

# Investigating the influence of anesthesia on resting state connectivity in rats using multiple analysis techniques

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## Introduction

Recent research has shown evidence of functional connectivity in rodents, indicating the potential of using rats as a model for human resting state connectivity<sup>1,2</sup>. A potential confound of using a rat model is the necessary use of anesthesia<sup>3,4</sup>. This study employs multiple analysis techniques, including cross-correlation, principle component analysis (PCA), and independent component analysis (ICA) to compare the connectivity between contralateral somatosensory cortices in rats anesthetized with  $\alpha$ -chloralose, isoflurane, and medetomidine hydrochloride.

## Method and Materials

Three groups of rats were imaged with a single-shot gradient-echo EPI sequence: (A)  $\alpha$ -chloralose anesthesia (n=7), 11.7 T Bruker scanner with TR/TE/FOV of 100 ms/20 ms/1.92 cm; (B) isoflurane anesthesia (n=6), 9.4 T Bruker scanner with TR/TE/FOV of 100 ms/20 ms/2.56 cm; and (C) medetomidine hydrochloride (n=3), 9.4 T Bruker scanner with TR/TE/FOV of 100 ms/20 ms/2.56 cm. A 2 mm coronal slice was positioned covering the bilateral primary somatosensory cortex. Each series consisted of 1200-3600 images with a matrix size of 64x64. Activation maps were also acquired during electrical stimulation of the forepaw in order to identify the primary somatosensory cortex (SI). In the unstimulated data, a region of interest (ROI = 2 x 2 pixels) was chosen in SI of a thresholded and low-pass filtered (cutoff = 0.14Hz) dataset, and the time course from the ROI was correlated with the time courses from all other pixels in the brain to make a resting-state correlation map. Pixels with cross-correlation values considered to be significant for resting state data ( $r \geq 0.35$ ) were counted from the cortex of each dataset and normalized by the total number of significant voxels in the brain. To remove user bias inherent in cross-correlation analysis, PCA and ICA were implemented on the datasets using FastICA<sup>5</sup>. PCA and ICA were implemented on the datasets on a temporal basis rather than a spatial basis because of the long time dimension of the datasets.

## Results

Cross-correlation maps were created for each group. Maps from group A consistently demonstrate increased correlation in the cortex (significant voxels = 79.0% $\pm$ 10). Correlation maps created from group B occasionally showed increased correlation in the cortex versus the rest of the brain, but often increased correlation occurred throughout the brain (significant voxels = 27.2% $\pm$ 7.7). Correlation maps created from group C demonstrate correlation within the cortex (significant voxels = 83.8% $\pm$ 23.2). PCA was used to reduce the dimensions of the datasets; the number of components chosen was based on a criterion of 90% variance explained. The remaining components were used for ICA. Group A results for ICA consistently generate a low-frequency component that maps to the somatosensory cortex (Fig. 1), as well as components whose power spectra peak around 1 Hz (respiratory rate) and 4-5 Hz (heart rate) and map accordingly to their physiological regions (ventricles, base of brain, not shown). Group B ICA results showed components that can be attributed to respiration and cardiac effects, but no clear low frequency component resulted (Fig. 1). Group C ICA results generate several components with low-frequency power contributions that map to SI (Fig. 1).

## Discussion

This study demonstrates the influence of anesthesia on resting state functional connectivity in rats using multiple analysis techniques. Rats anesthetized with  $\alpha$ -chloralose and medetomidine hydrochloride demonstrate the presence of resting state connectivity, but no data analysis technique found low-frequency synchrony in isoflurane-anesthetized rats. It has been shown that isoflurane affects phase synchrony between distant areas of the brain<sup>6</sup>, which may explain lack of evidence of resting state connectivity under such conditions. Because  $\alpha$ -chloralose does not permit survival experiments, in the interest of longitudinal studies, it is important to identify anesthetics that allow survival but do not disrupt spontaneous fluctuation. This research motivates further investigation of resting state connectivity using medetomidine hydrochloride.

**References:** 1. Williams, K.A. et al, *Proc ISMRM 2006*, 2119. 2. Lu, H., et al. *Proc ISMRM 2006*, 532. 3. Peltier, S.J., et al. *Neuroreport*, 2005.16(3):285-8. 4. Austin, V.C., et al. *NeuroImage*, 2005. 24:92-100. 5. Hyvarinen, A., E. Oja, *Neural Netw*, 2000.13(4-5):411-30. 6. Imas, O.A., et al. *Neuroscience Letters*, 2006. 402:216-21.

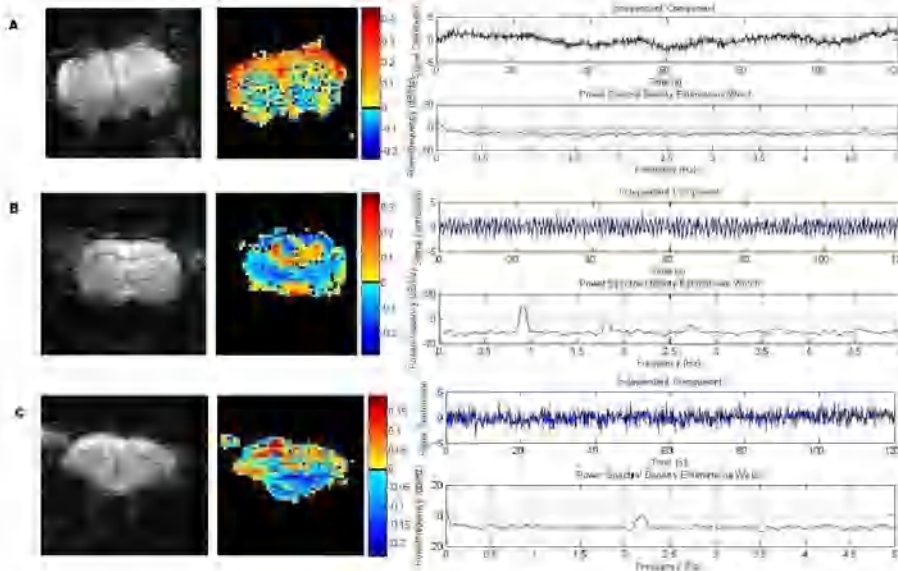


Figure 1. (A) Low frequency independent components (IC) map spatially to the somatosensory cortex for  $\alpha$ -chloralose data (time=120 s). (B) IC from isoflurane data whose power spectrum peaks at ~1Hz maps to regions that correspond to respiration effects. (C) An IC with low frequency contributions maps to the somatosensory cortex in medetomidine hydrochloride data. The time course of each IC and its power spectrum are displayed beside maps. EPI images are displayed on left for anatomical reference.