A non invasive experimental protocol for fMRI studies: Investigation of the Basal Ganglia-cortex circuit in a rat model

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Synopsis:

Combination of blood– oxygen level-dependent functional magnetic resonance imaging (BOLD fMRI) and electrical hindpaw stimulation has been used as a standard model to study the somatosensory pathway and brain rehabilitation in rats. In the present study, we examined the feasibility of performing BOLD fMRI experiments on rat to investigate the activity of the basal ganglia (BG)-cortex circuit associated to hindpaw sensitive stimulation. These findings will have relevance in the fMRI studies dealing with physiopathology of neurodegenerative diseases such as Parkinson.

Introduction:

Basal ganglia are involved in both the somatosensory and motor neural network of the rat [1]. These regions have direct connections to the somatosensory cortex and the thalamus. Actually, most fMRI studies are limited to cortical activity investigation. The absence of BOLD fMRI studies in deep brain regions could be explained by (i) neuronal activity depression due to anesthesia [2], (ii) a low Signal to Noise Ratio (SNR) [3] in these regions. Improving these two crucial parameters, we expect that a hindpaw sensitive stimulation could produce a detectable BG activation.

Animals and methods:

Ten male Wistar rats (280-300g) were used in this study. All animals were initially anesthetized with 4% isoflurane in O2:N2O (3:7), which was reduced to 2% isoflurane for maintenance during preparation. For all animals, the right femoral artery was catheterized with PE-50 tubing. Arterial blood gases (pH; pCO2 and pO2) were sampled before and after experiments. Rectal temperature was maintained at 37.5-C by a feedback-controlled, water-circulated heating pad during preparation and fMRI sessions. Of these ten rats, five were continuously anaesthetized with 1.5% isoflurane spontaneously breathing. The other five rats were anaesthetized with 1% isoflurane and Cisatracurium besylate (Nimbex®, 5mg/ml, i.v) was continuously administrated for muscle relaxation. The trachea was cannulated to allow for mechanical ventilation during the fMRI acquisition. For electrical stimulation, a pair of needle electrodes was inserted in the left hindpaw.

The fMRI experiments were conducted on a horizontal 4.7 T BioSpec animal scanner (Bruker BioSpin MRI GmbH, Ettlingen, Germany). Radiofrequency transmission was achieved with a volume coil (12 cm diameter) while the signal was detected using a 4 cm surface coil, positioned over the head of the animal. Both coils were actively decoupled from each other. Coronal multislice gradient-echo (GE) EPI images were acquired using the following parameters: two TE = 23.5 and 45 ms (echo position 50%); TR = 4000 ms; BW = 200 kHz; 14 consecutive slices of 1 mm thickness; FOV = 2.56 x 2.56 cm^2; matrix of 128 x 128 pixels. Hindpaw stimulation was performed using rectangular pulses (2.0 mA, 8 Hz, 10 ms; Pulsar 6bp bipolar stimulator, FHC, Bowdoinham, ME) in a paradigm of 4 blocks of 320s resting period (80 images, TR = 4000 ms) and 160s activation period (40 images, TR = 4000 ms), ending with an additional 320s resting period (4 x [80 OFF + 40 ON] + 80 OFF), thus resulting in a total experimental time of 37 min 20s.

Data analysis: All images processing were performed using SPM8b software (Wellcome Trust Centre for Neuroimaging, Institute of Neurology, UCL, London UK, http://www.fil.ion.ucl.ac.uk/spm).Sensitivity maps based on the minimum Signal to Noise Ratio required to detect an expected MR (BOLD) signal change were calculated and used as a mask of the activation maps to help remove false positive activations. Statistical parametric activation maps were generated using a combination of two thresholding levels, a high level ($p<0.0001$) to ensure the specificity and a lower level ($p<0.05$) to maximize the sensitivity.

Results and discussion:

Brain activation after left hindpaw stimulation was reproducibly observed in the contralateral somatosensory (Fig. a, b) for five rats. A robust negative BOLD response was observed in the caudate putamen region (Fig. c, d) for only three of these 5 rats with a high spatial variability. The limited reproducibility observed in the investigation of the BG activations in this study is probably due to the absence of a sufficient Signal to Noise Ratio, in these structures. Further improvements, especially a higher Signal to Noise Ratio would help with better reproducibility of the BG activations observed. This study describes a novel approach for mapping the BG-cortex circuit in the rat brain by hindpaw electric stimulation. The goal of developing a rodent fMRI model to investigate the BG activations appears within reach.

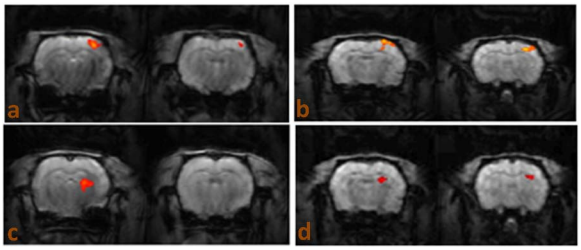

Fig. a : cortical activation(TE=45 ms ; 1.5% isoflurane).
Fig. b : cortical activation(TE=45 ms ; 1% isoflurane).
Fig. c : BG activation(TE=23.5 ms ; 1.5% isoflurane).
Fig. d : BG activation(TE=23.5 ms ; 1% isoflurane).

References:

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