

# Functional connectivity of the mouse brain is influenced by state of consciousness: a comparison of awake and differentially anesthetized mouse rsfMRI protocols.

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**Purpose:** The use of resting state functional MRI (rsfMRI) in preclinical research is expanding progressively, but the majority of resting state imaging in small animals is obtained under anesthesia. Since anesthesia may considerably change the physiology of the animal and hence may influence the functional connectivity (FC) outcome, we compared in this study rsfMRI data from awake mice with rsfMRI results obtained from mice anesthetized with  $\alpha$ -chloralose, urethane or isoflurane (1%).

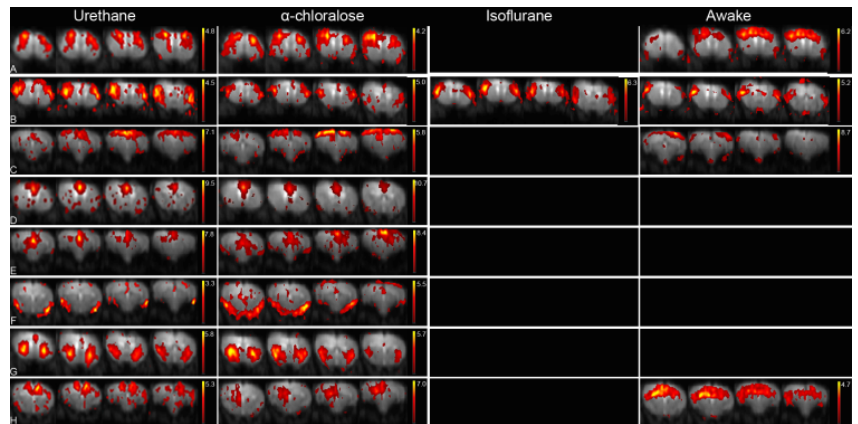
**Methods:** Twenty-seven male C57BL/6 mice (Charles River Laboratories, Wilmington, USA) (Body weight  $23 \pm 2$  g) of approximately 13 weeks of age were imaged. Animals were divided in three groups of nine. The first group was anesthetized with a low dose of isoflurane (1%) (IsoFlo, Abbott, Illinois, USA) [1]. Nine other animals were anesthetized using urethane (Sigma-Aldrich, Missouri, USA) (0.2 ml (2.5g/kg) IP) [2]. The last group was anesthetized using a bolus of 0.2 ml  $\alpha$ -chloralose (120mg/kg) (Sigma-Aldrich, Missouri, USA)[3]. Five of the nine animals measured under isoflurane anesthesia were measured again in awake conditions after an acclimatization protocol based on the method used for awake rat rsfMRI by King and colleagues [4].

**Imaging** was conducted on a 9.4T Biospec scanner (Bruker, Ettlingen, Germany). The resting state data was acquired using GE EPI with TR 2000ms and TE 15.2ms. Sixteen axial slices of 0.4mm (gap of 0.1mm) were recorded with a FOV of (20x20)mm<sup>2</sup>, a matrix size of 128x64 and a bandwidth of 400 kHz. Hundred fifty repetitions were taken, resulting in a 5 minute scan time for each dataset.

**Preprocessing** was done in SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>) using a common protocol for fMRI data consisting of realignment, normalization and in plane smoothing (FWHM twice voxel size).

To estimate FC, **Independent Component Analysis (ICA)** with a preset of 15 and 40 components was performed per group [5], using GIFT (<http://icatb.sourceforge.net/>). Resulting components were assigned to anatomical meaningful regions using the Paxinos atlas [6] and compared between different anesthesia regimes.

**Results:** 15 components ICA (each group separately) yields *bilateral* components which were strongly depending on the anesthesia protocol. The figure presents the spatial differences of the 15-ICA maps between the 4 regimes. 40 component ICA analysis resulted in components coinciding with similar anatomical regions and although the results were not completely overlapping, overall the same regions could be identified under all regimes. A detailed comparison of the different groups for both the 15 and 40 ICA is presented in the table. Awake data resulted in components co-localized with the same anatomical regions, nevertheless, the components were more globally distributed and less well aligned with anatomy.



Outcome of the 15 component analyses. Mean components (z-maps) coinciding with anatomical relevant regions are shown: motor cortex (A), somatosensory cortex (B), visual cortex (C), Cingulate cortex (D), retrosplenial cortex (E), Piriform cortex (F), striatum (G), hippocampus (H)

Table: Comparison of the components resulting from rsfMRI data obtained under different anesthesia regimes with a preset of 15 or 40 components.

	Urethane		$\alpha$ -chloralose		Isoflurane		Awake	
	40	15	40	15	40	15	40	15
motor cortex	B	B	B	B	B		B	B
primary somatosensory cortex	U	B	U	B	U	B	U	B
secondary somatosensory cortex	B		B		B			
somatosensory cortex barrel field	U		U		U		U	
visual cortex	U	B	U	B	U		U	B
cingulate cortex	B	B	B	B			B	
retrosplenial cortex	B	B	B	B	B		B	
piriform cortex	U	B	U	B	U		U	
striatum	U	B	U	B	U		U	
hippocampus	U	B	U	B	U		U	B

B = Bilateral, U = Unilateral

**Discussion and Conclusion:** Overall, we can conclude that using ICA to define regions and to perform a second level FC analysis with ICA based ROIs, a 40 component analysis can be performed for all anesthesia protocols and in awake mice. But to study interhemispheric FC using ICA in mice particularly, a 15 component analysis has to be used in urethane or  $\alpha$ -chloralose anesthetized animals which allow to detect interhemispheric FC in control animals and possible interferences in different models. Moreover awake mouse rsfMRI is proven to be possible and useful to study FC but needs to be optimized in future studies examining the best training or habituation protocol.

**References:** [1] Wang et al. 2011; [2] Janssen et al. 2004; [3] Ahrens et al. 2001 [4]; King et al. 2005; [5] Jonckers et al. 2010; [6] Paxinos and Watson 1982