Can recreational doses of THC produce significant dopamine release in the human striatum?

Paul R.A. Stokes a,⁎, Mitul A. Mehta a,b, H. Valerie Curran c, Gerome Breen d, Paul M. Grasby a

⁎ Corresponding author. Psychiatry Group, MRC Clinical Sciences Centre, Imperial College, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN, UK. Fax: +44(0)2083831783.
E-mail address: paul.stokes@imperial.ac.uk (P.R.A. Stokes).

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A B S T R A C T

Background: Cannabis use in early adolescence may be a risk factor for development of schizophrenia. In animals, Δ9-tetrahydrocannabinol (THC) increases the rate of dopamine neuronal firing and release in the striatum. Thus cannabis use may increase dopamine release in the human striatum leading to vulnerability to psychosis.

Aims: To investigate whether THC, the main psychoactive component of cannabis, can produce dopamine release in the human striatum.

Methods: Thirty healthy volunteers, with previous cannabis experience, underwent two [11C]-raclopride positron emission tomography (PET) scans to indirectly measure striatal dopamine levels following either 10 mg THC or placebo.

Results: Although THC markedly increased psychosis-like symptoms on the Psychotomimetic States Inventory (PSI), there was no significant effect of THC on [11C]-raclopride binding.

Conclusion: In the largest study of its kind so far, we have shown that recreational cannabis users do not release significant amounts of dopamine from an oral THC dose equivalent to a standard cannabis cigarette. This result challenges current models of striatal dopamine release as the mechanism mediating cannabis as risk factor for schizophrenia.

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Introduction

Cannabis sativa is the most widely abused recreational drug, used by an estimated 164 million people worldwide (Chawla, 2006). A recent prospective epidemiological study described cannabis use in early adolescence as a significant risk factor for development of schizophrenia. A systematic review also demonstrated a 1.4 fold increased risk of a psychotic outcome in individuals who had ever used cannabis and that this risk was highest in the most frequent users (Moore et al., 2007).

Although the epidemiological evidence of cannabis use as a risk factor for schizophrenia is emerging, the neurochemical mechanism mediating this risk is still unknown. Cannabis produces its behavioural effects by Δ9-tetrahydrocannabinol (THC), its main psychoactive constituent, binding at cortical cannabinoid CB1 receptors. In animal studies, THC both releases dopamine in the ventral striatum (Cheer et al., 2004) and increases the rate of dopamine neuronal firing (Tanda et al., 1997). In humans, a case report (Voruganti et al., 2001) showed a 20% decrease in [11C]-IBZM binding after one volunteer used cannabis and preliminary results from a study using inhaled THC (Bossong et al., 2009) showed a 3% decrease in [11C]-raclopride binding in the ventral striatum, indicating that dopamine release occurred. Taken together these studies imply that THC may increase dopamine release in the human striatum, which is highly relevant to the aetiology of schizophrenia as dysfunction of the striatal dopamine system in schizophrenia has been demonstrated (Abi-Dargham et al., 2000; Breier et al., 1997; Laruelle et al., 1996; McGowan et al., 2004).

To test the hypothesis that THC would produce significant in vivo dopamine release in the human striatum, we used [11C]-raclopride bolus–infusion positron emission tomography (PET) to measure striatal dopamine levels in healthy volunteers after a placebo and a THC challenge.

Materials and methods

Participants

Thirteen healthy volunteers [7 males; mean age 33 years (±7)], who had previous experience of using cannabis at least 20 times without significant adverse effects, were recruited to the study by means of public advertisement. The volunteers reported using cannabis 812 times (±671), ecstasy 18 times (±33), cocaine 5.3 times (±6.6) and amphetamine 6.9 times (±14.6) over their lifetime. All volunteers were assessed by a psychiatrist to exclude current or previous significant
mental health disorders and alcohol or drug dependency as defined by DSM-IV, serious physical illness, past neurological disorders or previous use of psychotropic medications. All volunteers gave written informed consent for the study, which was approved both by the Hammersmith Research Ethics Committee and the Administration of Radioactive Substances Advisory Committee, UK.

PET scanning and image acquisition

Volunteers underwent two [11C]-raclopride bolus plus constant infusion PET scans separated by at least 3 weeks. On each scan day, volunteers underwent urine drug screen analysis for cannabis, cocaine, methamphetamine, amphetamine, opiates and benzodiazepines. Any volunteer who produced a positive urine drug screen on the scan day was excluded from the study. Volunteers were also asked to abstain from alcohol for 24 hours prior to the scan day. 90 min before each scan volunteers consumed a capsule containing either 10 mg dronabinol (a synthetic form of THC) or placebo given in a randomised order. Volunteers were dosed at this time point so that the equilibrium period of subsequent scans would correspond to the known 2 hour plasma peak concentration of oral THC (Curran et al., 2002). During the study, although volunteers came into contact with various members of the research team, only one member knew the order of THC administration. The unblinded member of the team was responsible for insertion of the intravenous cannula, administration of the capsule prior to the scan, supervising scans, providing questionnaires to the volunteers and debriefing volunteers at the end of the second scan. Importantly, the radiographer running the scan was blind to the order of drug administration and during the scan the volunteer was alone in the scanner whilst the scan was monitored from an adjacent room. All questionnaires used were self-report with no interference from the unblinded investigator.

All PET scans were performed using an ECAT HR+ 962 scanner (CTI/Seimens) with an axial field of view of 15.5 cm. For each scan, volunteers were injected with [11C]-raclopride as a bolus and constant infusion, with a 400 μl/min rate, using the method described by Watabe et al. (2000). The bolus–infusion approach mitigates blood flow changes affecting binding potential (BPND) values by establishing a state of constant equilibrium (Carson et al., 1997). BPND is the ratio at equilibrium of specifically bound radioligand, in this case [11C]-raclopride, to that of the non-displaceable ligand in the tissue (Imnis et al., 2007). A 10 minute transmission scan was performed prior to each emission scan to measure tissue attenuation in two dimensional mode. Dynamic emission scans were then acquired in three dimensional mode using a standard acquisition protocol (28 time frames over 85 min).

Subjective measurements

Volunteers completed a Psychotomimetic States Inventory (PSI) at baseline and at the end of each scan. The PSI is a validated 28 item self-report questionnaire which assesses psychotic psychopathology produced by drugs of abuse in six subscales; delusory thinking, perceptual distortions, cognitive disorganisation, anhedonia, mania and paranoia (Mason et al., 2008). After completing both scans, volunteers were asked to assign the order in which they received THC and were asked about their subjective experiences during the THC scan day.

Plasma THC measurement

Plasma blood samples were taken pre-dose for baseline THC levels and at 100 min post dose for peak plasma THC levels. Plasma THC samples were analyzed using a method developed and validated by ABS Labs Ltd (London, UK). Briefly, samples were analyzed by extraction into hexane/ethyl acetate, derivatised to form the trimethyl silyl ether derivatives, separated using capillary gas chromatography and quantified by mass spectrometry. The limit of detection using this assay method is 0.1 ng/ml.

Image analysis

All scans were first corrected for head movement using frame by frame (FBF) realignment as previously described (Montgomery et al., 2006). Briefly, non-attenuation corrected PET images (which contain a significant scalp signal) were denoised using a 2/64 Battle LeMarie wavelet (Turkheimer et al., 1999). Frames from the denoised image were realigned to a single 5 min frame acquired 38 min post injection and the transformation parameters were then applied to the corresponding attenuation corrected dynamic image frame. This procedure was applied to all frames to generate a FBF corrected dynamic image, which was then analysed using both a region of interest (ROI) and a statistical parametric mapping (SPM) approach.

ROI analysis

Striatal and cerebellar ROIs were defined using an atlas comprised of the three functional subdivisions of the striatum; limbic, associative and sensorimotor striatum along with the cerebellum. These striatal subdivisions are anatomically analogous to the ventral striatum (limbic striatum), precommissural dorsal putamen, precommissural dorsal caudate and postcommissural dorsal caudate (associative striatum) and postcommissural putamen (sensorimotor striatum) (Martinez et al., 2003). An [11C]-raclopride template was spatially transformed to the individual PET space of each FBF corrected dynamic image within SPM2 (www.fil.ion.ucl.ac.uk/spm) and the resulting deformation matrix was then applied to the atlas. A weighted steady state average add image for the 38–85 min time period (frames 15–28) was then generated from each FBF corrected dynamic image using in house software written in Matlab (version 5; The MathWorks, Inc, Natick, Mass). The deformed striatal atlas was used to sample counts from the add image using Analyze 8.0 software (www.analyzedirect.com). BPND values were calculated for each striatal region as the ratio of striatal counts to cerebellar counts, minus 1, over the steady state time period.

SPM analysis

Each steady state FBF corrected add image was initially spatially normalised to an [11C]-raclopride template image. Parametric BPND maps were then generated, using image algebra within SPM5 by dividing counts for each voxel with cerebellar counts and subtracting one. The resulting images were then smoothed using a 10 mm (full-width-half-maximum) Gaussian kernel.

Statistical analysis

Differences across conditions were assessed using a repeated measures analysis of variance (ANOVA). Factors examined were drug (THC/placebo), striatal area and side. Covariates of interest included age, sex, previous cannabis exposure and scan order. In order to explore the effect of THC on individual subdivisions of the striatum a two tailed paired t-test was also performed to compare right and left limbic, associative and sensorimotor BPNDs before and after THC treatment. Correlations between continuous data were assessed using Pearson's product moment correlation coefficient and discontinuous data with Spearman's rho. For the SPM analysis, differences in [11C]-raclopride binding between the placebo and THC scans were compared using a paired t-test within SPM5, restricted to the striatum. A height threshold of p < 0.001 and corrected cluster level threshold of p < 0.05 were used for statistical significance. All statistical comparisons, with the exception of the SPM analysis, were performed using SPSS 15.0 (SPSS, Chicago, Illinois) and all values are expressed as mean ± standard deviation. All coordinates reported within this paper are MNI coordinates.
Results

Plasma THC levels

The mean baseline plasma THC level was <0.1 ng/ml and mean 100 minute post-dose plasma THC level was 2.54 ng/ml (±1.98). There was a significant difference between baseline and peak plasma THC levels \((F_{1,12} = 20.9, p < 0.001)\). One volunteer (volunteer 10) had a post dose THC level of <0.1 ng/ml. This volunteer was witnessed taking the THC capsule, showed a 12 point change in total PSI score, described feeling 'stoned' and was able to describe the correct scan order at the end of the study suggesting that their plasma THC level had degraded in storage. Exclusion of this volunteer did not alter the pattern of findings and thus their images were included in the ROI and SPM analyses, but excluded from analyses relating to plasma THC levels.

Subjective effects

Twelve out of 13 volunteers (92%) correctly assigned the order of administration of THC and commented they felt ‘stoned’ during the THC scan during debriefing. THC produced a highly significant increase in total PSI scores \((F_{1,12} = 18.9, p < 0.001)\) (Fig. 1).

The impact of THC on total and subscale PSI scores is shown in Table 1. THC produced a significant effect on the perceptual distortion \((F_{1,12} = 22.5, p < 0.001)\), cognitive disorganisation \((F_{1,12} = 21.6, p < 0.001)\) and mania \((F_{1,12} = 7.6, p < 0.02)\) subscales with a trend effect for the paranoia \((F_{1,12} = 4.3, p < 0.06)\) and anhedonia subscales \((F_{1,12} = 4.0, p < 0.07)\). There was no significant effect of THC on the delusory thinking subscale \((F_{1,12} = 0.3, p = 0.6)\). There was no significant correlation between post-dose plasma THC levels and change in either total PSI scores \((r\text{ and } p\text{ values})\) or any of the subscales.

ROI Imaging analysis

Post-hoc \(t\)-tests were performed to explore the effect of THC on [11C]-raclopride binding in each subdivision of the striatum (Table 2).

Overall there was no significant main effect of drug (THC/placebo) on [11C]-raclopride striatal BPND \((F_{1,12} = 0.4, p = 0.53)\). There was no interaction between drug and striatal subdivision \((F_{2,11} = 2.4, p = 0.13)\), drug and side \((F_{1,12} = 0.06, p = 0.8)\) or drug side and subdivision \((F_{2,11} = 0.08, p = 0.93)\) on [11C]-raclopride striatal BPND. Unrelated to drug effects, there was a main effect of both striatal side \((F_{1,12} = 27.4, p < 0.001)\) and area \((F_{2,11} = 64, p < 0.001)\) on [11C]-raclopride striatal BPND.

There was no significant interaction between drug effect on [11C]-raclopride binding and volunteer age \((F_{1,11} = 0.39, p = 0.54)\), volunteer sex \((F_{1,11} = 0.54, p = 0.48)\), scan order \((F_{1,11} = 0.18, p = 0.68)\) or extent of previous cannabis exposure \((F_{1,11} = 0.17, p = 0.68)\).

There was a significant inverse correlation between plasma THC level and percentage change in [11C]-raclopride binding in the whole striatum \((r = -0.78, p = 0.002)\) (Fig. 2). On a regional basis, there was a significant inverse correlation between post-dose THC level and percentage change in binding in the right associative striatum \((r = -0.63, p < 0.02)\) and a trend towards an inverse correlation in the left associative striatum \((r = -0.52, p < 0.07)\).

There was no significant correlation between percentage change in binding and change in total PSI score or any of the subscales.

SPM imaging analysis

At an uncorrected significance level \((p < 0.001)\) one cluster of voxels in the right caudate showed decreased [11C]-raclopride binding following THC exposure (peak coordinates: \(x = 12, y = 9, z = -16\), and cluster size = 10) however this did not survive multiple comparison correction for the volume of the striatum.

[11C]-raclopride equilibrium stability and radiochemistry

The degree of equilibrium stability over the equilibrium frames (frames 15–28) was 0.06% change per minute. Mean injected radioactive dose injected for placebo scans were 417 MBq \((± 42)\) and for THC scans 423 MBq \((± 20)\). The mean amount of cold raclopride ligand injected was 2.43 mcg \((± 1.06)\) for placebo scans and 2.86 mcg \((± 0.74)\) for THC scans. There were no significant differences between placebo and THC scans for either injected dose \((F_{1,12} = 0.14, p = 0.7)\) or the amount of cold ligand injected \((F_{1,12} = 1.6, p = 0.22)\).

Discussion

In this study we have shown that an oral dose of 10 mg THC produces significant behavioural effects in volunteers. All of the PSI subscales, except delusory thinking, were significantly increased after THC. The greatest changes occurred in the cognitive disorganisation and perceptual distortion subscales reflecting volunteer reports that their thoughts were often speeded up and were more creative, that lights

![Fig. 1.](image.png) The effects of THC on total PSI scores in 13 volunteers.

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<td><strong>Effects of THC on [11C]-raclopride BP</strong></td>
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![Image](image.png)
applied brighter and colours more vivid after THC. The smallest changes occurred in the paranoia and delusory thinking subscales which could either reflect the insensitivity of the subscale or perhaps suggest that whilst volunteers experienced changes in their perceptions and thought flow they did not experience significant paranoid or other delusional psychopathology at this dose of THC. While subjects reported being ‘stoned’ formal visual analogue scale assessments were not used limiting our description of behavioural effects to the PSI questions.

Despite clear behavioural changes we could not detect significant changes in [11C]-raclopride binding produced by an oral 10 mg dose of THC. Region of interest analysis results were non-significant with changes in all areas of the striatum falling within the test–retest variability of 4.7% for [11C]-raclopride bolus–infusion PET imaging at our unit (unpublished data). Although the SPM analysis did show a cluster of 10 voxels in the right caudate at p < 0.001 uncorrected, this did not survive multiple comparison correction across the striatal volume. Thus from these two independent analyses we conclude that at an oral dose of 10 mg, THC does not have a significant effect on dopamine release in the human striatum.

There are also two other possible explanations for the non-significant effect of THC on dopamine release described: the power of the study to detect change in binding and the mode of administration of THC. With 13 volunteers scanned and a power of 0.8 our study was powered to detect a 4% change in binding which is adequate given other studies showing a 16% decrease in binding with oral methylphenidate (Volkow et al., 2002) and an 11% decrease with intravenous ketamine (Breier et al., 1998). In our study THC was administered orally in contrast to the inhalational method which most cannabis users use to consume THC. Although smoking cannabis will produce a more acute pharmacodynamic effect, a previous study (Curran et al., 2002) described oral administration as producing a similar profile of behavioural effects to smoking and indeed that these effects were more pronounced at 2 hours post dose. This 2 hour behavioural and pharmacological peak was the time period specifically targeted by the [11C]-raclopride equilibrium phase of each scan and as such the design was optimised to detect changes in striatal dopamine levels had they occurred. Recently reductions of [11C]-raclopride binding following nasal administration of 8 mg THC have been described (Bossong et al., 2009) although the magnitude of change was modest (3.4%), that is within the test–retest reliability, and subject numbers (n = 7) were considerably smaller than our study. Nasal administration would provide a different THC kinetic profile with presumably faster changes in brain THC levels compared to the oral administration route we used. This may imply that the rate of increase of brain THC levels could be a contributing factor to striatal dopamine release. If true this could have implications for the route of administration chosen for medicinal preparations of cannabis.

Interestingly there was a highly significant correlation between plasma THC dose and [11C]-raclopride displacement in the whole striatum in our study. This may imply that higher average plasma THC levels than in our study are needed to produce significant striatal dopamine release. However this correlation should be treated with caution as if true it implies a biphasic effect of THC on raclopride binding (inspection of Fig. 2 suggests that lower levels of THC were associated with increased raclopride binding) over a narrow dose range. Furthermore a single measurement of plasma THC per individual, although validating drug exposure, may not be an adequate representation of total drug exposure during the PET scan. In addition, all subjects bar one had demonstrated clear behavioural effects of THC which did not correlate with measured plasma THC levels. Studies using larger doses of THC would be necessary to confirm or refute the correlation described in this study.

A recent study (Caspi et al., 2005) indicated that COMT val108/158met genotype, which has a functional impact on prefrontal dopamine catabolism, may influence whether adolescent cannabis users are at increased risk of developing a psychotic illness. The authors hypothesised that a combination of COMT val/val genotype and cannabis use may produce a state of increased mesolimbic dopaminergic transmission. Although our study was underpowered to formally evaluate a COMT effect, we were not able to demonstrate a significant interaction between COMT genotype and decreases in [11C]-raclopride binding after THC from our cohort of 13 volunteers [met/met mean change = −4.3% (SD = 2.0), val/met = 0.4 (4.6), val/val = −14 (3.2)]. Indeed the three volunteers with the greatest decreases in binding were not of a particular genotype group. Moreover the main mechanism for dopamine catabolism in the striatum is the dopamine transporter (Gogos et al., 1998). Thus further studies specifically focussing on the interaction between genetic polymorphisms, such as COMT and DAT1, and cannabis on striatal dopamine release in a larger cohort of volunteers seem warranted.

Given that 8 mg is approximately the amount of THC found in an average cannabis cigarette (Smart and Adlaf, 1986), and that nearly all volunteers displayed a behavioural response from 10 mg administered in this study, the non-significant result from both imaging analyses indicates that cannabis users may not release detectable amounts of dopamine when consuming a single cannabis joint. In addition, although this dose of THC produced clear behavioural effects, as measured by PSI changes, we can conclude from both categorical and correlation analyses that these effects are not mediated by striatal dopamine release. Indeed despite showing a small but significant decrease in [11C]-raclopride binding after inhaled THC Bossong et al. (2009) were also unable to explain subjective effects of THC, although they may have had limited power for this.

In summary we have shown, in the largest study of its kind so far, that an equivalent dose of THC found in a standard cannabis cigarette does not produce consistent and significant dopamine release in the human striatum. We have also shown that the behavioural states induced by THC are not a consequence of striatal dopamine release. This result challenges current models of striatal dopamine release as the causal mechanism underlying cannabis as an environmental risk factor for schizophrenia and instead implies that non-dopaminergic mechanisms, possibly through inhibition of GABA release, may be a more important causal factor (for review see Wilson and Nicoll, 2002).

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