Neuroelectrical Decomposition of Spontaneous Brain Activity Measured with Functional Magnetic Resonance Imaging

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Spontaneous activity in the human brain occurs in complex spatiotemporal patterns that may reflect functionally specialized neural networks. Here, we propose a subspace analysis method to elucidate large-scale networks by the joint analysis of electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) data. The new approach is based on the notion that the neuroelectrical activity underlying the fMRI signal may have EEG spectral features that report on regional neuronal dynamics and interregional interactions. Applying this approach to resting healthy adults, we indeed found characteristic spectral signatures in the EEG correlates of spontaneous fMRI signals at individual brain regions as well as the temporal synchronization among widely distributed regions. These spectral signatures not only allowed us to parcel the brain into clusters that resembled the brain’s established functional subdivision, but also offered important clues for disentangling the involvement of individual regions in fMRI network activity.

Keywords: band-limited power, functional networks, resting state, spectral clustering, subspace analysis

Introduction

Neuroimaging research with functional magnetic resonance imaging (fMRI) has suggested that, in the absence of overt behavior, the human brain is remarkably active. This so-called “spontaneous” or “resting-state” brain activity has been shown to covary among functionally related brain regions, for example, those involved with sensorimotor (Biswal et al. 1995; Lowe et al. 1998), attention (Fox et al. 2006), and default-mode (Raichle et al. 2001; Greicius et al. 2003) functions. The patterns that have been observed with resting-state fMRI are similar to those activated by various tasks (Smith et al. 2009), and generally consistent with those based on intracranial electro-optical and electrical recordings in animals (Kenen et al. 2003; Leopold et al. 2003), and on electrocorticography (He et al. 2008; Nir et al. 2008) and magnetoencephalography (MEG; de Pasquale et al. 2010; Liu et al. 2010; Brookes et al. 2011; de Pasquale et al. 2012; Hipp et al. 2012) in humans. For this reason, fMRI covariation patterns have been interpreted as representing covarying electrical activity in so-called “functional networks.”

While the nature, origin, and role of spontaneous brain activity remain incompletely understood, there have been many attempts at extracting information about functional networks from fMRI data (Smith et al. 2011), with the primary goal of better understanding normal brain function and abnormalities in disease (Zhang and Raichle 2010). For this purpose, the temporal correlation (Biswal et al. 1995) or spatial independence (Beckmann and Smith 2004) of fMRI data is often analyzed to infer the brain’s functional architecture. The putative functional networks resulting from such analyses have been found to involve most of the brain (Bullmore and Sporns 2009), be reproducible within and across healthy subjects (Damoiseaux et al. 2006), and partly reflect the underlying anatomical connectivity (Johnston et al. 2008; Honey et al. 2009; Krienen and Buckner 2009).

Despite these exciting advances, a fundamental limitation of resting-state fMRI studies is that they only indirectly measure neural activity (Logothetis 2008). One aspect of this limitation is that the fMRI signal receives nonneural contributions from cardiac and respiratory fluctuations (Glover et al. 2000; Birn et al. 2006; Shmuel et al. 2007) and involuntary head motion (Power et al. 2012; Van Dijk et al. 2012), all of which are difficult to correct for and may confound data interpretation. Another limitation of fMRI signals is that neurogenic contributions can originate from a multitude of underlying neural processes that can simultaneously, and sequentially or recurrently, emerge and travel along distinct neural pathways (Logothetis 2003; Douglas and Martin 2004). As such, a brain region may interact with other regions in a rather complex way that involves multiple functionally distinct yet spatially and temporally overlapping networks, supporting parallel and serial neural computation essential for the human behavior and cognition (Mesulam 1990).

Such overlapping functional networks cannot, in a straightforward manner, be disentangled by current analysis methods based on fMRI data alone. For example, independent component analysis (ICA) methods assume functional networks to be either spatially (Beckmann and Smith 2004) or temporally (Smith et al. 2012) independent. These assumptions, although advantageous from the signal processing perspective, appear too stringent and may not adequately capture the complex relationship between neural networks. Alternatively, it may be possible to extract network information from electrophysiological data. Previous electrophysiological studies have demonstrated the presence of “spectral fingerprints” in neuronal interactions and have proposed to use this spectral information to characterize large-scale functional networks (Buzsaki and Draguhn 2004; Siegel et al. 2012). Neuroelectrical signals sample the rich temporal dynamics of neuronal population activity with a spectral range, much broader than that accessible with fMRI, and therefore may improve the network analysis of resting-state data. In fact, recent MEG studies have reported a number of spectrally distinct and temporally synchronized resting-state networks (de Pasquale et al. 2010, 2012; Liu et al. 2010; Brookes et al. 2011; Hipp et al. 2012), some of which resemble those observed with fMRI (Brookes et al. 2011). Unfortunately, the poor spatial resolution of MEG (and also...
electroencephalography, EEG) severely limits the precision of the extracted network information.

To overcome these limitations, we propose to combine simultaneously acquired EEG and fMRI data to identify and characterize neurogenic functional networks in the resting state. This is achieved by combining spectral information from EEG with spatial information from fMRI based on a putative temporal coupling between the spontaneous fMRI signals and the power fluctuations of individual EEG spectral components (Goldman et al. 2002; Laufs et al. 2003; Leopold et al. 2003; Moosmann et al. 2003; Lu et al. 2007; Mantini et al. 2007; Goenese and Logothetis 2008; Scheeringa et al. 2011; Liu et al. 2012; Magri et al. 2012). Specifically, we developed a novel “subspace” analysis method to decompose each brain region’s fMRI signal into multiple component time-series signals that were temporally correlated with distinct EEG spectral components. By using this method, resting-state fMRI signals were analyzed in an EEG frequency band-specific manner, revealing informative spectral signatures that characterized fMRI activity locally and within networks. We hypothesized that these signatures would offer a new way to parcel the resting brain into spectrally defined clusters, and help disentangle the involvement of brain regions within and across overlapping functional networks.

**Methods and Materials**

**EEG-Informed Subspace Analysis Method for fMRI**

Distinct spectral components of neuroelectric activity collectively contribute to local fMRI signals and their large-scale correlation structures. To elucidate their differential contributions, we propose a subspace analysis method to decompose the fMRI signal at each voxel into multiple component time-series signals associated with different spectral components of EEG. Briefly, EEG band-limited power (BLP; Leopold et al. 2003) is extracted from all EEG sensors and used to span a temporal subspace, upon which the fMRI signal is projected. To generate this subspace, the EEG data are first band-pass filtered into nonoverlapping frequency bands. To remove correlation between signals caused by volume conduction effects (Buzsaki et al. 2012), the band-pass filtered signals are orthogonalized across sensors for each frequency band separately (Hipp et al. 2012). The power envelopes (i.e., the BLP signals) are extracted from the orthogonalized signals and then, for each frequency band, a temporal subspace is created based on the strongest principal components contributing to these signals. This subspace is further convolved with the hemodynamic response function (HRF) to account for the hemodynamic delay and smoothing. Subsequently, the fMRI signal at each voxel is projected separately onto each frequency band-specific subspace. As a result, the original fMRI signal is decomposed into multiple component time-series signals, each of which is associated with one of the EEG frequency bands. See Figure 1 for the schematic illustration of this method.

Using this approach, we set out to test the hypothesis that the neuroelectric activity underlying the fMRI signal has EEG spectral features that report on regional neuronal dynamics and interregional interactions.

**Experiments and Subjects**

To test this hypothesis, we applied the proposed subspace analysis method to EEG and blood oxygen level-dependent (BOLD) fMRI data simultaneously acquired from 15 healthy subjects (31 ± 11 years old, 8 females, all right handed) resting wakefully with eyes closed for 10 min. The scanner environment was darkened to minimize light exposure. Some subjects were scanned multiple times on the same or different days. In total, we collected 41 resting-state sessions. Seventeen sessions were excluded for either excessive head motion (7 sessions) or inability to sustain wakefulness (10 sessions). The former was judged by motion correction parameters used for retrospective realignment of fMRI images, with excessive motion defined as >2 mm translation or 1.5° of rotation; the latter was confirmed based on the EEG signature of drowsiness or wake-sleep transition (i.e., disappearing alpha power and increasing delta and theta power) and on the subject’s oral feedback after every scan. Data from 11 subjects were kept for further analyses. All subjects gave informed written consent in accordance with a protocol approved by the Institutional Review Board of the National Institute of Neurological Disorders and Stroke at the National Institutes of Health.

**Data Acquisition**

We acquired concurrent EEG (32-channel BrainAmp MR Plus, BrainProducts GmbH, Germany) and BOLD fMRI with a single-shot gradient-echo echo-planar imaging sequence on a 3-T MRI system (General Electric Health Care, Milwaukee, WI, USA). The EEG data were referenced to the FCz channel and sampled at 5 kHz with a resolution of 0.5 μV/bit and an analog bandwidth from 0.1 to 250 Hz. The fMRI data were acquired with 90° flip angle (FA), 30 ms echo time (TE), 1.5 s repetition time (TR), 30 axial 4 mm slices without gap, 220 × 165 mm² field of view, 2 mm slice thickness, 2012). The power envelopes (i.e. the BLP signals) are extracted from the orthogonalized signals and then, for each frequency band, a temporal subspace is created based on the strongest principal components contributing to these signals. This subspace is further convolved with the hemodynamic response function (HRF) to account for the hemodynamic delay and smoothing. Subsequently, the fMRI signal at each voxel is projected separately onto each frequency band-specific subspace. As a result, the original fMRI signal is decomposed into multiple component time-series signals, each of which is associated with one of the EEG frequency bands. See Figure 1 for the schematic illustration of this method.

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field of view for 64×48 matrix, and sensitivity-encoding (SENSE) parallel imaging with the acceleration rate of 2. T₁-weighted anatomical images covering the whole head were acquired with a three-dimensional (3-D) magnetization-prepared rapid gradient-echo sequence (725 ms inversion time, 12° FA, 2.25 ms TE, 5 ms TR, and 1 mm³ isotropic voxel size).

Data Analysis

EEG artifacts from gradient switching and cardiac pulsation were removed using an algorithm as previously described (Liu et al. 2012). fMRI data were preprocessed with a standard routine that excludes both global signal regression and low-pass filtering, and normalized to the Montreal Neurological Institute (MNI) space with 3-mm isotropic voxels.

The EEG data were filtered into 5 frequency bands (delta: 0.5–4 Hz, theta: 4–8 Hz, alpha: 8–13 Hz, beta: 13–30 Hz, and gamma: 30–70 Hz). For each frequency band, the band-pass filtered signals were orthogonalized using singular value decomposition. From the resulting orthogonal band-limited signals, the BLP signals were extracted using the Hilbert transform. We then used principal component analysis to identify a temporal signal subspace that explained a large fraction of variance and the correlation structure of the BLP signals. After further convolving this subspace with a canonical HRF, we projected the fMRI signal at every imaging voxel onto the EEG-derived subspace to obtain a projected time-series signal associated with the given EEG frequency band. By repeating this procedure over all frequency bands, we extracted multiple component time-series signals with distinct EEG spectral correlates.

Following the above subspace projection, we computed for each voxel the fraction of the fMRI signal variance projected onto the subspace of each frequency band and subtracted from it a trivial variance computed from the fMRI signal projected onto surrogate subspaces with a matched dimensionality but derived from convolving random noise with the HRF. The result was referred to as the fractional variance defined for each EEG frequency band and each fMRI voxel. Accordingly, we derived for each voxel a spectral profile describing the fractional variance of fMRI as a function of the EEG frequency band. We further used the k-means algorithm to group voxels in cerebral gray matter into spatial clusters based on their similarity in the spectral profile, while the optimal number of clusters was determined using the Bayesian information criterion (Zhang and Li 2012). For analysis at the group level, the fractional variance was averaged across subjects. The group-level statistical significance was assessed voxel-by-voxel through 2-sample paired t-tests, comparing the fMRI signal variance fitted by a given EEG frequency component on one hand and the variance fitted by the dimensionality matched random noise convolved with the HRF on the other hand. For all the significant voxels (P<0.05 corrected for false discovery rate), the group-averaged spectral profiles were used to extract spectrally distinct clusters.

Moreover, we conducted the seed-based correlation analysis based on the fMRI signals projected onto each frequency-specific subspace, as well as the original fMRI signals. The seed region of interest (ROI) was a cube of 3×3×3 voxels centered at a representative voxel within the primary auditory cortex or the posterior cingulate cortex (PCC). We selected the voxels where the original fMRI signals were correlated with the seed voxel with the z-transformed correlation >0.3. Then, we derived a spectral profile for describing the correlation between each selected voxel and the seed ROI as a function of the EEG frequency band. We further used such a spectral profile as the feature vector for classifying all selected voxels into clusters using the k-means algorithm, and consequently, subdivided the seed-based functional network into spectrally distinctive subnetworks. For group-level analysis, the z-transformed correlation was averaged across subjects and the group-averaged spectral profiles were clustered to subdivide functional networks.

See Supplementary Methods and Materials for more details.

Results

Frequency-Dependent Regional BOLD Signal Variance

The fraction of the BOLD signal variance projected onto each frequency band-specific subspace varied considerably across brain regions and frequency bands (Fig. 2, left). The frequency band accounting for the most variance at each voxel was used to form a map of the so-called “preferred frequency” (Fig. 2, right). This map showed similar spatial patterns as established functional systems or networks. For example, we observed the delta band for the auditory system, the theta band for the default-mode network, the alpha band for the visual system, the beta band for the motor system, and the gamma band for the lateral prefrontal cortex. The total amount of variance, collectively explained by all frequency bands, showed substantial differences between gray and white matter, as well as noticeable variations across cortical regions (Fig. 3).

Functional Clusters of Frequency-Dependent Feature

The fMRI signals from a specific network often not only preferentially projected onto a single preferred frequency, but also had similar spectral profiles (i.e. relative projection strengths onto the subspaces of distinct frequencies). For example, the...
spectral projection profile was found to be similar among regions within such functional systems as visual, motor, and default-mode networks, but not for others, for example, executive control and salience networks (Fig. 4). This led us to parcel the cerebral gray matter into divisions by grouping voxel-wise spectral features with the $k$-means clustering algorithm. On the basis of the minimum Bayesian information criterion (Supplementary Fig. 4), we obtained 11 spectrally informed clusters (Fig. 5b), which appeared well organized in the cortex and corresponded closely to functionally and structurally meaningful regions. Varying the number of clusters from 5 to 11 progressively revealed more subdivisions (Fig. 5a). For example, the visual cortex, which comprised 1 cluster when 5 clusters were resolved for the entire brain, was further subdivided into 2 clusters covering lower- and higher-order visual areas when a total of 10 clusters were resolved (Fig. 5a). For a comparison between the spectral clusters in the visual cortex and the established atlas of visual areas, we refer to (Supplementary Fig. 9). Some of these functional clusters found based on the frequency feature closely resembled those found based on the spatial ICA analysis of the fMRI data (Fig. 5b). This suggests that the EEG spectral signatures of spontaneous fMRI signals are functionally specific, allowing the classification of functional systems in a manner alternative or complementary to approaches based on the spatiotemporal structure of fMRI data alone.

**Frequency-Dependent BOLD Signal Correlation**

EEG spectral features may allow the extraction of functional network information not available with conventional methods. To evaluate this possibility, we computed the correlation between a seed voxel and every other voxel based on their BOLD signals projected onto each frequency-specific subspace. Figure 6 illustrates the geometric interpretation of such an analysis. The result of this analysis for a seed voxel in the left auditory cortex (A1) is shown in Figure 7. Both the correlation strength and pattern differed among individual frequency bands, each differing from the BOLD–BOLD correlation pattern (Fig. 7a). The interhemispheric correlation with the right A1 was strongest for the theta band; the cross-modal correlation with the primary visual cortex (V1) was strongest for the beta band. This result suggests that functional networks inferred from BOLD–BOLD correlations may be rather coarse and compound the contributions of multiple underlying neuronal networks with distinct spectral signatures. This notion led us to classify the electrical correlates of BOLD–BOLD correlations into clusters such that their spectral features appear similar within clusters but dissimilar across clusters. As a result, the A1-seeded correlation pattern was subdivided into 2 spectrally distinct clusters. In one cluster, the seeded correlations were dominated by the lower frequency bands, whereas in the other cluster the correlations were dominated by the higher frequency bands (Fig. 7b). The former covered the homologous area in the opposite hemisphere and appeared bilaterally symmetric; the latter extended to the visual cortex and tended to be located within the same hemisphere as the seed voxel (Fig. 7c). Similar frequency dependence was also observed for a seed located in the PCC (Fig. 8a). For this seed location, the spectral analysis allowed subdivision of the default-mode network into 5 spectrally distinct subnetworks (Fig. 8b). This result provides the electrophysiological evidence for a distributed modular organization within the default-mode network as previously suggested (Uddin et al. 2009; Andrews-Hanna et al. 2010; Leech et al. 2011).

**Discussion**

The integrated EEG–fMRI approach presented here allowed decomposition of the fMRI time-series signal at each brain region into multiple component signals with distinct EEG spectral correlates. This led to a novel interpretation of spontaneous brain activity and functional connectivity based on the presence of overlapping neural networks with distinct spectral characteristics. Specifically, it was found that: (1) Regional BOLD signals received differential contributions from individual frequency components of underlying electrophysiological signals, with a spectral signature that varied across brain regions. (2) This spectral signature allowed for the grouping of brain regions into functional clusters, some of which resembled functional networks extracted from fMRI data with conventional analysis methods (e.g. ICA). (3) Interregional
fMRI signal correlations were also frequency dependent, which allowed dividing a seed-based functional network into multiple subnetworks with distinct spectral properties. These results support the notion that neuroelectrical signals underlie resting-state fMRI activity patterns and their conjoint analysis provides network information not available with fMRI alone.

Spectral Signature of Regional BOLD Activity

Previous studies using either blind source separation (Beckmann and Smith 2004) or nuisance variable regression (Lund et al. 2006) have shown that a significant portion of resting-state fMRI signals can be attributed to nonneuronal fluctuations due to slow scanner drift, head motion, and respiratory and cardiac cycles.

Figure 4. Spectral profiles at selected ROIs: 6 (1–6) in the visual network, 3 (7–9) in the default-mode network, 2 (10–11) in the motor network, 4 (12–15) in the salience network, and 2 (16–17) from executive control network. For each ROI, the plot indicates the fractional variance of the regional fMRI signal explained by different EEG frequency bands; the circles indicate the average across subjects, and the error bars indicate the standard error of the mean. These ROIs are illustrated on the inflated cortical surfaces, and their MNI coordinates and anatomical labels are given as below. 1: primary visual cortex (V1) at (687 3); 2: secondary visual cortex (V2) at (30 0 3); 3: ventral occipital area (VO) at (–39 –72 15); 4: dorsal occipital area (V3) at (15 84 30); 5: intraparietal cortex (IPS) at (30 –72 42); 6: medial-temporal area (MT) at (54 –60 6); 7: PCC at (0 –53 26); 8: lateral parietal cortex (LPC) at (–42 –6 –36); 9: medial prefrontal cortex (mPFC) at (0 52 –6); 10: primary motor cortex (M1) at (–39 –30 57); 11: supplementary motor cortex (SMA) at (0 –33 57); 12: supramarginal gyrus (SMG) at (60 –30 30); 13: dorsal anterior cingulate cortex (dACC); 14: anterior prefrontal cortex (aPFC) at (36 39 36); 15: insula at (42 14 0); 16: dorsal lateral prefrontal cortex (dLPC) at (38 28 42); 17: anterior inferior parietal lobule (aIPL) at (46 –54 48).

Figure 5. Spectrally informed parcellation of brain regions. (a) 5 and 11 spectral clusters are color-coded and shown in axial slices. (b) Comparison between spectrally and spatially informed cortical parcellation with 11 clusters. The distinct clusters are color-coded with the color indicating the size of the cluster (i.e. the number of voxels belonging to each cluster).
butions of the various distinct networks that contribute to a...activity through integrated analysis of simultaneous EEG...sively probing the spectral composition of regional neural...sely related to the total synaptic delay accumulated along a...lar neural pathway embedded within a network, rather than the...chronizes over longer distances (Buzsaki and Draguhn 2004), we...oscillations are dominated by network activity that syn-

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Figure 6. Geometric interpretation for the effect of a subspace projection on the...signal correlation between a pair of regions. For a pair of regions (indexed by i and j),...their BOLD time-series signals (denoted by xi and xj and also illustrated as dashed arrows), can be viewed as 2 vectors in the time domain and the angle between them is denoted as αij. It is straightforward to see that the correlation between xi and xj is the cosine of αij. When a subspace is constructed from the BLP signals of the given frequency band, it can be viewed as a high-dimensional “plane” in the time domain. Projecting xi and xj onto this plane results in 2 “shadow” vectors (shown as solid arrows), denoted as yi and yj, respectively, which are separated by an angle denoted as βij. These 2 vectors, when represented in the time domain, are 2 component time courses of the original time courses xi and xj. Their correlation coefficient is the cosine of βij. From this geometric illustration, we can see that the subspace projection can increase or decrease the angle between the 2 projected vectors relative to that of the original vectors, depending on the relationship between each of the 2 vectors and the subspace.

(Bianciardi et al. 2009). In this study, we presented a technique to further separate and to quantify neurogenic contributions from various spectral components of neuroelectrical activity observed with concurrently acquired EEG. We report differential contributions of individual EEG frequency bands to the fMRI signal, as depicted by region-specific spectral profiles. Previous studies have suggested that local and network fMRI signals are associated with a mixture of electrophysiological processes underlying dynamic neuronal interactions at different temporal and spatial scales, which may be characterized by a varying interplay of distinct frequency components (Mantini et al. 2007; Goense and Logothetis 2008; Scheerlinga et al. 2011; Magri et al. 2012). Thus, the observed spectral profile may serve as an index, or “fingerprint” (Siegel et al. 2012) that reflects the relative contributions of the various distinct networks that contribute to a region’s activity. It also demonstrates the possibility of noninvasively probing the spectral composition of regional neural activity through integrated analysis of simultaneous EEG–fMRI. Note that although a common prediction is that higher frequency oscillations reflect more focal network activity and lower frequency oscillations are dominated by network activity that synchronizes over longer distances (Buzsaki and Draguhn 2004), we did not observe this specific relationship between frequency and distance. Instead, we speculate that the frequency may be inversely related to the total synaptic delay accumulated along a circular neural pathway embedded within a network, rather than the physical distance or scale. This speculation awaits validation through dedicated studies.

It is important to note that a region’s EEG-projected fMRI variance, combined over all frequency bands, accounted for only 27 ± 11% of its total signal variance (Fig. 3). The large fraction of unexplained fMRI variance may be the result of a number of causes. For example, it may reflect residual variance from confounds such as instrumental drift and motion that were not fully removed by fMRI preprocessing, or from other nonneurogenic sources. In addition, it may reflect other types of neuronal (or astrocytic) activity that produce little EEG but significant BOLD signals. Thirdly, it may result from the inadequacy of the empirical linear HRF in describing neurovascular coupling at a specific brain region. Lastly, it may also partly relate to the fact that EEG represents slow and synchronized events mostly reflecting the coordinated, possibly modulatory, activity of large neuronal populations, whereas it underestimates relatively asynchronous events associated with local processing.

Spectral Organization of Brain Regions

Importantly, the extracted location-specific spectral profile also showed informative distinction and similarity across regions, providing the basis for revealing the spectral organization of resting-state brain activity. Regions with the same preferred frequency band, in which the EEG–fMRI coupling appeared strongest, followed spatial divisions of the brain that closely resembled the established functional systems, such as vision (alpha), motor (beta), and cognition (gamma). This finding agrees with previous studies in which similar spatial patterns have been individually associated with specific electrophysiological rhythms, for example, the alpha rhythm in the visual cortex (Goldman et al. 2002; Moosmann et al. 2003; Liu et al. 2012), and the “mu” and beta rhythms in the motor cortex (Ritter et al. 2009). A unique finding in this study is that taking into account all EEG frequency bands in the region-specific spectral signature, instead of only the peak frequency, allowed for the identification of progressively refined subdivisions of fMRI-derived networks using a data-driven clustering algorithm. In particular, the finding of subclusters in the visual system suggests a hierarchical structure of the spectral organization in line with the hierarchical view of visual processing (Felleman and Van Essen 1991). The comparison between the 2 spectral clusters in the visual cortex and its established functional subdivisions suggests that higher-order processing areas (e.g. lateral occipital complex, MT+, V7, and most nonre
tinotopic areas) roughly fall into a single cluster, whereas lower-order visual areas (e.g. V1, V2, and V3) fall into the other spectral cluster (Supplementary Fig. 9).

It is important to note that the network parcellation presented here was based solely on region-specific spectral patterns, as opposed to spatial or temporal patterns revealed with standard resting-state analysis methods such as the spatial ICA (Damoiseaux et al. 2006). Despite this difference in underlying analysis methods, the spectrally defined clusters showed, at first glance, several similar features as those defined with spatial ICA (Fig. 5b), including bilaterally symmetric organization in the majority of the brain, the segregated lower- and higher-order visual areas, and lateralization in the lateral frontal cortex. Such similarities suggest that common anatomical and physiological substrates may underlie both of these...
characteristics. Among the candidates are distinct patterns in axonal and synaptic connectivity, cytoarchitecture, and neurotransmitter receptor distributions. A more detailed comparison between the spectral clusters and spatial ICA components (Supplementary Fig. 6) revealed some notable distinctions as well. For example, the somatosensory and motor cortices, which were grouped into a single spatial component by ICA, were found to be spectrally heterogeneous (Fig. 5b). This spectral heterogeneity may be related to the interplay of Rolandic alpha and beta rhythms (Salmelin and Hari 1994), both of which have been shown to be correlated with fMRI in somatosensory and motor cortices (Ritter et al. 2009) and react to motor tasks, motor imagery, and somatosensory stimulation in a rather complex fashion both spatially and temporally (Pfurtscheller 1981; Pfurtscheller et al. 1996). In addition, we also found that the spectral signatures within the frontal lobe, the medial-temporal lobe, and the lateral parietal cortex were highly variable across locations, which may be related to the integrative functions of these higher-order cortices in brain-wide network interactions (Hipp et al. 2012). However, these
interpretations are rather speculative. The source and role of the observed spectral heterogeneity at certain brain regions remain unclear and difficult to pinpoint within the scope of the present study. Full understanding of the relationship between patterns derived from spatial ICA of fMRI data and those derived from their spectral EEG correlates will require further research.

We have also investigated the minimum scan duration required to robustly perform the spectrally informed brain parcellation. For this purpose, the amount of data analyzed was varied from 2.5 to 10 min. The results suggested that, for scan durations >5 min, the parcellation converged to a stable pattern, whereas parcellations with shorter durations became unreliable. This is attributed to reduction in the degrees of freedom and higher noise level in the spectral signature of the shorter scans (Supplementary Fig. 7). Moreover, it appeared feasible to perform the spectrally informed brain parcellation in individual subjects (Supplementary Fig. 8). Despite a noticeable level of intersubject variation, generally consistent features (e.g., bilateral symmetry) and network patterns (e.g., visual and default-mode networks) could be observed for most of the subjects. The varying patterns for different subjects may be attributed to individual differences in spectral distribution and patterns of fMRI network activity or unidentified technical issues. The precise interpretation of such individual differences awaits future dedicated studies.

Spectral Signature of Interregional Functional Interactions

In brain networks, spontaneous activity fluctuations may occur over multiple time scales. For example, over time scales from milliseconds to seconds, network activity presents as synchronized neuronal oscillations at various frequencies, collectively spanning a wide spectral continuum (Buzsaki and Draguhn 2004). Part of this spectral range is accessible with EEG. Over a much longer time scale in the order of several seconds to tens of seconds, network activity presents as comodulations in the power envelop of specific spectral bands, to which neuronal oscillations contribute (Leopold et al. 2003; Lu et al. 2007; Nir et al. 2008) and which have an fMRI correlate (Magri et al. 2012). Although extensive efforts have been made to characterize these “fast” and “slow” network interactions separately, their relationship has rarely been studied and remains largely unknown.

The present study contributes to the understanding of the linkage between these 2 types of network analyses by revealing the correlation in the “slow” fMRI signals as a function of the frequency of the “fast” neuronal synchronizations underlying EEG. We found that fMRI signal correlations between specific brain regions varied across EEG frequency bands. This finding suggests that neuronal oscillations with different frequencies may play distinct roles in coordinating interactions between regions within a specific functional network or across different networks (Logothetis 2003; Siegel et al. 2012). Note that such frequency dependence is an extra degree of information inaccessible by fMRI alone and reflects the spectral signature of functional connectivity. Recent studies using MEG (Liu et al. 2010; Brookes et al. 2011; de Pasquale et al. 2012; Hipp et al. 2012) and transcranial magnetic stimulation combined with fMRI (Eldaief et al. 2011) have also shown evidence for the frequency dependence of functional connectivity.

Importantly, the new method allows for the division of a functional network revealed with fMRI into spectrally distinctive subnetworks, with spatial resolution and specificity much superior to MEG or EEG. This new possibility may lead to refined understanding of complex neuronal interactions in the resting and working brain. As a proof of principle, we have demonstrated here that the network interactions within the bilateral auditory system are spectrally different from those between the auditory and visual systems (Fig. 7). This finding emphasizes the frequency-dependent cortical interaction, and may contribute to the understanding of the large-scale brain circuits underlying multisensory processing and integration (Schroeder et al. 2008). The default-mode network was also found to consist of multiple subnetworks, showing different degrees of interplay among individual EEG frequency bands (Fig. 8). These network components may play distinct functional roles in passive and spontaneous cognition (Andrews-Hanna et al. 2010).

Neuroelectrical Oscillation Frequency-Dependent fMRI

Central to the current findings is the new approach for extracting from a brain region’s fMRI signal multiple component time series with distinct EEG spectral correlates. The value of this approach may be appreciated in the context of 2 “inverse problems.” The first pertains to the temporal decomposition of neuronal processes underlying spatially resolved fMRI signals; the second pertains to the spatial localization of neuroelectrical sources underlying temporally resolved EEG signals. Instead of solving these 2 very challenging inverse problems separately, our approach tackled both problems together by utilizing complementary spatial and temporal information from simultaneous fMRI and EEG. This approach assumes that regional oscillatory neural activity contributes remotely to a spatial component of band-limited EEG through linear volume conduction, and that its power fluctuation contributes locally to fMRI through linear neurovascular coupling. Using a novel subspace analysis, we obtained solutions in terms of region and frequency-specific hemodynamic signals, which were compatible with both region-specific fMRI signals and frequency-specific EEG signals.

In summary, the developed approach effectively expands conventional four-dimensional (4-D) fMRI data (i.e. 3-D in space and 1-D in time) to 5-D data with the added dimension representing the frequency of neuroelectrical oscillations observed with EEG. This comprehensive analysis may significantly enrich the information available with conventional fMRI by providing neuronal oscillation frequency contrast in addition to perfusion and oxygenation contrast. This additional contrast will allow frequency tags to be placed to distinct neuronal components of the fMRI signal, enabling the spatiotemporal imaging of neuroelectrical oscillations driven either externally by stimulation or internally through self-organized neural systems.

Limitation and Future Direction

Despite the potential significance of the proposed method, there remains ample room for technical improvement. For example, there likely are alternative ways to derive basis functions from the array of EEG sensors that may lead to improved performance and more informative interpretation. We also expect that increasing the number of electrodes would lead to more robust mapping of the spectral signatures of regional and network activities, whereas its precise effect is unclear and
awaits future dedicated studies to examine. Future studies may also be directed to assess the effect of potential confounds, such as head motion inside the scanner (Jansen et al. 2012).

To validate or evaluate the proposed method in a more rigorous way is non-trivial, simply because there is a lack of ground truth or unique definition of functional networks. Results from different analysis methods likely represent certain aspects of neural interactions, some of which may be coincident whereas others may not. Caution should be exercised when using the result of one analysis method to assess the accuracy or inaccuracy of other methods. As far as the proposed method is of concern, a more thorough evaluation might be possible in realistic, yet simplified to certain degree, large-scale simulation models of brain networks (Deco et al. 2011), or through comparison with invasive electrical recordings in animal models or patients with implanted electrodes for presurgical or surgical evaluation purposes.

Lastly, it is worth noting that the apparent correlation between electrophysiological spectral components and spontaneous hemodynamic fluctuations does not necessarily suggest that the former is the origin of the latter. The generation mechanism and functional significance of resting-state fMRI still remain largely unclear, despite tremendous growth in its applications.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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References


