

# Pharmacologic resting state-fMRI: effects of cannabis on functional brain connectivity ‘at rest’

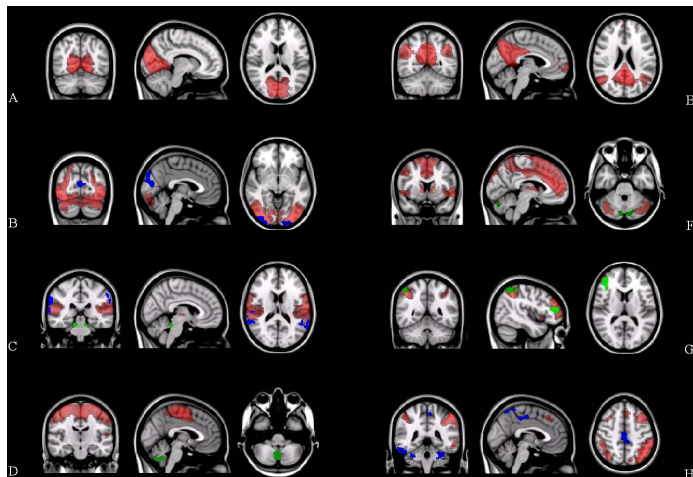
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**Introduction:** Pharmacological fMRI has often been used to study drug effects on task-related brain activity (Honey et al., 2004). However, the general applicability of this technique in drug research has been limited by the low availability of tasks that are drug-sensitive, repeatable and suitable for MRI. For drug studies, task-independent functional neuroimaging techniques would be very useful, if these would allow repeated measures of whole-brain activity. In this sense, ‘resting state’ fMRI (RS-fMRI) is a promising technique, because it allows a repeated task-independent assessment of functional interactions between brain regions (functional connectivity). However, the relatively novel technique has not been explored yet for this purpose. In this study we investigate the effects of  $\Delta$ -9-tetrahydrocannabinol (THC), the psychoactive compound of cannabis, on functional brain connectivity to: 1) study the feasibility of this fMRI technique for drug studies; 2) study whether multiple increasing doses of THC show dose-related changes in functional brain connectivity; 3) compare THC effects on functional connectivity to previously described effects using other neuroimaging techniques.

**Methods:** Nine healthy male volunteers, (age 18 - 30 years) participated in a randomised, double blind, placebo-controlled trial after giving written informed consent. The two treatment arms were three intrapulmonary administrations of 2 mg, 6 mg and 6 mg THC successively, versus three intrapulmonary administrations of a matched placebo. The intervals between administrations were 90 minutes. During each treatment occasion eight RS-fMRI scans of 8 minutes and 10 seconds were obtained (1 at baseline, 2 after the first and second administration and 3 after the third administration). Subjects rated their subjective feelings of drug effects on a Visual Analogue Scale (VAS) of “feeling high” directly after each scan. Drug induced effects on functional connectivity were examined using a double regression method (Beckmann OHBM 2009) with FSL (FMRIB analysis Group, Oxford UK). For each RS-fMRI series, this method provides (after spatial (8 regressors) and temporal (14 regressors) regression) eight maps of voxelwise connectivity throughout the entire brain, with eight predefined resting-state networks of interest. These maps were used in a mixed effects model group analysis, to determine brain regions that show a statistically significant drug-by-time interaction, and regions associated with concentration-dependent “feeling high” VAS scores ( $p < 0.05$ , cluster corrected).

**Results:** THC administration decreased connectivity in different brain regions, including cerebellum, cuneus and several cortical regions. (Fig. 1 and Fig. 2 left panel). No effects of increasing connectivity were found. Effects associated with “feeling high” VAS scores were found in the brainstem, cerebellum, medial frontal gyrus and the parietal lobe (Fig. 1 and Fig. 2 middle and right panel).



**Conclusion:** These results show that functional connectivity using RS-fMRI is a promising new technique to study pharmacologically induced changes in brain activity. Repeated RS-fMRI scans show clear THC related decreases in functional brain connectivity in various brain regions. Some changes were related to subjective feelings of cannabis effects. Connectivity changes occurred mainly in brain regions with high densities of CB1-receptors (the major THC binding receptor in the central nervous system) such as cerebellum and various cortical regions (Howlett et al. 2002). Decreased connectivity of the cuneus might be related to a disturbance in visual processing after THC administration (Koethe et al 2006), and cerebellar effects by a decrease in postural stability after THC administration (Zuurman et al. 2008). Most of the abovementioned effects were described in previous studies using SPECT, PET and task related fMRI to study the effect of THC on brain function (Martin-Santos et al., 2009)

Figure 1. Red indicates the mean 8 networks (A-H) over all RS-fMRI series. Blue: regions of decreased connectivity to the corresponding network. Green: regions associated with the VAS-high score. Resting state networks: A: visual; B: lateral visual; C: auditory; D: sensory motor; E: default mode; F: executive; G: right visual-spatial memory; H: left visual-spatial memory.

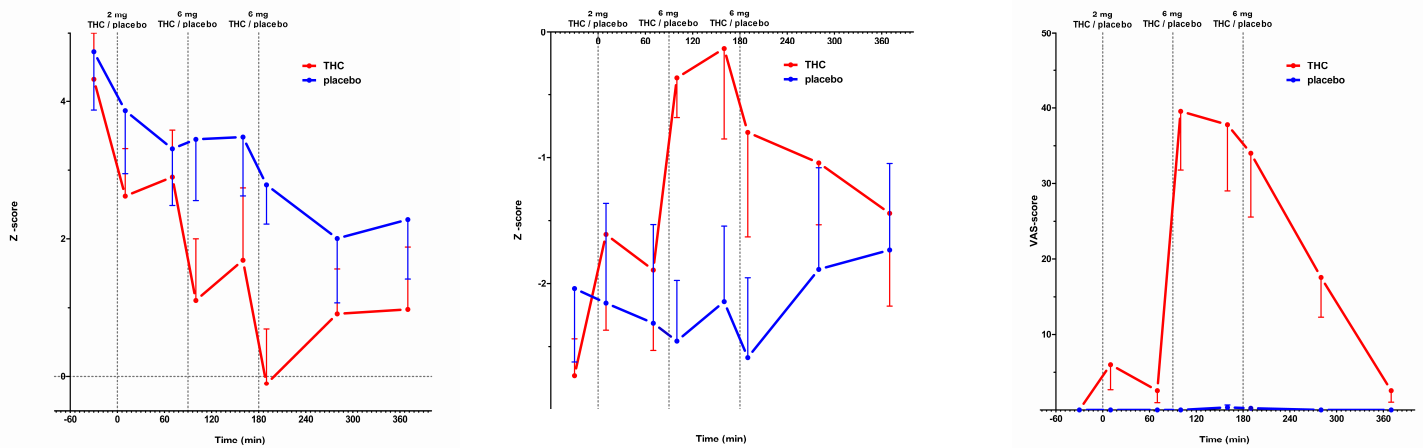


Figure 2. Left panel: plot of the average z-scores of connectivity of the cuneus to the lateral visual network (network B in Fig. 1) over time (in minutes). Each THC administration (vertical lines) is followed by a decrease in connectivity. Middle: Mean Z-scores of the cerebellar region (network D in Fig. 1) that is associated with VAS high scores (right panel). Error bars are indicating standard error of the mean.