dopaminergic input from the VTA to the NAc and further strengthen the disinhibition of thalamic signaling in the corticostriatal circuitry. The strong correlation between changes in striatal FC and striatal glutamate is likely to reflect their interrelatedness as well as the similarity of their impact in the circuitry.

Significant correlations were also found between FC from the NAc and changes in subjective experience and cognitive function. A negative correlation was found between changes in FC of the NAc and the left (pre)cuneus and ratings of significant high on the VAS. Specifically, as treatment induced changes in FC between the NAc and the (pre)cuneus increased, changes in subjective high decreased. It has been suggested that the precuneus is involved in various processes including self-consciousness and self-related mental representations during rest (Cavanna and Trimble, 2006). THC is known to lower levels of consciousness during a cannabis induced subjective high (Koethe et al., 2006; Vadhan et al., 2017). Level of consciousness and feeling of subjective high are therefore anti-correlated during cannabis intoxication. The negative correlation between changes in FC and subjective high supports the notion that decrements in consciousness are associated with increments in subjective high. The (pre)cuneus has also been implicated in the default mode network (Greicius et al., 2003; Utevsky et al., 2014), a network of interacting brain areas that are correlated with each other during rest. Similarly, the default mode network has been found to be altered in cannabis users (Cheng et al., 2014; Muetzel et al., 2013), as well as during acute THC intoxication (Bossong et al., 2013; Klumpers et al., 2012). Furthermore, altered precuneus activity has been found in users of other dopaminergic drugs of abuse (Moeller et al., 2016). Together this suggests that THC alters default mode network integrity via striatal dopaminergic activation, and that these changes may underlie subjective feelings of high.

A positive correlation was found between FC of the NAC and the left superior frontal gyrus (SFG) and the number of attentional lapses. Specifically, as treatment changes in FC between the NAC and the SFG increased, attentional performance decreased, as evidenced by an increase in number of attentional lapses. The SFG has been implicated in motor activity and higher cognitive functions, including working memory and attention (Boisgueheneuc et al., 2006; Nagahama et al., 1999). Previous studies suggest that altered levels of dopamine impair cognitive performance (Cools and D'Esposito, 2011; Prasad and Filbey, 2017), and studies assessing cannabis users have found altered activity in this region (Cheng et al., 2014; Orr et al., 2013; van Hell et al., 2010), as well as during acute THC intoxication (Ramaekers et al., 2016a; Weinstein et al., 2008). Taken together this suggests that THC alters activity in the SFG via striatal dopaminergic as well as glutamatergic activation, and that these changes can impair cognitive performance.

Significant subjective, cognitive, and biological changes were only found in the full dose group, who reached average serum THC concentrations of 7 ng/ml at T-max after administration of the full THC dose. This is in contrast to the divided dose group, who reached average serum THC concentrations of 1.6 ng/ml at T-max. Previous research has suggested serum THC concentrations of less than 2 ng/ml do not affect cognitive performance (Ramaekers et al., 2006). This study extends previous research in demonstrating that at serum THC concentrations of less than 2 ng/ml, brain functioning and metabolism are also not affected. As cannabis use becomes more popular, and access to medical cannabis grows, these findings are important in forensic settings when inferring impairment levels from THC levels in blood (Khiabani et al., 2006).

In conclusion, administration of 300 µg/kg THC increased striatal glutamate concentrations, and increased dopamine as measured by functional connectivity. Average glutamate changes correlated strongly with functional connectivity alterations between the NAC and cortical areas. Additionally, THC induced changes in FC correlated with feelings of subjective high and decreased performance on an attention task. Taken together, these findings suggest that THC elicits subjective and cognitive alterations via increased striatal dopaminergic activity and loss of corticostriatal connectivity, which is associated with an increase in striatal glutamate. Finally, alterations are dose dependent and only evident after serum THC concentrations exceed 2 ng/ml at T-max. Future studies should address long-term neuroadaptations in cannabis users, which may play a role in substance addiction.

## Conflict of interest

The authors declare no conflict of interest.

## Contributors

JG and ET designed the research. NM, NH, ET, and DT performed the research. NM, PS, and ST analyzed the data. NM, JG, and ET wrote the manuscript.

## Role of the funding source

Authors declare that the funding source had no influence on study design, interpretation of the results, neither in writing the manuscript, nor in the decision to submit the manuscript.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2018.12.003.

## References

- Bloomfield, M.A., Ashok, A.H., Volkow, N.D., Howes, O.D., 2016. The effects of Delta9-tetrahydrocannabinol on the dopamine system. *Nature* 539, 369-377.
- Boisgueheneuc, F.d., Levy, R., Volle, E., Seassau, M., Duffau, H., Kinkingnehun, S., Samson, Y., Zhang, S., Dubois, B., 2006. Functions of the left superior frontal gyrus in humans: a lesion study. *Brain* 129, 3315-3328.
- Bosson, M.G., Jansma, J.M., van Hell, H.H., Jager, G., Kahn, R.S., Ramsey, N.F., 2013. Default mode network in the effects of Delta9-Tetrahydrocannabinol (THC) on human executive function. *PLoS One* 8, e70074.
- Bosson, M.G., Mehta, M.A., van Berckel, B.N.M., Howes, O.D., Kahn, R.S., Stokes, P.R.A., 2015. Further human evidence for striatal dopamine release induced by administration of Δ9-tetrahydrocannabinol (THC): selectivity to limbic striatum. *Psychopharmacology* 232, 2723-2729.
- Bosson, M.G., van Berckel, B.N., Boellaard, R., Zuurman, L., Schuit, R.C., Windhorst, A.D., van Gerven, J.M., Ramsey, N.F., Lammertsma, A.A., Kahn, R.S., 2009. Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. *Neuropsychopharmacology* 34, 759-766.
- Brown, T.M., Brotchie, J.M., Fitzjohn, S.M., 2003. Cannabinoids decrease corticostriatal synaptic transmission via an effect on glutamate uptake. *J. Neurosci. Off. J. Soc. Neurosci.* 23, 11073-11077.
- Burns, H.D., Van Laere, K., Sanabria-Bohorquez, S., Hamill, T.G., Bormans, G., Eng, W.S., Gibson, R., Ryan, C., Connolly, B., Patel, S., Krause, S., Vanko, A., Van Hecken, A., Dupont, P., De Lepeleire, I., Rothenberg, P., Stoch, S.A., Cote, J., Haggmann, W.K., Jewell, J.P., Lin, L.S., Liu, P., Goulet, M.T., Gottesdiener, K., Wagner, J.A., de Hoon, J., Mortelmans, L., Fong, T.M., Hargreaves, R.J., 2007. [18F]MK-9470, a positron emission tomography (PET) tracer for in vivo human PET brain imaging of the cannabinoid-1 receptor. *Proc. Natl. Acad. Sci. USA* 104, 9800-9805.
- Cavanna, A.E., Trimble, M.R., 2006. The precuneus: a review of its functional anatomy and behavioral correlates. *Brain* 129, 564-583.
- Chang, L., Cloak, C., Yakupov, R., Ernst, T., 2006. Combined and independent effects of chronic marijuana use and HIV on brain metabolites. *J. Neuroimmune Pharmacol.* 1, 65-76 *Official Journal of Society NeuroImmune Pharmacology.*

- Chao-Gan, Y., Yu-Feng, Z., 2010. DPARSF: a MATLAB toolbox for "Pipeline" data analysis of resting-state fMRI. *Front. Syst. Neurosci.* 4, 13.
- Cheng, H., Skosnik, P.D., Pruce, B.J., Brumbaugh, M.S., Vollmer, J.M., Fridberg, D.J., O'Donnell, B.F., Hetrick, W.P., Newman, S.D., 2014. Resting state functional magnetic resonance imaging reveals distinct brain activity in heavy cannabis users - a multi-voxel pattern analysis. *J. Psychopharmacol.* 28, 1030-1040 Oxford, England.
- Colizzi, M., McGuire, P., Pertwee, R.G., Bhattacharyya, S., 2016. Effect of cannabis on glutamate signaling in the brain: a systematic review of human and animal evidence. *Neurosci. Biobehav. Rev.* 64, 359-381.
- Cools, R., D'Esposito, M., 2011. Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biol. Psychiatry* 69, e113-e125.
- Di Martino, A., Scheres, A., Margulies, D.S., Kelly, A.M., Uddin, L.Q., Shehzad, Z., Biswal, B., Walters, J.R., Castellanos, F.X., Milham, M.P., 2008. Functional connectivity of human striatum: a resting state fMRI study. *Cerebral Cortex* 18, 2735-2747 New York, N.Y.: 1991.
- Diana, M., Melis, M., Gessa, G.L., 1998. Increase in meso-pre-frontal dopaminergic activity after stimulation of CB1 receptors by cannabinoids. *Eur. J. Neurosci.* 10, 2825-2830.
- Dinges, D.F., Powell, J.W., 1985. Microcomputer analyses of performance on a portable, simple visual RT task during sustained operations. *Behav. Res. Methods Instrum. Comput.* 17, 652-655.
- EMCDDA, 2016. European Drug Report 2016: Trends and Developments. Publications Office of the European Union, Luxembourg.
- Fadda, P., Scherma, M., Spano, M.S., Salis, P., Melis, V., Fattore, L., Fratta, W., 2006. Cannabinoid self-administration increases dopamine release in the nucleus accumbens. *Neuroreport* 17, 1629-1632.
- Frahm, J., Bruhn, H., Gyngell, M.L., Merboldt, K.D., Hanicke, W., Sauter, R., 1989. Localized high-resolution proton NMR spectroscopy using stimulated echoes: initial applications to human brain in vivo. *Magn. Resonan. Med.* 9, 79-93.
- French, E.D., 1997. delta9-Tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. *Neurosci. Lett.* 226, 159-162.
- French, E.D., Dillon, K., Wu, X., 1997. Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. *Neuroreport* 8, 649-652.
- Fusar-Poli, P., Crippa, J.A., Bhattacharyya, S., Borgwardt, S.J., Allen, P., Martin-Santos, R., Seal, M., Surguladze, S.A., O'Carroll, C., Atakan, Z., Zuardi, A.W., McGuire, P.K., 2009. Distinct effects of {delta}9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Arch. Gen. Psychiatry* 66, 95-105.
- Galanopoulos, A., Polissidis, A., Papadopoulou-Daifoti, Z., Nomikos, G.G., Antoniou, K., 2011. Delta(9)-THC and WIN55,212-2 affect brain tissue levels of excitatory amino acids in a phenotype-, compound-, dose-, and region-specific manner. *Behav. Brain Res.* 224, 65-72.
- Gessa, G.L., Melis, M., Muntoni, A.L., Diana, M., 1998. Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. *Eur. J. Pharmacol.* 341, 39-44.
- Glass, M., Dragunow, M., Faull, R.L., 1997. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* 77, 299-318.
- Gonzalez, R., 2007. Acute and non-acute effects of cannabis on brain functioning and neuropsychological performance. *Neuropsychol. Rev.* 17, 347-361.
- Green, B., Kavanagh, D., Young, R., 2003. Being stoned: a review of self-reported cannabis effects. *Drug Alcohol Rev.* 22, 453-460.
- Greicius, M.D., Krasnow, B., Reiss, A.L., Menon, V., 2003. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proc. Natl. Acad. Sci. USA* 100, 253-258.
- Hall, W., Solowij, N., 1998. Adverse effects of cannabis. *Lancet* 352, 1611-1616 London, England.
- Iversen, L., 2003. Cannabis and the brain. *Brain: J. Neurol.* 126, 1252-1270.
- Johns, A., 2001. Psychiatric effects of cannabis. *Br. J. Psychiatry J. Ment. Sci.* 178, 116-122.
- Kelly, C., de Zubicaray, G., Di Martino, A., Copland, D.A., Reiss, P.T., Klein, D.F., Castellanos, F.X., Milham, M.P., McMahon, K., 2009. L-dopa modulates functional connectivity in striatal cognitive and motor networks: a double-blind placebo-controlled study. *J. Neurosci. Off. J. Soc. Neurosci.* 29, 7364-7378.
- Khiabani, H.Z., Bramness, J.G., Bjorneboe, A., Morland, J., 2006. Relationship between THC concentration in blood and impairment in apprehended drivers. *Traffic Injury Prev.* 7, 111-116.
- Clumpers, L.E., Cole, D.M., Khalili-Mahani, N., Soeter, R.P., Te Beek, E.T., Rombouts, S.A., van Gerven, J.M., 2012. Manipulating brain connectivity with delta(9)-tetrahydrocannabinol: a pharmacological resting state fMRI study. *NeuroImage* 63, 1701-1711.
- Koethe, D., Gerth, C.W., Neatby, M.A., Haensel, A., Thies, M., Schneider, U., Emrich, H.M., Klosterkotter, J., Schultze-Lutter, F., Leweke, F.M., 2006. Disturbances of visual information processing in early states of psychosis and experimental delta-9-tetrahydrocannabinol altered states of consciousness. *Schizophr. Res.* 88, 142-150.
- Lundqvist, T., 2005. Cognitive consequences of cannabis use: comparison with abuse of stimulants and heroin with regard to attention, memory and executive functions. *Pharmacol. Biochem. Behav.* 81, 319-330.
- Lupica, C.R., Riegel, A.C., Hoffman, A.F., 2004. Marijuana and cannabinoid regulation of brain reward circuits. *Br. J. Pharmacol.* 143, 227-234.
- Mathew, R.J., Wilson, W.H., Coleman, R.E., Turkington, T.G., DeGrado, T.R., 1997. Marijuana intoxication and brain activation in marijuana smokers. *Life Sci.* 60, 2075-2089.
- Mathew, R.J., Wilson, W.H., Humphreys, D.F., Lowe, J.V., Wiethel, K.E., 1992. Regional cerebral blood flow after marijuana smoking. *J. Cereb. Blood Flow Metab.* 12, 750-758 Official Journal of the International Society of Cerebral Blood Flow and Metabolism.
- Moeller, S.J., London, E.D., Northoff, G., 2016. Neuroimaging markers of glutamatergic and GABAergic systems in drug addiction: Relationships to resting-state functional connectivity. *Neurosci. Biobehav. Rev.* 61, 35-52.
- Muetzel, R.L., Marjańska, M., Collins, P.F., Becker, M.P., Valbrègue, R., Auerbach, E.J., Lim, K.O., Luciana, M., 2013. In vivo (1H) magnetic resonance spectroscopy in young-adult daily marijuana users ( ). *NeuroImage: Clin.* 2, 581-589.
- Nagahama, Y., Okada, T., Katsumi, Y., Hayashi, T., Yamauchi, H., Sawamoto, N., Toma, K., Nakamura, K., Hanakawa, T., Konishi, J., Fukuyama, H., Shibasaki, H., 1999. Transient neural activity in the medial superior frontal gyrus and precuneus time locked with attention shift between object features. *NeuroImage* 10, 193-199.
- Navarro, M., Fernandez-Ruiz, J.J., de Miguel, R., Hernandez, M.L., Cebeira, M., Ramos, J.A., 1993. An acute dose of delta 9-tetrahydrocannabinol affects behavioral and neurochemical indices of mesolimbic dopaminergic activity. *Behav. Brain Res.* 57, 37-46.
- Ng Cheong Ton, J.M., Gerhardt, G.A., Friedemann, M., Etgen, A.M., Rose, G.M., Sharpless, N.S., Gardner, E.L., 1988. The effects of delta 9-tetrahydrocannabinol on potassium-evoked release of dopamine in the rat caudate nucleus: an in vivo electrochemical and in vivo microdialysis study. *Brain Res.* 451, 59-68.
- O'Leary, D.S., Block, R.I., Turner, B.M., Koeppel, J., Magnotta, V.A., Ponto, L.B., Watkins, G.L., Hichwa, R.D., Andreasen, N.C., 2003. Marijuana alters the human cerebellar clock. *Neuroreport* 14, 1145-1151.

- Orr, C., Morioka, R., Behan, B., Datwani, S., Doucet, M., Ivanovic, J., Kelly, C., Weierstall, K., Watts, R., Smyth, B., Garavan, H., 2013. Altered resting-state connectivity in adolescent cannabis users. *Am. J. Drug Alcohol Abuse* 39, 372-381.
- Parsons, L.H., Hurd, Y.L., 2015. Endocannabinoid signaling in reward and addiction. *Nat. Rev. Neurosci.* 16, 579-594.
- Pertwee, R.G., 2008. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *Br. J. Pharmacol.* 153, 199-215.
- Pierce, R.C., Kumaresan, V., 2006. The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? *Neurosci. Biobehav. Rev.* 30, 215-238.
- Pistis, M., Ferraro, L., Pira, L., Flore, G., Tanganelli, S., Gessa, G.L., Devoto, P., 2002a. Delta(9)-tetrahydrocannabinol decreases extracellular GABA and increases extracellular glutamate and dopamine levels in the rat prefrontal cortex: an in vivo microdialysis study. *Brain Res.* 948, 155-158.
- Pistis, M., Ferraro, L., Pira, L., Flore, G., Tanganelli, S., Luigi Gessa, G., Devoto, P., 2002b.  $\Delta$ 9-Tetrahydrocannabinol decreases extracellular GABA and increases extracellular glutamate and dopamine levels in the rat prefrontal cortex: an in vivo microdialysis study. *Brain Res.* 948, 155-158.
- Prashad, S., Filbey, F.M., 2017. Cognitive motor deficits in cannabis users. *Curr. Opin. Behav. Sci.* 13, 1-7.
- Prescott, A.P., Locatelli, A.E., Renshaw, P.F., Yurgelun-Todd, D.A., 2011. Neurochemical alterations in adolescent chronic marijuana smokers: a proton MRS study. *NeuroImage* 57, 69-75.
- Prescott, A.P., Renshaw, P.F., Yurgelun-Todd, D.A., 2013. gamma-Amino butyric acid and glutamate abnormalities in adolescent chronic marijuana smokers. *Drug Alcohol Depend.* 129, 232-239.
- Puts, N.A.J., Edden, R.A.E., 2012. In vivo magnetic resonance spectroscopy of GABA: a methodological review. *Prog. Nuclear Magn. Reson. Spectrosc.* 60, 29-41.
- Quickfall, J., Crockford, D., 2006. Brain neuroimaging in cannabis use: a review. *J. Neuropsychiatry Clin. Neurosci.* 18, 318-332.
- Ramaekers, J., Evers, E., Theunissen, E., Kuypers, K., Goulas, A., Stiers, P., 2013. Methylphenidate reduces functional connectivity of nucleus accumbens in brain reward circuit. *Psychopharmacology* 229, 219-226 Berl.
- Ramaekers, J.G., Berghaus, G., van Laar, M., Drummer, O.H., 2004. Dose related risk of motor vehicle crashes after cannabis use. *Drug Alcohol Depend.* 73, 109-119.
- Ramaekers, J.G., Moeller, M.R., van Ruitenbeek, P., Theunissen, E.L., Schneider, E., Kauert, G., 2006. Cognition and motor control as a function of Delta9-THC concentration in serum and oral fluid: limits of impairment. *Drug Alcohol Depend.* 85, 114-122.
- Ramaekers, J.G., van Wel, J.H., Spronk, D., Franke, B., Kenis, G., Toennes, S.W., Kuypers, K.P.C., Theunissen, E.L., Stiers, P., Verkes, R.J., 2016a. Cannabis and cocaine decrease cognitive impulse control and functional corticostriatal connectivity in drug users with low activity DBH genotypes. *Brain Imaging Behav.* 10, 1254-1263.
- Ramaekers, J.G., van Wel, J.H., Spronk, D.B., Toennes, S.W., Kuypers, K.P.C., Theunissen, E.L., Verkes, R.J., 2016b. Cannabis and tolerance: acute drug impairment as a function of cannabis use history. *Sci. Rep.* 6, 26843.
- Rosner, B., 2006. *Fundamentals of Biostatistics*, Sixth ed. Thomson-Brooks/Cole, Belmont, CA.
- Sampedro, F., de la Fuente Revenga, M., Valle, M., Roberto, N., Dominguez-Clave, E., Elices, M., Luna, L.E., Crippa, J.A.S., Hallak, J.E.C., de Araujo, D.B., Friedlander, P., Barker, S.A., Alvarez, E., Soler, J., Pascual, J.C., Feilding, A., Riba, J., 2017. Assessing the psychedelic "After-Glow" in ayahuasca users: post-acute neurometabolic and functional connectivity changes are associated with enhanced mindfulness capacities. *Int. J. Neuropsychopharmacol.* 20, 698-711.
- Sano, K., Mishima, K., Koushi, E., Orito, K., Egashira, N., Irie, K., Takasaki, K., Katsurabayashi, S., Iwasaki, K., Uchida, N., Egawa, T., Kitamura, Y., Nishimura, R., Fujiwara, M., 2008. Delta 9-tetrahydrocannabinol-induced catalepsy-like immobilization is mediated by decreased 5-HT neurotransmission in the nucleus accumbens due to the action of glutamate-containing neurons. *Neuroscience* 151, 320-328.
- Schlicker, E., Kathmann, M., 2001. Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol. Sci.* 22, 565-572.
- Shungu, D.C., Mao, X., Gonzales, R., Soones, T.N., Dyke, J.P., van der Veen, J.W., Kegeles, L.S., 2016. Brain  $\gamma$ -aminobutyric acid (GABA) detection in vivo with the J-editing (1) H MRS technique: a comprehensive methodological evaluation of sensitivity enhancement, macromolecule contamination and test-retest reliability. *NMR Biomed.* 29, 932-942.
- Silveri, M.M., Sneider, J.T., Crowley, D.J., Covell, M.J., Acharya, D., Rosso, I.M., Jensen, J.E., 2013. Frontal lobe GABA levels during adolescence: associations with impulsivity and response inhibition. *Biol. Psychiatry* 74, 296-304.
- Sneider, J.T., Mashhoon, Y., Silveri, M.M., 2013. A review of magnetic resonance spectroscopy studies in marijuana using adolescents and adults. *J. Addict. Res. Ther.* 4, 010 Suppl.
- Sung, Y.-H., Carey, P.D., Stein, D.J., Ferrett, H.L., Spottiswoode, B.S., Renshaw, P.F., Yurgelun-Todd, D.A., 2013. Decreased frontal N-acetylaspartate levels in adolescents concurrently using both methamphetamine and marijuana. *Behav. Brain Res.* 246, 154-161.
- Theunissen, E.L., Kauert, G.F., Toennes, S.W., Moeller, M.R., Sambeth, A., Blanchard, M.M., Ramaekers, J.G., 2012. Neurophysiological functioning of occasional and heavy cannabis users during THC intoxication. *Psychopharmacology* 220, 341-350 Berl.
- UN, 2016. *World Drug Report 2016*. United Nations Publication.
- Utevsky, A.V., Smith, D.V., Huettel, S.A., 2014. Precuneus is a functional core of the default-mode network. *J. Neurosci.* 34, 932-940.
- Vadhan, N.P., Corcoran, C.M., Bedi, G., Keilp, J.G., Haney, M., 2017. Acute effects of smoked marijuana in marijuana smokers at clinical high-risk for psychosis: a preliminary study. *Psychiatry Res.* 257, 372-374.
- van Hell, H.H., Vink, M., Ossewaarde, L., Jager, G., Kahn, R.S., Ramsey, N.F., 2010. Chronic effects of cannabis use on the human reward system: an fMRI study. *European Neuropsychopharmacol.* 20, 153-163 The Journal of the European College of Neuropsychopharmacology.
- Weinstein, A., Brickner, O., Lerman, H., Greeland, M., Bloch, M., Lester, H., Chisin, R., Mechoulam, R., Bar-Hamburger, R., Freedman, N., Even-Sapir, E., 2008. Brain imaging study of the acute effects of Delta9-tetrahydrocannabinol (THC) on attention and motor coordination in regular users of marijuana. *Psychopharmacology* 196, 119-131 Berl.
- WHO, 2016. *The Health and Social Effects of Nonmedical Cannabis Use*. WHO.