Imaging brain activity: Longitudinal fMRI in mice using medetomidine sedation

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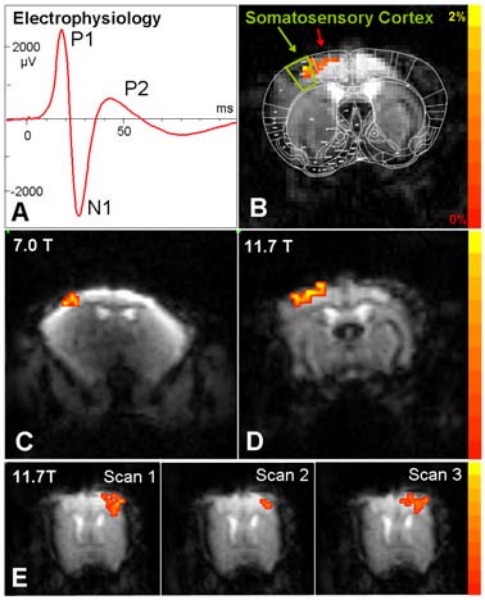
Introduction: Functional MRI (fMRI) using the endogenous blood oxygenation level-dependent (BOLD) contrast is a widely used technique to study brain activity non-invasively. It is the method of choice to assess damage and recovery processes in animal models of brain injury. In mice, fMRI has been performed under α-chloralose [1] or pure isoflurane [2]. Both approaches are unsuitable for longitudinal fMRI experiments due to severe side effects or the need of strong electrical stimulation, resulting in pain dependent brain activation. In rats, longitudinal fMRI has been successfully performed using the sedative drug medetomidine [3]. Here, we present the first longitudinal fMRI protocol for mice, which allows repetitive and non-invasive fMRI experiments in the somatosensory cortex of mice.

Methods: Male C57/Bl6 mice (n=14) were initially anesthetized with 3% isoflurane in O_2:N_2O (30:70). A bolus of medetomidine (Domitor®, Pfizer) was injected subcutaneously, isoflurane was discontinued after 15 min, followed by a subcutaneous infusion of medetomidine. Doses of bolus and infusion were varied to establish a dose response. Transcutaneous CO_2 was monitored (TCM4, Radiometer) and somatosensory evoked potentials (SSEPs) were recorded during electrical forepaw stimulation (I=2 mA, f=6 Hz). Animals were recovered from sedation with an intraperitoneal injection of the antidote Antisedan®.

fMRI experiments were conducted on a 7.0T Bruker system using a 8 cm Helmholtz coil for transmission and a 2 cm surface coil (Medres, Germany) for detection, as well as on a 11.7T Bruker system with a resonator for transmission and a quadrature surface receive coil (Bruker, Germany). Gradient-echo EPI images were acquired at a matrix of 64x64 pixels in 5 consecutive slices (1 mm thickness) with an in-plane resolution of 200x200µm² and TE/TR=16ms(7.0T), 17.5ms(11.7T)/3000ms. Each mouse received several fMRI scans per session. Images were analyzed with Stimulate [4] using a Student`s t-test with a confidence level of 97-99%.

Results: From the dose response study for medetomidine sedation, we determined the optimal dose to be 0.3 mg/kg, which resulted in sedation for at least 90 minutes. Doses higher than 0.5 mg/kg resulted in adverse behavior, and therefore do not qualify for longitudinal experiments. All transcutaneous CO_2 measurements showed an initial increase but were stable at approximately 20 min after the bolus injection. Normal SSEPs consisting of 3 significant peaks were recorded in all animals sedated with 0.3 mg/kg (Figure A). BOLD response was observed repetitively at 7T and at 11.7T (Figure C and D) in the brain regions known to be part of the somatosensory system (Figure B: red arrow indicates draining vein). The BOLD activation was reproducible during each session (Figure E). The activation occurred in up to 2 adjacent slices with an average BOLD change of 1-2%. At 11.7T 74% of all scans showed a BOLD response with an average of 11 activated pixels, whereas at 7.0T only 30% of all scans showed activation with an average of 4 activated pixels.



Conclusions: We have successfully established a medetomidine based fMRI protocol for mice, which can now be used for longitudinal studies of brain activity, e.g. in transgenic mice. It is possible to sedate mice sufficiently with medetomidine while preserving electrical activity as well as the hemodynamic response. Although doses higher than 0.5 mg/kg of medetomidine caused adverse behaviors, the evaluated dose of 0.3 mg/kg is well-tolerated by mice, and all recovered completely. More activated pixels can be detected at 11.7T than at 7.0T due to higher signal-to-noise ratio (SNR) of the higher field strength. Better SNR at 11.7T also made image analysis possible at a higher confidence level. However, SNR at 11.7T and shimming still requires improvement because of the small size of the mouse brain, which otherwise leads to deterioration in image quality.

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References: **[1]** Ahrens, *et al*. NMR Biomed. 2001. 14: 318-24. **[2]** Nair, *et al*. Magn Reson Med. 2004. 52: 430-4. **[3]** Weber, *et al*. NeuroImage. 2006. 29:1303-1310. **[4]** Strupp, NeuroImage. 1996. 3: 607.