Pharmacokinetic and behavioural profile of THC, CBD, and THC+CBD combination after pulmonary, oral, and subcutaneous administration in rats and confirmation of conversion *in vivo* of CBD to THC

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**Abstract**
Metabolic and behavioural effects of, and interactions between \(\Delta^9\)-tetrahydrocannabinol (THC) and cannabidiol (CBD) are influenced by dose and administration route. Therefore we investigated, in Wistar rats, effects of pulmonary, oral and subcutaneous (sc.) THC, CBD and THC+CBD. Concentrations of THC, its metabolites 11-OH-THC and THC-COOH, and CBD in serum and brain were determined over 24 h, locomotor activity (open field) and sensorimotor gating (prepulse inhibition, PPI) were also evaluated. In line with recent knowledge we expected metabolic and behavioural interactions between THC and CBD. While cannabinoïd serum and brain levels rapidly peaked and diminished after pulmonary administration, sc. and oral administration produced long-lasting levels of cannabinoïds with oral reaching the highest...
1. Introduction

Given the on-going research and use of cannabis and cannabis-derived products for medical purposes, it is particularly crucial to understand the nature of cannabinoid interactions within the organism. Even though more than 100 phytocannabinoids have been identified in cannabis plants (Bhattacharyya et al., 2010; Englund et al., 2016; Hanus et al., 2016; Mechoulam et al., 2014), the two major phytocannabinoids are Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), both of which have been shown to have distinct therapeutic and adverse effects (Alexander, 2016; Schubart et al., 2014; Whiting et al., 2015; Zhornitsky and Potvin, 2012). While THC is the primary psychoactive constituent of cannabis, CBD is primarily non-psychoactive and has been shown to attenuate the behavioural and metabolic effects of THC (Bhattacharyya et al., 2010; Englund et al., 2013). In relation to this, recent research on medical cannabis focuses on various THC: CBD ratios in order to target particular therapeutic requirements. Owing to the fact that there is great complexity in the nature of THC - CBD interactions (McPartland et al., 2015; Reid and Bornheim, 2001; Todd et al., 2016; Zuardi et al., 2012a), the pharmacokinetics and behavioural effects of THC and CBD, and especially their combination, across various forms of administration, are not yet fully described. Furthermore, the translation between pre-clinical and human data has frequently been problematic because the routes of administration typically used by humans for cannabinoids, i.e., pulmonary (e.g., smoking, vaporisation); dermal (e.g., application of ointment or lotion) and oral (e.g., consumption in food) are not often employed in animal studies.

In humans, pulmonary administration of cannabis produces the greatest bioavailability of THC, with serum concentrations peaking within minutes, and subjective effects apparent almost immediately (Huestis et al., 1992), similar to intravenous (IV) administration of THC (Bhattacharyya et al., 2010; Englund et al., 2016). By contrast, after oral administration of cannabinoids/cannabis preparations, the onset of subjective effects is typically delayed by 30 to 130 min and peak serum THC concentrations are lower and delayed for about 1 - 6 hours after ingestion, sometimes showing two peaks due to enterohepatic circulation. Due to first-pass liver metabolism, higher levels of the psychoactive metabolite 11-hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC) are also typical (for review see Huestis, 2007). Compared to pulmonary, IV and oral administration of cannabinoids, very little is known about the kinetics when administered via skin compartment (transdermal and subcutaneous administration; Paudel et al., 2010; Stinchcomb et al., 2004).

While THC binds to a number of non-cannabinoid receptors in the brain, the primary mechanism of action of THC responsible for its psychoactive effects is mediated via partial agonism at cannabinoid CB1 receptors (Campos et al., 2012; Mechoulam et al., 2014). It is also the major mechanism, via which THC mediates its pro-psychotic adverse effects (Bhattacharyya et al., 2010; Englund et al., 2013). In rodents, CB1 receptor agonists, like THC, induce what has been termed the “cannabinoid tetrad” which is characterised by anti-nociception, catalepsy, hypothermia and suppression of motor activity (El-Alfy et al., 2010; Katsidoni et al., 2013). The psychotomimetic-like effects of THC and other CB1 receptor agonists in preclinical experiments focusing on sensorimotor gating produced inconsistent findings, with some studies showing no effects, some a disruption of PPI and others a facilitation (Gomes et al., 2014; Gururajan et al., 2011; Levin et al., 2014; Long et al., 2010a; Long et al., 2010b, 2013; Malone and Taylor, 2006; Nagai et al., 2006; Peres et al., 2016).

Unlike THC, CBD lacks significant psychotomimetic effects; rather, it seems to counteract the psychotomimetic and behavioural effects of THC, as well as showing anxiolytic and antipsychotic properties in and of itself (Bhattacharyya et al., 2010; Englund et al., 2013; Long et al., 2010b; Morgan et al., 2010; Pertwee, 2008; Schubart et al., 2011; Varvel et al., 2006). In animal studies, again in contrast to THC, CBD does not elicit the classic CB1-mediated cannabinoid tetrad (in mice) and it produces minimal disruption of behavioural tasks in humans, monkeys and rodents (Lichtman et al., 1995; Winsauer et al., 1999). This is likely because CBD has a low affinity for, and only weakly antagonises CB1 and CB2 receptors (Pertwee, 2008; Thomas et al., 1998; Zuardi et al., 2012a, 2012b); more specifically at CB1 receptors it acts as a negative allosteric modulator (Laprairie et al., 2015). Instead, the major
effects of CBD are via negative modulation of endocannabinoid tone through the inhibition of fatty acid-binding proteins (FABPs) which transport endocannabinoids intracellularly to a metabolising enzyme fatty acid amidohydrolase (FAAH) (Elmes et al., 2015) and via its inhibitory action at anandamide transporters (Campos et al., 2013; Rakshshan et al., 2000; Watanabe et al., 1996). CBD has, however, further complex pharmacological actions involving other neurotransmitter systems and receptors (Campos and Guimarães, 2009; Campos et al., 2012; Kathmann et al., 2006; McPartland et al., 2015; Ryberg et al., 2007). At sufficiently high doses (120 mg/kg intraperitoneally in mice), CBD may also inhibit hepatic microsomal drug metabolism, which may lead to increased THC levels in blood owing to delayed hydroxylation of THC to 11-OH-THC. In consequence, at high doses, CBD can potentiate (rather than ameliorate) the aforementioned effects of THC (Bornheim et al., 1995).

The main aim of our study was to evaluate pharmacokinetic and behavioural effects of THC and CBD alone, and in combination, in rats across three routes of administration: sc., pulmonary and oral. Specifically, we aimed to compare 24 hour pharmacokinetic profiles of the two natural cannabinoids and their co-administration at a 1: 1 ratio in rat sera and brains and effects on locomotor behaviour in the open field and sensorimotor gating in the test of prepulse inhibition of acoustic startle reaction (PPI ASR). The profiles of the psychoactive metabolite 11-OH-THC, and non-psychoactive metabolite 11-nor-9-carboxy-THC (THC-COOH) were also evaluated. From the behavioural perspective we hypothesised that THC would have inhibitory effects on locomotion, that it would disrupt sensorimotor gating, and that CBD would counteract these effects. We also expected that the THC induced changes might be more pronounced after oral administration because of the expected presence of the potent psychoactive metabolite 11-OH-THC (Huestis, 2007).

Finally, since THC and CBD are chemically related compounds, it has been reported that under certain (acidic) conditions, CBD can be cyclised to THC in vitro: a partial cyclisation of CBD to THC was reported when CBD was machine smoked with tobacco, most likely due to acidic conditions produced by the burned tobacco and by the acidity of simulated gastric fluids (Merrick et al., 2016; Quarles et al., 1973; Watanabe et al., 2007). More recently, the important question has been raised as to whether CBD can also be converted to THC in vivo (Merrick et al., 2016). Therefore our last aim was to ascertain whether treatment with CBD (orally, or by any other route) can result in the presence of THC in serum, and if so, whether this is accompanied by THC-mediated behavioural effects.

2. Experimental procedures

2.1. Subjects

For all of the experiments, male Wistar rats (Velaz, Czech Republic) weighing 200-250 g were used. Animals were housed in standard laboratory cages in the animal facility with controlled temperature (22 ± 2 °C), humidity (30-70%), light/dark cycle (6 a.m. lights on / 6 p.m. lights off) and ad libitum access to water and standard diet. Before testing, the animals were acclimatised to the animal facility for 7-10 days, and all experiments were performed between 8:00 h and 13:00 h, during the light phase under standard temperature and humidity conditions already described. Unless otherwise stated, experimental groups (drug x route) for the behavioural experiments consisted of 10 animals and each subject was tested only once. Brains and sera of these rats were used also for kinetic analyses, with 6 animals per experimental group (see Section 2.4).

All of the experiments adhered to the Guidelines of the European Union (86/609/EU) and the directives of the Czech National Committee for the Care and Use of Laboratory Animals. Ethical approval for the studies was obtained from the Czech Ministry of Health.

2.2. Drug preparation and administration

THC and CBD were obtained from THC-Pharm GmbH in a powder form. For the pulmonary administrations, 20 mg of THC, CBD, or their combination (THC: CBD at 1: 1 dose ratio) were dissolved in 98% ethanol in a volume of 200 µl and dropped on the metal-wired liquid pad purchased with the vapouriser and dried for 1 min. New liquid pads were used for each administration. Cannabinoids were then delivered via an in-house set-up consisting of a Volcano® vapouriser and a hermetically closed plastic inhalation box (volume 9.5 L). Vapourisation was held at 226 °C for 45 sec; 4 animals were kept together in the box and inhaled the vapourised air for 5 min (including 45 sec of vaporization). Intact animals placed for the same period of time in the box, without vaporizing, served as controls. For sc., and oral administration, THC, CBD and THC+CBD were dissolved in pharmaceutical grade sunflower oil (oleum helanti) and administered at a dose of 10 mg/kg of each drug or a mixture of 10 mg/kg THC +10 mg/kg CBD in a volume of 0.5 ml/kg, which was then delivered by sc. injection, or directly to the stomach by oral gavage. Control animals were administered corresponding amounts of sunflower oil as vehicle. For oral administration, in order to control for effects of stomach contents on absorption, the rats were denied food for 12 h prior to drug administration. The doses used in the current study were selected according to animal and clinical studies in which: 1) THC shows behavioural locomotor effects and induces psychotic-like symptoms in animals (El-Alfy et al., 2010; Katsidoni et al., 2013; Nagai et al., 2006; Wiley and Burston, 2014); 2) doses of CBD that were effective in humans to treat schizophrenia, have shown some antagonising effects on THC and have also shown some antipsychotic-like properties in rodents (Bhattacharyya et al., 2010; Englund et al., 2013; Gomes et al., 2014; Leweke et al., 2012; Pedrazzi et al., 2015).

2.3. Blood and tissue collection

In order to minimise the number of animals used, the animals from behavioural experiments were subsequently used for the kinetic study. The rats were humanely killed at 0.5, 1, 2, 4, 8 and 24 h (in batches of six) after oral and sc. administration. After pulmonary administration, samples at two additional timings were also collected at 0 and 15 min after removal from the inhalation box. All experimental groups for kinetics comprised six animals per time point for each separate drug x route treatment. Additional animals had to be used for time points shorter than 1 hour, some of which came from the control group. To allow an interval between behavioural testing and kinetics data collection, these animals remained undisturbed in a home cage for at least two more days and subsequently they were exposed to inhalation of cannabinoids. Separated sera and brains were collected and kept at −20 °C until the toxicological analysis.
2.4. Quantification of THC, 11-OH-THC, THC-COOH and CBD

Cannabinoids were determined by an in-house validated and certified GC-MS method (certified by Police Presidium of the CR, ref. no.: PPR-31123-7/CZ-2015-990530 / evidence no.: 16/2015). A total of 10 μl of deuterated CBD-d3/THC-d3/ 11-OH-THC-d3 (5 ng/μl) internal standard solution was added to each 1.0 ml sample of serum or brain methanol homogenate (5 ml). For brain analysis, 1 g of brain tissue was homogenised in 5 ml of methanol. Homogenised brain samples were frozen at −20 °C in an ethanol bath for 10 min and then centrifuged at 4 200 rpm for 2 min. Supernatant (4 ml) was placed in a glass tube and evaporated to 200–300 μl. Serum and brain extracts were diluted with a 4 ml sodium acetate buffer with a pH of 4.0 (0.01 mol/l). Serum and brain cannabinoids were extracted with SPE columns (Bond-ELUT, 130 mg, Agilent Technologies) and eluted with hexan/ethyl acetate (1:4 v/v) and dried under a nitrogen gas stream in a 200 μl glass insert placed in a 1.5 glass vial. The samples were derivatised with 100 μl of N-formyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) for 20 min at 80 °C. Quantification of extracted cannabinoids was performed by gas chromatography-mass spectrometry (GC-MS) (GC7860/5742C MSD, Agilent Technologies) using electron impact ionization in the selective ion mode (CBD: m/z 391; CBD-d3: m/z 394; THC: m/z 386; THC-d3: m/z 389; 11-OH-THC: m/z 371; 11-OH-THC-d3: m/z 374; THC-COOH: m/z 371; THC-COOH-d3: m/z 374). Calibration curve ranges were prepared by spiking drug-free bovine serum or drug-free rat brain homogenate for serum and brain analysis, respectively, at concentrations of (i) 2-100 ng/ml CBD, THC, 11-OH-THC and THC-COOH; (ii) 100-1 000 ng/ml CBD, THC, 11-OH-THC and THC-COOH. Limits of detection (LOD) and quantification (LOQ) were 1 ng/ml and 2 ng/ml, respectively. The spiked samples were vortexed and treated identically to the experimental samples.

2.5. Behavioural testing

Behavioural testing was performed 5 min after inhalation, 60 min after sc. administration and 120 min after oral administration of the cannabinoids and vehicle according to the estimated maximal peak of cannabinoid serum levels (Huestis et al., 1992), the selection of which was subsequently confirmed by our kinetic findings. The general behavioural testing procedures were identical to our previous studies (Horsley et al., 2016; Palenicek et al., 2013; Palenicek et al., 2016), briefly:

Open field test: a square black plastic open field arena (68 × 68 × 30 cm) was placed in a soundproofed and diffusely lit room. Animals were placed into the centre of the arena and the length and spatial characteristics of their trajectory were registered for 30 min and pre-processed by an automatic video tracking system for recording behavioural activity (EthoVision Color Pro v. 3.1.1, Noldus, Netherlands). Locomotor activity was analysed in 5 min time intervals. The spatial characteristics of locomotor activity were recorded in 5 × 5 grid of virtual zones with 16 located peripherally and 9 centrally. Frequency (f) of line crossings into different zones of the arena was used to calculate thigmotaxis ( = $S_{peripheral zones}/S_{all zones}$) which indicates the probability of appearance in peripheral zones. Time spent in the centre of the arena (Tcentre) was calculated as a summation of the time spent in all 9 central zones ($\sum_{centre zones}$).

Prepulse inhibition of acoustic startle response: PPI ASR was tested in a startle chamber (SR-LAB, San Diego Instruments, California, USA). Two ventilated startle chambers (SR-LAB, San Diego Instruments, California, USA) were calibrated to ensure equivalent stabilimeter sensitivity between the chambers. The rats were acclimatised to the startle chamber two days before treatment with a short paradigm consisting of 5 min of background noise (75 dB white noise) and a subsequent presentation of 6 pulse alone stimuli (125 dB/20 ms). Startle data were not recorded for acclimatisation. On the day of the test, a total of 72 trials were held with an inter-trial interval (ITI) of 4-20 s (mean ITI: 12.27 s). Rats were acclimatised to the startle chamber for 5 min, during which time a 75 dB background white noise was continuously presented. Six 125 dB/40 ms duration pulse alone trials were then delivered to establish the baseline acoustic startle response (ASR) for the subsequent calculation of habituation. Subsequently, 60 trials were presented in a pseudorandom order as follows: (A) pulse alone: 40 ms 125 dB; (B) prepulse-pulse: 20 ms 83 dB or 91 dB prepulse, a variable (30, 60 or 120 ms) inter-stimulus interval (ISI: mean 70 ms), then 40 ms 125 dB pulse; (C) 60 ms no stimulus. Finally, six pulse alone (40 ms 125 dB) trials were delivered. Habituation was calculated by the percentage reduction in startle values from the initial six baseline trials to the final six trials. PPI was calculated as: [100 – (mean prepulse – pulse trials / mean pulse alone trials) %]. Mean ASR was derived from the pulse alone trials. The average startle response (the area under the curve in arbitrary units, AVG) was used for the calculation of the dependent variable. Animals with an ASR AVG response lower than 10 were excluded from further analysis as non-responders.

2.6. Statistical analysis of behavioural data

Analyses of behavioural data used IBM SPSS version 22 or MS Office Excel. In all cases, the criterion for rejection of the null hypothesis for p < 0.05, and all tests were two tailed. Factorial and one-way ANOVAs (Analysis of Variance) were used to analyse the data, according to the specific experimental design in use. Where time blocks was included in the ANOVA (locomotor trajectory data), pairwise comparisons of the overall behavioural curves were planned using contrast analyses according to the method described by (Abelson and Prentice, 1997), otherwise (for PPI, total locomotion, thigmotaxis and Tcentre), independent t-tests were planned to follow up significant main effects or interactions. Where Mauchly's test of sphericity (repeated measures ANOVA) or where Levene's test for unequal variances (independent t-tests) were significant, adjusted statistics are reported. For Abelson's contrasts, since a mixed design was used, the pooled error term and pooled degrees of freedom were calculated and are reported. Recalculated and adjusted degrees of freedom are rounded to whole numbers for presentational purposes.

2.6.1. Open field

An overall total locomotion was analysed by a 4 (drug) × 3 (route) independent ANOVA with drug (THC, CBD, THC+CBD or vehicle) and route (sc., pulmonary, oral) as independent factors. Locomotor trajectory data in 5 min time blocks were analysed using a 4 × 3 × 6 mixed factorial ANOVA with drug (THC, CBD, THC+CBD or vehicle) and route (sc., pulmonary, oral) independent factors, and time blocks (6 × 5 min) as a repeated measures factor, followed by Abelson's contrast analyses to compare (within each route) the characteristic patterns of locomotor effects (across time blocks) of the different cannabinoids with vehicle, and with one another. Tcentre and thigmotaxis data were each analysed using a 4 × 3 factorial ANOVA with drug (THC, CBD, THC+CBD or vehicle) and route (sc., pulmonary, oral) as independent factors.

2.6.2. Prepulse inhibition

ASR data were screened for non-responders (startle amplitude <10); 14 oral THC and THC+CBD rats were excluded on this basis. As a result, orally administered groups were excluded entirely from subsequent habituation and PPI analyses, and the oral route was dropped as a level of the route factor (leaving sc. and pulmonary administration groups for analysis). Habituation and PPI were therefore analysed using a 4 × 2 factorial ANOVA, with drug
(THC, CBD, THC+CBD, or vehicle) and route (sc. or pulmonary) as independent factors.

3. Results

3.1. Cannabinoid levels in blood and brain tissue

In the case of sc. administration, the concentration of THC was approximately four times higher and CBD two times lower during THC+CBD co-administration in both the serum and brain tissue compared to single cannabinoid administration. Administration by the sc. route also revealed two peaks, possibly indicative of a two-compartment model. CBD sc. resulted in a measurable THC serum concentration but only 4 h and 8 h after dosing (Figure 1).

After pulmonary administration of cannabinoids, their serum levels peaked just after removal from the inhalation box while the brain levels peaked at 15 min after dosing, and then gradually decreased. The pharmacokinetic profile after co-administration of THC+CBD was not different to the profiles of CBD or THC alone. The maximum brain cannabinoid concentrations were approximately three times lower in comparison with serum concentrations (Figure 2).

Following oral administration each of the cannabinoids or their combination, both serum and brain levels peaked at 2 h post administration and with THC alone the brain concentrations remained high for another 2 h. Serum and brain concentrations of CBD were approximately two to threefold lower during THC+CBD co-administration than in the case of administration of CBD alone. On the contrary, concentrations of THC were approximately two times higher during THC+CBD co-administration in comparison with THC alone. Importantly, oral administration of CBD again resulted in measurable THC serum concentrations along with CBD concentrations, but here were no detectable levels in the brain (Figure 3). To find out whether this was related to the fact that the levels in the brain were below the limit of detection (LOD) of the method, we administered an additional two animals with 60 mg/kg of oral CBD, and the sera and brains were analysed 2 h after administration. The concentrations of cannabinoïds detected were as follows: (A) for rat 1 serum CBD was 990.9 ng/ml and THC 19.1 ng/ml, brain levels were 1075.9 ng/g for CBD and 33.3 ng/g for THC, (B) for rat 2 serum CBD was 723.1 ng/ml and THC below the limit of detection, brain levels were 871.9 ng/g for CBD and 6.8 ng/g for THC. The oral administration also resulted in a significant accumulation of cannabinoïds in brain tissue, which in the case of THC was more than double the levels in the serum.

The pharmacokinetic profile of 11-OH-THC after each route of administration reached the highest concentrations after oral administration; it accumulated in the brain at concentrations of approximately 200 ng/g, which is comparable to THC brain levels after vaporisation (Figs. 1-3). It was not detected at measurable levels after administration of CBD alone.

The non-psychoactive metabolite THC-COOH had a delayed peak between 4-8 h after administration, remained detectable 24 h later and had very low levels compared to 11-OH-THC; after pulmonary and sc. administration it was not detected in the brain tissue (Table 1).

3.2. Behavioural tests

3.2.1. Open field

Total locomotion summed across the time blocks (Figure 4) showed a significant main effect of drug, route and a significant interaction, minimum \( F(2, 18) = 13.49, p = 0.001 \). Independent t-tests (within route) showed after sc. treatment, there was no significant difference between vehicle and each of the cannabinoids, but after pulmonary treatment, there were significant reduction of locomotion after each of the cannabinoids compared to vehicle, minimum \( t(18) = 2.96, p = 0.01 \). Similarly after oral treatment THC and THC+CBD significantly reduced total locomotion, but on the contrary

Figure 1  Pharmacokinetic profiles of CBD, THC and CBD+THC in serum and brain tissue after subcutaneous administration 10 mg/kg (six rats per time point). n.d. = not detected.
Figure 2  Pharmacokinetic profiles of CBD, THC and CBD+THC in serum and brain tissue after pulmonary administration of 20 mg vaporised per 4 rats, inhalation for 5 min (six rats per time point).

Figure 3  Pharmacokinetic profiles of CBD, THC and CBD+THC in serum and brain tissue after oral administration 10 mg/kg (six rats per time point). n.d. = not detected.
CBD increased it, minimum $t$ (18) = 3.92, $p = 0.001$. Comparisons of routes of administration within each drug treatment showed that THC and THC+CBD orally had significantly lower locomotion compared to sc. and pulmonary administration of these compounds, by contrast CBD increased locomotion, minimum $t$ (18) = 4.36, $p = 0.0004$.

The analysis of locomotor data in 5 min time blocks (Figure 5) showed significant main effects for blocks (shown as a typical progressive decline in locomotor activity over 30 min), for drug and for route, minimum $F$ (2, 108) = 13.49, $p = 0.001$. All possible two-way interactions were significant (drug $\times$ blocks, route $\times$ blocks, drug $\times$ route), minimum $F$ (10, 371) = 2.66, $p = 0.003$. The drug $\times$ route $\times$ blocks interaction was also significant: $F$ (21, 371) = 3.46, $p = 0.001$. Figure 6 shows characteristics trajectories.

Subsequent contrast analysis between cannabinoids and vehicle for the sc. route showed marginally significant differences between the behavioural curves of vehicle versus THC and CBD, $F$ (1, 256) = 3.01, $p = 0.08$, and THC (1, 256) = 3.23, $p = 0.07$, respectively; the THC+CBD combination did not differ from vehicle and no differences between the cannabinoids when delivered via the sc. route were observed.

Within the pulmonary route, THC was different to vehicle, contrast $F$ (1, 256) = 6.00, $p = 0.015$, but CBD and THC+CBD did not differ from vehicle. There were no significant differences between CBD versus THC, or CBD versus THC+CBD, although the difference between THC versus THC+CBD was marginally significant (contrast $F$ (1, 256) = 3.03, $p = 0.08$). This was manifested as slightly higher activity for THC in early blocks, and some minor fluctuations in responding from the THC+CBD group, whilst requiring comment, does not merit further discussion.

When administered orally, THC showed significantly reduced locomotor responding, which persisted over time compared to vehicle, contrast $F$ (1, 256) = 24.63, $p = 0.001$, and a similar pattern was shown after THC+CBD, $F$ (1, 256) = 22.87, $p = 0.001$. Even though the total distance travelled was longer in CBD than in vehicle, the contrasts for behavioural curves were not significantly different. Locomotor activity was also significantly reduced under THC and the THC+CBD combination compared to CBD alone, minimum contrast $F$ (1, 256) = 15.51, $p = 0.001$, and THC and THC+CBD did not differ significantly from one another.

Within drug contrasts of the curves over time showed that for CBD, there were significant differences between routes, where oral administration induced higher locomotor activity than sc. or pulmonary administration, minimum contrast $F$ (1, 256) = 4.10, $p = 0.05$. Oral THC and THC+CBD significantly reduced locomotion compared to sc. and pulmonary administration, minimum contrast $F$ (1, 256) = 10.23, $p = 0.001$, and there were no significant differences between the pulmonary and sc. routes.

For $T_{centre}$ (Figure 7), there were no significant main effects of drug or route, maximum $F$ (2, 108) = 1.02, $p = 0.37$, but there was

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**Table 1** Pharmacokinetic profiles of THC-COOH in serum and brain tissue after subcutaneous, pulmonary and oral administration of THC (20 mg vapourised per 4 rats, inhalation for 5 min and 10 mg/kg for oral and subcutaneous administration, six rats per time point). *n.d.* = *not detected.*

<table>
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<th>Route</th>
<th>Time of Sampling</th>
<th>0 min</th>
<th>15 min</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>8 h</th>
<th>24 h</th>
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<td>4.4</td>
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<td></td>
<td>brain (ng/g)</td>
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<td>-</td>
<td>n.d.</td>
<td>n.d.</td>
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<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
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<td>2.6</td>
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<tr>
<td></td>
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**Figure 4** Mean trajectory length over 30 min. after subcutaneous, pulmonary and oral administration of vehicle (VEH), THC, CBD, or THC+CBD. Error bars show $\pm$ 1 S.E.M. Asterisks indicate significant differences from corresponding vehicle, $p<0.05$. 
significantly affect this measure. Within the oral route, THC and CBD alone did not significantly affect thigmotaxis, however THC + CBD significantly reduced it, compared to vehicle, t(18) = 2.89, p = 0.010. When comparing cannabinoids with one another, there were no differences on thigmotaxis between THC or CBD versus THC + CBD, when treated sc. and via pulmonary administration. However after pulmonary administration, THC + CBD increased thigmotaxis compared to CBD alone and to THC alone, minimum t(18) = 3.42, p = 0.003. After oral treatment, THC + CBD decreased thigmotaxis compared to CBD and THC, t(18) = 2.51, p = 0.02, and there were no significant differences between THC and CBD.

3.2.2. Prepulse inhibition

ASR data were screened prior to analysis for non-responders (ASR < 10) and a number of rats were excluded on this basis, most notably from the groups that were orally administered THC (before exclusions mean ASR = 20.58, SEM = 6.69; 5 rats needed excluding) and THC + CBD (before exclusions mean ASR = 9.38 SEM = 3.13; 9 rats required excluding). Given the floor in responding (where any further decrease in startle would not be detectable: (Palenicek et al., 2013)) and the number of exclusions, the oral route was dropped from subsequent factorial analysis of habituation and PPI data. After excluding the oral group, ASR analysis showed no significant main effects or interactions, maximum F(3, 64) = 0.71, p = 0.55 (Table 2). Likewise, habituation data showed no significant main effects or interactions, maximum F(3, 63) = 0.32, p = 0.81. Therefore neither ASR nor habituation showed baseline differences that might confound interpretation of PPI data.

On PPI there was a significant main effect of drug, F(3, 64) = 4.13, p = 0.01 (Figure 8), but no significant main effect of route, or drug x route interaction. Independent t-tests by drug on the main effect (irrespective of route) showed that neither THC nor THC + CBD were different from vehicle (THC was marginally, maximum t(32) = 1.79, p = 0.08), but there was a significant difference between CBD and vehicle, t(33) = 2.30, p < 0.05. THC and CBD did not differ from one another, however, each was significantly different to THC + CBD, minimum t(34) = 2.64, p < 0.01.

Table 2 shows the descriptive statistics for the non-significant interaction, and also includes values (after exclusions) for the orally administered groups. Independent t-tests were used to compare useable oral PPI data; these showed that oral CBD did not differ to oral vehicle, however there were differences between the oral and the sc. and pulmonary routes, minimum t(18) = 2.09, p = 0.05.

4. Discussion

The main findings of the pharmacokinetic experiments were: 1) cannabinoids were best absorbed after oral administration, after which brain levels were several times higher compared to sc. and pulmonary administration; 2) pulmonary administration yielded an almost immediate peak in cannabinoid levels followed by their rapid elimination; in contrast, peaks appeared later after sc. and oral administration, and cannabinoids were still detectable 24 hours later; 3) sc. as well as oral, but not pulmonary, co-administration of THC with CBD yielded an increase in serum and brain levels of THC compared to THC alone; 4) the psychoactive metabolite 11-OH-THC accumulated in the brain tissue compared to sera.
and its abundance was highest after oral administration; and 5) the non-psychoactive metabolite THC-COOH was detected with a delayed peak but surprisingly only in very low concentrations. A novel and partly unexpected finding was the presence of THC in sera after oral and after sc. administration when only CBD alone had been administered. An additional experiment with high dose of oral CBD (60 mg/kg) confirmed the presence of THC also in the brain. The main behavioural effects of cannabinoids were: 1) moderate locomotor inhibition after pulmonary administration of cannabinoids, 2) strong sedation / catalepsy after oral administration of THC and THC + CBD; 3) mildly elevated locomotor activity after oral CBD; and 3) CBD disrupted PPI (main effect irrespective of pulmonary or sc. administration route) compared to vehicle.

4.1. Pharmacokinetics

Pulmonary administration is the most typical method of cannabis consumption in humans as either a recreational drug or experimental medicine. In accordance with our current findings, peak plasma THC concentrations in humans are usually reached within few minutes after smoking (Huestis, 2007; Manwell et al., 2014). While serum levels rapidly decreased, the brain levels, although having a maximum three times lower than sera, remained high for one hour after administration. This is consistent with the finding that peak behavioural/cognitive effects in humans appear with a delay compared to peak serum levels (Huestis, 2007; Manwell et al., 2014). CBD bioavailability was about two thirds of THC. One possible explanation may be the different thermodynamics of the two compounds with CBD having a slightly higher boiling point than THC (160–180 °C versus 157 °C, respectively; McPartland and Russo, 2001), which might, in turn, lead to a lower total amount of vaporised CBD. Pulmonary THC+CBD did not produce different pharmacokinetic profiles for the two compounds compared to when CBD or THC were administered alone. Finally, no THC levels were detected after pulmonary CBD administration, which is in line with (Quarles et al., 1973) who reported that CBD did not form Δ⁹-THC when marijuana cigarettes were smoked either by a human subject or by a smoking machine without concomitant use of tobacco.

As expected and contrary to pulmonary administration of cannabinoids, the oral administration of both compounds had much slower kinetics with a peak two hours after...
There are two very important issues of the oral administration. Firstly, co-administration of THC+CBD changed the pharmacokinetic profiles of both cannbinoids compared to administration of each cannbinoid separately. Specifically, the maximum mean peak serum concentration of CBD was three times lower during THC+CBD co-administration than after CBD alone, while the maximum mean peak concentration of THC was two times higher compared to THC alone. Similar pharmacokinetic profiles were established in brain tissue, whereby the maximum peak concentration of cannbinoids was approximately two times higher. The effect on THC is most likely related to CBD-induced inhibition of its liver metabolism, since CBD acts as a potent inhibitor of cytochrome P450 enzymes CYP1A2, CYP2B6, CYP2C9, CYP2D6 and CYP3A4 (Zhornitsky and Potvin, 2012). Even though the abundance of the main psychoactive metabolite 11-OH-THC was several times higher compared to pulmonary administration, almost no difference in its concentration was observed between THC alone and the THC+CBD groups indicating that this particular metabolic pathway was not affected. The presence of high levels of this potent metabolite most likely accounted for/added to the robust behavioural effects observed after oral administration. In contrast to THC, the lower CBD levels compared to CBD alone treatment are difficult to explain. Since CBD is metabolised by various enzymes at cytochrome P450 (Jiang et al., 2011), one of the possible explanations could be an induction of CBD metabolism by THC. However we did not find any literature to directly support this. Interestingly, a very similar finding has been shown in humans; when same doses of THC and CBD were co-administered via different routes of administration, CBD had lower plasma levels in comparison to administration of each cannabinoid separately. A very interesting finding is that despite the reported very low bioavailability of cannbinoids after oral administration (6-20% for THC and 6-19% for CBD (Huestis, 2007; Klumpers et al., 2012; Zhornitsky and Potvin, 2012)), in our case, we saw the best bioavailability compared to both alternative methods of administration. The fact that cannbinoids were dissolved in oil and that our animals fasted before administration may have led to better bioavailability compared to a situation when pure compounds, e.g., a solution encapsulated or dissolved in water, are administered. The very high brain levels were also reflected in the behavioural tests, as discussed below.

### Table 2

<table>
<thead>
<tr>
<th>Measure</th>
<th>Route</th>
<th>THC</th>
<th>CBD</th>
<th>THC+CBD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASR (arbitrary units)</td>
<td>Subcutaneous</td>
<td>162.57 (36.22)</td>
<td>189.73 (43.51)</td>
<td>213.06 (47.70)</td>
</tr>
<tr>
<td></td>
<td>Pulmonary</td>
<td>112.28 (25.79)</td>
<td>213.06 (47.70)</td>
<td>155.83 (31.25)</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>185.00 (39.75)</td>
<td>35.45 (9.47)</td>
<td>126.33 (22.23)</td>
</tr>
<tr>
<td>Percentage Habitation</td>
<td>Subcutaneous</td>
<td>59.93 (7.28)</td>
<td>58.67 (10.13)</td>
<td>60.53 (6.28)</td>
</tr>
<tr>
<td></td>
<td>Pulmonary</td>
<td>60.68 (6.17)</td>
<td>55.96 (.39)</td>
<td>57.68 (6.50)</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>51.71 (10.79)</td>
<td>71.11 (12.01)</td>
<td>44.31 (11.13)</td>
</tr>
<tr>
<td>Percentage PPI</td>
<td>Subcutaneous</td>
<td>51.91 (6.61)</td>
<td>39.13 (7.09)</td>
<td>30.23 (8.70)</td>
</tr>
<tr>
<td></td>
<td>Pulmonary</td>
<td>47.36 (6.25)</td>
<td>37.33 (4.54)</td>
<td>35.06 (7.60)</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>30.03 (5.39)</td>
<td>39.37 (18.07)</td>
<td>34.81 (8.12)</td>
</tr>
</tbody>
</table>

Figure 8

Mean percentage prepulse inhibition (AVG amplitude) for the significant main effect of drug treatment (vehicle (VEH), THC, CBD, or THC+CBD) collapsed across subcutaneous and pulmonary routes. Error bars show ± 1 SEM. Asterisk shows significant difference from vehicle, connecting lines indicate significant inter-cannabinoid differences, p<0.05.
tissue. This led us to add an additional study with two rats administered with a high oral dose of CBD (60 mg/kg) after which the THC was also detected in the brain two hours after treatment. Therefore it is possible that minimal levels of THC could have been present in the brain following treatment with CBD 10 mg/kg, but did not reach the limit of detection (LOD) of our analytical method. As noted in the introduction, previous experimental work indicated that when CBD is degraded in an acidic environment (artificial gastric juice), it rapidly cyclises to Δ⁹-THC (Watanabe et al., 2007). Recently, (Merrick et al., 2016) confirmed the formation of psychoactive cannabinoids when CBD is exposed to simulated gastric fluid and also clearly demonstrated acid catalysed cyclization to Δ⁹-THC. According to the first study, not only THC but also other related hexahydrocannabinols have been associated with catalepsy, hypothermia and antinociception in mice (Watanabe et al., 2007).

After an initial short-lasting and low amplitude peak, sc. administration of either THC or CBD resulted in relatively steady serum and brain concentrations. The concentrations of THC and CBD reached over the experimental interval were generally an order of magnitude lower than the respective pulmonary and oral administration of either of the cannabinoids. Nevertheless, after sc. administration of THC+CBD, the THC maximum mean peak concentration was five times higher and CBD concentration about half the value than administration of THC or CBD alone in both the serum and brain tissue. This presumably also indicates a significant inhibition of THC metabolism by CBD, as has been already discussed for oral administration. Important finding is that 11-OH-THC reached almost the same brain levels as of THC when THC was administered alone. At the same time these levels were approximately a half of THC levels when THC+CBD were co-administered indicating that there is an accumulation of this potent active metabolite in the brain. Even though we did not observe much behavioural changes, this may contribute to theoretical psychotomimetic effects of the combination. Another observation was a second peak of cannabinoids with a maximum after 8 h for THC and 11-OH-THC and 4 h for CBD, which was more prominent in the brain tissue. A theoretical explanation may be the two-compartment pharmacokinetics model, where the cannabinoids are initially released to the blood stream via certain carrier mechanisms directly after administration, while at the same time being depot bound in the skin and fat tissue from which they are subsequently released. Finally, sc. administration also yielded measurable levels of THC, indicating that cyclization may also occur in subcutaneous fat. Again, no measurable brain THC concentrations were observed.

The presence of THC in sera after oral as well as sc. administration of CBD may have important psychosocial and forensic consequences. (Merrick et al., 2016) stated that people who are treated with high CBD doses may theoretically experience some signs of THC intoxication, e.g., sedation, citing child CBD studies on epilepsy. However his paper induced vigorous opposition from other researchers against this suggestion (Grottenhermen et al., 2017; Russo, 2017). Whilst we agree that sedation is probably not linked to THC effects since other cannabinoids and terpenoids are more likely responsible, however our findings confirmed the conversion in vivo of CBD to THC (albeit in rats at doses several times higher compared to those typically used by humans). CBD has become widely used in various forms, including as a popular food supplement and a constituent of various dermatological preparations, therefore positive test results for THC cannot be excluded in subjects using these CBD-based products, with obvious legal ramifications (e.g., the capacity to drive a motor vehicle). Although the levels of THC seem to be quite low especially in brain tissue, it is very speculative if they might produce any objective signs of intoxication in adults even after very high doses of CBD. Moreover, conversion of CBD to THC in the stomach will very much depend on the formulation in which the drug is administered (Perez-Reyes et al., 1973) and we cannot exclude that the administration of CBD on an empty stomach might partially contribute to accelerated transformation of CBD to THC. A support for this can be extrapolated from human data where the pH of stomach has been reported to be the lowest in the morning after whole night fasting (Brooks, 1985). Nevertheless from a forensic perspective, in some places around the globe there may be zero tolerance to THC when driving and/or operating machines or performing activities requiring increased attention, thus CBD users may inadvertently break the law, even though the use of CBD may be legal.

Finally, very low concentrations of THC-COOH were detected in our samples (THC: THC-COOH ratio approximately 10: 1). This is surprising, since in humans this metabolite is the primary non-psychoactive metabolite formed from THC, typically displaying plasma levels several times higher than THC, especially in chronic cannabis users (Balíková et al., 2014; Karschner et al., 2011). We found only one study investigating at THC-COOH levels in rats (Sprague-Dawley strain), which was in agreement with our data and showed similar results. While THC-COOH was detected in high concentrations in the liver microsomes of an in vitro study, the in vivo experiments revealed 11-OH-THC as the major metabolite in the liver homogenates, while THC-COOH was much lower and only observed in female rats (Narimatsu et al., 1991). Similarly, the THC: THC-COOH ratio in Rhesus monkeys was also approximately 20: 1, thereby showing a similar trend as in rats (Ginsburg et al., 2014). A most recent finding has shown that in male and female Sprague Dawley rats, after intraperitoneal administration of THC and CBD, another important metabolite of THC, cannabiol (CBN) is produced; it especially occurs after co-administration of THC with CBD. Furthermore, 11-0H-THC and THC-COOH are much more likely to be detected in female compared to male rats (Britch et al., 2017). Therefore, significant interspecies and sex differences in the metabolism of THC may underlie this discrepancy between rats and other species.

4.2. Behavioural effects

The mild inhibitory effects on locomotion after pulmonary administration are in line with the reported cannabinoid tetrad (El-Alfy et al., 2010; Katsidoni et al., 2013). However the oral administration of THC either alone, or co-administered with CBD produced robust locomotor inhibition in the open field which reflected profound sedation/catalepsy.
(this was also seen in the PPI ASR study, where rats treated with oral THC or THC + CBD barely startled to unexpected auditory stimuli). Of interest is that only oral, but not pulmonary or sc. administration, yielded such robust effects which indicates that different metabolites might be responsible. In support of this, Marshall et al. (2014) found a lack of cataleptogenic effect and less pronounced inhibition of locomotion after pulmonary administration of THC and synthetic cannabinoids versus intraperitoneal administration (Marshall et al., 2014). CBD failed to ameliorate the observed sedation, even though after oral CBD alone increased locomotion compared to the control group. There was an effect of oral THC + CBD on the spatial characteristics of the trajectory; however, we assume that this is most likely confounded by the robust locomotor inhibition rather than related to attenuation of exploration or anxiety. None of the other treatments showed any significant effects either on locomotion or on its spatial characteristics. The locomotor inhibitory effect fits within the know cannabinoid tetrad and is presumably related to the high THC brain concentrations seen with the oral dose, as a dose dependency for THC induced sedation has already been described (Shi et al., 2005; Wiley and Martin, 2003). On the other hand, in some studies THC has been shown to have a dose dependent bidirectional effects i.e., hyperlocomotion in low doses and hypolocomotion or sedation at high doses (El-Alfy et al., 2010; Katsidoni et al., 2013). However, none of the THC treatments produced any sign of increased locomotion even though the brain concentrations varied widely across different routes of administration. Even though highly speculative, an exception could be the increase in locomotion after oral CBD. Low THC levels in the serum were present in this case and even though they were not detected in the brain because of the supposed higher LOD of the analytical method used, these low THC levels could theoretically be an underlying cause of the mild stimulatory effects (El-Alfy et al., 2010; Katsidoni et al., 2013). Even though the increase in locomotion after CBD was surprising, it has also been recently described in Sprague Dawley rats (Bricht et al., 2017). It is highly unlikely that increased locomotion under CBD was a result of anxiolysis, since we saw no evidence for this in our analysis of the spatial characteristics of locomotor behaviour.

After exclusion of the oral THC treated groups, we did not observe any significant baseline effects on ASR or habituation that might confound interpretation of effects of drug and route on PPI ASR. THC marginally disrupted PPI (two-tailed); it is of note that if one-tailed test would have been used (to test specific hypothesis that THC disrupts PPI), it would result in statistical significance (p = 0.04). Thus mild pro-psychotic effects of THC may have been detected. The disruptive effect of CBD alone was a surprise; usually the neurobiological basis of cannabinoid induced deficits in PPI, if present, seems to be attributed to CB1 as well as dopamine mediated mechanisms (Malone and Taylor, 2006; Nagai et al., 2006; Tournier and Ginovart, 2014), however in the case of CBD, with its wide pharmacological action, it cannot be explained so easily. As stated earlier, literature findings on PPI are inconsistent regarding the effects of CB1 agonists (Gomes et al., 2014; Gururajan et al., 2011; Levin et al., 2014; Long et al., 2010a; Long et al., 2010b, 2013; Malone and Taylor, 2006; Nagai et al., 2006; Peres et al., 2016). Most likely differences according species and strain, synthetic versus natural cannabinoids and their potency, partial versus full agonism underlie such inconsistencies (Levin et al., 2014; Malone and Taylor, 2006; Peres et al., 2016). This also seems to be the truth for CBD alone e.g., (Gururajan et al., 2011; Long et al., 2010b). Interestingly, even though CBD alone induced disruption in the PPI, when co-administered with THC, there was no difference from vehicle controls, and significant PPI improvement compared to both THC as well as CBD. This seems to be in accordance with CBD's capacity to reverse PPI deficits in various rodent models of psychosis (Gomes et al., 2014; Gururajan et al., 2011; Levin et al., 2014; Nagai et al., 2006; Pedrazzi et al., 2015; Peres et al., 2016) and might lead to the conclusion that a modest antipsychotic-like effect of CBD on THC induced deficit was observed.

In conclusion, in contrast to our predictions, CBD had only an isolated antagonising effect on THC induced sensorimotor deficits, while it left other THC behavioural effects unaffected and alone it produced sensorimotor gating disruption. The present study revealed remarkable differences between cannabinoid pharmacokinetics in relation to their route of administration and it also reveals important pharmacokinetic/metabolic interactions of co-administration of the two main constituents of cannabis, however these interactions were manifested only marginally in the behavioural tasks. Contrary to other studies, we observed good bioavailability of cannabinoids after oral administration in an oil solution, which will have applications in the development of formulas with high therapeutic efficacy. However, the detection of THC in the blood stream after oral and sc. administration of CBD alone, if confirmed in humans, may affect the development of therapeutic and medical CBD products as well as having potential legal and forensic implications.

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Contributors

Tomáš Hložek and Marie Baliková performed the analytical laboratory analyses and their subsequent evaluation as well as writing the manuscript.

Tomáš Páleníček designed the whole study and experimental protocol and participated in the behavioural and pharmacokinetic experiments and writing the manuscript.

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Kuchař performed the behavioural experiments, pharmacokinetic experiments and all contributed to writing and approving the present manuscript, Rachel Horsley contributed mainly with the statistical analysis of the data and writing of the manuscript.

Conflict of Interest

All of the authors declare that they have no conflicts of interest.

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