Simultaneous fMRI and local field potential measurements of epileptic seizures in medetomidine sedated and awake rats

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Introduction

Anesthetic drugs may alter normal brain physiology as well as modify cerebral hemodynamics. Therefore it is necessary to perform physiological and functional measurements both under awake and anesthetized conditions to understand the influence of anesthetics on neurovascular coupling and ultimately on fMRI signals. Recently, medetomidine has been increasingly used as a recovery anesthetic in experimental fMRI settings (1-3). In the present study, we focused on the effects of medetomidine sedation on simultaneous local field potential (LFP) and fMRI variables. First, we determined the baseline cerebral blood flow (CBF) in awake and anesthetized (medetomidine and isoflurane) rats using the continuous arterial spin labeling (CASL) technique. Second, simultaneous LFP and fMRI measurements were performed to compare BOLD signal changes, caused by kainic acid (KA) induced epileptic seizures in awake and medetomidine sedated rats.

Materials and Methods

The animal protocol was approved by the IACUC of Dartmouth College. The animals were divided in two subgroups: CASL (n=3) and simultaneous LFP and fMRI measurements (n=10). In all groups, the rats were anesthetized with isoflurane during the surgery. The femoral artery was cannulated for monitoring of blood gases and pH during the fMRI experiments. Non-invasive pulse oximetry was used to monitor oxygen saturation and heart rate during the entire experiment. The femoral vein was cannulated for the injection of pancoronium (2 mg/kg/h). Immediately before MRI scans, animals were tracheotomized and artificially ventilated using an MRI-compatible mechanical ventilator. CBF was studied using CASL with a fast spin echo read out (TR 15 s, echo spacing 10 ms, field of view of 3 x 3 cm, 64 x 64 points, and slice thickness 2 mm). The CASL imaging session was divided into three parts. First, CASL MRI was performed in isoflurane anesthetized (1-1.5 %) rats, thereafter isoflurane was discontinued. Second, CASL measurements in awake rats were started at least 15 min after terminating isoflurane. Third, after CASL recordings in awake animals, a bolus injection of medetomidine was given (Domitor®, bolus 0.05 mg/kg) followed by a continuous subcutaneous infusion (0.1 mg/kg/h) 5 min later. CASL MRI was performed under medetomidine sedation. For simultaneous LFP and fMRI measurements, a tungsten wire electrode was inserted into the right hippocampus (AP 3.6 mm and ML 2.5 mm from bregma, -2.5 mm from the cortical surface). After surgery, isoflurane anesthesia was discontinued, and rats were without anesthesia (n=5) or sedated with continuous infusion of medetomidine (n=5). 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 7 T horizontal scanner interfaced with a Varian UnityInova console. fMRI data were acquired using a single shot EPI sequence (TR 1 s, TE 60 ms, slice thickness 1 mm, image matrix of 64 x 64, and FOV of 3.0 x 3.0 cm). Simultaneous LFP and fMRI measurements were performed consisting of 450 images of baseline, thereafter; KA was injected in a dose of 10 mg/kg (i.p.). After KA injection, image acquisition was continued for 900 images in each rat. This was repeated at 3 times (total 2700 images). A model based on observed seizures in LFP signal was used to evaluate the BOLD fMRI signal. fMRI data were analyzed with the SPM5 program (Wellcome Department of Imaging Neuroscience, University College London, UK).

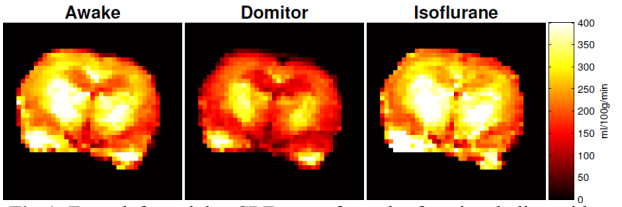

Fig 1. From left to right: CBF maps from the functional slice without anesthesia, in medetomidine (Domitor) and isoflurane anesthetized rats.

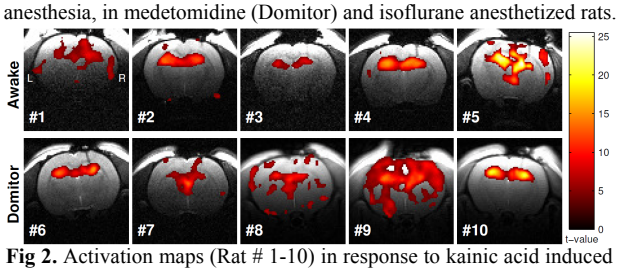

Fig 2. Activation maps (Rat # 1-10) in response to kainic acid induced seizures superimposed on the anatomical images. The threshold for statistical significance was set at $p \leq 0.05$ (FWE corrected).

Results

Fig. 1 shows CBF maps under the three conditions i.e. awake, medetomidine (Domitor) and isoflurane. The highest mean CBF values were observed in isoflurane anesthetized rats, the lowest ones were detected in medetomidine sedated animals. During simultaneous LFP and fMRI measurements in awake KA-injected rats, the LFP recordings showed recurrent epileptic seizures lasting from 10 sec to 4 min (43 ± 3 sec, Mean ± Sem) in the hippocampus due to KA injection. In contrast to awake rats, KA induced seizures in rats under medetomidine sedation lasted from 6 sec to 3 min (32 ± 1 sec). During seizures strong bilateral BOLD signal changes in the hippocampus were seen in all animals (Fig. 2). BOLD signal increases were also present in cortical regions (Rat # 1, 5, 8 and 9); however, substantial inter-animal variation in cortical activations was detected in both brain states. In awake rats, we found negative BOLD signal changes in the thalamic area of one rat (Rat # 5); however, four of five rats showed fMRI signal decreases in cortical areas as well. Under medetomidine sedation, negative BOLD signal changes (Rat # 6 and 10) were observed bilaterally in the thalamus; furthermore, negative cortical BOLD was detected from four sedated rats. Fig. 3 shows representative time courses from 2 x 2 pixel ROIs placed over the right hippocampus of an awake rat and a medetomidine sedated rat with the corresponding simultaneously recorded LFP signal from the right hippocampus during KA induced epileptic seizures.

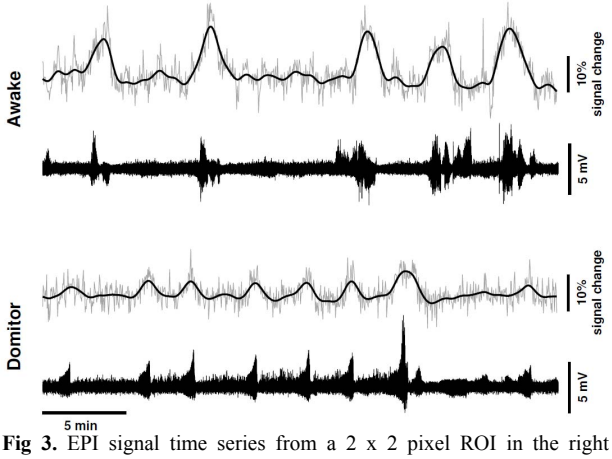

Fig 3. EPI signal time series from a 2 x 2 pixel ROI in the right hippocampus (upper signal) and the corresponding simultaneously recorded hippocampal LFP signal with gradient artifacts removed (lower signal) in awake and medetomidine sedated rats

Discussion

A common challenge for fMRI studies including animal models of epilepsy is the choice of an anesthetic agent. Many anesthetic agents suppress evoked responses (4) and/or modulate cerebral hemodynamics. The CASL measurements showed lower CBF in medetomidine sedated rats than in isoflurane anesthetized or awake rats. In medetomidine sedated rats, the low CBF means higher baseline oxygen extraction fraction which in turn will influence the BOLD signal amplitude (5). This would translate into a larger BOLD response to a given stimulation in rats under medetomidine than in rats under isoflurane. However, the KA induced seizures are more complex in nature than, for example somatosensory stimulus, so this could explain variations in BOLD signal between awake and medetomidine sedated rats. These CBF findings have strong implications for fMRI studies of anesthetized or sedated animals. In the present study, we demonstrate the feasibility of medetomidine sedation for simultaneous LFP and fMRI measurements. Furthermore, our LFP data show that medetomidine sedation has only negligible effect on brain activity and hemodynamic response induced by KA. We conclude that medetomidine anesthesia is well suited for studies of the normal and pathologic rat brain, but a basal CBF level that is lower than that of awake rats should be taken into account when interpreting the fMRI results.

References: [1] Weber et al. (2006) NeuroImage 29: 1303-1310. [2] Weber et al., (2008) The Journal of Neuroscience 28(5):1022–1029. [3] Pawela et al. (2009) NeuroImage 46: 1137-1147. [4] Rojas et al., (2006) The American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 291:189-196. [5] Lu et al. 2008 MRM 60:364-372.