NeuroImage 63 (2012) 1701–1711

Contents lists available at SciVerse ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Manipulating brain connectivity with δ^9-tetrahydrocannabinol: A pharmacological resting state FMRI study

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ARTICLE INFO

Article history:
Accepted 23 July 2012
Available online 1 August 2012

Keywords:
Resting state FMRI
THC
Pharmacokinetics
Brain connectivity
CNS effects
Pharmacodynamics

ABSTRACT

Resting state-functional magnetic resonance imaging (RS-FMRI) is a neuroimaging technique that allows repeated assessments of functional connectivity in resting state. While task-related FMRI is limited to indirectly measured drug effects in areas affected by the task, resting state can show direct CNS effects across all brain networks. Hence, RS-FMRI could be an objective measure for compounds affecting the CNS. Several studies on the effects of cannabinoid receptor type 1 (CB_1)-receptor agonist δ^9-tetrahydrocannabinol (THC) on task-dependent FMRI have been performed. However, no studies on the effects of cannabinoids on resting state networks using RS-FMRI have been published. Therefore, we investigated the effects of THC on functional brain connectivity using RS-FMRI.

Twelve healthy volunteers (9 male, 3 female) inhaled 2, 6 and 6 mg THC or placebo with 90-minute intervals in a randomized, double blind, cross-over trial. Eight RS-FMRI scans of 8 min were obtained per occasion. Subjects rated subjective psychedelic effects on a visual analog scale after each scan, as pharmacodynamic effect measures. Drug-induced effects on functional connectivity were examined using dual regression with FSL software (FMRIB Analysis Group, Oxford). Eight maps of voxel-wise connectivity throughout the entire brain were provided per RS-FMRI series with eight predefined resting-state networks of interest. These maps were used in a mixed effects model group analysis to determine brain regions with a statistically significant drug-by-time interaction. Statistical images were cluster-corrected, and results were Bonferroni-corrected across multiple contrasts.

THC administration increased functional connectivity in the sensorimotor network, and was associated with dissociable lateralized connectivity changes in the right and left dorsal visual stream networks. The brain regions showing connectivity changes included the cerebellum and dorsal frontal cortical regions. Clear increases were found for feeling high, external perception, heart rate and cortisol, whereas prolactin decreased. This study shows that THC induces both increases and (to a lesser extent) decreases in functional brain connectivity, mainly in brain regions with high densities of CB_1-receptors. Some of the involved regions could be functionally related to robust THC-induced CNS-effects that have been found in previous studies (Zuurman et al., 2008), such as postural stability, feeling high and altered time perception.

Introduction

Ideally, early clinical phase drug development for neurological and psychiatric indications should use tests that measure effects in an objective way and repeatedly over time across different species. These tests should also be able to distinguish unique effect profiles for different classes of drugs. Traditionally, measurements of drug effects on the central nervous system (CNS) in healthy volunteers include cognitive tasks, various questionnaires, neurophysiological measurements, and increasingly also neuroimaging. The wide diversity of these tests and their numerous variations limits their applicability for decision making in clinical practice or drug development. In addition, pharmacological studies can only include a limited number of pre-defined pharmacodynamic tests, which can easily miss drug effects in CNS domains that are not tested. Moreover, most CNS effects are influenced by various functions like attention and motor coordination, and therefore do not provide direct information on an exact site of drug action.

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http://dx.doi.org/10.1016/j.neuroimage.2012.07.051

Imaging techniques have the advantage of objectively assessing direct effects in the body. However, positron emission tomography (PET) studies have radiation dose restrictions that limit repeated measurements within subjects, and the targeted pharmacological or functional system is restricted by the availability of an appropriate imaging agent. Functional magnetic resonance imaging (FMRI) on the other hand is a non-invasive imaging technique based on blood-oxygen-level-dependent (BOLD) measurements that represent brain activity. Until recently, FMRI was applicable in task-related designs only, in which pharmacologically induced changes in BOLD signals were measured in response to a specific task. The application of FMRI in drug development has several restrictions, imposed by the need for a pre-defined hypothesis about how the drug affects the task, and by limitations related to the scanning environment and to repetitive testing.

Resting state (RS) FMRI is a recently developed imaging technique that measures spontaneous BOLD changes of subjects who are in a resting state, without the interference of any task or specific stimulus. This means that RS-FMRI can be applied in studies without *a priori* hypotheses on action site. The fact that RS-FMRI is non-invasive and not affected by variability or limitations of task performance and that it can be frequently and rapidly repeated, could make it a highly valuable technique in CNS drug development. Although experience is still limited, RS-FMRI could be applied in pre-clinical animal studies, healthy volunteers and patients, which could make it a suitable translational instrument in drug development.

Previous studies found that coherent resting state BOLD fluctuations form spatially correlated brain maps, or resting-state networks (RSNs) (Beckmann et al., 2005; Biswal et al., 2010). RSNs have shown to be consistently present across human subjects, and could represent brain regions that are anatomically and functionally connected, and related to behavioral outcomes and clinical conditions (Damoiseaux et al., 2006; De Luca et al., 2006; Fox et al., 2007; Greicius et al., 2004; Quigley et al., 2003; Smith et al., 2009). A previous study by Mennes et al. (2010) suggested that inter-individual differences in RS-FMRI could predict the response to task-induced BOLD activity. Only a few studies investigated the effects of pharmacologically active CNS compounds on the functional topography of RSNs. We recently conducted a study where RS-FMRI was repeated while plasma levels of morphine and alcohol were kept stable (Khalili-Mahani et al., 2011). In order to develop a broad basis for this technique by investigating reliability and reproducibility, more studies using different drug classes should be performed. This would provide important methodological information and reference data for the use of RS-FMRI as a biomarker for CNS drug research (Wise and Preston, 2010).

In the current study we investigated the effects of δ^9-tetrahydrocannabinol (THC) on the brain using RS-FMRI. THC is a major pharmacologically active constituent of the plant *Cannabis sativa* L. In the body, THC binds to two cannabinoid receptors (CB_1 and CB_2) of which CB_1 receptors are predominantly present in various brain areas (Herkenham, 1992). The action of THC on the CB_1 receptors is generally considered responsible for the commonly known pharmacodynamic effects, such as feeling high and postural instability (Zuurman et al., 2008a).

Previous PET and FMRI studies with THC that investigated regional cerebral blood flow and BOLD signal fluctuation found THC-induced effects on the limbic system (thalamus, amygdala, hippocampus, parahippocampal gyrus, cingulate cortex) and connected areas (basal ganglia, frontal cortex), which are involved in reward, emotion, memory, awareness, pain, and executive functions (Bhattacharyya et al., 2009; Mathew et al., 1998, 1999, 2002; Stokes et al., 2010; van Hell et al., 2011). THC also affects areas of sensory (insula, postcentral gyrus, superior temporal gyrus), and motor coordination systems (cerebellum). The functions associated with these regions are related to the behavioral effects after THC or cannabis use (Zuurman et al., 2009).

The primary aim of this study was to investigate the effects of THC on task-independent RS-FMRI functional connectivity patterns using repetitive measures in healthy volunteers. Based on previous studies using other psychopharmacological manipulations (Khalili-Mahani et al., 2011) we hypothesized that THC would induce changes in brain connectivity compared to placebo. In addition, we measured the plasma concentrations of THC and its active metabolite 11-hydroxy-THC (11-OH-THC) as well as a number of well-known THC-related CNS effects. Based on our previous studies, we expected to measure clear THC and metabolite plasma concentration profiles, and prominent pharmacodynamic effects, other than RS-FMRI (Strougo et al., 2008; Zuurman et al., 2008b).

Patients and methods

Design

This was a double-blind, randomized, placebo-controlled, two-way cross-over study with a wash-out period of at least 2 weeks.

Subjects

Healthy, right-handed male and female volunteers aged 18 to 45 years with a body mass index of 18.0 to 28.5 kg/m^2 were included in the study. Subjects with a history of psychiatric or neurological illness, or with a history of hereditary psychiatric illness in first degree relatives or neurological illness in first- or second degree relatives were excluded from participation. Subjects had to be cannabis users for at least 1 year with use frequency of no more than once a week, and had to be able to refrain from using cannabinoids from at least 2 weeks prior to the first treatment period up to the end of the study. They had to refrain from nicotine and caffeinated products on study days. Subjects were excluded if they used medication other than contraceptives, and if they were pregnant (as assessed by hCG urine test). They were not allowed to have a positive alcohol breath test or drug urine test at the screening visit or at the start of a study day, neither a history of alcohol or drug dependence. Subjects could not participate if they had metal body implants or claustrophobia.

As this was an explorative study, no sample size calculation could be performed. We planned a sample size of 12 volunteers (6 male and 6 female) who completed two occasions, since in all drug studies that we have performed so far, numbers of 12 subjects were found to be sufficient (Desmond and Glover, 2002; Strougo et al., 2008), and a similar number was also mentioned in a study about the power of FMRI and RS-FMRI.

Subjects who were not able to complete two occasions would be replaced.

Procedure

Subjects gave written informed consent before any study-specific procedure was performed. Eligible subjects were enrolled in the study after a general health screen within 3 weeks before the first study day. Subjects were acquainted with the visual analog scales questionnaire and the inhalation procedure using THC vehicle. At each study day, THC or placebo was administered at 0 min, 1 h 30 min and 3 h 00 min. Pharmacodynamic (PD) and pharmacokinetic (PK) measurements were frequently performed on all study days at fixed time points, as chronologically indicated in Fig. 1. At the beginning of each study day a venflon cannula was inserted intravenously for all blood samples that were drawn on both study days. Subjects were fasted for at least 4 h at arrival, and standardized meals were provided pre-dose, and at 3 h 40 min and after the last study day activity at 6 h 47 min. The wash-out period between study days was at least 2 weeks. The study protocol was approved by the Medical Ethics Review Board of Leiden