A robust non-invasive protocol for longitudinal fMRI studies in the rat

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Introduction

Functional magnetic resonance imaging (fMRI) allows the noninvasive assessment of brain activity and plasticity, adding important information to conventionally obtained anatomical MR imaging. Most of the previously performed fMRI studies in rats used α-chloralose to anesthetize the animals and invasive monitoring of blood gases. Because of the organ toxicity and severe acidosis caused by α-chloralose and because of the catheterization of the femoral blood vessels, animals had to be sacrificed after the fMRI experiment and were not available for longitudinal studies of brain activation under e.g. therapeutical intervention. We therefore sought to establish a new non-invasive fMRI protocol of rat forepaw stimulation using the potent α_2-agonist medetomidine in combination with transcutaneous monitoring of blood gases. This protocol led to map brain activation after forepaw stimulation in healthy rats, comparable with activation patterns using the standard of α-chloralose anesthesia, but permitted repetitive activation studies over weeks.

Materials and Methods

All experiments were performed in accordance with NIH guidelines and approved by local governmental authorities. Wistar rats (260-300g) were anesthetized with 1% halothane in O_2:N_2O (3:7). After intubation, placement of forepaw stimulation electrodes and a transcutaneous pCO_2 electrode (TCM4, Radiometer Copenhagen, Denmark), anesthesia was switched to the α_2-agonist medetomidine (Domitor, Pfizer, Karlsruhe, Germany; i.p. or s.c. injections of 0.15-0.3 mg/kg/h) or α-cloralose (ICN, Aurora, OH, USA; i.v. injections: bolus 50 mg/kg, infusion 36 mg/kg/h) and N_2O was replaced by N_2. Animals were mechanically ventilated during fMRI experiments.

Three different fMRI sessions were performed for each animal, with an interval of at least one week between sessions. In the first two sessions, medetomidine was used. After fMRI sessions, medetomidine effects were reversed by an i.p. injection (0.1 mg/kg) of atipamezole (Antisedan, Pfizer, Karlsruhe, Germany). In the last session, α-cloralose was used and animals were sacrificed thereafter. FMRI experiments were conducted on a 7T BioSpec animal scanner (Bruker BioSpin, Ettlingen, Germany) equipped with a 20 cm diameter autoshielded gradient insert (200 mT/m). For rf irradiation and signal detection custom-built coils were used. A 12 cm Helmholtz coil was used for rf excitation, whereas signal detection was achieved with a 3 cm surface coil. After pilot scans, SE-EPI images were acquired using the following parameters: FOV: 2.56x2.56 cm²; 64x64 points (giving an in-plane spatial resolution of 400 µm); TR=3 s, TE = 30 ms; BW=150 kHz; 1 slice of 2 mm thickness located –4.7 mm from the rhinal fissure.

Functional activation imaging was performed using BOLD contrast. Both forepaws were stimulated alternately with rectangular constant current pulses (1mA, 3Hz, 0.3ms). 115 EPI images were acquired using a paradigm in which ON vs. OFF stimulation periods were switched in a 60 s cycle (15 OFF + 5 ON), repeated 5 times, and ending with 15 OFF images.

Brain activation maps were constructed using STIMULATE (University of Minnesota, USA), using a t-test ($p<0.05$).

Results

All animals tolerated the non-invasive experimental procedure well, recovering fast (3-5 minutes) after reversal of medetomidine effects with atipamezole. Similar BOLD activation patterns and activation areas (Table 1) were observed in the somatosensory cortex of the rats during forepaw stimulation using medetomidine intraperitoneally or subcutaneously (Fig. 1), and α-chloralose (Fig. 2). Because of the achieved spatial resolution of our SE-EPI images, there was no need to overlay the activation maps over high resolution anatomical images (the standard procedure in BOLD imaging), avoiding potential errors in the co-registration process.

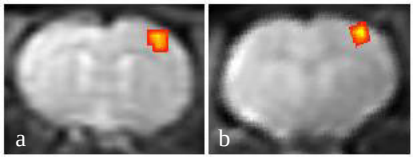

Fig. 1. Activation map of the right somatosensory cortex of one rat using medetomidine i.p. (a) and s.c. (b) at an interval of 4 weeks

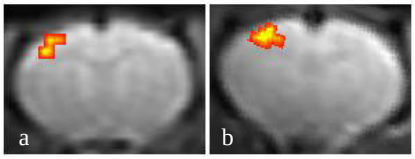

Fig. 2. Activation map of the left somatosensory cortex of one rat using medetomidine s.c. (a) and α-chloralose (b) at an interval of 1 week

	Medetomidine		**α -chloralose**
	i.p.	***s.c.***	***i.v.***
mean	9.49	5.56	5.56
SEM	5.94	2.3	2.58

Table 1. Activated area in mm^2 in all rats using medetomidine i.p. or s.c. and α-chloralose i.v.

Conclusions

To our knowledge, this is the first report of a successfully established protocol for longitudinal fMRI studies of rat forepaw stimulation. The α_2-agonist medetomidine can be used safely both subcutaneously and intraperitoneally as a repetitive anesthetic substance for fMRI studies in rats. Using medetomidine, we were able to produce robust and reproducible activation patterns after forepaw stimulation in healthy animals, which were comparable with activation patterns seen in rats anesthetized with α-chloralose, making this non-invasive fMRI protocol suitable for the assessment of time dependent changes of brain plasticity in the same animal.