Altered Baseline Oxygenation and Blood Flow Perturb Resting-state Functional Connectivity – a Nonhuman Primate Study

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Introduction Functional connectivity (fc) is inferred from spontaneous BOLD signal fluctuations arising from low frequency (<0.1Hz) brain activity. Spatially distinct regions with synchronized brain activity comprise intrinsic connectivity networks (ICNs) [1-3]. Although the underlying neurophysiology of such measure remains elusive, it has been repeatedly demonstrated in normal subjects and across species [3-5]. Recently, altered ICNs have been shown in many disorders, such as Alzheimer's, ADHD, stroke, epilepsy, among others. It is unclear how perturbed baseline physiology may impact fc measurements, especially those introduce large changes on baseline oxygenation and/or blood flow.

In this study, we aimed to systematically investigate the effects of different basal oxygenation and blood flow conditions on fc measurements. Such an experiment is difficult to perform on normal volunteers because of long scan time and multiple manipulations involved. Therefore, we carried out the study on nonhuman primates (baboons). We hypothesized that cerebral connectivity, when compared to normal air condition, is reduced under hypercapnia and hyperoxia and results in a reduction of fc indices, however, it remains comparable under hypoxia. These findings could have strong implications when applying resting-state fMRI in disease states.

Methods Four baseline conditions – *air, mild hypoxia (15% O_2), hyperoxia (100% O_2), and hypercapnia (5% CO_2)* – were introduced to the subjects within each session. A ten to fifteen min rest period was given between conditions to stabilize the physiology. Twelve resting-state fMRI scans were acquired under each condition (results in a total of *48 scans*) from five normal baboons (16-20 kg) in seven MRI sessions. Animals were anesthetized with 0.8~1.0% isoflurane with vecuronium (0.1mg/kg first dose, 0.02 mg/kg supplement dose as needed) and mechanically ventilated. End-tidal CO_2, O_2 saturation, heart rate, respiration rate, and rectal temperature were monitored continuously and maintained within normal ranges. All MRI studies were performed on a 3T Siemens TIM TRIO using an 8-channel coil. Gradient echo EPI was used for BOLD images with parameters: TR/TE=2000/30 ms, matrix = 64x64, field of view (FOV) = 12.8x12.8 cm (2 mm isotropic resolution), and 27 slices. Resting-state fMRI was acquired over 10 min.

Data were processed using FSL and AFNI. Image pre-processing includes motion correction, skull stripping, temporal band-pass filtering at 0.01-0.1 Hz, 4 mm spatial smoothing, and nine parameters nuisance extraction. All images were registered to high-resolution brain templates. Temporal-concatenated independent component analysis was performed using the MELODIC toolbox in FSL. Twenty independent components (IC) were pre-defined for the number of output networks. Dual regression analysis using the group IC maps generated was implemented for each subject and a one-way ANOVA was used for group comparisons. Cluster size and fractional amplitude of low frequency fluctuation (fALFF) was calculated for each ICN. Baseline CBF and percent BOLD and CBF changes in response to unilateral vibrotactile stimulation were also calculated.

Results and Discussion **Figure 1** shows three selected ICNs from the group results, including visual, somatosensory, default mode network under each baseline condition. The spatial extent of the network was larger under air than those under hypercapnia and hyperoxia, but comparable to hypoxia. Cluster sizes of these ICNs are summarized in Table 1. fALFF also showed a similar trend to cluster size. Our results comparing fc under air and hypercapnia are in agreement with an early study, which demonstrated reduced fc measurements and metabolism under hypercapnia, and suggest that subjects enter a lower arousal state with decreased oxygen metabolism [6]. Our results under hypercapnia also showed a reduced fc when compared to air, and such reduction is larger than that under hyperoxia. Under mild hypoxia, it is suggested that vascular reactivity is intact, while oxygen will likely being extracted more efficiently under compromised condition [8]. Our results seem to be in accordance with that study because stronger fc was found in a few ICNs (such as SM in fig 1), which might suggest higher oxygen utilization.

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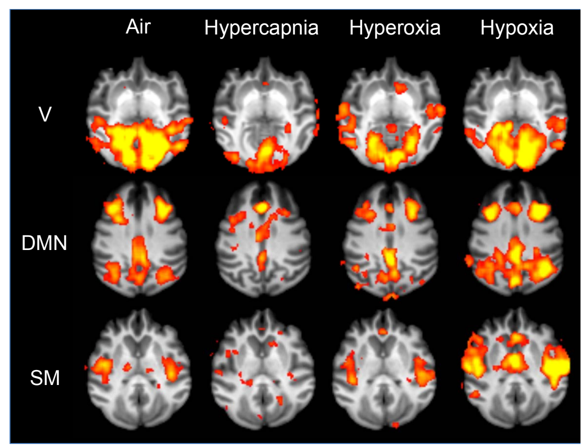

Figure 1. Group ICNs including visual (V), default mode network (DMN), and somatosensory-motor (SM) network under each condition. (color scale: Z: 3 – 8)

	Cluster (voxels)				
	V	SM	Aud	DMN	Sub
air	2204	94	193	220	150
hypercapnia	82	106	106	30	105
hyperoxia	145	76	174	257	45
hypoxia	606	383	779	591	138

Table 1. Group-averaged cluster size for six selected ICNs. V: visual, SM: sensory-motor, Aud: auditory, DMN: default mode network, Sub: subcortical network.

Conclusions We showed that the functional connectivity derived from BOLD fMRI could be affected by altered baseline oxygenation and blood flow. Cautions should be exercised when studying functional connectivity changes in disease states in which baseline oxygenation and blood flow are markedly altered.

References: [1] Biswal et al., MRM (1995). [2] Fox and Raichle. Nat Rev Neurosci (2007). [3] Smith et al., PNAS (2009). [4] Vincent et al. Nature (2007). [5] Biswal et al., PNAS (2010). [6] Xu et al., JCBFM (2011). [7] Kashikura et al., NeuroReport (2000). [8] Ho et al., NeuroImage (2008).