Imaging of pentylenetetrazol-induced seizures by fMRI: a novel method for detecting seizure onset zone in experimental post-traumatic epilepsy

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Introduction

Traumatic brain injury (TBI) is a major cause of death and disability worldwide with an estimated 10 million people affected annually [1]. Initial mechanical damage to the brain causes immediate brain damage and triggers a cascade of secondary damage as well as recovery processes which typically last from few weeks to even years. TBI is estimated to cause 10–20% of all symptomatic epilepsies and in the European Union (EU) and USA, at least 0.5 million individuals live with post-traumatic epilepsy (PTE). Animal models of TBI develop epilepsy, but identification of seizure onset zone has remained a major challenge even though such information would be critical, for example, for guiding the molecular analysis of ictogenic region. The aim of the present study was to investigate whether the onset zone for pentylenetetrazole (PTZ) induced seizures in the brain in animals undergoing epileptogenesis after TBI can be detected by using simultaneous local field potential (LFP) and BOLD fMRI measurements.

Materials and Methods

Adult male Sprague-Dawley rats (n=21) were used in this study. Severe TBI was induced for 14 animals using the lateral fluid-percussion injury model (FPI)[2]. Seven sham-operated animals served as controls. Two animals died during the TBI operation (1 sham, 1 TBI). The fMRI studies were performed 2 months after TBI (n=19, 439 ± 31 g). The femoral artery was cannulated for monitoring of blood gases and pH during the fMRI experiments. The femoral vein was cannulated for medetomidine and pancoronium bromide (2 mg/kg/h) administration. For simultaneous LFP and fMRI measurements, a tungsten wire electrode was inserted into the frontal cortex (AP 2 mm and ML 2 mm from bregma, -1 mm from the cortical surface). Animals were tracheotomized and artificially ventilated using a mechanical ventilator. After surgery, isoflurane anesthesia was discontinued and a bolus injection of medetomidine was given (i.v., bolus 0.05 mg/kg) followed by a continuous subcutaneous infusion (i.v., 0.1 mg/kg/h) 5 min later. The LFP signal was measured using a BrainAmp MR plus magnet compatible system. The signal from the electrode was low pass filtered at 1000 Hz (sampling rate 5000 Hz). The MRI experiments were performed in a 9.4 T horizontal scanner interfaced with a Varian DirectDrive console. Functional MRI data were acquired using a single shot SE-EPI sequence (TR 4 s, TE 40 ms, slice thickness 1.5 mm, 15 slices, image matrix of 64 x 64, and FOV of 2.5 x 2.5 cm). Simultaneous LFP and fMRI measurements were performed consisting of 1000 images of baseline, thereafter; PTZ was injected in a dose of 30 mg/kg (i.p.). After PTZ injection, image acquisition was continued for 1000 images in each rat. Anatomical images were acquired with fast spin echo sequence. All data analyses were conducted using Matlab. The fMRI data were analyzed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) along with in-house made Matlab code. LFP signal was analyzed using in-house made Matlab routines.

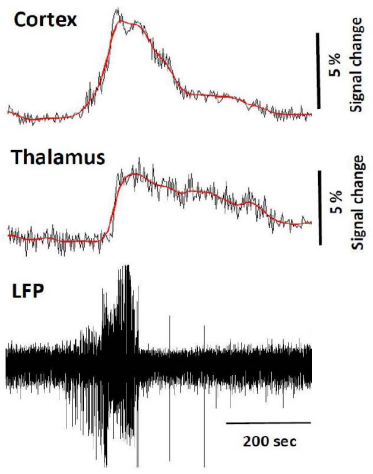

Figure 1. BOLD time courses from ROIs in the cortex and thalamus from a representative control animal and the simultaneously measured LFP signal.

Results

The size of the lesion including the adjacent ventricle was 95.9 ± 33.0 mm^3 in TBI animals. In all animals, PTZ caused detectable responses in BOLD and LFP signals (Figure 1). In all sham operated rats, the positive BOLD response started simultaneously bilaterally in the cortex (Figure 2 B). It spread rostro-caudally along the cortex and activated subsequently positively bilaterally the thalamus (6/6) and negatively the contralateral hippocampus (5/6). In 6 out of 13 TBI rats, the BOLD activation started bilaterally and spread similar to that in the sham group. However, in 7 TBI animals the BOLD activation started unilaterally near the trauma (Figure 1 A) and spread to the other hemisphere during following 20-100 s. The contralateral thalamus was activated in 6/7 animals and the ipsilateral in 2/7 rats. Positive contralateral hippocampal activation was detected in 2/7 rats.

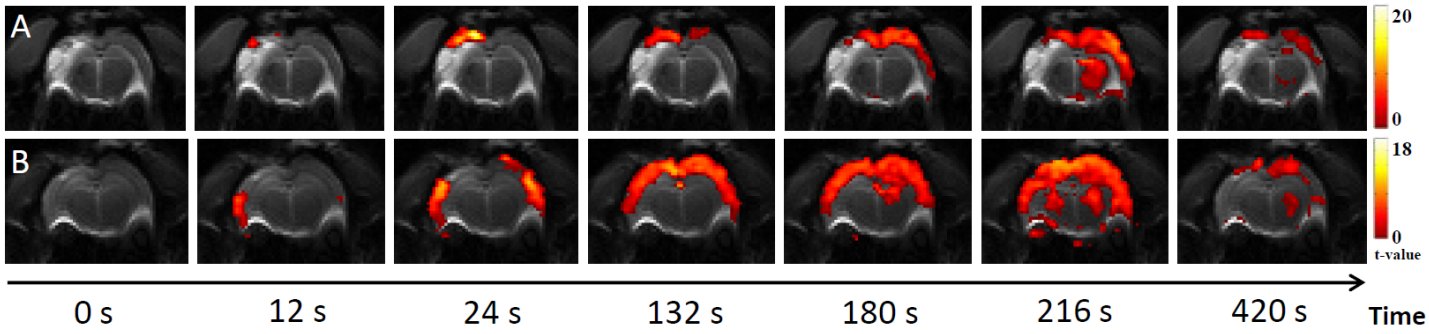

Figure 2. PTZ induced BOLD activations in selected time points from a TBI animal (A) and control animal (B) in a single slice. The zero time point denotes the seizure onset zone (not the injection time).

Discussion

Decreased threshold for PTZ induced seizures has been reported after lateral FPI induced TBI in rat [2]. In the present study, we addressed the spatial localization and spread of this activation. We demonstrate that PTZ induced seizures originate in the perilesional area in 7/13 animals at 2 months after TBI. This suggests that perilesional area is undergoing circuitry reorganization which eventually leads to the onset of spontaneous seizures. To avoid bias related to the PTZ administration, our data need to be confirmed in the same model by using other convulsants with different mechanisms of action. However, our results show that a combination of fMRI and pharmacological stimulation is a potential tool to study post-injury hyperexcitability.

References: [1] Hyder AA et al. NeuroRehabilitation, 22(5):341-53, 2007. [2] Kharatishvili I et al.Neuroscience, 140(2):685-97,2006.