Neuronal and Physiological Correlation to Hemodynamic Resting-State Fluctuations in Health and Disease

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Abstract

Low-frequency, spatially coherent fluctuations present in functional magnetic resonance imaging time series have had a tremendous impact on brain connectomics. This work aims to explore the degree with which hemodynamic connectivity is associated with neuronal, metabolic, and vascular connectivity measures. For this purpose, GCaMP and nontransgenic mice were used to image neuronal activity and oxidative metabolism activity, respectively, along with blood-oxygenation- and cerebral blood volume (CBV)–sensitive hemodynamic changes from the same animals. Although network clusters calculated using either GCaMP (neuronal activity) or optical imaging of intrinsic signal (OIS)–BOLD (blood oxygenation) data did not exhibit strong spatial similarity, the strengths of node-to-node connectivity measured with these modalities were strongly correlated with one another. This finding suggests that hemodynamic connectivity as measured by blood oxygenation measurements, such as functional connectivity magnetic resonance imaging, is a valuable surrogate for the underlying neuronal connectivity. In nontransgenic animals, greater connectivity correlation was observed between tissue oxidative metabolism (flavoprotein autofluorescence imaging [FAI]) and blood oxygenation measurements, suggesting that metabolic contributions to hemodynamic signals are likely responsible for its significant correlation with neuronal connectivity. Lastly, a mouse model of Alzheimer's disease was used to explore the source of decreases in connectivity reported in these mice, a finding that is thought to be associated with amyloid load-driven metabolic decline. The intercluster connectivity measured by metabolic-sensitive measurements (FAI and OIS-BOLD) was maintained while vascular-only signals (OIS-CBV) provided negligible correlation. Therefore, metabolism-sensitive measurements as used in this work are better positioned to capture changes in neuronal connectivity, such that decreases in hemodynamic connectivity likely reflect decreases in oxidative metabolic function.

Key words: calcium imaging; FAI; functional connectivity; GCaMP; neuronal activity; optical imaging; oxidative metabolism; resting state

Introduction

LOW-FREQUENCY, SPATIALLY coherent fluctuations present in functional magnetic resonance imaging (MRI) time series have had a tremendous impact on brain connectomics. In short, slow (<0.1 Hz) changes in blood oxygenation that take place while the brain is at “rest” (not performing any particular task) show specific bilateral patterns that outline known connectivity within brain networks (Biswal et al., 1995; Cordes et al., 2000; Fox and Raichle, 2007; Lowe et al., 1998; Smith et al., 2009). Biswal and colleagues (1995) first demonstrated the physiological relevance of these fluctuations by showing their strong

correlation between left and right motor cortices. Although these resting fluctuations were initially regarded as a source of noise, the importance of these fluctuations has been recognized and they have become the primary subject of research for many groups. This technique is commonly referred to as functional connectivity MRI (fcMRI) and it has been used in concert with diffusion imaging to provide new insights into the relationship between function and structure in the human brain (Greicius et al., 2009; Honey et al., 2009; Wang et al., 2013). Further, alterations in functional connectivity are under ongoing investigation for their potential use as a biomarker of a number of diseases, including but not limited to Alzheimer's disease (AD) (Vemuri et al., 2011),

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schizophrenia (Woodward et al., 2012), and autism (Stigler et al., 2011).

fcMRI is sensitive to changes in blood oxygenation that are known to stem from regional changes in cerebral blood flow (CBF), cerebral blood volume (CBV), and the cerebral metabolic rate of oxygen consumption (CMR_{O2}) (Davis et al., 1998; Kim et al., 1999). These physiological parameters serve as surrogate markers of underlying neural activity, such that fcMRI measurements may reflect large changes in neural activity, vascular function, and/or oxidative metabolism. Changes in vascular function or oxidative metabolism may occur with or without changes in neural activity. Although some studies have explored physiological correlates of low-frequency fluctuations (De Luca et al., 2006; Miao et al., 2014; Wu et al., 2009; Zou et al., 2009), fundamental gaps remain regarding the relationship between these fluctuations and brain function, including the relative contributions of neuronal and vascular factors to the measured fcMRI time series. A complicating factor is that vascular smooth muscle is known to have slow contractile rhythms, termed vasomotion, around this frequency range (Mayhew et al., 1996). Nonetheless, there is electrophysiological evidence that shows that low-frequency neural activity patterns are related to fcMRI fluctuations at the electrode location in monkey visual cortex, showing a neuronal contribution (Shmuel and Leopold, 2008). In addition, resting-state networks measured in humans with electrocorticography and magnetoencephalography were similar to those measured by fcMRI (Brookes et al., 2011; de Pasquale et al., 2010; Mantini et al., 2007). Since these techniques bypass the vascular system, these findings lend further support to a neuronal basis for the detected resting-state networks.

Studies in rodents have shown the presence of these fluctuations in blood oxygenation using fcMRI and analogous optical methods under awake and lightly anesthetized conditions (Drew et al., 2011; Liu et al., 2012; Magnuson et al., 2014; Pawela et al., 2008; White et al., 2011; Williams et al., 2010). White and colleagues (2011) and others have shown distinct, bilateral sensory networks in the mouse and rat cortices with similar frequency as those observed in human studies (Majeed et al., 2009; Pawela et al., 2008). Similarly, rodent electroencephalography and intracortical electrode recordings have shown that electrical and hemodynamic signals are correlated at specific locations over a relatively large range of frequencies (Liu et al., 2011; Lu et al., 2007; Pan et al., 2011, 2013; Thompson et al., 2013). Mohajerani and colleagues (2010) used voltage-sensitive-dye imaging, a technique that is sensitive to changes in cell membrane potential, such as local field potentials, to show similar spatial network features to those observed using hemodynamic methods.

Although functional connectivity observations have been reported in animals and humans using hemodynamic methods, their neurophysiological relationship remains elusive across the brain and their ultimate utility and interpretability remain unclear due to the complex nature of the fcMRI signal. Therefore, the goal of this work is to explore the neurophysiological contributions of low-frequency fluctuations across much of the mouse cortex such that the changes in brain connectivity observed in disease (e.g., AD) can be more appropriately interpreted. Specifically, this work aims to (1) determine the degree with which hemodynamic con-

nectivity is associated with neuronal connectivity, (2) determine the degree with which hemodynamic connectivity is driven by vascular and metabolic signals, and (3) determine the relative impact of physiological factors behind decreases in hemodynamic connectivity in a model of AD (i.e., whether decreases in low-frequency fluctuations reflect decreases in vascular function or oxidative metabolism).

Robust and suitable animal models will be used for this purpose. Transgenic mice expressing GCaMP3, a fluorescent calcium indicator that reports changes in intracellular calcium concentration that accompany spiking activity (Chen et al., 2012), were used to image low-frequency changes in neuronal activity as well as hemodynamic imaging measurements sensitive to blood oxygenation (analogous to fcMRI measurements) and CBV (to assess vascular contributions). GCaMP3 (or GCaMP) is widely expressed across the cortex, avoiding the use of point measurements (e.g., electrodes) or potentially toxic neural reporters (e.g., voltage-sensitive dyes), and therefore presents an ideal model to investigate the neuronal contribution to low-frequency hemodynamic oscillations. Nontransgenic mice were used to image changes in oxidative metabolism using flavoprotein autofluorescence imaging (Reinert et al., 2004; Shibuki et al., 2003; Vazquez et al., 2012) as well as hemodynamic measurements of blood oxygenation and CBV. Lastly, a mouse model of AD was previously used to show decreases in hemodynamic connectivity that are similar to those observed in human populations (Bero et al., 2012). This mouse model of AD was used to investigate the physiological interpretation of decreases in hemodynamic connectivity.

Methods

A total of 13 male mice were used for this study: seven adult transgenic mice expressing GCaMP3 throughout the central nervous system (strain B6.CBA(Thy1-GCaMP3)8Gfng; 23–29 g, 2–4 months old), three adult nontransgenic mice (strain C57BL6; 28–32 g; 3–4 months old), and three adult AD mice (strain B6C3.Tg(APPswe-PSEN1d9); 33–39 g, 12–14 months old). All mice were obtained from the Jackson Laboratory (Bar Harbor, ME). All procedures performed followed an experimental protocol approved by the University of Pittsburgh Institutional Animal Care and Use Committee in accordance with the standards for humane animal care and use as set by the Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animals were initially anesthetized using a cocktail of ketamine (75 mg/kg) and either xylazine (10 mg/kg) or dex-dormitor (0.5 mg/kg) administered intraperitoneally (IP) for surgery. An IP line was then inserted to administer fluids (5% dextrose in saline) as well as supplementary anesthesia throughout the experiment, which consisted of only ketamine at 30 mg/(kg·h), typically commencing about 1 h after induction. The animals were then placed in a stereotaxic frame (Narishige, Tokyo, Japan) and supplementary oxygen was administered blow-by using a cannula at a rate of 500 mL/min. Heart rate was continuously monitored throughout the experiment using metal leads placed subcutaneously in the abdomen to assess the physiological condition of the animal and depth of anesthesia. Body temperature was maintained at 38°C using a thermal probe and heating pad controlled by a DC feedback unit

(40-90-8C; FHC, Inc., Bowdoinham, ME). The thermal probe was placed under the abdomen to maintain the animal's temperature throughout the experiment. Heart rate and body temperature were recorded using a polygraph data acquisition software (Acknowledge; Biopac Systems, Inc., Goleta, CA). The skull was then exposed over both hemispheres. A well was made using acrylic cement surrounding an area about $9 \times 7 \text{ mm}^2$, centered about 1 mm posterior to Bregma. The skull in this area was then removed using a dental drill. The well area was then filled with 1% agarose gel at body temperature. At least 15 min was allotted for the setup to settle prior to commencing experimental data collection.

Data acquisition

Images sensitive to neuronal activity were acquired from GCaMP mice using an epifluorescence microscope (MVX-10; Olympus, Tokyo, Japan) over a field-of-view of $11.9 \times 8.9 \text{ mm}^2$ (Fig. 1). GCaMP was excited using a mercury lamp light source coupled to a low-noise power supply (Opti-Quip, Highland Mills, NY) and an appropriate excitation filter ($470 \pm 20 \text{ nm}$). The fluorescence emission over $525 \pm 25 \text{ nm}$ was captured using a digital-cooled CCD camera (Cool-Snap HQ2; Photometrics, Princeton, NJ) at a frame rate of 10 Hz in 5-min recording periods. The exposure time of the camera was set to 100 msec with a bin factor of 3 to increase signal-to-noise ratio (SNR) for an effective pixel resolution of $26 \mu\text{m}$. The same microscope, optical path, optical filters, and acquisition parameters were used to record images sensitive to changes in the TCA cycle rate (oxidative metabolism) from nontransgenic and AD mice using flavoprotein autofluorescence imaging (FAI). In addition, the same microscope and optical path were used for optical imaging of intrinsic signal (OIS) sensitive to either blood oxygenation (OIS-

BOLD) or CBV (OIS-CBV) from all mice strains (Fig. 1). Oblique light guides transmitting filtered light ($600 \pm 50 \text{ nm}$) connected to a halogen light source (Thermo-Oriel, Stratford, CT) were used for illumination and an appropriate barrier filter was placed prior to the camera ($572 \pm 7 \text{ nm}$ for OIS-CBV, which corresponds to an isosbestic point for hemoglobin making these images sensitive to total amount of hemoglobin and therefore representative of CBV, and $620 \pm 7 \text{ nm}$ for OIS-BOLD, which corresponds to a spectral region dominated by deoxygenated hemoglobin light absorption) (Horecker, 1943). The OIS data were acquired at 30 Hz using an analog CCD camera (Sony XT-75, Tokyo, Japan) and an analog-to-digital frame-grabbing board (Matrox, Inc., Dorval, Quebec, Canada) over the same field-of-view. The OIS pixel size was $14 \mu\text{m}$. GCaMP and OIS-BOLD as well as the GCaMP and OIS-CBV images were recorded simultaneously from all GCaMP mice using these light-splitting optics shown in Figure 1. Since the light-splitting optics reduce the amount of light captured by each camera, this setup was not used in nontransgenic and AD mice to maintain a reasonable SNR for FAI recordings. In these experiments, FAI, OIS-BOLD, and OIS-CBV images were obtained sequentially.

Data analysis

After all the experimental data were collected, the SNR of the data was increased by binning the GCaMP and FAI images by a factor of 2 and the OIS images by a factor of 4 for a final in-plane pixel resolution of 52 and $56 \mu\text{m}$, respectively. In addition, the OIS data were temporally binned by a factor of 3 to match the sampling rate of the GCaMP and FAI data (10 Hz). The images were processed according to the pipeline depicted in Figure 2 using custom routines implemented in Matlab (Mathworks, Natick, MA). In summary, the images were first realigned for small amounts of

FIG. 1. (A) Sample imaging setup. An epifluorescence microscope coupled to two light sources and a two-camera system was used to image neuronal (GCaMP) or oxidative metabolism (flavoprotein autofluorescence imaging [FAI]) fluorescence along with hemodynamic images of blood oxygenation (using optical imaging of intrinsic signal [OIS]-BOLD) or cerebral blood volume (OIS-cerebral blood volume [OIS-CBV]). A mercury light source (blue bulb) was used for fluorescence imaging (470 nm excitation, 525 nm emission), while a halogen light source (red bulb) was used for OIS experiments (620 nm for OIS-BOLD, 572 nm for OIS-CBV) transmitted via the oblique light guides. Images from cameras 1 and 2 were obtained simultaneously for all GCaMP mice and sequentially for nontransgenic and Alzheimer's disease (AD) mice to improve sensitivity. (B) Sample GCaMP (top) and OIS-BOLD (bottom) images obtained from a GCaMP animal.

FIG. 2. Diagram of the processing pipeline. The images were first corrected for small amounts of motion (mostly due to respiration) by realigning them relative to the first image of the acquisition. Then, fluctuations from the light source were regressed out of each pixel's time series using a region-of-interest positioned over the cranial bone. These data were then filtered and a *k*-means clustering algorithm was used to delineate six clusters over one hemisphere. A correlation analysis was performed using the mean time series from each cluster to examine the bilateral character and spatial confinement from the respective correlation images.

motion due mostly to breathing using a two-dimensional rigid body model with two degrees of freedom (*x*- and *y*-translations). A region-of-interest was then placed over the skull posterior to lambda to extract unwanted fluctuations in the light source and regress this contribution from each pixel's time series. Then, the data were band-pass filtered from 0.02 to 0.2 Hz in frequency domain using a Fermi filter with spread parameter of 0.005 and 0.05 Hz at each band, respectively, to maintain low-frequency fluctuations while removing bulk baseline drifts. Masks were outlined for each visible brain region in a hemisphere while excluding large pial vessels from further analysis. A *k*-means algorithm was then used to outline six clusters from one hemisphere (typically the left hemisphere) based on the correlation distance between the pixel's time series for each imaging data set (clustering was performed for each imaging modality separately). The number of clusters (6) was chosen based on a previous report on the number of identifiable areas over the superior surface of the mouse brain (White et al., 2011). The clusters were ordered first from medial to lateral and then from anterior to posterior. Then, correlation images for each imaging modality were generated by computing the average time series from each cluster and correlating it with the time series of each pixel in the image. The correlation images were inspected to ensure that strong bilateral cor-

relations were observed in the data from GCaMP and nontransgenic mice.

The following metrics were generated to investigate correspondence between neural and hemodynamic connectivity based on blood-oxygenation- and CBV-sensitive OIS data.

Overlap coefficient. The overlap or dice coefficient of the cluster maps obtained from the GCaMP and OIS data was computed for each animal. That is, the overlap coefficient of clusters *i* and *j* from data sets *A* and *B*, respectively, is $OC_{ij} = 2(\#A_i - \text{intersection} - B_j) / (\#A_i + \#B_j)$, where $\#$ is the number of pixels. A 6×6 overlap coefficient matrix was computed for each comparison (e.g., GCaMP vs. OIS-BOLD and GCaMP vs. OIS-CBV). A high overlap coefficient would be expected over the diagonal of the overlap coefficient matrix for data sets with highly overlapping clusters with very similar size. Hence, the average over the diagonal of the overlap coefficient matrix was calculated and reported. To ensure both GCaMP and OIS were in the same space for comparison, a five-degree-of-freedom affine transformation (*x*-translation, *y*-translation, rotation, *x*-linear scaling, and *y*-linear scaling) was estimated based on the average acquired image from each modality being compared. These images were histogram equalized and normalized between 0 and 1 before solving for the transformation that minimized the sum of the squared residuals. The GCaMP cluster map was then resampled using this transformation and nearest-neighbor interpolation. A paired *t*-test was performed between the compared modalities containing data from more than three animals to determine whether the overlap fraction is significantly different ($p < 0.05$) between imaging modalities.

Connectivity correlation. The connectivity matrix (6×6) for the GCaMP, OIS-BOLD, and OIS-CBV data was calculated using the cluster average time series from each respective imaging modality while holding the spatial extent of the clusters constant. The GCaMP cluster map was chosen as the standard of reference because of its sensitivity to neuronal activity. The connectivity correlation was then calculated as the correlation between corresponding off-diagonal connectivity matrix elements obtained from the GCaMP and OIS data. Since the connectivity matrix is symmetric along its diagonal, only the upper-triangular elements were used. This metric was used as an indication of the fidelity of hemodynamic data in reporting neuronal connectivity. The significance of this relationship was determined using a one-sided *t*-test with $p < 0.05$ for each animal. In addition, a group comparison of the connectivity correlation was also performed using a paired *t*-test as described above.

Spatial correlation. Six correlation images were calculated for each of the GCaMP, OIS-BOLD, and OIS-CBV data, again using only the GCaMP cluster map to hold the spatial extent of the clusters constant. Corresponding correlation images were obtained by calculating the temporal correlation between each pixel in the brain and the average time course from a given cluster. A 6×6 spatial correlation matrix was generated where the diagonal elements indicate the similarity between the same clusters across modalities and the off-diagonal elements provide a measure of the overlap in correlation between clusters. The mean of the diagonal elements of the spatial correlation matrix was calculated and

reported to provide a measure of the similarity between correlation maps between modalities and complement the connectivity correlation metric. A group comparison of the spatial correlation was performed using a paired *t*-test as described previously.

Spectral power. The power spectral density of mean normalized and subtracted cluster average time series was calculated from the processed data. The GCaMP cluster map was used as the reference map between modalities. The spectra across clusters were averaged and the energy over three frequency bands was reported: 0.02–0.08 Hz, 0.08–0.14 Hz, and 0.14–0.25 Hz. This metric was used to explore spectral differences between low-frequency neuronal and hemodynamic signals. Paired *t*-tests were performed between modalities containing data from more than three animals to determine significant differences in the spectral energy between groups ($p < 0.05$).

These metrics were also computed using the FAI and OIS data obtained from the nontransgenic (and AD) mice to investigate the similarity of vascular (OIS-CBV) and metabolic (FAI) signals on hemodynamic (OIS-BOLD) connectivity. For these data, the FAI cluster maps were used as reference. Measurements obtained from AD mice were used to explore the physiological impact of observed decreases in hemodynamic connectivity in terms of whether they reflect decreases in vascular or metabolic connectivity.

Results

Neuronal (GCaMP) versus hemodynamic (OIS) data

GCaMP, OIS-BOLD, and OIS-CBV images were acquired from the seven mice studied. The GCaMP data were realigned and filtered as described in the “Methods” section and the data were then clustered into six regions over the superior surface of one hemisphere. Distinct and contiguous clusters were consistently obtained over anatomical areas that approximately correspond to cingulate, frontal, motor, somato-sensory, retrosplenial, and visual cortices (clusters labeled 1–6, respectively; see *k*-means image shown in Figure 3A, C, and E for sample cluster maps obtained from one animal). The overall correspondence of the cluster maps across modalities was generally good although some deviations were observed in all animals. The average time series from each cluster was extracted and used to generate correlation images from each data set (Fig. 3B, D, F). Prominent and consistent bilateral networks were observed around each cluster area and their contralateral location. The most striking difference between the GCaMP and OIS correlation maps is that the spatial extent of the networks appeared to be significantly larger for the OIS-BOLD and OIS-CBV data. The overlap fraction, connectivity correlation, and spatial correlation were then computed and sample results obtained from one of the animals are shown in Figure 4. The overlap fraction (Fig. 4A) shows that some of the clusters did not match well (e.g., 2 and 4) for OIS-BOLD data, whereas the OIS-CBV data show slightly better overlap in this subject. The cluster connectivity matrices show a greater degree of connectivity between the clusters from the OIS-BOLD and OIS-CBV data compared with the GCaMP data due to their larger spatial extent (Fig. 4B). However, the intercluster connectivity was higher between GCaMP and OIS-BOLD

data compared with GCaMP versus OIS-CBV (Fig. 4C). In addition, the spatial correlation was also slightly higher between GCaMP and OIS-BOLD compared with GCaMP and OIS-CBV.

A summary of the results obtained from all animals is presented in Table 1. Since two GCaMP resting-state runs were always obtained from each animal (each in conjunction with OIS-BOLD and OIS-CBV), GCaMP-versus-GCaMP results were included to provide an estimate of the test–retest variability of these metrics. As expected, these data show consistently high overlap fraction, consistently high and significant connectivity correlation, and high spatial correlation. Paired *t*-tests performed on the overlap fraction and spatial correlation indicated that these metrics from GCaMP-versus-OIS-BOLD and GCaMP-versus-OIS-CBV data were significantly lower from the GCaMP-versus-GCaMP data with $p < 0.001$. However, these metrics from GCaMP-versus-OIS-BOLD and GCaMP-versus-OIS-CBV were not significantly different from each other. As depicted in Figure 4, the connectivity correlation findings in Table 1 show that the OIS-BOLD data represent well the neuronal (GCaMP) intercluster connectivity, while this relationship was significantly lower between GCaMP and OIS-CBV with $p < 0.05$. In addition, the GCaMP-versus-OIS-BOLD connectivity correlation was significant in every animal whereas it was not for GCaMP-versus-OIS-CBV. The superior performance of OIS-BOLD compared with OIS-CBV in this regard suggests that its sensitivity to brain metabolism is an important contributor to the fMRI signal’s ability to report neuronal connectivity. The overlap fraction and spatial correlation were not as high probably due to various sources of noise, such as vascular factors (draining and vasomotion) inherent in these measurements.

The average power spectrum density was calculated for the processed GCaMP, OIS-BOLD, and OIS-CBV data from one hemisphere in GCaMP mice (Fig. 5). The average power spectrum from one animal is shown in Figure 5A. Although the filter’s pass-band is evident in the spectral shape, it is clear that the GCaMP data have higher overall energy than OIS-CBV and OIS-BOLD data (in that order). To compare the shape of the power spectra, these were normalized by their total energy (Fig. 5B). Although small deviations are present, no significant differences were observed in the spectra. To investigate spectral differences further, the energy over three different bands was extracted. The average power spectrum energy and normalized power spectrum energy from all GCaMP mice are shown in Figure 5B and C, respectively. The most noticeable finding is that the OIS-BOLD data have the lowest overall energy and that the GCaMP data have the highest normalized average spectral energy over the 0.14–0.25 Hz bands with $p < 0.05$. Note that even if the overall energy of OIS-BOLD is lower than that of OIS-CBV, OIS-BOLD has a significantly larger connectivity correlation with the GCaMP data compared with OIS-CBV.

Metabolic (FAI) versus hemodynamic (OIS) data from nontransgenic and AD mice

The same cluster analysis was also performed in the FAI and OIS data from nontransgenic mice. Similar results were obtained using oxidative-metabolism-sensitive FAI as the reference modality. That is, distinct clusters with strong bilateral correlation were observed; however, the spatial

FIG. 3. GCaMP, OIS-BOLD, and OIS-CBV results obtained from one GCaMP animal. (A) Sample image (top) and k -means clusters ($k=6$; bottom) obtained from a 5-min GCaMP resting-state run. The cluster numbers are indicated in the cluster image. The same number system has been applied to all cluster maps (midline-to-lateral and anterior-to-posterior). (B) Correlation images obtained using the average time series from each cluster. (C, D) Sample image, calculated clusters, and correlation images for each calculated cluster from OIS-BOLD data in the same animal. (E, F) Sample image, calculated clusters, and correlation images for each calculated cluster from OIS-CBV data. Note that although some of the hemodynamic correlation images (e.g., cluster six in the OIS-BOLD and OIS-CBV data) do not show a strong bilateral character, this decreased bilateral character is also present in the corresponding GCaMP correlation images from the same area.

extent of the cluster correlation images was not as spatially defined as that of GCaMP and more closely resembled the OIS correlation images (Fig. 6, top row shows FAI from a nontransgenic animal). The connectivity correlation findings from nontransgenic (non-Tg) mice in Table 1 suggest that the OIS-BOLD connectivity is more closely related to FAI connectivity compared with OIS-CBV. This provides additional support to the notion that connectivity measured by OIS-BOLD is driven predominantly by metabolism, which reinforces that OIS-BOLD would be preferred to OIS-CBV for reporting neuronal connectivity. The overlap fraction and spatial correlation metrics were similar to those obtained in the GCaMP mice and did not show a significant tendency.

Lastly, the same analysis was performed in AD mice. Although distinct clusters were obtained using k -means in a hemisphere, the cluster correlation images show significant interhemispheric decreases in correlation (Fig. 6, bottom three rows). The interhemispheric correlation was observed

to be weakest for the OIS-CBV data compared with the FAI and OIS-BOLD data. Notwithstanding, the intercluster connectivity reported by OIS-BOLD was more strongly correlated with FAI measurements compared with OIS-CBV data (Table 1). This suggests that changes in intercluster connectivity reported by OIS-BOLD data likely result from differences in oxidative metabolism between these areas and not vascular differences.

The average power spectrum density and normalized power spectra were also calculated for the processed FAI, OIS-BOLD, and OIS-CBV data from nontransgenic and AD animals (Fig. 5). The FAI energy across frequency bands in nontransgenic mice is similar to the GCaMP energy distribution across frequency bands obtained from GCaMP mice (Fig. 5C, D). In addition, the normalized spectral energy across frequency bands was found to be very similar to that of GCaMP (data not shown). The most significant difference in the power spectrum from nontransgenic and AD

FIG. 4. Overlap fraction, connectivity correlation, and spatial correlation metrics calculated from one GCaMP animal. **(A)** Calculated overlap fraction of the clusters obtained from GCaMP, OIS-BOLD, and OIS-CBV data. GCaMP versus OIS-BOLD (left matrix) and GCaMP versus OIS-CBV (right matrix) overlap fraction for each corresponding cluster number (sample cluster arrangement presented on the left) shows relatively strong overlap along the diagonal, as expected, with some overlap along off-diagonal elements. This metric is also commonly referred to as the dice coefficient. **(B)** The cluster connectivity was obtained for the GCaMP (left), OIS-BOLD (middle), and OIS-CBV (right) data from this animal using the GCaMP cluster as reference, extracting the average time series from each cluster and each modality, and computing the cross correlation (connectivity matrix). **(C)** The off-diagonal elements (upper triangular) from the cluster connectivity matrix were then used to compute their connectivity correlation between GCaMP and OIS-BOLD (left) as well as GCaMP and OIS-CBV (right). Although both connectivity correlation metrics were high for this animal, the connectivity correlation between GCaMP and OIS-BOLD was higher than that of GCaMP versus OIS-CBV. **(D)** The spatial correlation metric was calculated by extracting the average time series of each cluster (using the GCaMP cluster map as reference) for each modality and calculating correlation images for each cluster. The correlation images for each cluster and modality were then correlated to construct the GCaMP versus OIS-BOLD (left) and GCaMP versus OIS-CBV (right) spatial correlation matrix. High values along the off-diagonal indicate broader high-correlation areas between modalities.

TABLE 1. OVERLAP FRACTION, CONNECTIVITY CORRELATION, AND SPATIAL CORRELATION METRICS OBTAINED FROM ALL ANIMALS STUDIED

Comparison	Overlap fraction	Connectivity correlation	Spatial correlation
GCaMP vs. GCaMP			
GCaMP mouse 1	0.594	0.912*	0.705
GCaMP mouse 2	0.567	0.914*	0.667
GCaMP mouse 3	0.559	0.650*	0.561
GCaMP mouse 4	0.609	0.896*	0.865
GCaMP mouse 5	0.610	0.884*	0.857
GCaMP mouse 6	0.618	0.871*	0.762
GCaMP mouse 7	0.690	0.904*	0.836
Average $\pm$ SD	0.607 $\pm$ 0.043	0.862 $\pm$ 0.095	0.750 $\pm$ 0.113
GCaMP vs. OIS-BOLD			
GCaMP mouse 1	0.365	0.958*	0.559
GCaMP mouse 2	0.459	0.775*	0.485
GCaMP mouse 3	0.262	0.750*	0.193
GCaMP mouse 4	0.297	0.581*	0.540
GCaMP mouse 5	0.371	0.811*	0.332
GCaMP mouse 6	0.269	0.779*	0.528
GCaMP mouse 7	0.315	0.801*	0.626
Average $\pm$ SD	0.334 $\pm$ 0.070	0.780 $\pm$ 0.111	0.466 $\pm$ 0.151
GCaMP vs. OIS-CBV			
GCaMP mouse 1	0.473	0.914*	0.421
GCaMP mouse 2	0.334	0.425	0.410
GCaMP mouse 3	0.254	0.386	0.366
GCaMP mouse 4	0.245	0.417	0.463
GCaMP mouse 5	0.381	0.557*	0.438
GCaMP mouse 6	0.377	0.439	0.387
GCaMP mouse 7	0.264	0.900*	0.284
Average $\pm$ SD	0.333 $\pm$ 0.084	0.577 $\pm$ 0.231	0.396 $\pm$ 0.059
FAI vs. OIS-BOLD			
Non-Tg mouse 1	0.419	0.469	0.255
Non-Tg mouse 2	0.319	0.677*	0.326
Non-Tg mouse 3	0.250	0.704*	0.496
Average	0.329	0.617	0.359
FAI vs. OIS-CBV			
Non-Tg mouse 1	N/A	N/A	N/A
Non-Tg mouse 2	0.350	0.650*	0.370
Non-Tg mouse 3	0.395	0.232	0.482
Average	0.373	0.441	0.426
FAI vs. OIS-BOLD AD			
AD mouse 1	0.283	0.690*	0.313
AD mouse 2	0.390	0.715*	0.341
AD mouse 3	0.374	0.714*	0.396
Average	0.349	0.706	0.350
FAI vs. OIS-CBV AD			
AD mouse 1	0.355	0.299	0.214
AD mouse 2	0.163	0.673*	0.278
AD mouse 3	N/A	N/A	N/A
Average	0.259	0.486	0.246

Significant connectivity correlation metrics with $p < 0.05$ are indicated by *.

FAI, flavoprotein autofluorescence imaging; OIS, optical imaging of intrinsic sign; CBV, cerebral blood volume; AD, Alzheimer's disease; non-Tg, nontransgenic; N/A, not available.

mice is an overall decrease in spectral energy in AD mice. The ratio in AD-to-non-Tg spectral energy (Fig. 5F) was calculated to explore whether the decrease in energy is uniform across modalities. This calculation shows the largest decreases in the 0.02–0.08 and 0.08–0.14 Hz bands from OIS-CBV data, suggesting that overall vascular function is impaired in AD mice across the brain. This might be due to age and/or cerebral amyloid angiopathy (CAA), which is the deposition of amyloid plaques in vascular smooth muscle. An image of amyloid deposition from one AD mouse

(Fig. 7) obtained using Methoxy-X04 shows prevalent amyloid deposition in all visible pial arteries over both hemispheres as well as tissue plaques.

Discussion

Blood oxygenation connectivity reflects neuronal and metabolic connectivity

This work aimed to investigate the degree with which hemodynamic connectivity measures are associated with neuronal, metabolic, and vascular measures. For this purpose, GCaMP and nontransgenic mice were used to image neuronal activity and oxidative metabolism activity, respectively, along with blood-oxygenation- and CBV-sensitive hemodynamic changes from the same animal. While overlap fraction and spatial correlation metrics did not show significant agreement between these measurements, the connectivity correlation metric showed significant agreement between GCaMP (neuronal activity) and blood oxygenation (OIS-BOLD) signals across the brain. This finding suggests that hemodynamic connectivity—as measured by blood oxygenation measurements, such as fMRI—is a valuable surrogate for the underlying neuronal connectivity. Nontransgenic animals were used to explore which physiological process most significantly reflects hemodynamic (blood oxygenation) connectivity. Greater connectivity correlation was also observed between FAI (tissue oxidative metabolism) and blood oxygenation measurements, further suggesting that metabolic contributions to hemodynamic signals are likely responsible for its significant connectivity correlation with neuronal activity. Overall, metabolism-sensitive measurements are better positioned to capture changes in connectivity, such that decreases in hemodynamic connectivity likely reflect decreases in metabolic, and hence, neural function.

GCaMP-expressing mice are ideally positioned to investigate fundamental questions behind resting-state oscillations, especially since neuronal and hemodynamic changes can be imaged simultaneously. Previous work has investigated neuro-hemodynamic relationships using a multimodal approach usually involving electrophysiology. In this work, focus was placed on comparisons between neuronal, metabolic, and hemodynamic signals measured using a comparable/state-of-the-art imaging strategy on a cortical system scale in the same animals. The transgenic GCaMP mice used express GCaMP3 throughout the cortex in a large proportion of layer 2, 3, and 5 pyramidal neurons, such that the changes in fluorescence are directly related to excitatory neuronal spiking activity (Chen et al., 2012). The temporal kinetics of GCaMP3 have been characterized and, although the changes in fluorescence are slow relative to the changes in intracellular calcium (~ 0.3 sec and ~ 2.0 sec rise and fall time, respectively), the changes in intensity are linearly related to the spike rate (Chen et al., 2012). In addition, GCaMP kinetics preserve enough energy over the selected low-frequency bands to simplify the data processing and analysis steps while maintaining a significant amount of spiking information energy. It is worth noting that GCaMP3 reports mostly spiking activity and not really subthreshold synaptic activity. Higher-frequency activity, such as gamma-band activity, is more sensitive to spiking activity and the results obtained from GCaMP mice are in line with resting-state electrophysiological reports where the energy across broad frequency bands is correlated with low-

FIG. 5. (A) Sample GCaMP, OIS-BOLD, and OIS-CBV power spectra obtained from the processed data of a GCaMP animal. (B) Normalized power spectrum of the data in (A). (C) Average energy across three bands 0.02–0.08 Hz, 0.08–0.14 Hz, and 0.14–0.25 Hz, outlined in (A), for all GCaMP animals. GCaMP signal had the largest overall energy while OIS-BOLD signal had the lowest overall energy. (D) Average normalized spectral energy for GCaMP, OIS-BOLD, and OIS-CBV for all GCaMP animals. (E) Average spectral energy across the selected bands for FAI, OIS-BOLD, and OIS-CBV for the nontransgenic animals studied. (F) Ratio of the average spectral energy between the nontransgenic and AD animals studied across selected bands for FAI, OIS-BOLD, and OIS-CBV data. The normalized power spectra of nontransgenic and AD mice showed similar residual energy distribution across frequency bands (data not show). Error bars were provided for data with more than three samples and indicate the standard error ($n=7$).

FIG. 6. Sample correlation images generated using the average time series extracted for each cluster from each indicated modality and animal model. The scale bar indicates the magnitude of the correlation coefficient. The FAI correlation images (top row) from one nontransgenic (non-Tg) mouse show high-correlation areas with larger extent as those seen in Figure 3 for GCaMP and closer in size to those obtained using OIS. In general, the correlation images obtained from an AD mouse show much smaller high-correlation areas compared with nontransgenic animals. Scale bars indicate the correlation coefficient.

frequency hemodynamic oscillations (Liu et al., 2011; Lu et al., 2007; Pan et al., 2011, 2013; Thompson et al., 2013). Comparisons between the GCaMP runs obtained in the same animal (GCaMP-to-GCaMP in Table 1) show great agreement across the metrics used. There are several potential factors to consider that can contribute to imperfect agreement in GCaMP-to-GCaMP comparisons beyond physiological (cardiac and respiratory cycles) and random noise, especially away from the cluster locations. It is possible that the activity over different networks was not sampled a sufficient number of times to generate identical networks. Including data from longer time periods would mitigate this potential issue. Anesthetic depth is another factor that might impact the in-

teraction between networks, especially over very long recording periods. However, significant agreement was found in the metrics used, suggesting that the identified networks were stable over 5-min acquisition periods. The largest factor influencing relatively lower agreements in overlap fraction and spatial correlation metrics between GCaMP and OIS was the fundamental differences in their correlation images. OIS correlation images showed significantly larger areas of high correlation compared with GCaMP. This may be due to the larger spectral energy of GCaMP, especially over higher-frequency bands. In addition, the hemodynamic character of the OIS data is influenced by vascular and physiological artifacts (cardiac and respiratory cycles), including

FIG. 7. Amyloid plaque deposition from one of the AD mice studied over the exposed brain (top). Plaques in brain tissue (bright dots in tissue) as well as in arterial smooth muscle (bright layer surrounding pial arteries) over both hemispheres are evident. A section of the right hemisphere was expanded to better visualize plaque deposits (bottom). Methoxy X-04 was used to stain and image amyloid plaque deposits (Klunk et al., 2002).

draining effects. Some of these artifacts were minimized by masking out large vascular areas from the analysis, but it is still influenced by subcortical and small pial vasculature areas. Nonetheless, similar cluster sizes and locations were generally obtained from the OIS data. More importantly, the intercluster connectivity was largely preserved across modalities.

FAI is a fluorescence imaging technique that has been used to measure changes in the rate of oxidative metabolism (Reinert et al., 2004; Shibuki et al., 2003; Vazquez et al., 2012). This method was shown to be highly correlated with evoked neuronal activity. Also, it possesses similar temporal kinetics as GCaMP3 in the rodent brain. Its emission in the green portion of the visible light spectrum prevents it from being recorded simultaneously with GCaMP; hence, these data were obtained from a nontransgenic mouse group. In general, the FAI correlation images showed highly corre-

lated areas that spanned a similar extent to that of OIS-BOLD. This is surprising since a spatial extent similar to GCaMP was expected but it may be due to contributions from broader synaptic activity. Interestingly, the overlap fraction and spatial correlation metrics did not improve between FAI and OIS-BOLD. This is likely due to larger noise inherent in these measurements (the FAI effect size is smaller than GCaMP) and the sequential acquisition of these measurements. Other potential factors include known vascular artifacts in the FAI signal stemming from hemoglobin absorption of the autofluorescence emission. Although the GCaMP data are also potentially impacted by this source noise, its effect size is larger and vascular artifact contributions were not significant. Nonetheless, the connectivity correlation did show a relatively high correlation between FAI and OIS-BOLD. Collectively, these findings suggest that blood oxygenation measurements reflect neuronal connectivity between brain areas due to the metabolic nature of blood oxygenation signals.

These findings are in line with resting-state MRI measurements of blood flow and blood volume (Miao et al., 2014; Zhu et al., 2013; Zou et al., 2009), whereby typical low-frequency and connectivity patterns were observed. The findings obtained in this work indicate greater value in blood-oxygenation-based fMRI compared with CBF- or CBV-sensitive fMRI for reporting neuronal connectivity. Still, CBF- and CBV-sensitive fMRI techniques are important to explore since they can image brain regions prone to susceptibility artifacts. Similar functional clusters were also obtained using CMRO₂-sensitive fMRI (Wu et al., 2009), which is in line with the GCaMP and FAI findings of this work. More importantly, CBF- or CBV-based fMRI techniques might not be appropriate for certain patient populations, such as AD, since vascular function might be impaired due to CAA and may improperly indicate loss in connectivity.

AD decreases vascular correlation more than metabolic correlation

Perhaps the most important aspect of fMRI is its sensitivity to specific changes in network function associated with particular neurological conditions or diseases. AD pathology is generally characterized by amyloid and tau protein accumulation, glycolytic hypometabolism, and cognitive impairment. Previously, Bero and colleagues (2012) used a mouse model of AD to show that the degree of regional, age-related bilateral connectivity decline was associated with the amount of amyloid deposition across cortical areas, and suggested that connectivity strength predicts vulnerability to subsequent amyloid deposition. Specifically, retrosplenial cortex (an active participant in learning and memory processing in mice, similar to the default-mode network [DMN]) underwent the largest amyloid deposition and subsequent declines in connectivity, while the connectivity within sensory areas, such as visual and somato-sensory, did not change significantly (Bero et al., 2012). A simple seed-based correlation analysis over retrosplenial and somato-sensory cortices was used to verify this finding in the AD mice used (data not shown). It is possible that the sensitivity of blood-oxygenation-based methods to decreases in connectivity stems from early decreases in oxidative metabolism that precede glycolytic hypometabolism. Therefore, this

mouse model of AD was used to explore whether decreases in connectivity reported from this model might stem from decreases in metabolic activity (and hence decreases in neuronal activity) or decreases in vascular function. FAI connectivity correlation was found to be very similar to OIS-BOLD for AD and nontransgenic mice on average, suggesting that metabolic declines underlie decreases in connectivity between regions. On the other hand, vascular-only measurements (OIS-CBV) provided negligible correlation. This was found to be probably due to overall decreases in the energy of vascular measurements, suggesting strong declines in overall vascular function regardless of neural activity. Although additional experiments are necessary to establish significance, these findings support the notion that metabolism-sensitive measurements are better positioned to capture changes in connectivity, such that decreases in hemodynamic connectivity likely reflect decreases in metabolic function.

Methodological considerations

The resting-state data were collected under ketamine anesthesia. In general, anesthesia is known to have an impact on network dynamics. However, the anesthetic plane used was relatively light and network dynamics have been reported to be relatively stable between awake and lightly anesthetized conditions (Mohajerani et al., 2010). In addition, the GCaMP-to-GCaMP comparison of different acquisitions in the same animal shows great agreement lending confidence to the results obtained.

Data processing and analysis are important aspects of fMRI, where at a minimum, low-pass filtering of the resting-state blood oxygenation data is typically performed prior to seed point, cluster, or connectivity analysis. Removal of global mean signals prior to data analysis has been controversial and its inclusion in the processing pathway did not appear to have a significant impact on the results obtained. Hence, it was not implemented in the processing pipeline. Instead, focus was placed on a simple data-processing strategy that only removed known sources of error due to small light source fluctuations followed by traditional low-pass filtering of the data. It was found that this processing strategy was sufficient to delineate known networks in the mouse brain. A standard common map was not used across animals because the collection of GCaMP and FAI data required removal of the skull for imaging with acceptable SNR. It was difficult to consistently remove near-identical bilateral skull regions in every animal for imaging; therefore, all analyses were performed relative to cluster maps obtained from each animal. A thin-skull preparation that is adequate for fluorescence imaging (GCaMP and FAI) is being currently tested to avoid this source of variability and ensure the same brain areas are imaged in all animals. Data analysis then consisted of three metrics: overlap fraction, connectivity correlation, and spatial correlation. Overlap fraction aimed to quantify the overlap in clusters obtained from each measurement and it is therefore sensitive to variations in the calculated clusters. Because the denominator of the overlap fraction considers all total pixels, values over 0.5 typically indicate very good overlap between maps. In practice, most comparisons yield values between 0.3 and 0.5, indicating that there is overlap but not complete or cogenerated. Connectivity correlation and spatial correlation metrics were

used based on a reference cluster map to ensure the same brain regions are being compared. Spatial correlation is sensitive to sources of noise described earlier as well as differences in the size of highly correlated areas. Relatively small spatial correlation metrics were obtained mostly stemming from differences in the correlation extent. Connectivity correlation is probably the most important metric since it reflects the agreement in connection strength between clusters for the modalities compared. Lastly, since these metrics were computed using data from individual modalities prior to comparisons with hemodynamic data, accounting or correcting for hemodynamic lag was not necessary.

Conclusions

This work explored the degree with which hemodynamic connectivity is associated with neuronal, metabolic, and vascular connectivity measures. Although network clusters calculated using either GCaMP or OIS-BOLD data did not exhibit high spatial correlation, the connectivity correlations obtained were high and significant. Therefore, blood-oxygenation-based hemodynamic connectivity is a valuable surrogate for the underlying neuronal connectivity. In nontransgenic animals, greater connectivity correlation was observed between tissue oxidative metabolism and blood oxygenation measurements, suggesting that metabolic contributions to hemodynamic signals are likely responsible for its significant correlation with neuronal connectivity. Lastly, a mouse model of AD was used to explore the source of decreases in connectivity reported in these mice. The intercluster connectivity measured by FAI and OIS-BOLD was maintained while vascular-only signals (OIS-CBV) provided negligible correlation. Therefore, metabolism-sensitive measurements, such as BOLD-based fMRI, are better positioned to capture changes in neuronal connectivity compared to purely hemodynamic measurements.

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References

- Bero AW, Bauer AQ, Stewart FR, White BR, Cirrito JR, Raichle ME, Culver JP, Holtzman DM. 2012. Bidirectional relationship between functional connectivity and amyloid-deposition in mouse brain. *J Neurosci* 32:4334–4340.
- Biswal B, Zerrin Yetkin F, Haughton VM, Hyde JS. 1995. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med* 34:537–541.
- Brookes MJ, Woolrich M, Luckhoo H, Price D, Hale JR, Stephenson MC, Barnes GR, Smith SM, Morris PG. 2011. Investigating the electrophysiological basis of resting state networks using magnetoencephalography. *Proc Natl Acad Sci U S A* 108:16783–16788.
- Chen Q, Cichon J, Wang W, Qiu L, Lee S-JR, Campbell NR, DeStefino N, Goard MJ, Fu Z, Yasuda R, Looger LL,

- Arenkiel BR, Gan W-B, Feng G. 2012. Imaging neural activity using Thy1-GCaMP transgenic mice. *Neuron* 76:297–308.
- Cordes D, Haughton VM, Arfanakis K, Wendt GJ, Turski PA, Moritz CH, Quigley MA, Meyerand ME. 2000. Mapping functionally related regions of brain with functional connectivity MR imaging. *AJNR Am J Neuroradiol* 21:1636–1644.
- Davis T, Kwong K, Weisskoff R, Rosen B. 1998. Calibrated functional MRI: mapping the dynamics of oxidative metabolism. *Proc Natl Acad Sci U S A* 95:1834–1839.
- De Luca M, Beckmann CF, De Stefano N. 2006. fMRI resting state networks define distinct modes of long-distance interactions in the human brain. *Neuroimage* 29:1359–1367.
- de Pasquale F, Penna Della S, Snyder AZ, Lewis C, Mantini D, Marzetti L, Belardinelli P, Ciancetta L, Pizzella V, Romani GL, Corbetta M, Raichle ME. 2010. Temporal dynamics of spontaneous MEG activity in brain networks. *Proc Natl Acad Sci U S A* 107:6040–6045.
- Drew PJ, Shih AY, Kleinfeld D. 2011. Fluctuating and sensory-induced vasodynamics in rodent cortex extend arteriole capacity. *Proc Natl Acad Sci U S A* 108:8473–8478.
- Fox MD, Raichle ME. 2007. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci* 8:700–711.
- Greicius MD, Supekar K, Menon V, Dougherty RF. 2009. Resting-state functional connectivity reflects structural connectivity in the default mode network. *Cereb Cortex* 19:72–78.
- Honey CJ, Sporns O, Cammoun L, Gigandet X, Thiran JP, Meuli R, Hagmann P, Raichle ME. 2009. Predicting human resting-state functional connectivity from structural connectivity. *Proc Natl Acad Sci U S A* 106:2035–2040.
- Horecker BL. 1943. The absorption spectra of hemoglobin and its derivatives in the visible and near infra-red regions. *J Biol Chem* 148:173–183.
- Kim SG, Rostrup E, Larsson H, Ogawa S. 1999. Determination of relative CMRO₂ from CBF and BOLD changes: significant increase of oxygen consumption rate during visual stimulation. *Magn Reson Med* 41:1152–1161.
- Klunk WE, Bacskaï BJ, Mathis CA, Kajdasz ST, McLellan ME, Frosch MP, Debnath ML, Holt DP, Wang Y, Hyman BT. 2002. Imaging A[β] plaques in living transgenic mice with multiphoton microscopy and methoxy-X04, a systemically administered congo red derivative. *J Neuropathol Exp Neurol* 61:797.
- Liu X, Zhu XH, Zhang Y, Chen W. 2011. Neural origin of spontaneous hemodynamic fluctuations in rats under burst-suppression anesthesia condition. *Cereb Cortex* 21:374–384.
- Liu X, Zhu X-H, Zhang Y, Chen W. 2012. The change of functional connectivity specificity in rats under various anesthesia levels and its neural origin. *Brain Topogr* 26:363–377.
- Lowe MJ, Mock BJ, Sorenson JA. 1998. Functional connectivity in single and multislice echoplanar imaging using resting-state fluctuations. *Neuroimage* 7:119–132.
- Lu H, Zuo Y, Gu H, Waltz JA, Zhan W, Scholl CA, Rea W, Yang Y, Stein ES. 2007. Synchronized delta oscillations correlate with the resting-state functional MRI signal. *Proc Natl Acad Sci U S A* 104:18265–18269.
- Magnuson ME, Thompson GJ, Pan W-J, Keilholz SD. 2014. Time-dependent effects of isoflurane and dexmedetomidine on functional connectivity, spectral characteristics, and spatial distribution of spontaneous BOLD fluctuations. *NMR Biomed* 27:291–303.
- Majeed W, Magnuson M, Keilholz SD. 2009. Spatiotemporal dynamics of low frequency fluctuations in BOLD fMRI of the rat. *J Magn Reson Imaging* 30:384–393.
- Mantini D, Perrucci MG, Del Gratta C, Romani GL, Corbetta M. 2007. Electrophysiological signatures of resting state networks in the human brain. *Proc Natl Acad Sci U S A* 104:13170–13175.
- Mayhew J, Askew S, Zheng Y, Porcill J, Westby G, Redgrave P, Rector D, Harper R. 1996. Cerebral vasomotion: a 0.1-Hz oscillation in reflected light imaging of neural activity. *Neuroimage* 4:183–193.
- Miao X, Gu H, Yan L, Lu H, Wang DJJ, Zhou XJ, Zhuo Y, Yang Y. 2014. Detecting resting-state brain activity by spontaneous cerebral blood volume fluctuations using whole brain vascular space occupancy imaging. *Neuroimage* 84:575–584.
- Mohajerani MH, McVea DA, Fingas M, Murphy TH. 2010. Mirrored bilateral slow-wave cortical activity within local circuits revealed by fast bihemispheric voltage-sensitive dye imaging in anesthetized and awake mice. *J Neurosci* 30:3745–3751.
- Pan W-J, Thompson G, Magnuson M, Majeed W, Jaeger D, Keilholz S. 2011. Broadband local field potentials correlate with spontaneous fluctuations in functional magnetic resonance imaging signals in the rat somatosensory cortex under isoflurane anesthesia. *Brain Connect* 1:119–131.
- Pan W-J, Thompson GJ, Magnuson ME, Jaeger D, Keilholz S. 2013. Infralow LFP correlates to resting-state fMRI BOLD signals. *Neuroimage* 74:288–297.
- Pawela CP, Biswal BB, Cho YR, Kao DS, Li R, Jones SR, Schulte ML, Matloub HS, Hudetz AG, Hyde JS. 2008. Resting-state functional connectivity of the rat brain. *Magn Reson Med* 59:1021–1029.
- Reinert K, Dunbar R, Gao W, Chen G, Ebner T. 2004. Flavoprotein autofluorescence imaging of neuronal activation in the cerebellar cortex *in vivo*. *J Neurophysiol* 92:199–211.
- Shibuki K, Hishida R, Murakami H, Kudoh M, Kawaguchi T, Watanabe M, Watanabe S, Kouuchi T, Tanaka R. 2003. Dynamic imaging of somatosensory cortical activity in the rat visualized by flavoprotein autofluorescence. *J Physiol* 549:919–927.
- Shmuel A, Leopold DA. 2008. Neuronal correlates of spontaneous fluctuations in fMRI signals in monkey visual cortex: implications for functional connectivity at rest. *Hum Brain Mapp* 29:751–761.
- Smith SM, Fox PT, Miller KL, Glahn DC, Fox PM, Mackay CE, Filippini N, Watkins KE, Toro R, Laird AR, Beckmann CF, Raichle ME. 2009. Correspondence of the brain's functional architecture during activation and rest. *Proc Natl Acad Sci U S A* 106:13040–13045.
- Stigler KA, McDonald BC, Anand A, Saykin AJ, McDougle CJ. 2011. Structural and functional magnetic resonance imaging of autism spectrum disorders. *Brain Res* 1380:146–161.
- Thompson GJ, Merritt MD, Pan W-J, Magnuson ME, Grooms JK, Jaeger D, Keilholz SD. 2013. Neural correlates of time-varying functional connectivity in the rat. *Neuroimage* 83C:826–836.
- Vazquez AL, Fukuda M, Kim S-G. 2012. Evolution of the dynamic changes in functional cerebral oxidative metabolism from tissue mitochondria to blood oxygen. *J Cereb Blood Flow Metab* 32:745–758.
- Vemuri P, Jones DT, Jack CR. 2011. Resting state functional MRI in Alzheimer's Disease. *Alzheimers Res Ther* 4:2.
- Wang Z, Chen LM, Négyessy L, Friedman RM, Mishra A, Gore JC, Roe AW. 2013. The relationship of anatomical and functional connectivity to resting-state connectivity in primate somatosensory cortex. *Neuron* 78:1116–1126.
- White BR, Bauer AQ, Snyder AZ, Schlaggar BL, Lee J-M, Culver JP. 2011. Imaging of functional connectivity in the mouse brain. *PLoS One* 6:e16322.

- Williams KA, Magnuson M, Majeed W. 2010. Comparison of α -chloralose, medetomidine and isoflurane anesthesia for functional connectivity mapping in the rat. *Magn Reson Imaging* 28:995–1003.
- Woodward ND, Karbasforoushan H, Heckers S. 2012. Thalamo-cortical dysconnectivity in schizophrenia. *Am J Psychiatry* 169:1092–1099.
- Wu CW, Gu H, Lu H, Stein EA, Chen J-H, Yang Y. 2009. Mapping functional connectivity based on synchronized CMRO2 fluctuations during the resting state. *Neuroimage* 45:694–701.
- Zhu S, Fang Z, Hu S, Wang Z, Rao H. 2013. Resting state brain function analysis using concurrent BOLD in ASL perfusion fMRI. *PLoS One* 8:e65884.
- Zou Q, Wu CW, Stein EA, Zang Y, Yang Y. 2009. Static and dynamic characteristics of cerebral blood flow during the resting state. *Neuroimage* 48:515–524.

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