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Vascular Involvement with Bilateral Connectivity in BOLD and CBV-Weighted Functional Magnetic Resonance Imaging of the Rat Somatosensory System

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Abstract

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By Rahul M. Varman

Functional magnetic resonance imaging (fMRI) can reflect measures of brain activation but can also show discrepancies due to underlying physiological processes. Previous studies have suggested that vasculature can play a large role in causing such variance. Scans using different modalities of fMRI such as blood oxygen level dependent (BOLD) or cerebral blood volume (CBV) can sometimes show differential results due to the underlying vascular topography. Recent studies have shown that there exists differential bilateral connectivity in the brain and that BOLD and CBV-weighted scans register similar patterns showing this pattern, albeit with different magnitudes. It remains to be examined how underlying vascular profile can affect bilateral connectivity at different regions of the brain. We compared resting state bilateral connectivity between various regions associated with the somatosensory system from both BOLD and CBV acquisitions from five rats with vascular density profile rendered from functional ultrasound. We observed that there exists a correlation between how strongly a region of the brain showed bilateral connectivity over time and the vascular density evident in that region. BOLD and CBV scans showed similar patterns of activation and connectivity, albeit with different magnitudes as shown before. We conclude that vasculature may in fact play an important role in bilateral connectivity in resting state rats. Further experiments should address whether such correlations exist in other regions of the brain and whether BOLD and CBV continue to show similar yet different magnitude results.

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Table of Contents

INTRODUCTION

Background

Functional magnetic resonance imaging (fMRI) is a procedure that reflectively measures brain activation by detecting associated changes in blood flow (Heeger 2002). Neurons require energy in the form of glucose and oxygen which are transported in blood. Haemodynamic response to active neural assemblies is to increase blood flow to those areas while decreasing blood flow to inactive assemblies (Huettel 2004). The mechanism for this neurovascular coupling is still not fully understood, and thus the exact relationship between the measured fMRI signal and associated neural activity remains unclear (Logothetis 2001).

Various signals including changes in cerebral blood flow (CBF), cerebral blood volume (CBV), and oxygenation in the brain vasculature accompany changes due to neural activity. These components coupled with changes in oxygen metabolism comprise the blood oxygen level dependent (BOLD) signal (Shen 2008). fMRI machines can detect changes of such signals by measuring variations in magnetization of various endogenous and exogenous moieties within the blood. Although these various modalities can suggest similar activations, they often exhibit different spatial and temporal responses (Kim 2008). Studies examining stimulus-induced activation have suggested that CBV-weighted imaging may confer more localized and sensitive results than BOLD in certain areas since the CBV is less sensitive to larger blood vessels near activated areas (Mandeville 2001).

Subsequent research on low-frequency fluctuations in fMRI activity suggest that there exist areas of brain through which brain activity propagates through time (Majeed 2009). There exists strong bilateral symmetry correlation for activation between left and right areas of interest during these spontaneous fluctuations (Kannurpatti 2008). Recent studies show that although BOLD and CBV suggest similar patterns of spatial correlation, BOLD-weighted scans exhibit higher cross-correlation values. Interestingly, both aforementioned modalities confer distinctive power spectra during resting state scans (Magnuson 2010).

We hypothesize that vascular topography can confer differential cross-correlation symmetries. Comparing strength of symmetry with coronal vascular architecture of the brain could support a strong neurovascular involvement in functional connectivity paradigms. In this study, differences between BOLD and CBV-weighted activations for whole-brain fMRI of the rat were compared during forepaw stimulation and resting state scans.

fMRI Modalities

Blood Oxygen Level Dependent (BOLD)

Hemoglobin carries oxygen in arteries towards active neurons and releases the oxygen through capillaries in the active site. Deoxygenated hemoglobin subsequently leaves the active area through veins. Oxyhemoglobin is diamagnetic and deoxyhemoglobin is paramagnetic (Lores 2005). The change in magnetic properties of hemoglobin when it loses its oxygen can be measured by fMRI machines. Increased measure of paramagnetism due to increased deoxyhemoglobin concentration is thought to correspond to increased neural activity.

Cerebral Blood Volume (CBV)

Ultrasmall paramagnetic iron oxide (USPIO) is super paramagnetic, has a long half-life, and maintains a relatively constant concentration throughout the brain during typical fMRI experiments (Leite 2002). Thus when there is increased blood volume to an activated region, the MRI signal decreases beneath post injection baseline. Increased blood volume to a region corresponds to increased hemoglobin content also. Thus, cerebral blood volume change is intricately concomitant with BOLD signal changes also. However, the converse is not necessarily true—there can be changes in oxygen levels in a region with no accompanying changes in blood volume.

Rat Experimental Conditions

Forepaw Stimulation

Rat fMRI of somatosensory pathway in response to forepaw stimulation is an interesting animal model and is convenient due to how well it has previously been studied (Kolb 1990). Previous work has shown that in the rodent motor/sensory system, corpus callosum and thalamic regions can activate regions of the primary somatosensory cortex, secondary somatosensory cortex and motor regions. The caudate putamen in the striatum can then receive signals from the cortex and become activated. During stimulation, the SI region seems to show the most activation, and further studies in rats have shown that robust activation can also be observed in the SII and cerebellum during the forepaw stimulation protocols (Keilholz 2004). Connectivity evident during forepaw stimulation can also be seen even in the absence of such forepaw stimulations, albeit to a lesser degree.

Resting State

Resting state functional Magnetic Resonance Imaging (rsfMRI) is commonly used to study functional connectivity in the brain (Pawela 2008). Low frequency fluctuations (LFF) in fMRI signals can show contralateral and ipsilateral connectivity in the brain (Williams 2006). Interestingly, such LFFs in the somatosensory region seem to originate from same SI region as forepaw stimulated fMRI responses (Peltier 2002). Bilaterally symmetric regions of the brain show strongest connectivity. However, different analogous regions do not exhibit the same strengths of connectivity (Bifone 2010). It would be of interest to examine why different regions may show higher or lower symmetrical connectivity. Such an analysis could hold implications for analysis of ipsilateral connectivity pathways. Furthermore, if BOLD or CBV are more sensitive in different regions, image acquisition protocols could be altered to accommodate for these nuances.

METHODS

Experimental Setup

Full setup in Magnuson et al. 2010 (summarized below)

Animal Preparation

All experiments in compliance with Institutional Animal Care and Use Committee (IACUC). Five male Sprague-Dawley rats (200-300 g) were initially anesthetized with1.5% isoflurane during the experiment. Body temperature was maintained approximately 37°C while in the magnet. Heart rate, blood oxygen saturation, body temperature, and respiratory rate were all monitored throughout the fMRI acquisitions.

Rat tail vein catheterized to allow for iron oxide contrast agent injection during experiment. Subcutaneous bolus injection of 0.05 mg/kg medetomidine was administered then isoflurane was discontinued after three minutes. Fifteen minutes postbolus, 0.1 mg/kg/hr medetomidine subcutaneous infusion was initiated.

Forepaw Stimulation Protocol

Forepaw stimulation provided with needle electrodes in rat's left forepaw between digits 2 and 3 and digits 3 and 4. Total of 180 imaging volumes were collected (30 imaging volumes stimulation off – 20 on – 30 off – 20 on – 30 off – 20 on – 30 off).

CBV Infusion Protocol

After BOLD forepaw stimulation and resting state scans, 2% isoflurane was administered to further sedate the rat, then 5 mg/kg USPIO iron oxide contrast agent infused over approximately a minute. Isoflurane discontinued after completion of USPIO infusion and forepaw stimulation and resting scans acquired thirty minutes after USPIO injection.

Machine Settings

MRI Parameters

Images from a 9.4 T/20 cm bore Bruker BioSpec magnet with AVANCE console and with gradient coil capable of providing 20 G/cm with a rise time of 120 μs. Two-coil actively decoupling imaging setup used to achieve maximum signal-to-noise-ratio (SNR). A single-shot, multislice gradient echo EPI sequence was used for the fMRI studies. Setup for paradigms included shimming of SI region using FASTMAP.

fMRI Parameters

BOLD Forepaw Stimulation

Effective echo time [TE] = 15 msec, Repetition time [TR] = 1500 msec, Field of view [FOV] = 2.56 × 2.56 cm, Matrix size = 64 × 64, Slice thickness = 2 mm, Effective bandwidth = 200 kHz, Flip Angle = 31°, Time series repetitions: 180 images

BOLD Resting State

Effective echo time [TE] = 15 msec, Repetition time [TR] = 300 msec, Field of view [FOV] = 2.56 × 2.56 cm, Matrix size = 64 × 64, Slice thickness = 2 mm, Effective bandwidth = 200 kHz, Flip Angle = 31°, Time series repetitions: 1000 images

CBV Forepaw Stimulation

Effective echo time [TE] = 15 msec, Repetition time [TR] = 300 msec, Field of view [FOV] = 2.56 × 2.56 cm, Matrix size = 64 × 64, Slice thickness = 2 mm, Effective bandwidth = 200 kHz, Flip Angle = 31°, Time series repetitions: 180 images

CBV Resting State

Effective echo time [TE] = 15 msec, Repetition time [TR] = 300 msec, Field of view [FOV] = 2.56 × 2.56 cm, Matrix size = 64 × 64, Slice thickness = 2 mm, Effective bandwidth = 200 kHz, Flip Angle = 31°, Time series repetitions: 1000 images

Data Analysis

Forepaw stimulation and resting state fMRI scans were repeated three times from each of the five rats using both BOLD and CBV-weighted scans, producing sixty separate acquisitions for analysis. Changes in signal were registered onto a standard somatosensory template 40 × 40 × n matrix, with n representing number of subsequent

images taken from the slice of interest (n=180 for stimulation paradigms, n=1000 for resting state).

Acquisitions were blurred and overlaid to visualize a threshold for the brain since different slices depicted differential activations. Paxinos rat brain atlas (Coronal Bregma -0.30 mm) was proportionately fit over the acquisitions matrix to assign specific regions of interest to different pixels as motivated by previous studies (Paxinos 2007). Similarly, the rat brain atlas was fit over a Power Doppler Image of rat somatosensory region with activated regions proportional to cerebral blood volume.

Data analysis employed custom developed routines and procedures written in Matlab (Math-Works, Natick, MA). Location of SI region was double checked by using an unpaired t-test comparison of forepaw stimulation on and off periods followed by comparing topography of threshold t-value maps with rat brain atlas matrix.

Four additional 3 × 3 pixel ROIs were selected based off previous examinations of cross-correlation analysis and functional connectivity, namely, primary somatosensory cortex (SI), secondary somatosensory cortex (SII), caudate putamen dorsal region (CPuD), and caudate putamen ventral region (CPuV).

Cross-correlation was examined between the right and left hemispheres of bilaterally symmetric regions. Activation topography of relevant voxels was compared between the four ROIs. Furthermore, strength of symmetry was examined by looking at cross-correlations of time series between ROIs. ROI functional connectivity strength was then compared to a Doppler Power Image of the rat brain. Doppler image depicting vasculature was processed into a 40 × 40 2D matrix showing approximate prominence of

vasculature at various pixels. The vascular architecture of this region of the brain was then probed alongside ROI activation patterns.

RESULTS

Activation from Forepaw Stimulation

Pixel clusters showing the strongest correlation with the forepaw stimulation paradigm were localized to determine an initial seed region to be used with the resting state data. This procedure is possible since scans were taken from the same region for both the forepaw stimulation and resting state acquisitions. The area of strong cross-correlation over the stimulation scans in each of the five rats was in the right primary somatosensory cortex as expected since the left forepaw was stimulated in the paradigm (**Figure 1**). By examining previously studied connectivity associated with forepaw stimulation, approximate locations of various seed regions of the brain were determined (**Figure 2**).

Cross-correlation maps associated with seed regions were then examined in resting state rats. Localized spontaneous correlation areas were demarcated and examined alongside the rat brain atlas. These cross correlation maps were morphed over the rat brain atlas and voxel-grid to create a 2D matrix displaying cross correlations ROI associated voxels on a 40 × 40 template (**Figure 3**).

Resting State Symmetry Analysis

The anatomically based template was then used to reselect 3 × 3 seed regions. Larger seed regions were used to allow for averaging when time course analyses were performed. Previous studies have shown that cross-correlation is strongest between

analogous contralateral regions of interest (**Figure 4**). Cross-correlations between bilaterally symmetrical seeds were examined for both BOLD and CBV acquisitions (**Table 1**). The general pattern of contralateral analog correlations is similar for both BOLD and CBV scans (**Figure 5**).

The primary somatosensory cortex displayed the highest connectivity correlation, followed by the dorsal caudate putamen, secondary somatosensory cortex, and ventral caudate putamen, respectively. Interestingly, although the general patterns of bilateral connectivity strengths were similar, CBV correlation coefficients were less in magnitude for each seed region than similar BOLD connectivity strengths.

Vascular Correlation

Doppler power data for vascular architecture in the rat brain was converted to a 2D matrix array (**Figure 6**). Threshold for vasculature was taken to be 60 ADU. Regions with Doppler intensities above 120 ADU were considered to have strong vascular innervation. Previously established seed regions were overlaid onto the vascular matrix to determine average vasculature in each anatomical region of interest.

Strengths of cross-correlations from BOLD and CBV scans were compared to vascular densities from the Doppler vascular matrix (**Figure 7**). The areas of the brain with the most vasculature were the primary somatosensory cortex, dorsal somatosensory cortex, secondary somatosensory cortex, and ventral somatosensory cortex, respectively. This trend correlates with the general trend of connectivity strength. The Pearson correlation for this correlation was strong with a magnitude of 0.8787.

DISCUSSION

Differences between fMRI Modalities

When comparing fMRI imaging modalities, it is important to differentiate between sensitivity and prevalence. CBV imaging is more sensitive to smaller changes in field strengths but may not display strong prevalence of signals in certain regions since it may not respond as strongly to certain moieties such as large draining veins (Nencka 2007). Other studies showed CBV modulation can be relatively stronger in deeper cortical layers compared with BOLD modulation (Harel 2006). It should be noted that functional connectivity measurements by a modality could certainly be mutually exclusive from a modalities specificity or prevalence in picking up a signal.

Various ideas exist as to why these differences in modalities exist. In addition to reflecting neural responses, fMRI signals also contain physiological variances (Di 2012). Inflow effects from blood flowing from upstream can contribute and contaminate signals and alter spatiotemporal measurements of magnitude and time courses (Gao 2011). CBV modality is not are not susceptible to such effects, whereas CBF can show higher signals from such effects. Since BOLD comprises of CBF and CBV, such effects can confer variance in measurements. Many of the theories of variance suggest a strong influence coming from vasculature supporting the problem of "brain versus vein" ambiguity in using fMRI methods (Marota 1999).

Our results corresponded with previous studies that BOLD and CBV display similar activation patterns in the rat somatosensory system (Keilholz 2006). Forepaw stimulation indicated similar degrees of activation (**Figure 1**). This was probably evident

since forepaw stimulation paradigm is very specific with regards to showing elevated activation in the primary somatosensory cortex. Previous literature suggests that SIFL forelimb region is initially activated, our activation occurred more between the SIFL and SIBF barrel flow region. Previous studies suggest that differences in frequency can contribute to visualized activation regions of fMRI scans (Sanganahalli 2008).

Exploring how blood flow can affect different modalities can allow for better correction of fMRI collected data (Vazquez 2006). In humans, BOLD modality is almost exclusively used to collect functional brain data. Thus by examining component modalities such as CBV in the rodent model, it may be possible to calibrate BOLD signals to better indicate activities in neural activity in humans (Magnuson 2010). Further studies of regional differences can suggest that BOLD or CBV can show differential results in different secondary areas, motivating combined modality studies.

Variance in Symmetrical Bilateral Connectivity

Symmetrical bilateral resting state connectivity can vary due to neural modulation or from inherent neurophysiological processes (Tucker 1986). Spatial distributions of the interhemispheric regions in a fluctuation pathway can be found by looking into the appropriate activation pathways (van de Ven 2004). A caveat when examining pathways within a brain during resting state is that metabolism or local blood changes can contribute to the fluctuations in addition to postulated changes in neural activity (Mitra 1997).

In the somatosensory pathway we examined, it was evident that the primary somatosensory cortex displayed the strongest bilateral connectivity. This seems to correspond with the idea that this pathway originates at this location. We hypothesized

that the signal may diminish both in time and in regions with less vascular density. Interestingly, dorsal caudate putamen displayed higher connectivity than secondary somatosensory cortex; however, the ventral caudate putamen displayed less functional connectivity. Previous studies did not separate caudate putamen into regions, yet this separation suggests that even specific areas within a functional region can display differences in connectivity.

Previous studies suggest that CBV may be more sensitive to deeper cerebral blood changes (Zhao 2006). This could still correspond to our results showing that CBV connectivity correlations were lower in magnitude than BOLD scans. Since we measured correlations in time course, sensitive measures from CBV could show more frequent fluctuations over time. Although the general trends in CBV matched BOLD, CBV correlations were low enough that trials could be repeated in the future using more ROIs even within specific functional regions of the brain.

Exploring Cerebral Vascular Influences

The exact mechanism between the intrinsic relationship between neural activity and associated vasculature remains unclear. Various fMRI modalities work by measuring changes in regional blood contents. However, vascular density does not necessarily correlate with neuronal density on a micro scale (Tsai 2009). Often, regions of the brain that are symmetrical do not exhibit assumed underlying patterns (Oostenveld 2003). Studies have shown that as volumetric asymmetry increase, the average amount of axonal termination decreased due to less amounts of callosal axonal termination (Rosen 1989). We had sought to examine whether vascular density may correlate with strength of symmetrical connectivity.

fMRI has excellent depth penetration but does not provide good spatial and temporal resolution of microvasculature, but functional ultrasound (fUS) can provide better spatiotemporal resolution of rat brain than other functional brain imaging modalities (Macé 2011). Previous analysis of spatial configuration of micro vessels corresponds with the profile from the Doppler based matrix. Examining the detailed microvascular profiles can be helpful to investigate causal relationships between microvascular structure and functional activities in the cerebral cortex (Masamoto 2004). Experiments involving agenesis of the corpus callosum suggest that functional connectivity may be linked with interhemispheric blood flow (Quigley 2003).

SI region displayed highest density of microvasculature and highest symmetry correlation. However, high symmetry could also have been because this spontaneous fluctuation arises from the SI region and diminishes as it progresses in its circuit. However, it was interesting that the dorsal caudate putamen displays higher symmetrical connectivity than the secondary somatosensory cortex even though it follows after SII in the schematic fluctuation circuit (**Figure 2**). Likewise, it is interesting to note the difference between dorsal and ventral caudate putamen. It suggests that connectivity within smaller functional regions can vary as is not identical as one would assume. Further experiments could more systematically probe differential connectivity within a functional area of the brain.

CONCLUSION

Strong correlation between vascular density and bilateral connectivity strength suggests that vasculature may play an important role in bilateral connectivity in resting

state rats. Furthermore, although magnitudes of BOLD and CBV activations and correlations differed in this study, both modalities displayed similar patterns of activation and connectivity in forepaw stimulation paradigm and resting state analyses. A limitation that can be addressed in future studies is to coordinately measure changes in vasculature from functional ultra sound alongside BOLD and CBV fMRI acquisitions. Also, more regions of interest can be examined in future studies to better correlate bilateral connectivity with vascular density.

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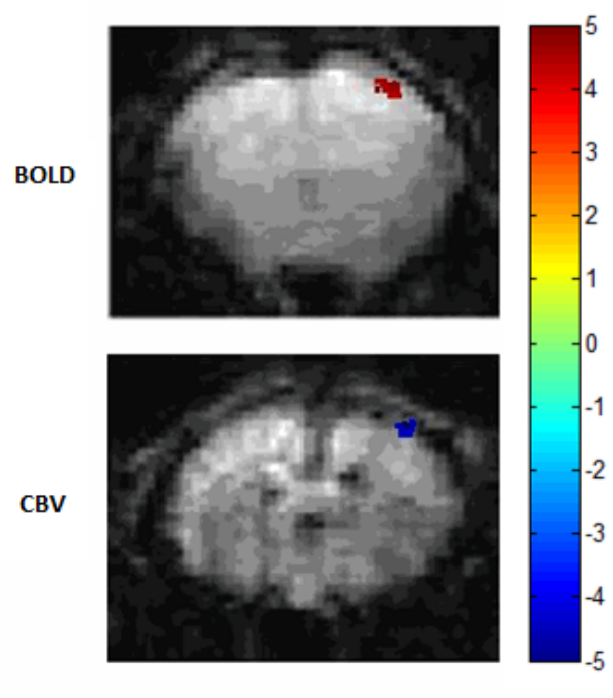

Figure 1. Activation of primary somatosensory cortex using BOLD (top) and CBV (bottom) weighted scans in a single rat from electrical stimulation of the left forepaw. Cluster of most highly activated pixels with t-values greater than three units from zero were superposed on corresponding EPI images.

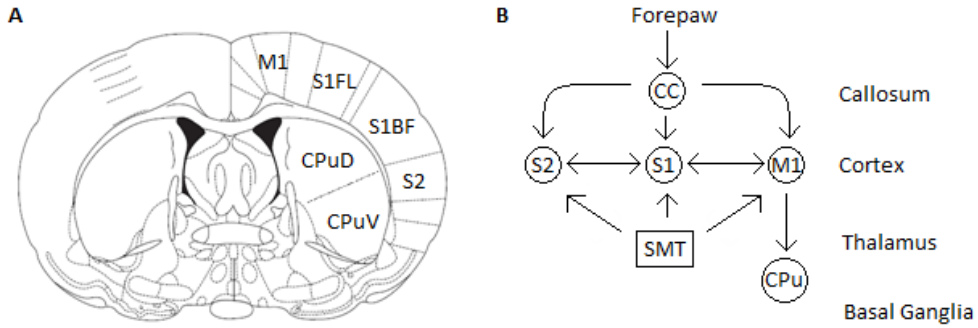

Figure 2. Diagrams of previously studied rat somatosensory system. (A) Spatial profile of various regions associated with the forepaw stimulation. (B) Simplified connection flowchart of the forepaw stimulation pathway.

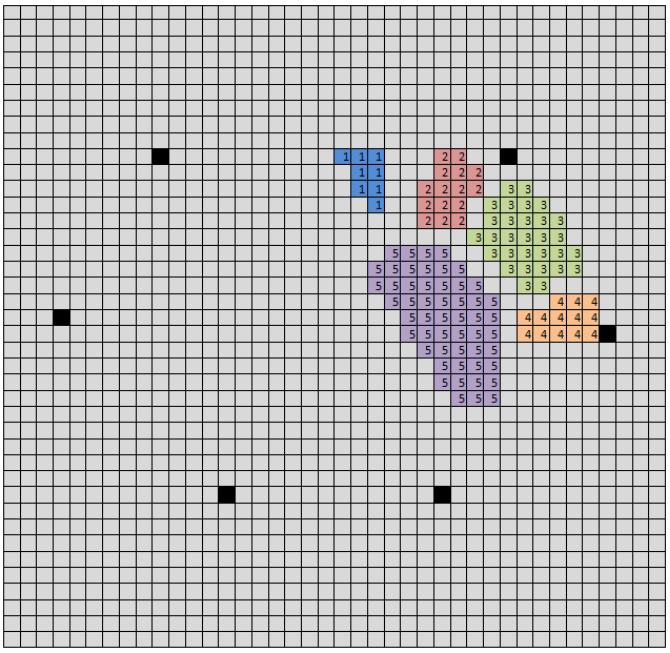

Figure 3. 64 × 64 2D matrix with corresponding pixels in the right hemisphere highlighted (blue-M1, red-S1FL, green-S1BF, orange-S2, and purple-CPu). Seed regions were then selected by selecting 3× 3 regions from an area of interest.

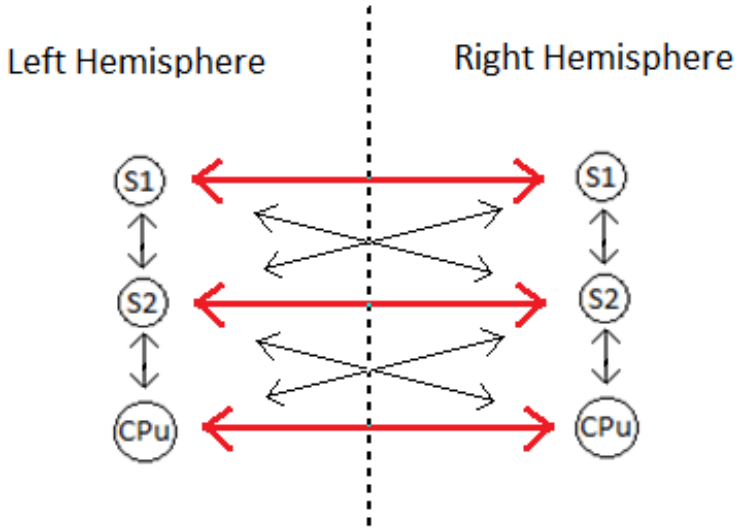

Figure 4. Diagrams displaying low frequency fluctuation correlations between various regions of interest. Contralateral connections between analogous regions display strongest correlations in connectivity strength.

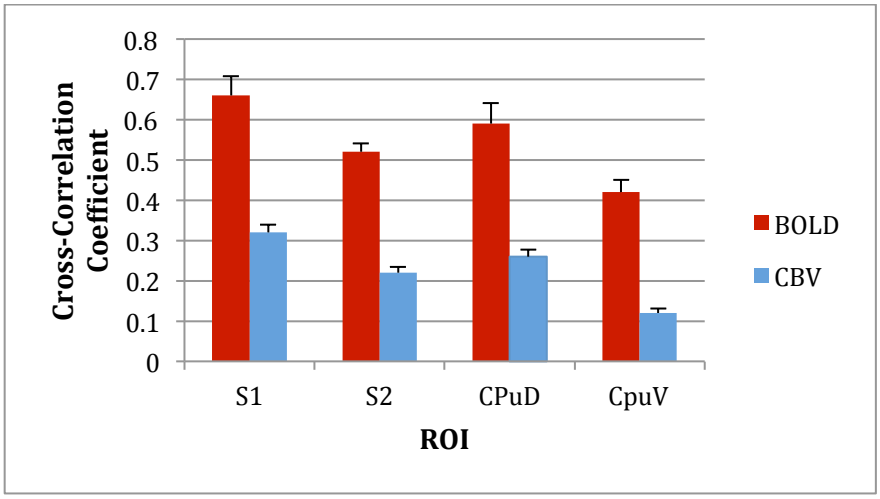

Figure 5. Average cross-correlation strengths between bilateral somatosensory system regions of interest. Red bars indicate BOLD acquisitions and blue bards indicate CBV acquisitions, with both modalities showing similar patterns of connectivity strength.

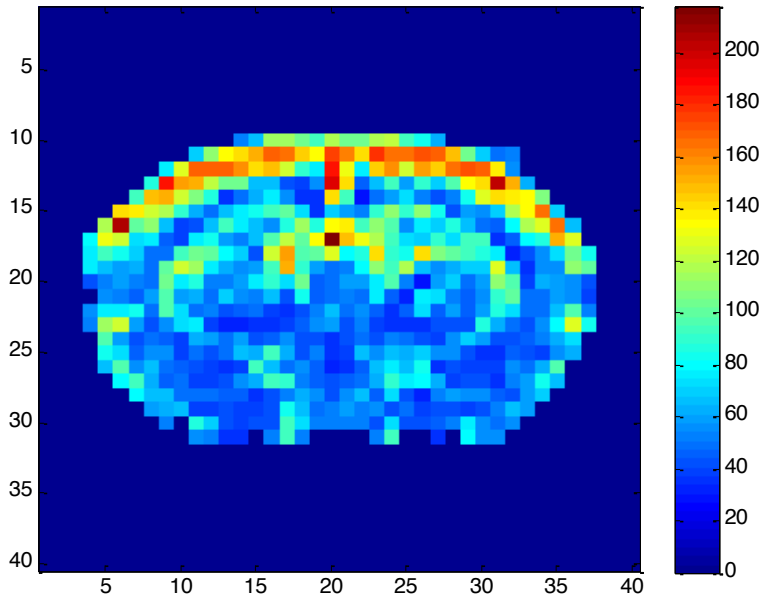

Figure 6. A 64 × 64 2D matrix rendered from functional ultrasound Doppler Power image of rat microvasculature in the rat somatosensory system. Scale indicates intensity of Doppler scan, which corresponds to vascular volume in a given region.

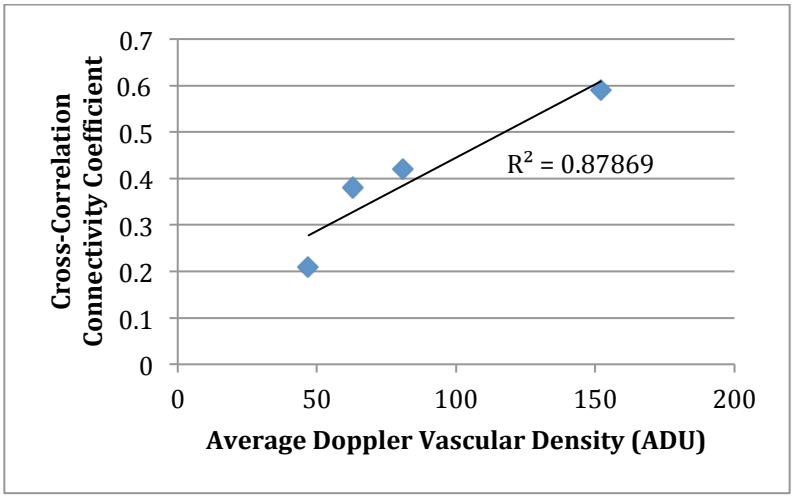

Figure 7. Correlation between vascular density from Doppler power image and connectivity strengths between bilateral regions of interest from rat resting state condition across BOLD and CBV modalities. Pearson correlation attained from regression between these two variables.

	BOLD		CBV	
	CPuD	**CPuV**	**CPuD**	**CPuV**
Rat 1	0.615	0.446	0.254	0.112
Rat 2	0.546	0.506	0.231	0.123
Rat 3	0.689	0.319	0.258	0.228
Rat 4	0.607	0.341	0.224	0.088
Rat 5	0.523	0.483	0.312	0.145
Mean	0.596	0.419	0.2558	0.1392
SEM	0.052	0.063	0.021	0.034

Table 1. Table comparing dorsal and ventral cross-correlation connectivity in the rats during both BOLD and CBV scans. Average was taken across all rats with standard error of the mean also indicated.

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