Dual-Echo ASL Contributes to Decrypting the Link between Functional Connectivity and Cerebral Blood Flow

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Abstract: Arterial spin labeling (ASL) MRI with a dual-echo readout module (DE-ASL) enables noninvasive simultaneous acquisition of cerebral blood flow (CBF)-weighted images and blood oxygenation level dependent (BOLD) contrast. Up to date, resting-state functional connectivity (FC) studies based on CBF fluctuations have been very limited, while the BOLD is still the method most frequently used. The purposes of this technical report were (i) to assess the potentiality of the DE-ASL sequence for the quantification of resting-state FC and brain organization, with respect to the conventional BOLD (cvBOLD) and (ii) to investigate the relationship between a series of complex network measures and the CBF information. Thirteen volunteers were scanned on a 3 T scanner acquiring a pseudocontinuous multislice DE-ASL sequence, from which the concomitant BOLD (ccBOLD) simultaneously to the ASL can be extracted. In the proposed comparison, the brain FC and graph-theoretical analysis were used for quantifying the connectivity strength between pairs of regions and for assessing the network model properties in all the sequences. The main finding was that the ccBOLD part of the DE-ASL sequence provided highly comparable connectivity results compared to cvBOLD. As expected, because of its different nature, ASL sequence showed different patterns of brain connectivity and graph indices compared to BOLD sequences. To conclude, the resting-state FC can be reliably detected using DE-ASL, simultaneously to CBF quantifications, whereas a single fMRI experiment precludes the quantitative measurement of BOLD signal changes. *Hum Brain Mapp* 38:5831–5844, 2017. © 2017 Wiley Periodicals, Inc.

Key words: DE-ASL; BOLD; resting state; functional connectivity; graph theory

INTRODUCTION

Additional Supporting Information may be found in the online version of this article.

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In the past decades, the discovery of consistent intrinsic metabolic and perfusion patterns in the brain has led to a change in traditional neuroimaging methods of acquisition and analysis of the brain function [Raichle, 2011]. The increasing and widespread use of resting-state functional magnetic resonance imaging (rs-fMRI) can be mostly attributable to the studies of Biswal et al. [1995], which proved for the first time and noninvasively the existence of a spontaneous activity in the brain. In few years, the scientific community focus shifted from simple regional activations or deactivations toward a deeper understanding

of the communication patterns between brain regions and network organization, that is, the so-called “functional connectivity” (FC).

Assessment of FC from neuroimaging data has revealed fundamental information about the brain organization even at rest. Complex spontaneous dynamics were observed in resting-state condition with intermittent spatio-temporal fluctuation patterns [Hansen et al., 2015]. Some authors observed modular structures in the resting-state brain networks [Achard and Bullmore, 2007; Yan and He, 2011] and similar organization between task-related and resting-state networks [Smith et al., 2009], demonstrating that an “intrinsic” standard architecture of functional brain organization persists also in rest conditions [Cole et al., 2014]. Recent studies have also established that interregional correlations between regions of interest (ROIs) involved in the visual, language, motor, and working memory systems can be detected in resting state [Rogers et al., 2007].

To date, the majority of rs-fMRI studies have been based on the blood oxygen level-dependent (BOLD) contrast [van Dijk et al., 2010; Friston et al., 2014]. However, despite its success and undoubted advantages, BOLD rs-fMRI suffers from several limitations. First, the signal is only an indirect measure of the neural activity due to several physiological and noise contributions to its contrast mechanism. It does not provide any quantitative measure and consists of only indirect contributions from cerebral blood flow (CBF), cerebral blood volume (CBV) and, when applicable, the cerebral metabolic rate of oxygen consumption. Second, the technique offers limited spatial specificity to the site of neuronal activity due to the contribution of draining veins to the BOLD contrast [Chen et al., 2015].

Nevertheless, thanks to its high signal-to-noise ratio (SNR) and temporal resolution compared to other functional MRI techniques as arterial spin labeling (ASL), BOLD is still most frequently used to investigate the whole-brain FC [Smith et al., 2013], as a biomarker for various diseases [Fox and Greicius, 2010] and to assess physiologic parameters with clinical relevance such as the cerebrovascular reactivity (CVR) [Faraco et al., 2015]. On the other hand, over the past years, many efforts have been devoted to solve specific technical issues and improve still suboptimal aspects of the different ASL sequences, typically related to reduced temporal and spatial resolution, limited brain coverage, and low SNR [Alsop et al., 2015], to make ASL more suitable for characterizing brain perfusion and to increase its sensitivity to different brain states or pathological mechanisms.

An excellent alternative to fully assess the brain mechanisms is given by the ASL with a dual-echo readout module (DE-ASL). This technique enables the noninvasive simultaneous acquisition of CBF-weighted images and BOLD contrast [Ghatriq et al., 2014]. Images acquired at a short echo time (TE) can be used to produce perfusion time series with low BOLD weighting, while images acquired at long TE can be used to form BOLD time series [Liu et al., 2002; Woolrich et al., 2006]. The main advantage of this combined sequence

is given by the complementary nature of the BOLD and perfusion contributions that characterize the spatiotemporal properties of each single voxel. While BOLD signal is sensitive to blood oxygenation, CBF and CBV, ASL can offer absolute CBF quantification in mL/g/min allowing the detection of global and regional altered perfusion and, therefore, a more direct quantitative correlate of neural activity in comparison to BOLD [Luh et al., 2000]. Furthermore, the concomitant acquisition provides information about the cerebral microvasculature functioning and reduces significantly the scan time when compared to the BOLD and ASL sequences separately acquired.

Although a general consensus on the most accurate or efficient method of measuring FC at rest [Rogers et al., 2007] is still lacking, the most frequently used approach is to extract the time-series of the BOLD response from multiple ROIs [Fox and Raichle, 2007]. The extracted time-series are then correlated (or partial correlated) with the time-series of other regions to determine connectivity among the different ROIs. An additional level of analysis that has been recently introduced [Bullmore and Sporns, 2009] relies on graph theory. The ROI-based approach described above results in a connectivity matrix reflecting the brain functional/structural organization that can be expressed in the form of a graph. Even though graph theory is a relatively old branch of mathematics, the extraction of the most significant features from brain connectivity patterns is a recent challenging topic [Bullmore and Sporns, 2009; De Vico Fallani et al., 2010]. Graph analysis can be used to study complex systems ranging from micro (genes, neurons) to macroscopical level (brain regions) [De Vico Fallani et al., 2010]. Theoretically, nodes correspond to individual neurons, neuronal populations, or brain regions, while edges are connections that relate pairs of nodes, and the set of nodes and edges are represented in a connectivity matrix. Among all the characteristics that can be extracted from the network, of great importance are how a node interacts with its immediate neighbors and how a node transfers or processes information (e.g., highly influential nodes are referred to as “hubs”) [Bullmore and Sporns, 2009].

To date, it is still also unclear how those graph indices could express FC based on CBF. Only recently, advancements in ASL have enabled the assessment of FC as in fMRI. This article allows moving a step forward in this direction providing some evidence of the relation among functional brain mapping as revealed by different imaging modalities and the first assessment of the relation between FC and CBF.

In particular, ASL showed equivalent results in terms of resting-state networks (RSNs) as the canonical BOLD sequence [Liang et al., 2012a] and the static and dynamic characteristics of the CBF at rest had high correlation in terms of FC between nodes in the default mode network [Zou et al., 2009]. The overall FC in BOLD RSNs was demonstrated to be stronger than that of ASL RSNs, but ASL networks showed higher FC in specific brain regions (e.g., orbitofrontal cortex). Indeed, the brain regions inside the default mode network

(DMN) along with others (temporal cortex, frontal cortex, and occipital cortex) were classified as hub regions [Achard et al., 2006; Liang et al., 2012b].

The overall aim of this work was to demonstrate that the DE-ASL sequence, which allows the concomitant acquisition of ASL and BOLD data, could be a valid substitute of conventional BOLD (cvBOLD) delivering comparable results over a spectrum of different analyses while providing new and complementary insights based on the assessment of the CBF. This goal was achieved through (i) the assessment of the potentiality of the DE-ASL sequence for the quantification of FC changes and brain organization in resting-state condition with respect to cvBOLD following the same analysis; (ii) the investigation of the relationship between a series of complex network measures and the CBF information as derived from the ASL data. This last grounded on the hypothesis that the network specific quantitative CBF values may indicate the baseline perfusion activity and may be associated with the strength of FC of the same network.

MATERIALS AND METHODS

Image Acquisition and Protocol

Thirteen right-handed volunteers (mean age 32.5 ± 8.6 , 8 males) were scanned on a 3T Philips Achieva scanner equipped with 8-channel head coil. For DE-ASL data, interleaved control and label images were acquired using a 2D echo-planar imaging (EPI) readout. A pseudocontinuous (pCASL) labeling was applied in combination with the following parameters: $TR = 3,800$ ms; $TE_1 = 10$ ms for ASL data set and $TE_2 = 35$ ms for concomitant BOLD [ccBOLD] data set; label duration/post-label delay = 1,500/1,000 ms; 26 slices, $3 \times 3 \times 3$ mm³, slice gap = 1 mm, $FOV = 240 \times 240$ mm²; two background suppression pulses at 1,530 and 2,250 ms from the start of the scan; flip angle = 90°. For cvBOLD data, the following parameters were applied: $TR = 2,200$ ms; $TE = 35$ ms; 36 slices, $3 \times 3 \times 3$ mm³, slice gap = 1 mm, $FOV = 240 \times 240$ mm²; flip angle = 90°. A 3D T1-weighted turbo field echo anatomical scan was also acquired for each subject (MPRAGE sequence, $TR = 8.16$ ms; $TE = 3.73$ ms; 180 slices, $1 \times 1 \times 1$ mm³, $FOV = 256 \times 256$ mm²; flip angle = 8°). cvBOLD and DE-ASL sequences (256 volumes each) were acquired in resting-state conditions. The volunteers did not perform any task and were instructed to lie still in the scanner, to keep their eyes closed, but not to fall asleep while images were collected.

FC Analysis

All data sets (cvBOLD, ccBOLD, and ASL) were standardly preprocessed, denoised, that is, corrected for nuisance effects by using linear regression, and band-pass filtered as described hereafter. FSL 5.0.5 (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>) and MatlabR2012b (MathWorks,

Natick, MA) with in-house scripts specific for this study were employed for the analyses.

Preprocessing

The brain tissue was extracted from T1 anatomical images using Brain Extraction Tool (BET) [Smith, 2002] and registered to the standard Montreal Neurological Institute (MNI) space by using FLIRT (affine registration, 12 DOF). FMRIB's Automated Segmentation Tool (FAST) was used to segment the spatially normalized brain images into white matter (WM) and cerebrospinal fluid (CSF) tissues, whose time courses were then used for nuisance correction. Functional images were preprocessed using FEAT to correct for subject motion (MCFLIRT) [Jenkinson et al., 2002] and to extract the brain. Spatial smoothing was applied using an isotropic Gaussian filter with a full-width-at-half-maximum of 6 mm³. FLIRT was then used to register functional images to anatomical images (rigid body registration, 6 DOF) which were finally normalized to MNI space (affine registration, 12 DOF).

Denoising: Removing residual motion artifacts and CSF and WM fluctuations

In BOLD fMRI datasets, several nuisance signals are typically regressed out from each voxel time series to reduce the effects of non-neuronal fluctuations such as scanner instabilities, subject motion, and physiological artifacts [Birn et al., 2008; Dagli et al., 1999; Windischberger et al., 2002]. As the same correction was previously proven to be appropriate also for ASL data [Wang 2012], all preprocessed data sets were corrected for nuisance effects (head motion profiles, CSF, and WM signals) by linear regression that minimizes the sum of squares of the residuals.

The head motion regressors consisted of the six motion parameters (three translations and three rotations) obtained from rigid-body head motion correction as implemented in FSL (MCFLIRT), whereas the CSF and WM regressors were calculated as average of time courses within the corresponding tissue masks generated from the previous segmentation.

For ccBOLD and ASL data, CSF and WM regressors were orthogonalized with respect to the ideal label/control paradigm before entering the regression model [Wang, 2012]. Of note that denoting label by -1 and control by 1 , the zig-zagged label-control paradigm is a binary numerical series consisting of oscillating -1 and 1 values.

The "global signal"—i.e., the average signal across all brain voxels—was not regressed out from the time series. The reason behind this conservative choice is that global signal regression is still a controversial issue in preprocessing [Murphy et al., 2009; Saad et al., 2012]. As this strategy could lead to the introduction of spurious negative measures and the underestimation of true positive ones, global signal regression was not taken into account to minimize errors in functional connectivity results and avoid ambiguous conclusions [Weissenbacher et al., 2009].

Band-pass filtering

Resting-state fMRI data were band-pass filtered to reduce the effects of low-frequency drift and high-frequency physiological noise. Considering that the resting-state networks are characterized by slow fluctuations of functional imaging signals, the residuals derived from the nuisance regression were bandpass filtered between 0.008 and 0.1 Hz [Cordes et al., 2001; Damoiseaux et al., 2006; De Martino et al., 2006; Mantini et al., 2007]. As suggested by Hallquist et al., the motion-related fluctuations and other nuisance signals, corrected by bandpass filtering followed by regression, reintroduces nuisance variation at frequencies that were previously bandpass filtered, and under-corrects for nuisance variation in the passband. Conversely, the appropriate application of a regression followed by bandpass filtering was not associated with the introduction of nuisance artifacts and did not suffer from the same suboptimal attenuation of nuisance variation [Hallquist et al., 2013].

FC estimation and graph theoretical analysis of network topology

Using in-house Matlab scripts, cleaned data from each subject and sequence were used for the connectivity analysis. First, brain parcellation, based on the Harvard-Oxford Probabilistic MRI Atlas (HOA) [Makris et al., 1999] and the cerebellar Atlas [Diedrichsen et al., 2009] as included in FSL, was performed. From these atlases, 139 regions were extracted. In details: cortical and subcortical regions, considering separately the right and left hemispheres, and cerebellar ROIs divided into left, vermis, and right regions. For each of these regions and for the different datasets (cvBOLD, ccBOLD, and ASL), a representative mean time series of 256-volumes length was extracted by averaging the time series of all voxels within the area and used as reference.

A 139×139 symmetric connectivity matrix was then derived for each subject and sequence by calculating the Pearson correlation coefficient (r value) between pairs of nodes. To avoid the issue of arbitrary thresholding and given the lack of agreement in how treating negative correlations [Achard and Bullmore, 2007; Fair et al., 2009], our network analyses were conducted on fully connected graphs [Storti et al., 2016] with only positive weights. For the sake of matrices representations, regions of cerebral and cerebellar left hemisphere (and cerebellar vermis) were indexed in the first part of the matrix and regions of right hemisphere in the second one, respectively. The 2D-correlation between the mean connectivity matrices (cvBOLD vs ccBOLD and ccBOLD vs ASL) was calculated to better elucidating their global similarity in terms of resting-state FC.

Graph theoretical analysis was performed by using the open-source BCT toolbox (Brain Connectivity Toolbox, BCT, <https://sites.google.com/site/bctnet/Home>). The local topological properties of the brain networks (strengths S , clustering coefficient CC , betweenness centrality BC , and modularity) were calculated. In particular, S of the i th node

is defined as the sum of weights along edges connecting to the node i , CC measures the tendency of the nodes to segregate into separate subnetworks and BC is an indicator of a node centrality in a network. As graph properties are highly dependent on the average weight of a network (which is a variable independent of network architecture), the connection weights of all edges were normalized before the statistical assessment. The normalization consisted in dividing each individual connectivity matrix by the mean weight of the whole network to keep the network cost at the same level for all subjects [Chen et al., 2013]. Finally, the indices of the graph were averaged across subjects obtaining a mean value for each condition and each sequence.

Communities or modules are defined as subgroups of densely interconnected nodes in a network [Newman and Girvan, 2004]. The network modularity was computed for the mean cvBOLD and ccBOLD matrices by using the Louvain algorithm [Blondel et al., 2008], which is a greedy method trying to optimize a quality function of clusters to identify community structures of the brain network. To overcome the heuristic nature of the algorithm for community detection, the partition of the network was performed 1,000 times. An agreement matrix, whose elements indicate the number of times any two nodes were assigned to the same class, was constructed on these independent realizations. The Louvain algorithm was then applied to this agreement matrix to generate a consensus partition for each sequence. The similarity of the modular community structure of cvBOLD and ccBOLD sequences was quantified in terms of normalized mutual information (NMI) [Danon et al., 2005]. The NMI ranges between [0–1]: if the two partitions obtained from cvBOLD and ccBOLD are identical, NMI is 1, whereas NMI is 0 if they are completely dissimilar.

Statistical analysis of FC matrices and graph indices

To assess the statistical significance of between-sequence differences (cvBOLD vs ccBOLD, ccBOLD vs ASL and cvBOLD vs ASL) in terms of FC and regional network measures, a nonparametric method, specifically a permutation-based inference, was used [Bassett et al., 2008; Bernhardt et al., 2011; He et al., 2008; Lynall et al., 2010]. This analysis was conducted to examine whether the role of region i in the overall organization of the brain network was changed using the different techniques. As the permutation process empirically generates the distribution under the null hypothesis from the data by permuting data labels, the test requires no knowledge of how the test statistic of interest is distributed under the null hypothesis [Simpson et al., 2013]. Of note that the comparisons between real networks do not require normalization for the random network to test statistically whether the graph properties are different between the three sequences.

Specifically, for permutation testing, the FC values (or the network measures) of each individual are randomly reassigned to one of the two groups in each repetition, so

that each randomized group has the same number of subjects as the original group. In a pair-wise test, as in our scenario, the pairing information needs to be maintained during the permutation process and the exchangeability is limited to the same subject acquired using the different MRI sequences. In our case, there are 13 different blocks. To do so, the group label is flipped within each subject [Simpson et al., 2013]. Then, the differences in FC or network measures between randomized groups are computed resulting in a permutation distribution of difference under the null hypothesis. The procedure results in a sampled between-group difference null distribution for FC and each graph metric. Finally, the actual between-group difference in network measures is placed in the corresponding permutation distribution and a two-tailed P value with a significance threshold of 0.05 (family-wise error, FWE, corrected) is calculated based on its percentile position (de Reus and van den Heuvel, 2013). This testing was carried out with the “randomise” function of FSL with 5,000 permutations.

CBF Estimation

ASL data were preprocessed as described in the section “Preprocessing,” that is denoised to remove residual motion artifacts but maintained in the native space for the purpose of quantifying the CBF. Then, a high-pass filtering with a cutoff of 0.008 Hz was applied.

After these preprocessing steps, control and label pCASL volumes were pair-wise subtracted and averaged to obtain perfusion-weighted images. These perfusion-weighted maps were quantified into CBF [ml/100 g/min] applying the general kinetic model [Buxton et al., 1998] as follows:

$$\text{CBF} = \frac{6000 \cdot \lambda \cdot \Delta M \cdot e^{\frac{\text{PLD}}{T_{1b}}}}{2 \cdot \alpha \cdot T_{1b} \cdot M_{0t} \cdot (1 - e^{-\frac{\tau}{T_{1b}}})} \quad (1)$$

where λ is the brain-blood partition coefficient (0.9 mL/g), ΔM represents the difference images (perfusion-weighted maps), PLD is the postlabeling delay, T_{1b} is the longitudinal relaxation time of arterial blood (1,650 ms), α is the labeling efficiency (0.85 for pCASL), M_{0t} is the tissue equilibrium magnetization (voxel-wise estimated from the calibration scan), and τ represents the labeling duration [Alsop et al., 2015]. The increase in label decay in the ascending slices acquired with 2D readout was accounted for.

For each subject, these CBF maps in native space were affine-registered to the individual high-resolution anatomical images by applying the previously estimated transformation matrix (see section “Preprocessing”). Then, the joint ASL/T1-weighted and T1-weighted/MNI space transformation parameters were used to spatially normalize the CBF maps. Finally, the mean and standard deviation (SD) of CBF across all subjects were calculated and represented as maps.

In addition, mean and SD of CBF within the grey matter voxels of each ROI were extracted.

Regional CBF versus Graph Indices

To investigate the relationship between CBF and graph indices (BC, S, and CC) at group level, a polynomial regression of first and second order were employed to fit a predictive model to observed region-wise network indices versus CBF. A F test on the regression model was applied to test for a significant linear regression relationship between each of the indices (BC, CC, and S) and the CBF. The F statistic was then compared with the intercept-constant model: if the P value for the F test was <0.05 , the null-hypothesis was rejected since our model provided a better fit than the intercept-only model. R^2 , root-mean-square error (RMSE), and P values resulting from testing the significance of the polynomial fitting for each of the three indices (BC, CC, and S) and for the three comparisons (cvBOLD vs CBF, ccBOLD vs CBF, and ASL vs CBF) were reported. It is worthy noting that other models were also investigated but they did not improve the distribution of the residuals. In detail, CBF values were extracted for each subject and mean CBF was obtained by averaging CBF across all subjects; network metrics were calculated for single subject first, and then averaged to obtain mean network indices at group level.

RESULTS

Figure 1 displays the FC between ROIs, averaged over subjects in each sequence. The similarities between cvBOLD and ccBOLD FC matrices and between ccBOLD and ASL FC matrices were high ($r = 0.72$ and $r = 0.80$, respectively), as expected. FC analyses revealed common links in cvBOLD and ccBOLD with a high level of spatial overlap, as assessed by the statistical permutation test ($P < 0.05$, FWE corrected). In detail, cvBOLD $>$ ccBOLD did not show any significant difference, while ccBOLD $>$ cvBOLD showed differences only in few connections, which mainly were affecting cerebellum and right and left temporal lobe, as illustrated in Figure 2. Comparing the ASL sequence with the other two, some interesting observations can be made. In particular, keeping in mind the different nature of the ASL signal, we found that the ASL connectivity values were never significantly lower than the connectivity values from BOLD data. Conversely, statistically higher FC values were found in multiple and widespread connections across networks for ASL versus cvBOLD and the same pattern was also reflected in numerous connections for the ASL versus ccBOLD comparison (Fig. 2).

Nodes exhibiting significant group differences in CC, BC, and S are illustrated in Figure 3. Considering the statistical comparisons between the two BOLD methods, group analyses did not show strong differences in BC, cvBOLD being significantly greater than ccBOLD only in

Figure 1.

Functional connectivity in resting-state condition averaged across all the subjects ($n = 13$) for cvBOLD, ccBOLD, and ASL. Region labels correspond to the Harvard–Oxford atlas (Supporting Information, S1). The ROIs are sorted according to anatomical lobe information: FL, frontal lobe; TL, temporal lobe; PL, parietal lobe; OL, occipital lobe; LL, limbic lobe; SUB, subcortical cortex; CER, cerebellum; LH, left hemisphere; RH, right hemisphere.

Regions are the row and column indices of the connectivity matrices and the corresponding matrix element provides the color-coded Pearson correlation value, as indicated by the color bar on the right. [Color figure can be viewed at wileyonlinelibrary.com]

the subcallosal cortex. For the CC, cvBOLD was significantly higher in regions principally involved in the motor circuit as well as in few subcortical regions, while no differences were detected between S values. Regarding the cvBOLD–ASL comparison, decreased BC for the left insula (INS) and angular gyrus (AG) and an increase BC in the right supplementary motor cortex (SMC) were highlighted.

Similarly to the cvBOLD–ccBOLD comparison CC values from cvBOLD were significantly higher in regions principally involved in the motor circuit, compared to ASL. For the S values, the differences were limited to the motor circuit and few subcortical regions (PT.1 and H.1). Finally, the ccBOLD–ASL comparisons did not show any significant difference for the three metrics.

Figure 2.

Significant functional connectivity links ($P < 0.05$, FWE corrected) are illustrated between cvBOLD versus ccBOLD, ccBOLD versus ASL, and cvBOLD versus ASL. Each matrix element provides the color-coded P value, as indicated by the hot-color bar on the right. In each panel, only the lower triangle part of the matrix—i.e., excluding self-connections and redundant connections—is shown

due to the symmetry of the connectivity matrix. Region labels correspond to the Harvard–Oxford atlas (Supporting Information, S1). FL, frontal lobe; TL, temporal lobe; PL, parietal lobe; OL, occipital lobe; LL, limbic lobe; SUB, subcortical cortex; CER, cerebellum; LH, left hemisphere; RH, right hemisphere. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 3.

Nodes exhibiting significant group differences in betweenness centrality (BC) (A), cluster coefficient (CC) (B), and strengths (S) (C). The sizes of the spheres are based on *P*-corrected values, red color represents significantly higher BC, SS, and CC in cvBOLD compared to ccBOLD (or cvBOLD vs ASL) (Contrast

1); blue color represents significantly lower BC, SS, and CC in the cvBOLD compared to ccBOLD (or cvBOLD vs ASL) (Contrast 2). Region labels correspond to the Harvard-Oxford atlas (Supporting Information, S1); r, right hemisphere; l, left hemisphere. [Color figure can be viewed at wileyonlinelibrary.com]

The network modularity calculation resulted in the same number of communities for the two group-based community structures (cvBOLD and ccBOLD). Specifically, eight predominant modules were identified comprising the fronto-parietal, visual, orbitofrontal/limbic, subcortical, sensorimotor, and temporal systems. Figure 4 shows the modular structure of group averaged functional brain network for cvBOLD and ccBOLD data. The NMI between cvBOLD and ccBOLD was 0.63, indicating a reasonable degree of similarity between sequences in modular organization of functional networks. The common modules are the occipital, temporal, and fronto-parietal. The main difference between techniques is that ccBOLD can distinguish between sensori-motor (orange) and temporal (green) module and create a subcortical isolate (yellow) module, whereas cvBOLD separates the cingulate Gyrus, posterior division and precuneous (yellow) in a different module and the fronto-parietal in right (orange) and left (red) modules, respectively.

The quantitative CBF values were consistent with the results on normal groups obtained in a previous work [Boscolo Galazzo et al., 2015]. The mean CBF maps across subjects for some slices of interest are reported in Figure 5, along with the corresponding SD maps. The mean CBF value

inside each ROI was also calculated to investigate the relationship with the graph indices. The mean value can be considered as representative of the central location of the data because they exhibited good spatial uniformity. The mean CBF map showed an overall mean of 46.6 ml/100 g/min with a ROI range of [13.4–75.0] with the minimum value in the right cerebellum VIIIb and the maximum in the intracalcarine cortex and a mean ROI SD of 14.2 ml/100 g/min with a ROI range of [4.5–30.3], with the minimum value in the cerebellum crus II and the maximum in the right superior temporal gyrus, posterior division.

For all subjects, all measures were within the range of normal perfusion levels. Higher than average CBF + SD was found in INS, inferior frontal gyrus pars opercularis (F3o), intracalcarine cortex (CALC), planum polare (PP), supracalcarine cortex (SCLC), paracingulate gyrus (PAC), cingulate gyrus anterior and posterior division (CGa and CGp), cuneal cortex (CN); parahippocampal gyrus posterior division (PHp), lingual gyrus (LG), planum polare (PP), heschls gyrus (H), planum temporale (PT), and cerebellum V–VI.

When CBF and graph indices were compared at group level, a general second-order polynomial regression provided a better fit than a first-order polynomial regression,

Figure 4.

Partition of the cerebral cortex into network communities (modules) based on functional connectivity derived from cvBOLD (A) and ccBOLD (B). R, right; L, left. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 5.

Mean (A) and standard deviation (B) maps of CBF values in physiological units (ml/100 g/min) calculated across a group of 13 subjects in axial view. R, right; L, left. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 6.

Scatterplots of CBF values for each region versus corresponding BC indices calculated from the three sequences (cvBOLD, ccBOLD, and ASL). A first-order polynomial (red line) and a second-order polynomial (green line) were fitted to the data. Each symbol represents data from one region of the atlas (139 in total), averaged over all subjects. [Color figure can be viewed at wileyonlinelibrary.com]

although all the results were quite similar. Figure 6 shows the scatter plot between the BC and CBF for the group-averaged data. Each symbol represents data from one HOA region, averaged across all subjects. The mean BC values of the different cortical and cerebellar regions were positively correlated with the mean CBF values across subjects.

In detail, the BC index derived from ccBOLD and ASL sequences versus region-wise CBF mean value showed positive linear trend with P values consistently lower than 0.05 for both datasets. This demonstrates that BC of both ccBOLD and ASL data can be significantly fitted with the polynomial

model (first or second order). For example, in ccBOLD sequence high BC (>0.6) and high CBF (>60 ml/100 g/min) mean values were found in regions principally involved in the resting state networks, such as the LG (CBF = 69 ml/100 g/min, BC = 0.81) and SCLC (CBF = 72 ml/100 g/min, BC = 0.63) related to the occipital network or PP, PT, and INS involved in the motor network as well as F3o, CGa, CGp, and SCLC. For the cvBOLD versus CBF low values of R^2 were detected. The CC index showed almost no correlation between CC-cvBOLD and CBF, while quite high values of R^2 were found between CC-ccBOLD and CBF and between CC-ASL and CBF ($P < 0.05$). Finally, the S index

TABLE I. R^2 , root-mean-square error (RMSE) and P values that result from testing the polynomial fitting of first and second order for each of the three indices (BC, CC, and S) and for the three comparisons: cvBOLD versus CBF, ccBOLD versus CBF, and ASL versus CBF

	cvBOLD vs CBF		ccBOLD vs CBF		ASL vs CBF	
	Order 1	Order 2	Order 1	Order 2	Order 1	Order 2
BC						
R^2	0.083	0.009	0.308	0.316	0.252	0.253
RMSE	12.2	12.2	10.6	10.58	11.02	11.05
P	0.000571	0.00168	1.31E-12	6.23E-12	3.13E-10	2.38E-09
CC						
R^2	0.0002	0.0007	0.287	0.288	0.137	0.175
RMSE	12.74	12.79	10.76	10.79	11.84	11.62
P	0.878	0.955	1.06E-11	9.47E-11	7.63E-06	2.10E-06
S						
R^2	0.047	0.047	0.131	0.146	0.129	0.161
RMSE	12.44	12.48	11.88	11.82	11.89	11.72
P	0.0102	0.0369	1.19E-05	2.12E-05	1.42E-05	6.69E-06

showed low R^2 values between all the sequences versus CBF, although significant ($P < 0.05$) (Table I).

DISCUSSION

Over the last two decades, perfusion MRI has received particular attention in neuroscience due to its quantitative and noninvasive nature. The recent development of more advanced ASL sequences, characterized by higher spatial resolution, good brain coverage, and higher SNR, enabled the investigation of FC and graph indices with this technique, in addition to its established ability to locate the brain activity due to blood flow changes [Chuang et al., 2008]. Recently, the DE-ASL sequence was introduced for simultaneously measuring BOLD and CBF signals, but its potential to reliably measure connectivity at rest is still unproved. To the best of our knowledge, only one study of rs-fMRI exploited the DE-ASL sequence to determine its potential in the FC investigations [Tak et al., 2015]. In this work, the authors explored the regional associations between resting-state functional connectivity MRI estimates and dynamic coupling between BOLD and CBF, as well as resting macrovascular volume fraction in some RSNs. Using seed-based connectivity analyses and anatomical MNI coordinates from literature for identifying the seed regions within three predefined functional networks (default mode, frontoparietal, and primary sensory-motor networks), they found in all cases that the functional connectivity strength was significantly correlated with the regional increase in CBF-BOLD coupling strength, and inversely proportional to macrovascular volume fraction.

Differently, this technical report explored the potentialities of using DE-ASL rather than cvBOLD to estimate the FC patterns in resting state. In the proposed comparison, a brain parcellation scheme based on the cortical and sub-cortical HOA and cerebellum atlas was employed to study

the functional brain organization of the cvBOLD versus the ccBOLD and ASL derived from DE-ASL sequence. The main finding is that the ccBOLD part of the DE-ASL sequence provides highly comparable connectivity results to conventional BOLD in resting-state condition, featuring only some minor differences in BC without any difference in S. As expected, ASL, compared to cvBOLD and ccBOLD, showed different patterns of brain connectivity and graph indices. In addition, the cvBOLD and ccBOLD sequences showed the same hub patterns, whereas the ASL sequence showed again a different behavior, probably related to its different nature and to the information that it conveys.

Globally, the cvBOLD and ccBOLD sequences showed modular organization of FC within specific brain regions. NMI between cvBOLD and ccBOLD was relatively high (0.63) suggesting that cvBOLD and ccBOLD tended to produce similar community structures. The common spatial overlap between cvBOLD and ccBOLD matrices suggested a comparable behavior, despite the FC values of ccBOLD were generally higher. Also, the ASL showed globally higher values of FC, and significant differences with respect to the other techniques were observed in numerous connections. The larger correlation values exhibited by ASL could be due to the fact that the ASL signal depends only on CBF changes. In fact, our results showed that in resting state, the HOA cortical areas have all similar values of CBF (around 60 ml/100 g/min) and therefore would subtend similar signals.

The first two BOLD sequences and the ASL one are likely associated with distinct aspects of regional FC, as expressed by the fact that different regions are involved showing different connectivity properties, and thus are described by different graph indices. One possible explanation of this behavior is that these techniques may reveal different aspects of brain activity. The BOLD technique is sensitive to the relative level of oxy- and deoxy-hemoglobin concentrations in the blood as a result of brain activity induced changes in blood flow, blood volume, and metabolic rate of

oxygen extraction. ASL, instead, has a quantitative nature and can provide a more focal localization of brain activity [Boscolo Galazzo et al., 2014] and signals that are more directly related to the underlying neuronal activity, being flow-dependent only. Another possible explanation of this difference could be related to the hemodynamic response shape variability [Storti et al., 2013]. It is well known that vascular contributions could influence this variability and the different macro vascular architecture could introduce spurious differences and delays that are differently detected by the two methods (BOLD and ASL). Our data showed that for resting-state connectivity, the sensitivity of the two BOLD sequences was the same and the similarity between techniques was further confirmed by the statistical test with permutations. Differently, the observed differences between BOLD and ASL sequences in connectivity topology and local descriptors reflect the differences observed in the FCs.

Under normal physiological conditions, the brain derives most of its energy requirements from the metabolism of glucose and energy reserve glucose equivalents [Tomasi et al., 2013]. In PET imaging studies with [18F] fluorodeoxyglucose (FDG), high baseline glucose metabolic rates in ventral posterior regions of the brain were detected [Langbaum et al., 2009]. The high degree of connectivity of the same region with other brain regions has suggested an association between energy consumption and rs-fMRI data [Fukunaga et al., 2008]. Voxelwise correlations across subjects between the cerebral metabolic rate of glucose (CMRGlu) and the amplitude of the rs-fMRI signal in the brain revealed that higher metabolism was associated with higher rs-fMRI signal amplitude in cerebellum, occipital, and parietal regions [Tomasi et al., 2013].

In this study, we assessed whether a connectivity–flow relationship was present as well. To deeply explore the nature of the DE-ASL and its double contribution in terms of blood flow and synchronization in neuronal activity, we have introduced the quantification of CBF and a regression analysis with graph indices. Some previous promising results have shown a spatial similarity between connectivity hubs and metabolism distribution suggesting a relationship between intrinsic network connectivity and metabolic demands of the human brain [Liang et al., 2014]. In addition, a correspondence between BOLD and CBF connectivity maps was found in a large sample of subjects ($N = 265$) using a set of seed structures such as the intraparietal sulcus, middle temporal region, and the posterior cingulate/pre-cuneus [Viviani et al., 2011]. It was hypothesized that in normal subjects during spontaneous activity, the energy consumption is almost identical to that of a task-related state [Fox and Raichle, 2007] and the brain regions are highly organized (brain hubs) [Liang et al., 2014]. This could suggest that spontaneous activity demands a large amount of energy consumption and cerebral perfusion that could reflect an efficiently organized network. In particular, we observed high coupling between ccBOLD centrality and CBF that is likely to underlie higher neuronal involvement. In detail, the

correlation analysis between the CBF and the graph indices (BC, S, and CC) indicates a relationship between the ASL and ccBOLD-based BC in key regions and its baseline perfusion suggesting that high-perfused tissue had a large influence on the transfer of information through the network, under the assumption that item transfer follows the shortest paths. In the regression analysis, the results support the hypothesis of a linear relationship between CBF and BC of ccBOLD and ASL, although fMRI-derived parameters using graph theory cannot directly refer any physiological meaning as baseline CBF does. The relationship that exists between the two parameters may provide a physiological significance to the graph indices.

This study also has some limitations.

First, the flip angle of 90° with a TR of 2,200 ms for cvBOLD could determine suboptimal signal intensity and image contrast and it does not correspond to the Ernst angle. In this case the optimal flip angle should be around 82° , while for long TRs (>3 s), as in the DE-ASL sequence, 90° is recommended.

Second, the reduced volume coverage in the cerebellum can strongly impair the sensitivity values, and therefore suggests that an accurate optimization and planning of the acquisition volume is necessary when the cerebellum is the main focus of the study.

Third, although the data analyses (i.e., resting-state related, functional connectivity, and graph analysis) were conducted in common line for all the sequences to enable a direct comparison between results, some differences due to the acquisition of sequence-specific parameters are present. A direct comparison of ccBOLD and cvBOLD with identical parameters is not possible. In DE-ASL with pCASL labeling, the minimum TR possible is almost twice as long as in standard BOLD mainly due to the long labeling and PLD required by this acquisition scheme and the time necessary for whole brain coverage with 2D readout. The DE-ASL acquisition had the same TE and the same number of time-points of the cvBOLD acquisition for the ccBOLD part, but a lower temporal resolution. Nonetheless, it has been noted that the temporal resolution has only minor effects on FC estimation, while the total scan length is a critical factor affecting FC reliability [Birn et al., 2013; Jann et al., 2015; Smith et al., 2011]. In particular, it has been suggested that longer duration acquisitions can maximize similarity of overall whole-brain connectivity, with clear stability benefits for 10 min rather than 5 min scans [Gonzalez-Castillo et al., 2014; Birn et al., 2013], and can yield more power for detecting smaller correlations while providing more precise estimates of the size of correlations [van Dijk et al., 2010]. Considering that in our experiment acquisitions lasted around 9 min for cvBOLD and 16 min for DE-ASL, the lower temporal resolution of DE-ASL should not have a significant impact on the FC. In addition, it was compensated by a longer sequence duration that increases the reliability of FC. On the other hand, a significant advantage of the DE-ASL sequence is that

BOLD and ASL parts can be acquired within a single sequence in ~16 min for more than 250 volumes, saving acquisition time with respect to the acquisition of cvBOLD and ASL separately.

Finally, a short PLD value (1,000 ms) was chosen for the pCASL labeling instead of the recommended PLD value for young controls, that is 1,800 ms [Alsop et al., 2015]. The practical problem behind this choice is that in case of PLD = 1,800 ms, $\tau = 1,500$ ms, and 26 slices, a long TR (of about 4,600 ms) would be necessary for acquiring the whole brain with a 2D-EPI readout, resulting in a very low time resolution with a long time of acquisition. Conversely, a PLD of 1,000 ms and a TR of 3,800 ms would allow a fairer comparison between sequences. We recognize that a shorter than normally recommended PLD could generate suboptimal CBF estimates with possible over/underestimation in some regions due to the contamination from residual labeled blood in arteries and the incomplete delivery to the tissue. However, a short PLD would result in less T1 decay and thus higher SNR which is clearly advantageous for connectivity studies. Noteworthy, short PLDs with pCASL sequences (1,000–1,200 ms) have already been adopted by some researchers for concomitant connectivity analyses and CBF quantification [Cohen et al., 2016; Jann et al., 2015; Liang et al., 2013; Tak et al., 2015].

CONCLUSION

The resting-state FC can be reliably detected using DE-ASL, simultaneously to CBF quantifications, whereas a single fMRI experiment precludes the quantitative measurement of BOLD signal changes. As ccBOLD and cvBOLD sequences showed very similar patterns of FC and graph parameters in resting-state condition, DE-ASL could be a powerful technique for research and clinical applications allowing to extract quantitative physiological parameters and to characterize functional connectivity.

DISCLOSURE OF CONFLICTS OF INTEREST

None of the authors has any conflict of interest to disclose.

REFERENCES

- Achard S, Bullmore E (2007): Efficiency and cost of economical brain functional networks. *PLoS Comput Biol* 3:e17.
- Achard S, Salvador R, Whitcher B, Suckling J, Bullmore E (2006): A resilient, low-frequency, small-world human brain functional network with highly connected association cortical hubs. *J Neurosci* 26:63–72.
- Alsop DC, Detre JA, Golay X, Günther M, Hendrikse J, Hernandez-Garcia L, Lu H, MacIntosh BJ, Parkes LM, Smits M, van Osch MJ, Wang DJ, Wong EC, Zaharchuk G (2015): Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. *Magn Reson Med* 73:102–116.
- Bassett DS, Bullmore E, Verchinski BA, Mattay VS, Weinberger DR, Meyer-Lindenberg A (2008): Hierarchical organization of human cortical networks in health and schizophrenia. *J Neurosci* 28:9239–9248.
- Bernhardt BC, Chen Z, He Y, Evans AC, Bernasconi N (2011): Graph-theoretical analysis reveals disrupted small-world organization of cortical thickness correlation networks in temporal lobe epilepsy. *Cereb Cortex* 21:2147–2157.
- Birn RM, Molloy EK, Patriat R, Parker T, Meier TB, Kirk GR, Nair VA, Meyerand ME, Prabhakaran V (2013): The effect of scan length on the reliability of resting-state fMRI connectivity estimates. *Neuroimage* 83:550–558.
- Birn RM, Smith MA, Jones TB, Bandettini PA (2008): The respiration response function: The temporal dynamics of fMRI signal fluctuations related to changes in respiration. *NeuroImage* 40:644–654.
- Biswal B, Yetkin FZ, 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.
- Blondel VD, Guillaume JL, Lambiotte R, Lefebvre E (2008): Fast unfolding of communities in large networks. *J Stat Mech* P10008.
- Boscolo Galazzo I, Storti SF, Formaggio E, Pizzini FB, Fiaschi A, Beltramello A, Bertoldo A, Manganotti P (2014): Investigation of brain hemodynamic changes induced by active and passive movements: A combined arterial spin labeling-BOLD fMRI study. *J Magn Reson Imaging* 40:937–948.
- Boscolo Galazzo I, Storti SF, Del Felice A, Pizzini FB, Arcaro C, Formaggio E, Mai R, Chappell M, Beltramello A, Manganotti P (2015): Patient-specific detection of cerebral blood flow alterations as assessed by arterial spin labeling in drug-resistant epileptic patients. *PLoS One* 10:e0123975.
- Bullmore E, Sporns O (2009): Complex brain networks: Graph theoretical analysis of structural and functional systems. *Nat Rev Neurosci* 10:186–198.
- Buxton RB, Wong EC, Frank LR (1998): Dynamics of blood flow and oxygenation changes during brain activation: The balloon model. *Magn Reson Med* 39:855–864.
- Chen JJ, Jann K, Wang DJ (2015): Characterizing resting-state brain function using arterial spin labeling. *Brain Connect* 5:527–542.
- Chen Z, Liu M, Gross DW, Beaulieu C (2013): Graph theoretical analysis of developmental patterns of the white matter network. *Front Hum Neurosci* 7:716.
- Chuang KH, van Gelderen P, Merkle H, Bodurka J, Ikonomidou VN, Koretsky AP, Duyn JH, Talagala SL (2008): Mapping resting-state functional connectivity using perfusion MRI. *NeuroImage* 40:1595–1605.
- Cole MW, Bassett DS, Power JD, Braver TS, Petersen SE (2014): Intrinsic and task-evoked network architectures of the human brain. *Neuron* 83:238–251.
- Cordes D, Haughton VM, Arfanakis K, Carew JD, Turski PA, Moritz CH, Quigley MA, Meyerand ME (2001): Frequencies contributing to functional connectivity in the cerebral cortex in “resting-state” data. *AJNR Am J Neuroradiol* 22:1326–1333.
- Dagli MS, Ingeholm JE, Haxby JV (1999): Localization of cardiac-induced signal change in fMRI. *NeuroImage* 9:407–415.
- Damoiseaux JS, Rombouts SA, Barkhof F, Scheltens P, Stam CJ, Smith SM, Beckmann CF (2006): Consistent resting-state

- networks across healthy subjects. *Proc Natl Acad Sci USA* 103: 13848–13853.
- Danon L, Díaz-Guilera A, Duch J (2005): Comparing community structure identification. *J Stat Mech* 2005:P09008.
- De Martino F, Gentile F, Esposito F, Balsi M, Di Salle F, Goebel R, Formisano E (2006): Classification of fMRI independent components using IC-fingerprints and support vector machine classifiers. *NeuroImage* 34:177–194.
- de Reus MA, van den Heuvel MP (2013): Rich club organization and intermodule communication in the cat connectome. *J Neurosci* 33:12929–12939.
- De Vico Fallani F, Costa L, da F, Rodriguez FA, Astolfi L, Vecchiato G, Toppi J, Borghini G, Cincotti F, Mattia D, Salinari S, Isabella R, Babiloni F (2010): A graph-theoretical approach in brain functional networks. Possible implications in EEG studies. *Nonlinear Biomed Phys* 4: S8.
- Diedrichsen J, Balsters JH, Flavell J, Cussans E, Ramnani N (2009): A probabilistic atlas of the human cerebellum. *NeuroImage* 46: 39–46.
- Fair DA, Cohen AL, Power JD, Dosenbach NU, Church JA, Miezin FM, Schlaggar BL, Petersen SE (2009): Functional brain networks develop from a “local to distributed” organization. *PLoS Comput Biol* 5:e1000381.
- Faraco CC, Strother MK, Dethrage LM, Jordan L, Singer R, Clemmons PF, Donahue MJ (2015): Dual echo vessel-encoded ASL for simultaneous BOLD and CBF reactivity assessment in patients with ischemic cerebrovascular disease. *Magn Reson Med* 73:1579–1592.
- Fox MD, Greicius M (2010): Clinical applications of resting state functional connectivity. *Front Syst Neurosci* 4:1–13.
- Fox MD, Raichle ME (2007): Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci* 8:700–711.
- Friston KJ, Kahan J, Razi A, Stephan KE, Sporns O (2014): On nodes and modes in resting state fMRI. *NeuroImage* 99:533–547.
- Fukunaga M, Horovitz SG, de Zwart JA, van Gelderen P, Balkin TJ, Braun AR, Duyn JH (2008): Metabolic origin of BOLD signal fluctuations in the absence of stimuli. *J Cereb Blood Flow Metab* 28:1377–1387.
- Ghahriq E, Chappell MA, Schmid S, Teeuwisse WM, van Osch MJ (2014): Effects of background suppression on the sensitivity of dual-echo arterial spin labeling MRI for BOLD and CBF signal changes. *NeuroImage* 103:316–322.
- Gonzalez-Castillo J, Handwerker DA, Robinson ME, Hoy CW, Buchanan LC, Saad ZS, Bandettini PA (2014): The spatial structure of resting state connectivity stability on the scale of minutes. *Front Neurosci* 8:138.
- Hallquist MN, Hwang K, Luna B (2013): The nuisance of nuisance regression: Spectral misspecification in a common approach to resting-state fMRI preprocessing reintroduces noise and obscures functional connectivity. *NeuroImage* 82: 208–225.
- Hansen EC, Battaglia D, Spiegler A, Deco G, Jirsa VK (2015): Functional connectivity dynamics: Modeling the switching behavior of the resting state. *NeuroImage* 105:525–535.
- He Y, Chen Z, Evans A (2008): Structural insights into aberrant topological patterns of large-scale cortical networks in Alzheimer’s disease. *J Neurosci* 28:4756–4766.
- Jann K, Hernandez LM, Beck-Pancer D, McCarron R, Smith RX, Dapretto M, Wang DJ (2015): Altered resting perfusion and functional connectivity of default mode network in youth with autism spectrum disorder. *Brain Behav* 5:e00358.
- Jenkinson M, Bannister P, Brady M, Smith S (2002): Improved optimization for the robust and accurate linear registration and motion correction of brain images. *NeuroImage* 17:825–841.
- Langbaum JB, Chen K, Lee W, Reschke C, Bandy D, Fleisher AS, Alexander GE, Foster NL, Weiner MW, Koeppe RA, Jagust WJ, Reiman EM Alzheimer’s Disease Neuroimaging Initiative (2009): Categorical and correlational analyses of baseline fluorodeoxyglucose positron emission tomography images from the Alzheimer’s Disease Neuroimaging Initiative (ADNI). *NeuroImage* 45:1107–1116.
- Liang X, Connelly A, Calamante F (2014): Graph analysis of resting-state ASL perfusion MRI data: Nonlinear correlations among CBF and network metrics. *NeuroImage* 87:265–275.
- Liang X, Tournier JD, Masterton R, Connelly A, Calamante F (2012a): A k-space sharing 3D GRASE pseudocontinuous ASL method for whole-brain resting-state functional connectivity. *Int J Imaging Syst Technol* 22:37–43.
- Liang X, Wang J, Yan C, Shu N, Xu K, Gong G, He Y (2012b): Effects of different correlation metrics and preprocessing factors on small-world brain functional networks: A resting-state functional MRI study. *PLoS One* 7:e32766.
- Liang X, Zou Q, He Y, Yang Y (2013): Coupling of functional connectivity and regional cerebral blood flow reveals a physiological basis for network hubs of the human brain. *Proc Natl Acad Sci U S A* 110:1929–1934.
- Liu TT, Wong EC, Frank LR, Buxton RB (2002): Analysis and design of perfusion-based event-related fMRI experiments. *NeuroImage* 16:269–282.
- Luh WM, Wong EC, Bandettini PA, Ward BD, Hyde JS (2000): Comparison of simultaneously measured perfusion and BOLD signal increases during brain activation with T(1)-based tissue identification. *Magn Reson Med* 44: 137–143.
- Lynall ME, Bassett DS, Kerwin R, McKenna PJ, Kitzbichler M, Muller U, Bullmore E (2010): Functional connectivity and brain networks in schizophrenia. *J Neurosci* 30:9477–9487.
- 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 USA* 104:13170–13175.
- Makris N, Meyer JW, Bates JF, Yeterian EH, Kennedy DN, Caviness VS (1999): MRI-Based topographic parcellation of human cerebral white matter and nuclei II. Rationale and applications with systematics of cerebral connectivity. *NeuroImage* 9:18–45.
- Murphy K, Birn MR, Handwerker AD, Jones TB, Bandettini PA (2009): The impact of global signal regression on resting state correlations: Are anti-correlated networks introduced? *NeuroImage* 44:893–905.
- Newman ME, Girvan M (2004): Finding and evaluating community structure in networks. *Phys Rev E Stat Nonlin Soft Matter Phys* 69:026113.
- Raichle ME (2011): The restless brain. *Brain Connect* 1:3–12.
- Rogers BP, Morgan VL, Newton AT, Gore JC (2007): Assessing functional connectivity in the human brain by fMRI. *Magn Reson Imaging* 25:1347–1157. Review.
- Saad ZS, Gotts SJ, Murphy K, Chen G, Jo HJ, Martin A, Cox RW (2012): Trouble at rest: How correlation patterns and group differences become distorted after global signal regression. *Brain Connect* 2:25–32.
- Simpson SL, Lyday RG, Hayasaka S, Marsh AP, Laurienti PJ (2013): A permutation testing framework to compare groups of brain networks. *Front Comput Neurosci* 7:171.
- Smith SM (2002): Fast robust automated brain extraction. *Hum Brain Mapp* 17:143–155.

- Smith SM, Beckmann CF, Andersson J, Auerbach EJ, Bijsterbosch J, Douaud G, Duff E, Feinberg DA, Griffanti L, Harms MP, Kelly M, Laumann T, Miller KL, Moeller S, Petersen S, Power J, Salimi-Khorshidi G, Snyder AZ, Vu AT, Woolrich MW, Xu J, Yacoub E, Ugurbil K, Van Essen DC, Glasser MF WU-Minn HCP Consortium (2013): Resting-state fMRI in the Human Connectome Project. *NeuroImage* 80:144–168.
- Smith SM, Fox PT, Miller KL, Glahn DC, Fox PM, Mackay CE, Filippini N, Watkins KE, Toro R, Laird AR, Beckmann CF (2009): Correspondence of the brain's functional architecture during activation and rest. *Proc Natl Acad Sci USA* 106:13040–13045.
- Smith SM, Miller KL, Salimi-Khorshidi G, Webster M, Beckmann CF, Nichols TE, Ramsey JD, Woolrich MW (2011): Network modelling methods for FMRI. *Neuroimage* 54:875–891.
- Storti SF, Formaggio E, Bertoldo A, Manganotti P, Fiaschi A, Toffolo GM (2013): Modelling hemodynamic response function in epilepsy. *Clin Neurophysiol* 124:2108–2118.
- Storti SF, Formaggio E, Manganotti P, Menegaz G (2016): Brain network connectivity and topological analysis during voluntary arm movements. *Clin EEG Neurosci* 47:276–290.
- Tak S, Polimeni JR, Wang DJ, Yan L, Chen JJ (2015): Associations of resting-state fMRI functional connectivity with flow-BOLD coupling and regional vasculature. *Brain Connect* 5:137–146.
- Tomasi D, Wang GJ, Volkow ND (2013): Energetic cost of brain functional connectivity. *Proc Natl Acad Sci USA* 110:13642–13647.
- Van Dijk KR, Hedden T, Venkataraman A, Evans KC, Lazar SW, Buckner RL (2010): Intrinsic functional connectivity as a tool for human connectomics: Theory, properties, and optimization. *J Neurophysiol* 103:297–321.
- Viviani R, Messina I, Walter M (2011): Resting state functional connectivity in perfusion imaging: Correlation maps with BOLD connectivity and resting state perfusion. *PLoS One* 6:e27050.
- Yan C, He Y (2011): Driving and driven architectures of directed small-world human brain functional networks. *PLoS One* 6:e23460.
- Wang Z (2012): Improving cerebral blood flow quantification for arterial spin labeled perfusion MRI by removing residual motion artifacts and global signal fluctuations. *Magn Reson Imaging* 30:1409–1415.
- Weissenbacher A, Kasess C, Gerstl F, Lanzenberger R, Moser E, Windischberger C (2009): Correlations and anticorrelations in resting-state functional connectivity MRI: A quantitative comparison of preprocessing strategies. *NeuroImage* 47:1408–1416.
- Windischberger C, Langenberger H, Sycha T, Tschernko EM, Fuchsjaeger-Mayerl G, Schmetterer L, Moser E (2002): On the origin of respiratory artifacts in BOLD-EPI of the human brain. *Magn Reson Imaging* 20:575–582.
- Woolrich MW, Chiarelli P, Gallican D, Perthen J, Liu TT (2006): Bayesian inference of hemodynamic changes in functional arterial spin labeling data. *Magn Reson Med* 56:891–906.
- 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.