Effect of four commonly used anesthetics on stimulation-induced and resting state fMRI signal in mice

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INTRODUCTION: With the advent of transgenic technology and its potential for biomedical research, functional MR imaging in mice is receiving particular interest. Anesthesia is a recurring issue in animal imaging, in particular when assessing the brain function. A significant number of studies characterizing the influence of different anesthetics on functional readouts have been carried out (for review see [1]). It was found that fMRI signals depend on the choice of anesthetic and level of sedation. Given species differences, results obtained in rat studies cannot be translated to mice in an unreflected manner. Therefore, we investigated the influence of four commonly used anesthetics - isoflurane, urethane, medetomidine, and propofol - on temporal and spatial dynamics of stimulation-induced fMRI signal changes, as well as on resting state in the mouse brain.

METHODS: Animal preparation and Anesthesia: Female C57Bl/6 mice were intubated, artificially ventilated with an air/oxygen mixture (90%/10%), stereotactically fixated; physiological parameters (temperature, blood gas level) were monitored. N=6-8 mice were used per anesthetic regime. After an initial induction with 3% isoflurane, the following doses of anesthesia were used: (A) 1% isoflurane, (B) 1.5g/kg urethane (i.p.), (C) Bolus of 0.1mg/kg medetomidine with subsequent infusion of 0.2mg/kg/h (i.v.), (D) Bolus of 30mg/kg propofol with subsequent infusion of 150mg/kg/h (i.v.). All mice were paralyzed using pancuronium bromide (i.v.). FMRI: Experiments were carried out on a Bruker BioSpec 94/30 (Bruker BioSpin MRI, Ettlingen, Germany) horizontal bore MR system using a four-element receive-only cryogenic phased array coil (Bruker BioSpin AG, Fällanden, Switzerland) in combination with a linearly polarized room temperature volume resonator for transmission. For the acquisition of BOLD responses a GE-EPI sequence with the following parameters was applied: 12 axial slices; STH/ISD=0.5/0.5mm, in-plane spatial resolution=185x312µm^2; TE/TR=10/1000ms; NA=1; temporal resolution=1s. Electrical stimulation: Bipolar needle electrodes were placed subcutaneously in the right plantar hindpaw. After a baseline of 180s a repetitive block design was used comprising 4 cycles, that each consisted of an on-period of 20s and an off-period of 120s, applying electrical pulses of 0.5ms duration at a frequency of 5Hz with amplitudes of either 0.5mA, 0.7mA or 1.0mA, respectively. Data analysis: Stimulation-induced and resting state fMRI signal data were analyzed using different MR image data processing software and Matlab.

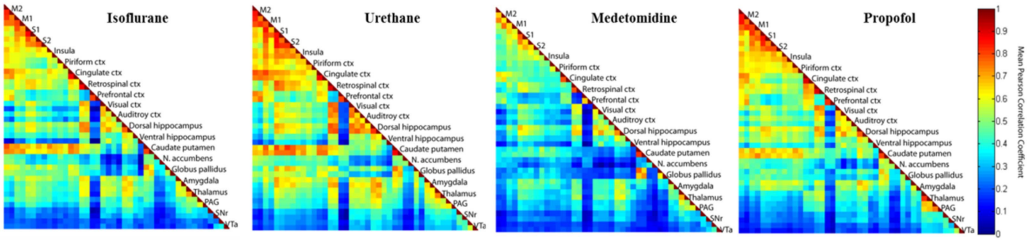

Fig. 1: Maps show similar correlation of network activation for isoflurane, urethane and propofol anesthesia, whereas the data recorded from medetomidine-anesthetized mice show smaller values for the Pearson's correlation coefficient.

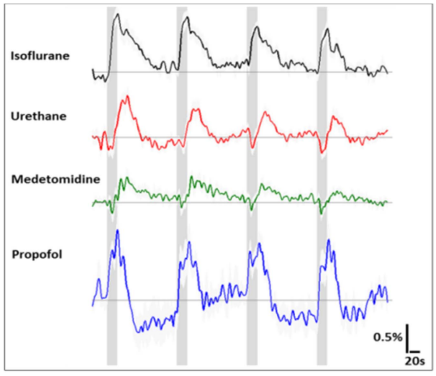

Fig. 2: Averaged BOLD signal change when stimulated with 1mA extracted from the contralateral S1 hindpaw area for each anesthesia group.

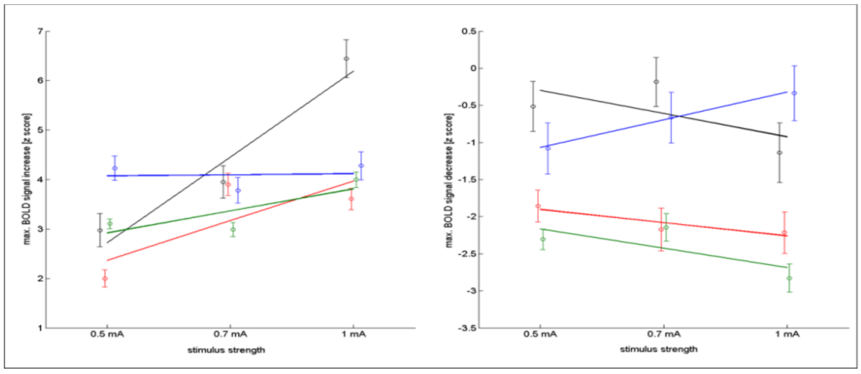

Fig. 3: The left plot depicts the dependency of BOLD signal change on the stimulus strength. With increasing stimulus amplitude all except the propofol-anesthetized mice show a larger BOLD signal intensity. The right plot depicts the occurrence of an initial dip depending on the stimulus strength. With increasing stimulus amplitude, urethane- and medetomidine-anesthetized mice show a larger decrease of BOLD signal preceding the increase.

RESULTS AND DISCUSSION: The analysis of resting state fMRI data resulted in network correlation maps that were rather similar for the three anesthetics isoflurane, urethane, and propofol, while the map generated for the medetomidine group deviated significantly (Fig.1). These data might allow a distinction of different states of anesthesia and can be correlated with the outcome of the stimulus-based functional activation experiment.

Electrical hindpaw stimulation induced different BOLD signal responses depending on the anesthetic used. Under isoflurane, urethane and medetomidine anesthesia the BOLD signal was found to depend on the stimulus intensity, with 1mA exerting the biggest response (Fig. 3). In contrast, the BOLD response under propofol did not change upon increasing the current amplitude from 0.5 to 1mA (Fig. 3). For all anesthetics tested except propofol, the first of the four stimulation blocks always showed the strongest change in signal intensity irrespective of stimulation amplitude used (Fig. 2). For propofol we did not observe any decrease in BOLD amplitude for subsequent stimulation cycles (Fig. 2). Urethane- and medetomidine-anesthetized animals showed a pronounced initial dip preceding the positive BOLD signal change, which became more prominent with increasing stimulation strength and for later stimulation blocks, being largest for the last block (Fig. 3). This might indicate an increasing delay in the onset of the vascular response. The peak time of positive signal change was shortest under propofol anesthesia, followed by isoflurane (Fig. 2). When analyzing different regions involved in the processing of the sensory stimulus on the contra- and ipsilateral side of the brain (such as S1, M1, S2, Thal), isoflurane and propofol showed a homogeneous profile of the BOLD signal response corresponding to that in the contralateral S1 hindpaw area. However, the profile of the BOLD signal change in the urethane and medetomidine-anesthetized animals was found dependent on the brain region. This non-uniformity of the hemodynamic response across different brain areas will impact the computation of activation maps. We aim to characterize the temporal and spatial dynamics of the BOLD signal response by also including perfusion measurements, as CBV and CBF data will help explaining characteristics of onset, peak times and of the decay, or the observed initial dip. Measurement of the hemodynamic response function (HRF) for the different anesthetics will allow modeling the BOLD signal change under a particular anesthetic regime and allow generating representative activation maps.

Overall, the cascade of neurophysiological, metabolic and hemodynamic processes that link the effects of a sensory stimulus to measurements of a change in the BOLD signal is obviously highly complex and susceptible to the differing effects of the various anesthetic agents. The inferences that can be made from the fMRI signal strongly depend on the type of anesthesia applied.

REFERENCES: [1] Masamoto et al. Journal of Cerebral Blood Flow & Metabolism 32, 1233–1247 (2012).