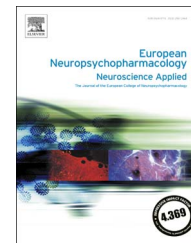




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Probing the endocannabinoid system in healthy volunteers: Cannabidiol alters fronto-striatal resting-state connectivity

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Abstract

Tetrahydrocannabinol (THC) and Cannabidiol (CBD) are two substances from cannabis sativa that have been implicated in the treatment of mental and neurological disorders. We concentrated on a previously validated neuroimaging phenotype, fronto-striatal connectivity across different striatal seeds, because of this loop's relevance to executive functioning, decision making, salience generation and motivation and its link to various neuropsychiatric conditions. Therefore, we studied the effect of THC and CBD on fronto-striatal circuitry by a seed-voxel connectivity approach using seeds from the caudate and the putamen. We conducted a cross-over pharmaco-fMRI study in 16 healthy male volunteers with placebo, 10 mg oral THC and 600 mg oral CBD. Resting state was measured in a 3 T scanner. CBD led to an increase of fronto-striatal connectivity in comparison to placebo. In contrast to our expectation that THC and CBD show opposing effects, THC versus placebo did not show any significant effects, probably due to insufficient concentration of THC during scanning. The effect of CBD on enhancing fronto-striatal connectivity is of interest because it might be a neural correlate of its anti-psychotic effect in patients.

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1. Introduction

The endocannabinoid system has been discussed in mental and neurological disorders, for example, patients suffering from schizophrenia show increased cerebrospinal levels of endocannabinoids (Leweke et al., 2007). The exogenic modulation of the endocannabinoid system has been investigated related to therapeutic and disease-mediating mechanisms for some time (Schubart et al. 2013). The consumption of tetrahydrocannabinol (THC) has been linked to psychotic symptoms in healthy volunteers (D'Souza et al., 2005). The effect of THC consumption on an epidemiological level is still a matter of debate. Several epidemiological studies on psychotic experiences and psychosis underscore the negative influence of THC on psychosis-prone individuals (Arseneault et al., 2004; Moore et al., 2007; Schubart et al., 2011; van Gastel et al., 2013). However, a twin study points to a genetic cause of cannabis consumption which is genetically correlated with psychosis risk (Nesvag et al., 2016). This genetic risk is reflected in an interaction between cannabis exposure and genetic risk for schizophrenia leading to a delayed brain maturation in adolescence (French et al., 2015).

THC is not the only potent phytocannabinoid in cannabis sativa. From several hundred active ingredients, cannabidiol (CBD) has received recent interest because of its opposite effects on psychosis in comparison to THC. Animal studies in psychosis models, pharmacologic psychosis-models in humans and treatment studies in schizophrenic patients point to an antipsychotic effect of CBD (Schubart et al., 2014; Zhornitsky and Potvin, 2012).

Cannabinoid receptor type 1 (CB1) receptors are found in the central and peripheral nervous system. There is strong expression of the CB1-receptor in the cerebellum, the limbic system (amygdala and hippocampus) and the basal ganglia (Glass and Felder, 1997; Herkenham et al., 1991; Mailleux et al., 1992). Especially in striatal neurons, dopaminergic signalling and endocannabinoid signalling are tightly linked (Glass and Felder, 1997).

Therefore, the fronto-striatal circuit makes a plausible target for assessment of systemic neural effects for THC and CBD. Recent studies identified a decrease in fronto-striatal coupling as an intermediate phenotype in schizophrenia (Dandash et al., 2014) with patients suffering from schizophrenia, their unaffected siblings as well as at-risk individuals showing a decrease in fronto-striatal coupling. These studies provide the rationale to examine the basal-ganglia as seed regions for studying connectivity.

In addition, neuroimaging of THC and CBD effects was done before by McGuire and colleagues in a neuroimaging study of 15 male subjects using a within-subject comparison. An overview of this study and subsequent analyses is given in (Colizzi and Bhattacharyya, 2017). Main finding of the authors was that THC and CBD had opposite effects in several different neuroimaging tasks capturing different aspects of emotion and cognition e.g. activation relative to placebo in the striatum during verbal recall, in the hippocampus during the response inhibition task or in the amygdala when subjects viewed fearful faces (Bhattacharyya et al., 2010). Therefore, we wanted to replicate these findings of opposite effects, and the previously reported impact on fronto-striatal

connectivity (Bhattacharyya et al., 2015) and broaden these studies' perspective by using a hypothesis driven striatal seed-voxel resting-state approach.

Therefore, the present study aimed at identifying whether a well-known psychosis-related intermediate phenotype can (I) be linked to a low dose THC induced disturbance in healthy humans and (II) whether CBD shows the opposite pattern in resting-state fMRI (functional magnetic resonance imaging) underscoring the translational validity of functional connectivity measures in the THC/CBD-challenge pharmacological model.

2. Experimental procedures

2.1. Subjects

Subjects were recruited via local advertisement; those with psychiatric or other medical disease and medication were excluded (except for stable thyroid substitution). Positive drug urine test or regular drug use were other exclusion criteria. The human fMRI study took place in a single centre and was conducted as subject- and observer-blind, placebo-controlled, randomized, three-period cross-over study in healthy male subjects. The subjects received counterbalanced, single dose administrations of either saline (placebo condition), tetrahydrocannabinol (THC) 10 mg or cannabidiol (CBD) 600 mg. Scanning of resting state took place about 75 min after oral capsule intake. Approval was given by the local ethics committee (Medical Faculty Mannheim, University of Heidelberg, Germany). The study was registered as clinical study in the German clinical study registry (<https://drks-neu.uniklinik-freiburg.de/study-ID: DRKS00005442>). Subjects underwent three consecutive fMRI sessions over the course of three weeks (max. 8 weeks apart). The Dissociative Symptoms Scale (DSS) was used to assess levels of depersonalization, derealization or gaps in awareness and memory (Carlson et al., 2018). The state anxiety inventory (STAI) was used to assess state dependent anxiety levels (Spielberger, 1973) and the positive and negative affect schedule (PANAS) was used to assess positive and negative affect, valence and arousal (Janke and Glöckner-Rist, 2014; Watson et al., 1988). Rating was done 120 min after drug intake. Nineteen participants completed the study. Two subjects were dismissed because of lack of THC in blood samples, one subject did not complete the study because of side-effects (nausea). Therefore, the analysis was done with $n = 16$.

2.2. Drug application

On each day of the experiment, subjects received a light standardized meal (sandwich ~350 kcal) before capsule intake. Scanning took time 75 min later. For avoidance of order effects, the order of substance application was randomly permuted across all participants. The participants were supervised by a certified psychiatrist. Blinding took place at the University Pharmacy at the University of Heidelberg. Unblinding took place after all participants completed the study and the data were analysed. At the end of each session the participant, physician and experimenter had to indicate which substance they believed to have been administered. Pharmacokinetics were measured by HPLC-chromatography of blood samples before and after intake.

2.3. Data acquisition

Data measurement took place using a 3 T MR system (Siemens Trio, Erlangen, Germany), a 32-channel head-coil and an echo-planar imaging (EPI) sequence for functional imaging. The EPI sequence specifications were: TR = 1960 ms, TE = 30 ms, 36 oblique slices (aligned to the AC-PC plane), 3 mm slice thickness, FA = 76°, FOV = 192 mm, 64 * 64 matrix, total measurement time 7 min 9 s. Head movement was restricted by foam cushions. We set a movement threshold of < 2 degrees and < 2 mm. None of the data sets used had volumes exceeding these limits.

2.4. Data processing and analysis

Data were processed using statistical parametric mapping (SPM12) (<http://www.fil.ion.ucl.ac.uk/spm/>) and the complementary CONN toolbox v15 (Whitfield-Gabrieli and Nieto-Castanon, n.d.). Images were realigned, slice-time corrected, spatially normalized to standard stereotactic space (Montreal Neurological Institute [MNI] template), resampled to 3 mm isotropic voxels, and smoothed with 8 mm full-width at half maximum (FWHM) Gaussian kernel. A band-pass filter reduced frequency bands to 0.01-0.1 Hz. Further noise correction was done by regressing out motion parameters (from rigid-body transformation), their 1st order derivative and correcting for cerebrospinal-fluid signal and white-matter-signal with an aCompCor-strategy (5 dimensions). This method (Behzadi et al., 2007; Muschelli et al., 2014) takes the principal components (usually 5 PCs) of white matter/cerebrospinal fluid (WM/CSF) regions as regressors as nuisance regressors and is able to avoid the global-scaling related anticorrelation issues (Chai et al., 2012). This method is not prone to acquire the anti-correlated networks of global signal regression.

For seed-voxel connectivity, we used four a priori region-of-interest (ROI) masks from the AAL-atlas (Tzourio-Mazoyer et al., 2002) to model the striatum: the caudate (left or right) and the putamen (left or right). For correction of multiple testing during second-level statistics we used cluster-wise whole-brain analysis with topological FDR correction (cluster-threshold $p < 0.001$) (Chumbley et al., 2010). In a recent paper, cluster-wise correction was critiqued as too lenient (Eklund et al., 2016). In a consecutive analysis a cluster-threshold of $p = 0.001$ was found to set a reasonable threshold for false positive (Kessler et al., 2017).

Clusterwise seed-to-voxel analysis can lead to clusters which do not align well to classic anatomic boundaries. We complemented this analysis by extracting mean values from 90 AAL ROI masks (Destrieux et al., 2010) and calculated a bivariate correlation as implemented in the CONN-toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012). Multiple comparison correction for ROI-to-ROI analyses with multiple target regions was done with a threshold of $p < .05$ false discovery rate (FDR) as described in (Whitfield-Gabrieli and Nieto-Castanon, 2012).

For second-level analysis in the CONN-toolbox we used a 2 × 2 within-subjects / repeated measures ANOVA model. Different Drugs were entered as sessions, aka drug was treated as between-conditions contrast. Subsequent contrast vectors define comparisons between THC, CBD and placebo. No further between-subjects covariates were used.

To demonstrate the main effect of caudate or putamen connectivity we calculated during the placebo condition a ROI-to-ROI analysis (thresholded with $pFDR < 0.05$). To demonstrate where the seed-to-voxel connectivity of seeds caudate or putamen show significantly stronger connectivity, we calculated a between-sources contrast for the comparison between caudate and putamen. Results were thresholded with $p < 0.001$ and corrected for multiple comparisons with cluster-wise $pFDR < 0.05$. and.

3. Results

3.1. Behavioural effects, self-reported symptoms and responses

One subject reported uneasiness after substance administration, namely vertigo which lasted for several hours. Subjects were not able to guess whether they received THC, CBD or placebo (chi-square 3.36, $df = 4$, $p = 0.49$), thus blinding was maintained. We compared state anxiety, positive and negative affect, subjective valence and arousal ratings as well as dissociative symptoms. No significant effect on these scales was found for the comparison of THC with placebo or of CBD with placebo (all $p > 0.15$, $df = 15$, $t < 1.05$). Three hours after intake of $\Delta 9$ -THC and placebo there were no significant differences for any of the state variables. Nevertheless, higher state anxiety was significant ($p = 0.03$, $df = 15$, $t = 2.03$) and a trend towards significance for higher scoring on the DSS acute items ($p = 0.07$, $df = 15$, $t = 1.56$) was found for $\Delta 9$ -THC in comparison to the placebo condition.

3.2. Pharmacokinetics

In the $\Delta 9$ -THC condition two subjects had no detectable plasma level concentrations of $\Delta 9$ -THC and were excluded from the analysis. For the remaining 16 subjects in the $\Delta 9$ -THC condition, the mean (SD) blood levels of $\Delta 9$ -THC before, 62 min and 206 min after drug administration were 0.00 pmol/ml (0.00), 1.31 pmol/ml (5.25), and 19.94 pmol/ml (13.20), respectively. For the subjects in the CBD condition, the mean (SD) blood levels of CBD before, 62 min, and 208 min after drug administration were 0.00 pmol/ml (0.00), 5.49 pmol/ml (16.62), and 283.63 pmol/ml (373.77), respectively. Increase of CBD and THC level between pre-intake and last blood sample was significant ($p < 0.001$, $df = 15$, $t > 3.8$).

3.3. Main effect connectivity fMRI

To demonstrate the validity of our connectivity analysis with caudate and putamen seed ROIs and to enable comparability with previous connectivity studies using slightly different ROI's (Di Martino et al., 2008; Pauli et al., 2016), we calculated first the significant ROI-to-ROI correlations based on the AAL atlas (Supplemental Figure 1). As several studies used different striatal ROIs and parcellations to demonstrate a specific topological pattern of striatal connectivity, we calculated a seed to voxel contrast comparing caudate versus putamen (see Supplemental Figure 1, Supplemental Table 1). Caudate and putamen did not vary significantly

between left and right hemisphere and showed clearly distinct connectivity patterns. While both regions showed strong frontal connectivity, the link connectivity between the anterior cingulate and caudate was strong. In contrast to the caudate, putamen seeds showed connectivity with insula and amygdala and to a broad frontal network.

An illustrative depiction of all ROI-wise connectivity patterns is shown in [Supplemental Figure 1](#), statistics of ROI-to-ROI correlation and seed-to-voxel correlation is provided in [Supplemental Table 1](#).

3.4. Pharmacological fMRI

We tested two within-subject contrasts for each seed ROI from the caudate and putamen (AAL atlas) with each hemisphere separately. First, THC versus placebo and second, CBD versus placebo. THC did not show a significant effect when we corrected stringently for multiple testing ($pFDR > 0.45$).

CBD versus placebo gave significant results for the right putamen seed ($pFDR < 0.03$). This seed showed an increase in connectivity to three clusters, situated mainly in the right prefrontal cortex. The CBD plasma concentration at 1 and 3 h after intake were not significantly correlated with the extracted beta connectivity from the significant cluster ($p > 0.7$, $df = 14$) ([Figure 1](#)).

The direct comparison between THC and CBD yielded a significantly ($pFDR < 0.001$) higher connectivity between the right putamen and a large cluster of the frontal pole (left > right) in the contrast CBD > THC (details for all contrasts with the seed right putamen are given in [Table 1](#)).

Connectivity from other seeds did not show a significant change in correlation strength between our basal ganglia-seeds and other whole-brain areas ($pFDR > 0.35$).

In addition, we analyzed ROI-to-ROI-connectivity from the right putamen to the brain for the CBD > placebo contrast. While strict control for multiple comparisons does not give other results than the seed-voxel approach, it gave a trend towards significance for a decrease in connectivity

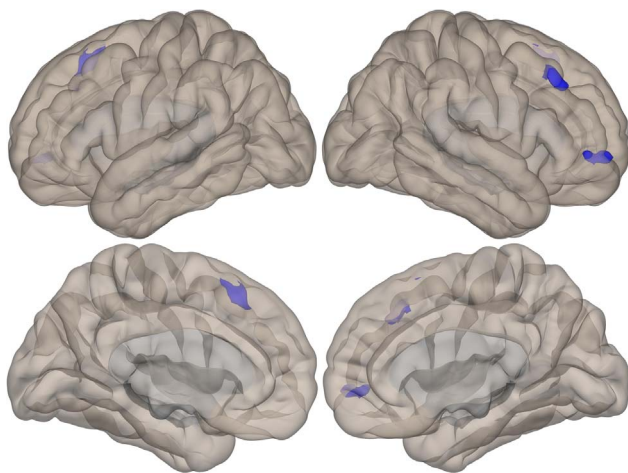


Figure 1 Connectivity between the extracted right Putamen seed mean time course. Translucent surface model of the brain shows in blue FDR-corrected clusters ($pFDR < 0.035$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to the amygdala and various parts of the visual system in the occipital lobe ([Figure 2](#)).

Our primary hypothesis of an opposite effect between THC and CBD was not confirmed. Fronto-striatal connectivity was increased in CBD, but not significantly different in THC ([Table 1](#) and [Figure 3](#)).

4. Discussion

In our resting-state fMRI analysis, we detected a significant increase in fronto-striatal coupling during intake of 600 mg CBD. This increase was seen for the right putamen as seed region, but not for other parts of our a priori ROIs from the striatum. The striatum includes anatomically the caudate nucleus and the putamen. The striatum is a major hub in orchestrating motor, limbic and cognitive function, mainly mediated by dopamine. The success of blocking striatal dopamine in schizophrenia lead to the idea of a distinct dopaminergic striatal dysfunction in schizophrenia ([Howes and Kapur, 2009](#); [Jensen and Kapur, 2008](#)). Indeed, altered fronto-striatal connectivity has been described as an intermediate phenotype in schizophrenia. Fronto-striatal connectivity was not only linked to genetic risk ([Fornito et al., 2013](#)) but lower functional connectivity in fronto-striatal circuitry was correlated with more severe positive symptoms in patients ([Fornito et al., 2013](#)). This is generally interpreted from the background of the dopamine-hypothesis in schizophrenia ([Fusar-Poli and Meyer-Lindenberg, 2013a,b](#)). Connectivity changes in the fronto-striatal circuitry might be a consequence of altered dopamine levels in patients. An increase or a decrease in dopamine is thought to increase noise in prefrontal information-processing systems ([Seamans et al., 2008](#)), which can be understood as moving away from a maximum or optimum point on an inverted U-shape-like function. Dopamine is not the only neurotransmitter which plays a role in regulating striatal motor, affective and cognitive function: Within the striatum, CB1 receptors have been shown to be localized on the same neurons as the inhibitory G-coupled dopamine D2 receptor ([Glass and Felder, 1997](#)). This might suggest a common pathway for the antipsychotic effects seen in CBD ([Leweke et al., 2012](#)) which might be mediated through the same mechanism, a regulation of fronto-striatal circuitry.

Indeed, we found such an increase in fronto-striatal connectivity both for the contrast CBD > Placebo and for the contrast CBD > THC. However, the plasma concentration after intake of THC before scanning was low. We doubt that THC reached a sufficient concentration in the brain during resting state fMRI measurement. On the other side, there was a hint of THC effect in the behavioural variables. We prefer the cautious interpretation of a clear CBD > placebo effect. The CBD > THC effect might be just a CBD > placebo effect. Nevertheless, both contrasts document a significant influence of CBD on fronto-striatal connectivity. The observed increase in the connectivity of the fronto-striatal circuit might reflect the antipsychotic effect of cannabidiol reported in previous studies ([Leweke et al., 2012](#)).

When looking in more detail into the fronto-striatal circuitry seen in our main CBD effect, we refer to behaviourally relevant fronto-subcortical connectivity networks: a dorsolateral prefrontal circuit that mediates "executive"

Table 1 Results of the right Putamen AAL seed connectivity for the contrasts CBD > Placebo, CBD > THC and THC > Placebo. Table gives significant clusters, their size and location in the MNI space. MNI-coordinates are given in the order X,Y,Z. Brodmann area refers to the area with the biggest overlap with the cluster. T-values refer to given MNI-coordinate. To enable comparability with FWE-based analysis in other studies, we report additional cluster-corrected pFWE significance in the right column. Threshold for cluster-correction was set to $p < 0.001$. Degrees of freedom $df = 15$ for comparisons.

Brain region	Overlap with brodmann area	(peak voxel MNI coordinate)	Cluster size	pFDR	pFWE
Contrast CBD > Placebo					
Right middle frontal gyrus	8/9	(+38 +34 +38)	176	0.022	0.026
Bilateral Superior frontal gyrus and paracingulate gyrus	8	(-04 +30 +58)	170	0.022	0.029
Right frontal pole	8/6	(+44 +56 -04)	129	0.039	0.076
Contrast CBD > THC					
Frontal Pole and paracingulate gyrus	10	(+08 +52 +14)	633	0.000017	0.000013
Contrast THC > Placebo					
-	-	-	-	Not significant	

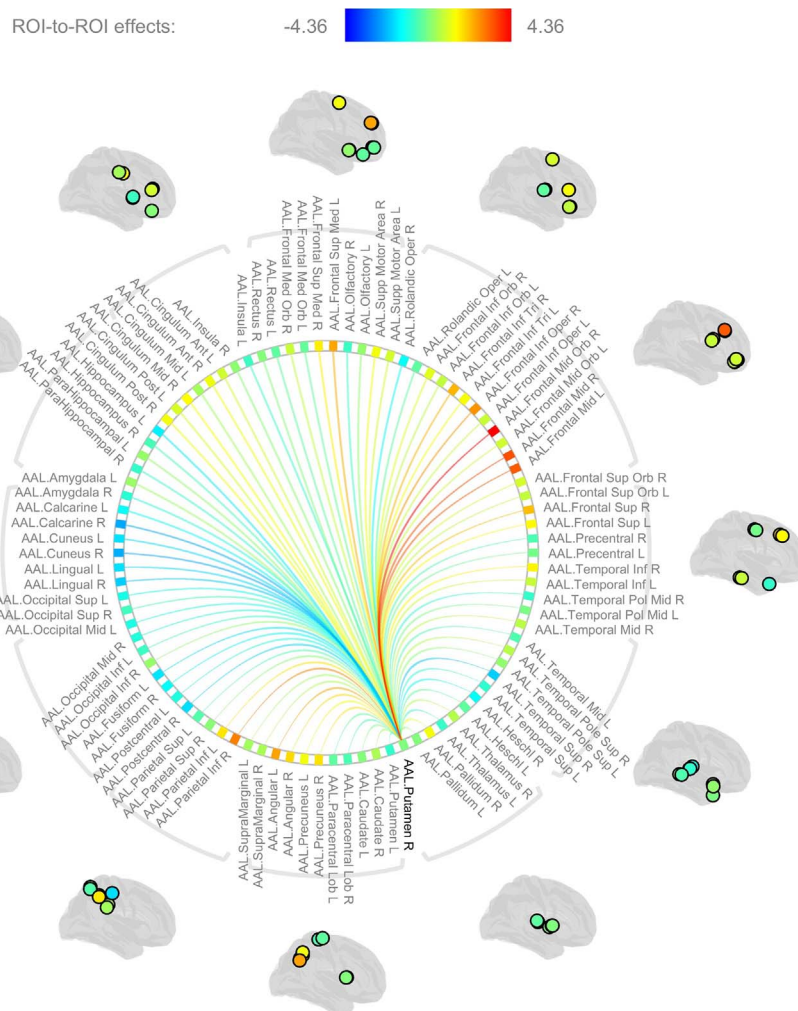


Fig. 2 Connectogram of the CBD versus Placebo comparison. The connectivity between the right Putamen (mean value from the ROI-mask of the AAL-atlas) and all other ROI sis shown. Only the three (red) connections to the frontal cortex survived FDR-correction for multiple testing. A trend for lower connectivity with the parieto-occipital region (blue lines) should be noted. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

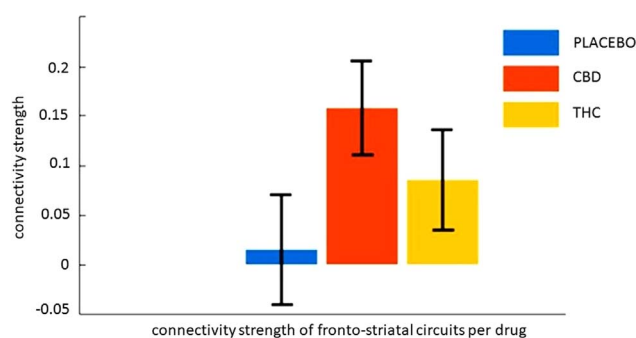


Figure 3 Boxplots of connectivity strength of fronto-striatal circuits per drug. The y-axis gives correlation strength between the mean timecourse of the right AAL putamen seed and the significant cluster from the right middle frontal gyrus (mainly brodmann BA10). Colours depict drug type, the errorbar gives the 95% confidence interval. Significant differences were found between CBD and Placebo, but not between THC and placebo (note the overlapping CI-interval).

functions; an anterior cingulate circuit that mediates reward and saliency; and an orbitofrontal circuit that is involved in integrating limbic and emotional information into appropriate behavioral patterns (Bonelli and Cummings, 2007). All circuits have in common a connection of the frontal lobe with a specific area of the striatum as shown in our study by the different ROIs. This results in a prefrontal projection onto the striatum, the pallidum, specific thalamic nuclei and back to the frontal lobe. When looking in more detail into the fronto-striatal circuitry seen in our main CBD effect, we refer to behaviourally relevant fronto-subcortical connectivity networks: a dorsolateral prefrontal circuit that mediates "executive" functions; an anterior cingulate circuit that mediates reward and saliency; and an orbitofrontal circuit that is involved in integrating limbic and emotional information into appropriate behavioral patterns (Bonelli and Cummings, 2007). All circuits have in common a connection of the frontal lobe with a specific area of the striatum as shown in our study by the different ROIs. This results in a prefrontal projection onto the striatum, the pallidum, specific thalamic nuclei and back to the frontal lobe.

While we found an increase of fronto-striatal connectivity during CBD for the putamen seed ROI, we did not observe significant coupling from caudate seeds to PFC. APFC straightforward interpretation would be that CBD alters predominantly emotional (ventral putamen) in contrast to more cognitive (dorsal caudate) connectivity. As can be seen in our "main effect connectivity"-analysis, the main effect of the putamen was predominantly limbic (except for the anterior cingulate) and the caudate had more prefrontal connections than the putamen (especially anterior cingulate).

However, the significant effect of increased putamen - prefrontal connectivity during CBD intake was found for three clusters, namely in the right middle frontal gyrus, the frontal pole and the superior frontal gyrus. According to the model described above (Bonelli and Cummings, 2007), these clusters overlap with parts of the dorsolateral and the anterior cingulate-circuit but not with the orbitofrontal circuit... We believe this result is convincing in its spatial specificity as we did not restrict our analysis to a-priori region-of-interest masks but conducted a whole brain

search. The right frontal pole (BA 10), the middle frontal gyrus (BA 8) and more medial areas containing the paracingulate gyrus have been found to be relevant for various aspects of executive functioning. A coupling of these areas found in our study and parts of striatum have been previously demonstrated in connectivity studies (Pauli et al. 2016; Orr et al. 2015; Moayed et al. 2015). When comparing our results with a meta-analysis of NeuroSynth-data, the fronto-striatal coupling implicated in our study suggests an involvement of cannabidiol in nonverbal executive functions in the support of incentive behavior (Pauli et al., 2016).

The exact pharmacodynamic interpretation is hampered by a lack of insight into CBD's mode of action: In contrast to rimonabant, CBD is not a direct CB1-R antagonist (Laprairie et al., 2015). CBD is a very low-affinity CB1 ligand that can nevertheless affect CB1 receptor activity in vivo in an indirect manner. In some studies, the effects of CBD could be reversed by CB1 receptor inverse agonists and were absent in CB1 receptor knockout mice. This points to an indirect agonism at the CB1 receptor by augmenting CB1 constitutive activity or augmenting endocannabinoid tone. However, CBD seems to show a very heterogeneous, even contrasting, response in pharmacological animal models depending on the dose and brain region (Pertwee, 2008; Schubart et al., 2014). This makes it difficult to compare to our study, where different brain regions were affected by an oral, one-time dose. This pattern might even be different in patients taking CBD for a longer time. Nevertheless, it is plausible to assume that the change in fronto-striatal coupling is related to CBD-induced modulation of striatal CB1-receptors because CB1R is expressed in the striatum and influences dopaminergic activity (Glass and Felder, 1997).

In a previous THC-CBD-placebo-study by McGuire and colleagues (Bhattacharyya et al., 2009; Borgwardt et al., 2008; Fusar-Poli et al., 2009), several neuroimaging analyses were done in a sample size of $n = 15$ in an almost identical within-subject design. While this study used a 1.5-T GE scanner and 1-month intervals between sessions, we used a 3-Tesla Siemens Trio and 1-week intervals. Apart from these technical differences, most paradigms used by McGuire and colleagues do not align easily to our striatal resting-state connectivity approach. Their main finding, opposite effects of THC and CBD, was not replicated in our study. This might be due to true non-replication, differences in neuroimaging modalities (resting state versus task fMRI) with different sensitivity, missing opposite effects in the striatum due to biological mechanisms or methodological limitations as discussed separately below. However, another result of these studies more clearly recalls the results of our study, an increase in fronto-striatal connectivity during CBD intake. In an oddball task, CBD attenuated prefrontal activity, but increased activity in the caudate (Bhattacharyya et al., 2012). A subsequent seed-cluster analysis found decreased striatal-frontal connectivity during THC, and an increase in striatal-frontal connectivity during CBD intake. Comparing our connectivity analysis with (Bhattacharyya et al., 2015) is difficult not only because of task versus resting-state connectivity but because these authors used different seeds (inferior frontal, dorsal striatal and posterior hippocampi) than our more fine-grained striatal seeds. Nevertheless, while connectivity during the oddball

task is not exactly the same as our resting-state analysis, the parallels are striking. Therefore, our data replicates at least partly, this aspect of CBD's impact on fronto-striatal connectivity. In another resting-state study in healthy volunteers, the CB1 antagonist tetrahydrocannabinarin (vTHC) demonstrated increased connectivity in the cognitive control network. While this study used different seeds than our study, it nevertheless points to a general increase of fronto-subcortical connectivity due to CB1 blockage as both vTHV and cannabidiol antagonize CB1 functioning (Laprairie et al., 2015; McPartland et al., 2015; Rzepa et al., 2015).

Cortico-striatal connectivity is not specific to psychosis but are involved in other pathological states of the brain e.g. pain. Studies have shown that cortico-striatal resting-state connectivity predicts transition from acute to chronic back pain (Baliki et al., 2012) and neuropathic pain is linked to a reduced cortico-striatal connectivity (Chang et al., 2014). This indicates that CBD might be useful in several neuropsychiatric disorders based on a mechanistic account of cortico-striatal networks.

Some limitations of our study should be kept in mind:

Our sample size is small. Since pharmaco-fMRI studies with substances like CBD and the scheduled drug THC pose an enormous logistic and legal task. We tried to counter this by using a within subject design which has a better power than a between subject design.

In contrast to previous studies, we did not observe an opposite effect of THC on fronto-striatal connectivity. In fact, THC did not differ at all from placebo. This might be linked to the difficult pharmacokinetics of the THC preparation. While we used a very similar formula as previous studies, it might be that uptake of THC was slower and less intensive. In fact, plasma concentrations of THC just before scanning were very low. It might be that THC reached sufficient concentration only after the resting state scanning took place. This low level might explain why we did not find a significant effect for the comparison THC > placebo. The pharmacokinetics is a clear strength of our study, even if this led to a detection of very low THC levels.

The plasma concentration of CBD was not significantly correlated with the connectivity pattern. It might be that the diffusion between the compartment brain and plasma is slower or more complicated. Additionally, we did not take samples exactly at the time of the measurement. Sophisticated analysis of the dosage-brain function relationship might be more feasible in the animal model (e.g. by injection of a specific dose) or by use of different doses in a within subject design. Nevertheless, we believe that it is a strength of our study that we assessed pharmacokinetics at all.

The connectivity measure we used here is simply correlational. This has the advantage that it is well comparable with previous resting-state data, but as a consequence we are not able to assess the direction of the effect and cannot address the question if the connectivity between PFC and putamen is more top-down or bottom-up. However, complex top-down models (e.g. dynamic causal modelling) lacks convincing model validation methods, as well as a reliable model selection procedure (Lohmann et al., 2012), so we feel it premature to use them in complex pharmacological fMRI-studies with cross-over design.

Obviously, we did not study connectivity in patients. Therefore, our interpretation that CBD's increase of fronto-striatal connectivity is related to its antipsychotic effect is speculative. Our observed increase in fronto-striatal connectivity might be a phenomenon in healthy volunteers which is not at all related to psychosis and its treatment. Therefore, future clinical studies of CBD might study its influence on fronto-striatal connectivity and relate it to treatment success. Second, studies in healthy volunteers should not only replicate our findings but verify whether CBD elicits behavioural changes related to fronto-striatal connectivity (e.g. executive functions). This should be verified by a more extensive characterization of cognitive performance as in our study.

In conclusion, we have found evidence that cannabidiol, a modulator of the endocannabinoid system, leads to stronger connectivity between the putamen and the prefrontal cortex. Before we conclude that this enhancement of connectivity is the basis of a potential therapeutic use of CBD in patients, we should i) replicate this effect in patients and ii) understand the behavioural correlate of this enhanced connectivity. Nevertheless, we believe these results opens several interesting aspects of CBD pharmacodynamics and highlight the importance of the fronto-striatal circuit in understanding CBDs action.

Role of funding source

The funding source has been not involved in the study design, in the collection and interpretation of data, in the writing of the report, or in the decision to submit this study for publication.

Contribution statement

HF and OG were responsible for the conception and design of the study. OG was the study physician. SK, ML and AH executed the experiments and MRI scanning. SK did behavioural analysis. ML and CR helped with clinical trial documents, study medication and analysed substance's plasma levels. Paper draft and neuroimaging analysis was done by OG. All authors revised the manuscript critically and gave approval of the final version published.

Conflict of interests

All authors have no financial interests or potential conflicts to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.euroneuro.2018.04.004>.

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