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 pancuronium (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 transferred to the MRI scanner. Medetomidine was also present in cortical regions (Rat # 1, 5, 8 and 9); however, no 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 BOLD signal increases 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.

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, KA injection also decreased CBF (n=5); however, KA induced seizures were 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 baseline CBF level that is lower than that of awake rats should be taken into account when interpreting the fMRI results.