Spontaneous depolarization waves in medetomidine sedated Sprague-Dawley rats detected by fMRI

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Introduction. In animal fMRI, anesthesia is one of the key factors influencing physiology and responsiveness to presented stimuli, therefore playing an important role in overall quality of the experiment. Medetomidine is a potent and highly specific alpha-2-adrenoreceptor agonist that has recently been increasingly used as an anesthetic in experimental fMRI of rodents [1] due to controlled dose-dependent sedation, general safety and reversibility. Spreading depolarization (SD), a wave of neuronal and glial depolarization moving across the cortex with characteristic speed of 2-6 mm/min, is known to take place in number of neurological diseases including migraine, brain trauma and ischemia [2]. Here we report our findings of spontaneous SDs registered in medetomidine sedated Sprague-Dawley rats during prolonged (1h) BOLD fMRI measurement. To our knowledge there are no previous reports of spontaneously occurring SD's detected by fMRI.

Materials and Methods. Adult male Sprague-Dawley rats (n=9, 455 ± 65 g) were used in this study. The femoral artery and vein were cannulated under isoflurane anesthesia for monitoring of blood gases and pH during the fMRI experiments, and for medetomidine and pancuronium bromide (2 mg/kg/h) administration, respectively. We used 7 animals which served as sham control in another study and had ∅5mm opening in the parietal skull bone. In addition, two intact animals were measured with identical protocol. Rats 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 (0.1 mg/kg/h) 5 min later. 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 spin-echo echo planar imaging (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). fMRI measurements were performed consisting of 1000 images (1 h 7 mins). The fMRI data preprocessing and GLM-based analysis performed using in-house made Matlab routines.

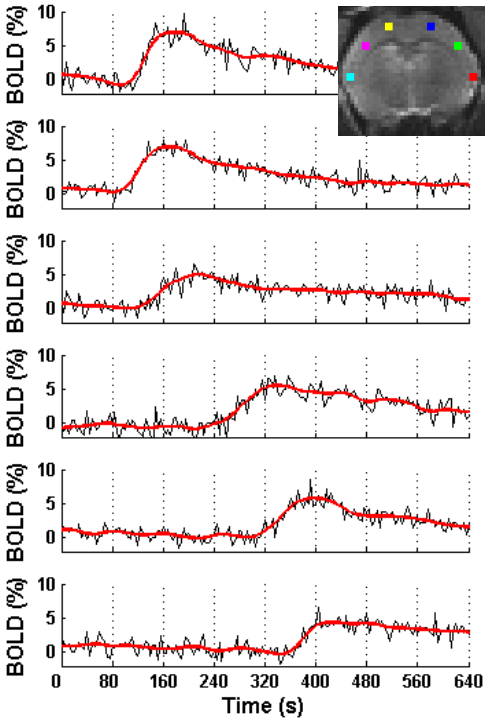

Figure 1. BOLD timecourses from 6 ROIs shown in upper right box. ROIs placed sequentially from right to left correspond to timecourses from top to bottom.

Results. Temperature and the blood gases of the animals were within normal physiological limits. We observed SDs in all animals including the naïve animals. We recorded total of 17 spreading depolarizations, in average 2.1 ± 1.4 (mean ± std) SDs per animal, during one hour follow up. Site of the onset and propagation patterns were heterogeneous, varying even within one animal between repeated SDs. Most of the waves originated unilaterally from inferior cortical areas in medial and posterior parts of rat brain (8 waves in the left and 5 in the right hemispheres and rest in frontal cortex and subcortically). Mean duration of activation in a ROI was 193.3 ± 58.8 sec propagating in cortical regions with speed of 2.93 ± 0.6 mm/min. Propagation was restricted to one hemisphere in 4 cases but 10 waves were freely crossing hemispherical border also involving subcortical regions. Other 3 waves were partially caught due to their presence in the beginning or the end of the recordings and were excluded from further propagation analysis.

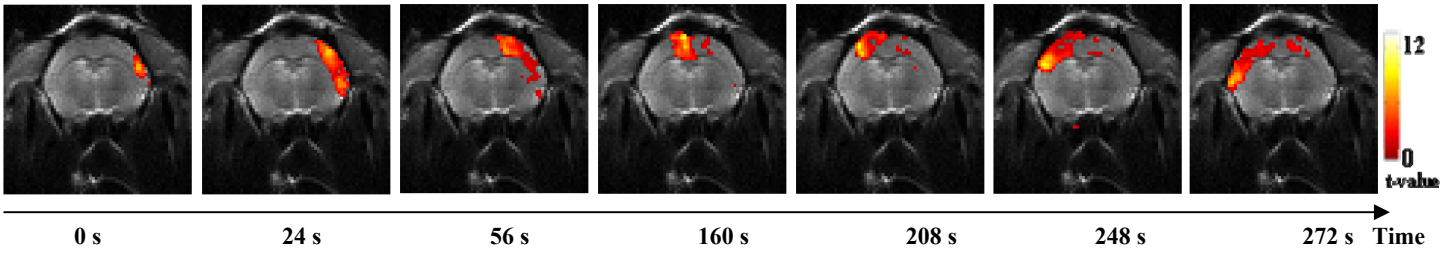

Figure 2. BOLD activation maps of one spreading depolarization in one representative case.

Discussion. We observed spontaneous depolarizations with features (spreading pattern and involvement of subcortical regions) that are not typical for commonly reported spreading depression both in sham operated an in naïve animals. There are currently only a few fMRI studies with medetomidine sedation in Sprague-Dawley rats and none of those report similar phenomenon. Duration of a single experiment in those studies is much shorter – 15 minutes maximum, which might be too short to detect described condition, which in our case occurred with a rate of 1 to 5 per hour. There are reports of increased acoustic hyperexcitability in medetomidine sedated animals which can cause a SD upon strong auditory stimulation [3]. Therefore continuous acoustic stimulation by loud gradient switching during multislice EPI fMRI imaging can be a triggering factor for spreading depression. Another possible candidate for trigger role is peripheral nerve stimulation effect. Spontaneous depolarizations are clearly a potential confounding factor for fMRI studies of rodents and origin of them should be studied in more detail.

References [1] Weber et al., (2006) Neuroimage 29(4):1303-10. [2] Dreier et al., (2011) Nat Med. 17(4):439-47. [3] Vinogradova et al., (2009) Brain Res. 1286:201-7.