Design and importance of a continuous physiologic monitoring for fMRI in rats at 7T and first results with the novel anesthetic Sevoflurane.

G.-K. Elbel[1], R. Kalisch[1], M. Czisch[1], R. Hipp[2], D.P. Auer[1]
[1]Max-Planck-Institute of Psychiatry, Magnetic Resonance Study Group, Munich, and [2]Technical University Munich, Dept. of Anesthesiology, Munich, Germany

Introduction:

Functional MRI based on the BOLD contrast could be a very powerful tool for animal studies in neurobiology, esp. in developmental and behavioral studies, where MRI offers non-invasive measurements for follow-up designs. Although first reports of fMRI in "awake" rodents exist with somewhat contradictory results [1,2], it is generally preferable to study animals under well defined and closely monitored conditions (⇨ reproducibility and validity, animal safety).

Anesthesia, however, obviously has to interfere with neuronal activity. In addition, undesired side-effects of general anesthesia further reduce the observable BOLD response upon stimulation by alteration of

(i) CBF and cerebral autoregulation
(ii) the general cardio-vascular situation (heart rate, blood pressure, vascular tonus)
(iii) the respiratory status (respiratory depression / artificial ventilation), which is reflected best by
(iv) the arterial carbon-dioxide tension p_aCO_2.

Whereas neuronal depression and effects (i) can only be controlled by the proper selection (and dose) of the anesthetic agent, effects (ii) through (iv) can be assessed and controlled during the experiment. Esp. the p_aCO_2 is most important for fMRI-experiments, as the basal cerebral blood flow CBF is closely linked to p_aCO_2 (ΔCBF=1-2 ml·100g^{-1}·min^{-1} p.1 mmHg CO_2).

The anesthetic agents in use so far in fMRI experiments all show major limitations in regard to the needs of neuro-developmental studies (sensitivity for neuronal activation, safe recovery from anesthesia):

- *α-chloralose* and *urethane* show only a minor reduction of neuronal activity and BOLD response [3,4] but due to long elimination times do not allow neither fine adjustments of anesthesia levels during the experiment nor safe animal recovery.
- *Halothane* and *Isoflurane* allow quick adjustments and safe recovery but reduce the observable BOLD response due to marked increase in CBF [5,6].

As inhalation anesthetics are generally very safe and convenient, the novel agent *Sevoflurane* might be a promising candidate for animal fMRI, as it causes remarkably little alteration of cerebral blood flow and systemic blood pressure[7].

Aim:

This work was performed to establish a reliable animal monitoring during fMRI experiments, which will allow to optimize the anesthetic regime in order to separate effects of anesthesia and stimulation. Here we report technical details of the monitoring setup as well as first experiences with *Sevoflurane*.

Methods:

MRI was performed on a BIOSPEC 7 Tesla experimental scanner (BRUKER, Ettlingen, Germany). A standard Helmholtz volume coil (inner diameter. 7.1 cm) was used for excitation, a sattle-shaped surface coil for detection.

A BOLD-sensitive T2*w FLASH sequence (TE/TR=20/93.8ms, α=22.5° , FOV 38x38mm, matrix 128^2, thickness 0.8mm) was used, 3 coronal slices were acquired every 12 sec. A trigger was provided to synchronize image acquisition and respiratory cycle.

Stimulation: CO_2 was added to the inspired gas-mixture for 2 minutes per run to achieve a defined elevation of expired CO_2 tension (Et CO_2) of 40 and 70 mm Hg (baseline 23 mmHg).

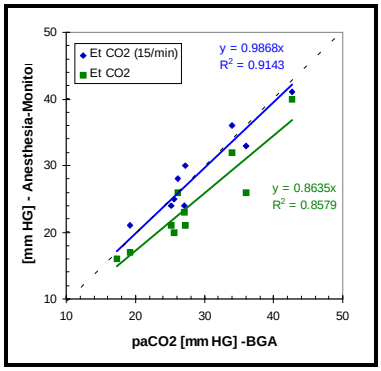
***Data analysis*:** The serial MRI data were analyzed using the FUN-Tool program of the PARAVISON software package (BRUKER). A large ROI was placed in the brain, and for all scans the averaged signal time course and the BOLD amplitude in this ROI were calculated (inter-scan displacement was absent due to fixation).

***Animal preparation*:** Male wistar rats (200-450g) were pre-anesthetized with halothane, endotracheally intubated and artificially ventilated by a pressure controlled ventilator (FMI, Germany). The tail vein and the tail artery were canulated for drug administration and blood sampling. The arterial connected to a standard pressure transducer for blood pressure measurements. Finally the animals were placed in a custom-made frame with built-in water heating, and the surface and volume coils were mounted. For ECG recording three MR-compatible electrodes were afixed to the skin of the paws.

Monitoring: the following equipment was combined: AS/3 anesthesia monitor (DATEX, Finland) for gas-analysis, invasive blood pressure and temperature, NONIN 8600V pulse oximeter with a fiberoptic sensor, Physiogard (BRUKER MEDICAL) for ECG.. In addition, optional arterial blood gas (BGA) analysis was performed (sample 350µl).

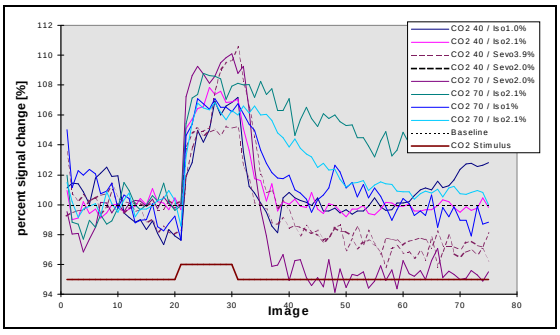
Results:

1. Continuous physiologic monitoring of rats was achieved during MRI at 7T without deterioration in signal-to-noise.
2. The pulse oxymeter did not deliver stable readings due to instable pulse wave detection. Thus the heart rate must be determined by ECG or invasive pressure monitoring.
3. Determination of the correct end tidal CO_2 (gas monitor) as compared to p_aCO_2 (BGA) was hindered by the mixing of inspiratory and expiratory gases in the sampling line from the animal to the monitor (approx. 4m). Here, reducing the respiratory rate down to 15 per minute for 3 cycles allows proper measurement .



4. With this continuous and reliable monitoring a *stable anesthesia up to 14 hours* (with quick recovery) was achieved allowing complex experimental designs with multiple intraindividual testing or pharmacological MRI.
5. Preliminary results with *Sevoflurane* anesthesia show larger and clearly graded BOLD amplitudes (see table) upon different stimulus (CO_2) and anesthesia (MAC value) levels:

EtCO₂	40 mmHg		70 mmHg	
MAC	0.75	1.5	0.75	1.5
Isoflurane	6.5	6.8	7.2	6.3
Sevoflurane	7.5	5.1	***9.2***	7.2



Discussion & Conclusion:

Continuos monitoring of all relevant physiological parameters is feasible- pCO_2 and blood pressure data should be considered during data analysis of BOLD experiments. In addition, *Sevoflurane* seems to be a potential anesthetic agent for fMRI that might allow for both proper and quick control of anesthesia depth and high sensitivity for neuronal activation.

References:

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