# Hypoxia Decreases Resting-state Functional Connectivity in Anesthetized Rats

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## INTRODUCTION:

Resting-state functional MRI (rsfMRI) utilizing blood-oxygen-level-dependent (BOLD) contrast has increasingly used to map inter and intra-hemispheric connectivity in normal and diseased brains [1]. BOLD is a complex function of changes in oxygen metabolism, cerebral blood flow (CBF), and cerebral blood volume (CBV). While the effect of hypoxia on baseline and stimulus-evoked BOLD signal were documented [2], how BOLD synchronization or rsfMRI can be affected by hypoxia has not been investigated. This study aimed to assess the effect of mild and severe hypoxia on BOLD rsfMRI connectivity in an animal model.

## METHODS:

***Animal preparation:*** Adult male SD rats (n=5, 200-250g) were anesthetized with isoflurane under ventilation (3% for induction and 1.5% for maintenance) and kept warm with a water pad. Heart rate, $SpO_2$, and rectal temperature were monitored. For each rat, normoxia, mild and sever hypoxia (air, 15% $O_2$ and 8% $O_2$ (balanced $N_2$)) inspiration were studied sequentially. Before scanning each hypoxia condition, 3 mins of rest were allowed for the rats' physiology to stabilize. Respiration rate was set as 60 during normoixa and mild hypoxia, but as 80 during severe hypoxia.

***MRI Protocols:*** All MRI experiments were conducted using a 7T Bruker scanner with a surface coil. For each condition, three rsfMRI acquisitions were performed using a single-shot GE-EPI sequence with TR/TE=1000/18ms, flip angle=$30^0$, FOV=32×32mm$^2$, 64×64 matrix, 9 1-mm-thick contiguous slices and a total of 280 data points. RARE T2W images were acquired using TR/TE=4200/36ms as an anatomic reference for EPI data.

***Data analysis:*** All rsfMRI data was slice-timing corrected, coregistered,detrended and temporally low-pass filtered. Group independent component analysis (ICA) was performed using GIFT v1.3h Toolbox. The number of components was set at 20 and the spatial maps of independent components were scaled to z scores with a threshold of z>2 (correlation coefficient >0.35). Strength of an identified resting-state network (RSN) was quantified as averaged z-score and statistical tested between conditions using paired test with $p<0.05$ considered to be significant.

## RESULTS:

Fig.1 shows the group ICA maps of RSNs during each inspiration condition. Caudate putamen (CPu), secondary somatosensory cortex (S2) and primary somatosensory cortex (S1) maps exhibited diminished rsfMRI connectivity during mild hypoxia. During severe hypoxia, only a very weak RSN was observed covering the aforementioned brain structure. Statistical tests confirmed the significant reduction of mean z-score in primary and secondary somatosensory cortex but not in caudate putamen (Fig 2).

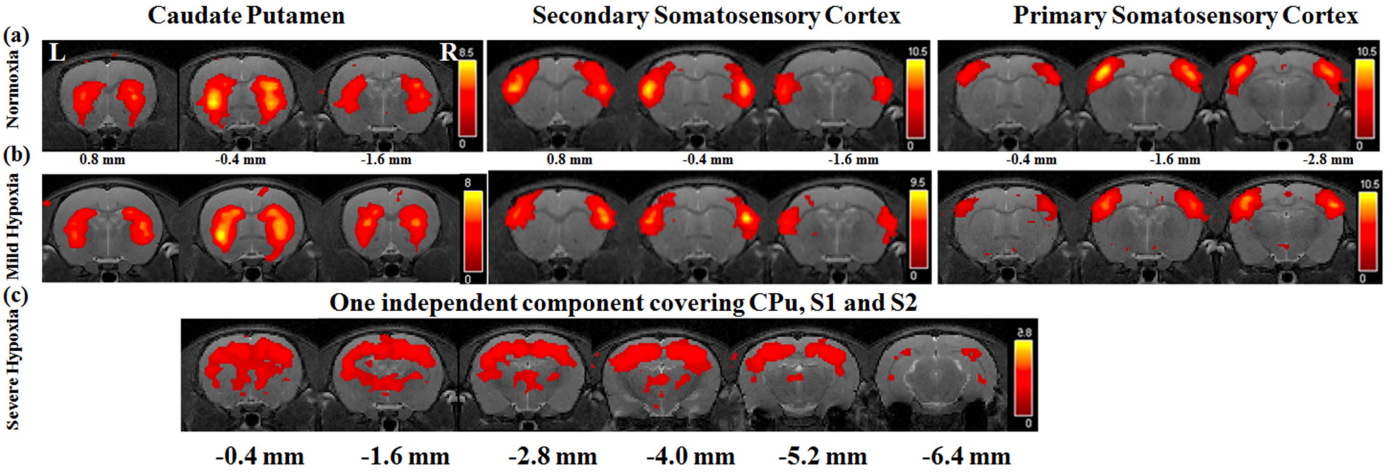


**Fig 1:** Typical rsfMRI connectivity maps with the components covering caudate putamen (CPu), secondary somatosensory cortex (S2) and primary somatosensory cortex (S1), respectively, from animals under normoxia (a), mild hypoxia (b), and sever hypoxia (c). Distance to Bregma for each slice is labeled at bottom.

## DISCUSSIONS AND CONCLUSIONS:

This study demonstrated that both mild (15% $O_2$) and severe (8% $O_2$) hypoxia diminished rsfMRI connectivity significantly. This is supported by electrophysiology studies in anesthetized animals showing a decrease in the total power of EEG during acute hypoxia [3, 4]. Microstructural deterioration such as cytotoxic edema, vasogenic edema and vessel wall damage result from intravascular pressure might also impair the functional connectivity [5]. Furthermore, CBF and CVB could significant increase during hypoxia as compensation[6]. These findings indicated that alterations in physiologic conditions, vascular characteristics and hemodynamic regulations can affect rsfMRI. Therefore, caution much be taken in designing experiments and interpreting rsfMRI.

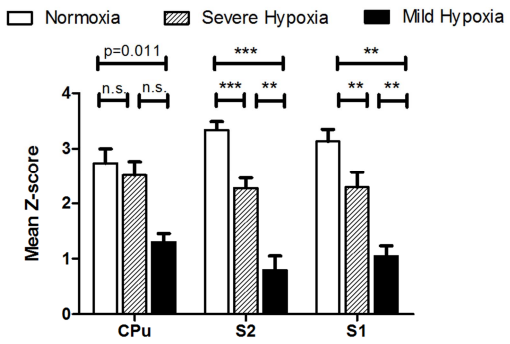


**Fig. 2:** Mean and standard error of the mean (SEM) of averaged z-score of each independent component reflecting the strength of rsfMRI networks. *(n.s. no significance, ** p<0.005, *** p<0.001)*

## REFERENCES:

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