Hemodynamic changes can be detected in rat white matter using a hypercapnic challenge

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Introduction

We have recently reported evidence for white matter (WM) functional magnetic resonance imaging (fMRI) activation in humans (e.g., [1, 2]). WM fMRI activation has potential to expand current approaches for studying brain connectivity by measuring activation patterns in the connections themselves, rather than inferring the functional involvement of connections on the basis of functional connectivity analyses and/or structural connectivity approaches such as tractography. WM fMRI activation also has potential clinical applications, such as improving the diagnosis and assessment of WM disorders. For example, current clinical MRI measures for multiple sclerosis (MS) often do not correlate well with the functional deficits experienced by patients (i.e., the clinico-radiological paradox [3]). By evaluating the functional status of WM regions, WM fMRI activation may provide a metric that is more closely related to the patient's functional deficits.

The neurophysiologic bases of fMRI activation in WM are not understood, which currently limits interpretation and application of WM fMRI activation. The first step towards investigating the neural and hemodynamic events that underlie WM fMRI activation is to confirm that hemodynamic changes can be detected in the WM of an animal model. To do this, we used a hypercapnic challenge to elicit whole brain activation in the rat. Previous work has demonstrated hemodynamic changes in both gray matter (GM) and WM associated with hypercapnia in humans (e.g., [4]), but only GM activation has been examined in rats. In addition to blood oxygen level dependent (BOLD) fMRI, we acquired cerebral blood volume (CBV) fMRI to evaluate the contribution of blood volume changes to WM fMRI activation.

Methods

Acquisition: The experiment used a Magnex Scientific 3T magnet with 0.2T/m gradients, interfaced with a Varian DirectDrive Console. A 52mm inner diameter quadrature radiofrequency coil (built in-house) was used for transmit/receive. Anatomic images with 200µm isotropic resolution were acquired using 3D TrueFISP (TR = 10, TE = 5ms, 50˚ flip, 160×160×160 matrix, 32mm field of view (FOV), 8 averages). Spiral fMRI data were collected (TR = 600ms, TE_{BOLD} = 20ms, TE_{CBV} = 3ms, 4 shots, 60˚ flip, 128×128 matrix, 32mm FOV, 12 1mm interleaved coronal slices, no gap). Given the small size of WM structures (e.g., the corpus callosum), high spatial resolution (250×250µm in-plane) was required to reduce partial volume effects. CBV fMRI was acquired after intravenous administration of a contrast agent (28mg/kg, Molday ION super paramagnetic iron oxide [SPIO]). Long-Evans rats (N=3, 263-300g) were anesthetized via an intraperitoneal injection of urethane (1.6g/kg). Rats were immobilized using a head holder with ear bars (built in-house). A nose cone was placed over the snout for administration of gases. The hypercapnic challenge was preceded by a 2min baseline during which the rat breathed medical air. This was followed by 6min of 5% CO_2 (balance air) alternated with 6min of medical air (repeated thrice).

Analysis: The anatomic images were segmented in Statistical Parametric Mapping (SPM) [5] using the SPMMouse toolbox [6] to create GM and WM probability maps, which were used to create GM and WM regions of interest (80% probability threshold). The olfactory bulb, midbrain, and brainstem were excluded from the analysis. The brain was extracted by creating a mask from the BOLD images using a 10% intensity threshold (manually corrected). The mask was subsequently registered and applied to the CBV images. FMRI motion correction was performed in SPM. After temporal (720s cutoff) and spatial (375µm full width at half maximum) smoothing, fMRI statistical analysis was performed in FMRIB Software Library [7] using the general linear model. Voxels containing large vessels were removed by creating a vessel map from the difference of 3D TrueFISP images acquired before and after SPIO injection. A threshold was applied to the resulting image such that voxels with intensity differences below the 5th percentile were considered large vessels (Figure 1). Activation was modeled as a boxcar function representing the hypercapnia paradigm convolved with a sine basis function (120s window). Z scores were calculated using a cluster-level correction for multiple comparisons ($z > 2.3$, $p < 0.05$). Percent signal change was calculated for significantly activated voxels.

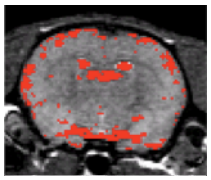

Figure 1. Example vessel map.

Table 1. Grand average (N=3) mean percent signal change (± standard deviation) for significantly activated voxels.

	BOLD	CBV
GM	3.2 ± 1.6	3.4 ± 1.4
WM	3.3 ± 1.2	3.8 ± 1.5

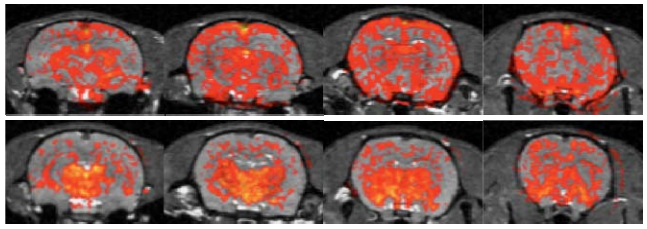

Figure 2. Coronal sections of BOLD (top) and CBV (bottom) activation during a hypercapnic challenge (representative animal).

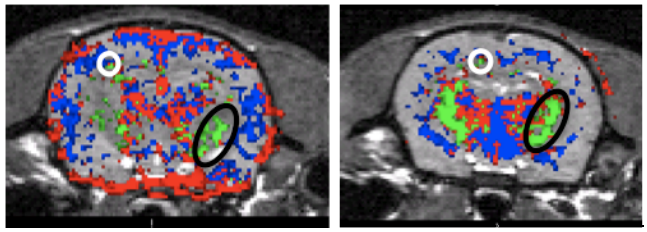

Figure 3. GM (blue) and WM (green) activation associated with a hypercapnic challenge for BOLD (left) and CBV (right) fMRI in a representative animal. White circles highlight example corpus callosum activation; black ovals highlight example internal capsule activation. Red indicates activated voxels that were either 1) not identified as GM or WM, or 2) associated with large vessels (see Methods).

Results and Discussion

Activation was reliably elicited using a hypercapnic challenge in both GM and WM (Figure 2). Percent signal change for BOLD and CBV are presented in Table 1 for GM and WM separately. The results established that hemodynamic changes can be detected in rat WM (including regions such as the corpus callosum and the internal capsule) using both BOLD and CBV fMRI (Figure 3). Future work will investigate whether BOLD and CBV changes are also associated with neural activity in rat WM (e.g., by stimulating the corpus callosum with intracortical electrodes while simultaneously acquiring fMRI data).

In the CBV data, the large dose of SPIO may have reduced signal in regions with extensive vasculature (e.g., the pial surface of the cortical GM) such that hypercapnia-related signal changes were not measurable. Future work will evaluate SPIO dose to determine if the optimal dose (in terms of the resulting percent signal change) depends on the tissue type and/or brain region under investigation.

Conclusion

We have shown that rat WM has the capacity to support hemodynamic changes due to hypercapnia. To our knowledge, this is the first report of BOLD fMRI activation in the WM of the rat, as well as the first demonstration of CBV fMRI activation in the WM of any species. These findings will serve as the foundation for future work investigating the neurophysiologic bases of WM fMRI activation.

References

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