Frequency distribution of causal connectivity in rat sensorimotor network: resting-state fMRI analyses

Woo H. Shim,1,2 Kwangyeol Baek,1,2 Jeong Kon Kim,2,3,4 Yongwook Chae,1 Ji-Yeon Suh,2,3 Bruce R. Rosen,2 Jaeseung Jeong,1 and Young R. Kim1,5

1Department of Bio and Brain, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea; 2Department of Radiology, Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, Massachusetts; 3Department of Radiology, Asan Medical Center, Ulsan University, Seoul, South Korea; 4Division of Magnetic Resonance Research, Korea Basic Science Institute, Ochang, Cheongwon, Chungbuk, South Korea; and 5Ulsan National Institute of Science and Technology, Ulsan, Republic of Korea

Submitted 23 April 2012; accepted in final form 20 September 2012

Shim WH, Baek K, Kim JK, Chae Y, Suh J-Y, Rosen BR, Jeong J, Kim YR. Frequency distribution of causal connectivity in rat sensorimotor network: resting-state fMRI analyses. J Neurophysiol 109: 238–248, 2013. First published September 26, 2012; doi:10.1152/jn.00332.2012.—Resting-state functional MRI (fMRI) has emerged as an important method for assessing neural networks, enabling extensive connectivity analyses between multiple brain regions. Among the analysis techniques proposed, partial directed coherence (PDC) provides a promising tool to unveil causal connectivity networks in the frequency domain. Using the MRI time series obtained from the rat sensorimotor system, we applied PDC analysis to determine the frequency-dependent causality networks. In particular, we compared in vivo and postmortem conditions to establish the statistical significance of directional PDC values. Our results demonstrate that two distinctive frequency populations drive the causality networks in rat; significant, high-frequency causal connections clustered in the range of 0.2–0.4 Hz, and the frequently documented low-frequency connections <0.15 Hz. Frequency-dependence and directionality of the causal connection are characteristic between sensorimotor regions, implying the functional role of frequency bands to transport specific resting-state signals. In particular, whereas both intra- and interhemispheric causal connections between heterologous sensorimotor regions are robust over all frequency levels, the bilaterally homologous regions are interhemispherically linked mostly via low-frequency components. We also discovered a significant, frequency-independent, unidirectional connection from motor cortex to thalamus, indicating dominant cortical inputs to the thalamus in the absence of external stimuli. Additionally, to address factors underlying the measurement error, we performed signal simulations and revealed that the interactive MRI system noise alone is a likely source of the inaccurate PDC values. This work demonstrates technical basis for the PDC analysis of resting-state fMRI time series and the presence of frequency-dependent causality networks in the sensorimotor system.

resting-state connectivity; sensorimotor system; partial directed coherence; postmortem; frequency-domain analysis

Based on the coupling between neural and hemodynamic responses, blood oxygen level-dependent (BOLD) functional MRI (fMRI) has been long established as a means to evaluate stimulus-specific activity in the brain. Spatiotemporal properties of the fMRI signals are used to assess the neural activation generated in response to the externally applied stimulation or task. In the absence of stimulation or task, investigators recently observed the presence of low-frequency fluctuations (LFFs) in the resting-state BOLD fMRI time series (Biswal et al. 1995), which exhibit temporal synchronicity across functionally related brain regions (Hampson et al. 2002; Lowe et al. 1998; Thirion et al. 2006). Several studies have substantiated the neural basis of LFFs (Fransson 2005; Lowe 2010; Lowe et al. 2000), and correlations between fMRI time series have since expanded the applicability of BOLD fMRI from a tool to assess task-elicited brain activity to one that can be used to construct global maps of neural connectivity networks (Deco et al. 2011; Margulies et al. 2010; Rogers et al. 2007). In turn, a number of analysis techniques have also been developed to interpret better the resting-state fMRI data and improve understanding of the association patterns among brain regions.

Of particular interest, the causality between fMRI time series has been investigated to describe preceded or lagged interdependencies in brain connectivity networks (Abler et al. 2006; Daunizeau et al. 2009; Frioston 2009; Goebel et al. 2003). As a means to quantify causal influences, thus to identify brain regions initiating the neural activity, various analysis techniques have been proposed to determine the directional relationship of one brain region to another. Most of earlier techniques such as dynamic causal modeling (Frioston et al. 2003) or structural equation modeling (McIntosh 1998) used advanced statistical approaches to detect directional flows in the previously specified connections. To explore the directional connectivity in structurally unknown networks, vector autoregressive (VAR) models were introduced to estimate the causal influence by observing predictability and temporal precedence (Goebel et al. 2003; Roebroek et al. 2005). Therefore, causal connectivity analyses based on the VAR model (e.g., Granger causality) have been often utilized to calculate the order of interactions among unidentified neural circuits (Abler et al. 2006; Deshpande et al. 2009; Eichler 2005; Goebel et al. 2003; Roebroek et al. 2005; Valdes-Sosa et al. 2005).

In addition to delineating temporal traits, frequency decomposition of the functional connectivity has become of high interest for effectively dissociating interactions be-
between neural correlates and other indirect factors such as physiological and measurement noise (Havlíček et al. 2010; Sato et al. 2009; Sun et al. 2004). Compared with the commonly used cross-correlation coefficient in the time domain, frequency analyses such as partial coherence provide each functional association with the coherence level at a specific frequency, thus narrowing down the frequency range for constructing neural connectivity networks (Salvador et al. 2005; Sun et al. 2004). Several studies have demonstrated diverse distributions of frequency components among functionally connected brain regions (Leopold et al. 2003; Salvador et al. 2005; Sun et al. 2004). Combining causality and frequency-domain analyses, one approach, the partial directed coherence (PDC) method (a VAR method in the frequency domain), was developed to determine unassumed directional connectivity in the frequency domain (Baccalá and Sameshima 2001). By estimating the linear causal influence among frequency components, the PDC technique provides an effective frequency range of the causal flows without prior knowledge of the network structure. Unlike other causality methods such as the directed transfer function and relative power contribution, the PDC method can distinguish direct pathways from indirect pathways, offering more selective filtering for construction of true causal pathways (Baccalá and Sameshima 2001; Yamashita et al. 2005).

Despite the important potential of PDC as a tool to illustrate directional neural connectivity, incomplete exclusion of noise arising from physiology and/or the imaging equipment can limit the measurement accuracy (Deshpande et al. 2010; Kalthoff et al. 2011). To confirm the significance of directional connectivity, here we used animal models to refine experimentally the PDC analysis using both in vivo and postmortem brain imaging and determined criteria needed to establish the frequency-specific significance of the calculated PDC values. With such extensive threshold, we hypothesize that the significant causal connections are frequency-dependent and also that the frequency distribution of causal connections provides an independent means to isolate the association of neural networks. The direct importance of the current study is threefold, enabling us to: 1) determine the PDC value threshold for establishing meaningful causal connectivity; 2) demonstrate the frequency dependence of causal connectivity in spontaneous BOLD fluctuations; and 3) identify sensorimotor neural networks based on frequency distribution of significant causal connections.

MATERIALS AND METHODS

Animal Surgery and Treatment

A total of 13 male Sprague-Dawley rats (Charles River, Wilmington, MA), weighing 280–320 g, were studied under experimental protocols approved by the Institutional Subcommittee on Research Animal Care, in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. The animals were initially anesthetized with 1.5% isoflurane in a mixture of O2 and N2 gases (3:7). Polyethylene catheters were inserted into the right femoral artery, for monitoring of arterial blood pressure, and into the right vein, for infusion of anesthetics. Before the MRI experiment, isoflurane was disconnected, and a continuous infusion of α-chloralose (~30 mg·kg⁻¹·h⁻¹) with pancuronium (~1.25 mg·kg⁻¹·h⁻¹) was administered, preceded by a loading bolus of ~20 mg/kg. Body temperature was maintained by a water-circulated heating pad, whereas blood oxygen saturation, blood pressure, and heart rate were continuously monitored throughout the MRI experiments. Each rat was mechanically ventilated at a rate of 40–50 intakes per minute throughout the experiment.

For acquisition of postmortem data, we administered a high dose (5%) of isoflurane for 30 min and, while maintaining the isoflurane level, euthanized the animal with phenobarbital intravenous infusion (1 mg/kg). The postmortem echoplanar imaging (EPI) data recordings were performed 30 min after the heart had completely stopped (i.e., 0 blood pressure and heart rate), during which the mechanical ventilator was either turned on or off.

Phantom

A uniform cylindrical phantom filled with saline solution was imaged in transverse slices (perpendicular to the axis of the phantom). Regions of interest (ROIs), which covered ~50 voxels, were randomly drawn in 18 nonoverlapping areas. Averaged time series for each ROI were used for power spectra analysis and connectivity calculations between all possible combinations of 2 ROIs.

MRI Data Acquisition

MR images (gradient EPI, repetition time/echo time = 1,000/12.89 ms, field of view = 2.5 × 2.5 cm²; 9 contiguous 1-mm slices, and 64 × 64 matrix) were acquired on a horizontal-bore 9.4-T Bruker/Magnex system equipped with a home-built head surface radio-frequency transmit-and-receive coil with a diameter of ~3 cm. The in vivo EPI data were collected for 10 min (600 acquisitions) as the animal breathed a 1:1 mixture of O2 and room air ~1 h after discontinuation of isoflurane. EPI parameters were the same for all in vivo and postmortem acquisitions as well as for the phantom data acquisitions.

Data Analysis

The time series for each voxel were detrended to the second order and band-pass-filtered between 0.01 and 0.5 Hz using Analysis of Functional Neuroimages (AFNI) software (Cox 1996). Afterward, the average time course of each ROI was corrected for zero mean and unit variance and used for connectivity calculation. Data analyses were processed using MATLAB (The MathWorks, Natick, MA) and BioSig toolbox (see APPENDIX for details; Schlögl and Brunner 2008).

ROI Delination

ROIs were drawn freehand using AFNI software (NIH/National Institute of Mental Health) based on the rat brain atlas defined by Paxinos and Watson (2007). Brain regions associated with the sensorimotor system and spatially corresponding areas in the phantom were used as ROIs for analyses. As shown in Fig. 1, we selected eight ROIs based on the rat brain atlas: motor (M1/M2), primary sensory (S1), caudate and putamen (CPu), and thalamus (TA) regions in bilateral hemispheres.

Cluster Analysis and Construction of Connectivity Diagram

To describe the frequency distribution of significant connection values based on the partial coherence and PDC methods, we plotted the number of significant connections against the frequency. In this occurrence histogram, cluster analysis of the frequency components was performed, assuming a Gaussian mixture distribution for each spectral group by using expectation maximization algorithm (Moon 1996). To summarize the results, we created connectivity diagrams for all of the analysis methods. In these diagrams, connection strength,
process of order tests of PDC (Baccalá and Sameshima 2001; Schelter et al. 2006).

The signal, we modified a model that has been extensively used in previous

Simulation of PDC Analysis

To illustrate the performance of PDC when patterned noise is added to

Set 1: signal with white noise. We tested the five-dimensional VAR

process of order $p = 4$ for signal $s_i(t)$. $\mathbf{s}_i(t) = [x_1(t), x_2(t), \ldots, x_5(t)]$

\[
x_1(t) = 0.95 \sqrt{2} x_1(t-1) - 0.9025 x_1(t-2) + w_1(t)
\]

\[
x_2(t) = 0.5 x_1(t-2) + w_2(t)
\]

\[
x_3(t) = -0.4 x_1(t-3) + w_3(t)
\]

\[
x_4(t) = -0.5 x_1(t-2) + 0.25 \sqrt{2} x_2(t-1) + 0.25 \sqrt{2} x_3(t-1) + w_4(t)
\]

\[
x_5(t) = -0.25 \sqrt{2} x_2(t-1) + 0.25 \sqrt{2} x_3(t-1) + w_5(t)
\]

Set 2: signal with patterned noise. Patterned noise $\mathbf{p}(t)$ was generated and added to signal $\mathbf{s}_2(t)$ by the equations:

\[
\mathbf{p}(t) = [p_1(t), p_2(t), \ldots, p_5(t)]
\]

\[
p_1(t) = 0.2 \sqrt{2} p_1(t-2) + 0.1 p_2(t-4) - 0.1 \sqrt{2} p_3(t-3) + w_1(t)
\]

\[
p_2(t) = -0.07 \sqrt{2} p_3(t-3) + 0.1 p_3(t-4) + w_2(t)
\]

\[
p_3(t) = 0.1 \sqrt{2} p_4(t-1) - 0.15 p_4(t-3) + w_3(t)
\]

\[
p_4(t) = -0.1 p_4(t-3) + 0.05 \sqrt{2} p_4(t-6) + 0.07 \sqrt{2} p_5(t-4) + w_4(t)
\]

\[
p_5(t) = -0.15 p_2(t-3) + 0.15 p_3(t-9) + w_5(t)
\]

\[
\mathbf{s}_2(t) = [y_1(t), y_2(t), \ldots, y_5(t)]
\]

\[
y_1(t) = 0.95 \sqrt{2} y_1(t-1) - 0.9025 y_1(t-2) + p_1(t)
\]

\[
y_2(t) = 0.5 y_1(t-2) + p_2(t)
\]

\[
y_3(t) = -0.4 y_1(t-3) + p_3(t)
\]

\[
y_4(t) = -0.5 y_1(t-2) + 0.25 \sqrt{2} y_2(t-1) + 0.25 \sqrt{2} y_3(t-1) + p_4(t)
\]

\[
y_5(t) = -0.25 \sqrt{2} y_2(t-1) + 0.25 \sqrt{2} y_3(t-1) + p_5(t)
\]

where $w_i(t)$ are zero-mean uncorrelated white processes with identical variances. The number of simulated data points was 600 for each component of the VAR process.

RESULTS

We compared the MRI time series and calculated power spectra for the phantom and in vivo (anesthetized) and postmortem rat brain data. Figure 2 shows the left $S_1$ region as a representative ROI to demonstrate comparison between the time course signals obtained from the in vivo and postmortem data. Spatially similar areas in the phantom were chosen for the comparison. Power spectra acquired in vivo show apparent peaks in the low-frequency range ($<0.15$ Hz), resembling 1/f characteristics within this range, whereas such obvious peaks
were not observed in the postmortem data (either with or without mechanical ventilation) or in the phantom (Fig. 2). Mechanical ventilation postmortem did not alter the power spectra. The average power spectra of the S1 area for all 13 rats are shown in Fig. 2B.

The cross-correlation matrices for both the in vivo and postmortem data were generated as shown in Fig. 3A. We considered all connections above the correlation threshold of 0.35 meaningful, as previous fMRI connectivity studies have generally considered this value as the connection threshold (Biswal et al. 1995; Pawela et al. 2008). Compared with the postmortem values, all meaningful pairwise in vivo connections were statistically significant (pairwise t-test, \( P < 0.05 \)). High interhemispheric cross-correlations were observed between all ROIs and the homologous counterparts in the opposite hemisphere. We also found strong intrahemispheric connectivity between M1/M2 and S1. We found rather weak but meaningful connections between M1/M2 and CPu, S1 and CPu, as well as M1/M2 and thalamus. All connection strengths were dismissed as negligible for the postmortem condition. The partial coherence matrix was plotted in Fig. 3B, showing the frequency distribution in significant connections. Only the connections that showed the statistically significant difference between in vivo and postmortem data were considered meaningful (pairwise t-test, \( P < 0.05 \)). The connectivity pattern was similar to that demonstrated in the cross-correlation matrix. Strong interhemispheric coherence was detected between all the bilaterally homologous ROI pairs for a wide frequency range (0.01–0.3 Hz) except for the bilateral S1 areas, the connection between which was only significant within a very low frequency range (<0.05 Hz). Relatively weak but significant coherence connections between motor cortex and thalamus were also detected in the low-frequency range. In general, significant in vivo coherence values were mostly concentrated in the low-frequency range (<0.15 Hz).

Figure 3C shows a direct connectivity matrix derived from the PDC analysis of the in vivo time courses. PDC values between 0.01 and 0.5 Hz depicted the directionality from source ROI to target ROI as off-diagonal components of the matrix. Note that the cross-correlation and partial coherence matrices are symmetric with respect to the main diagonal. A pairwise t-test was performed to validate the significance of the difference between PDC values at fixed frequencies in the in vivo and postmortem images. The connectivity pattern of the PDC matrix generally resembles those found in cross-correlation and coherence matrices, as strong bidirectional interactions were detected in the interhemispheric cortical (M1/M2 and S1) and subcortical (CPu and TA) regions. We also found significantly strong directional influences from M1/M2 to TA and from S1 to CPu. Significant PDC values were mostly concentrated in the low-frequency (<0.15 Hz) range even though some significance also existed in the high-frequency band (>0.2 Hz; Fig. 3C).

To elucidate the frequency components responsible for driving significant connectivity, we constructed occurrence histograms for all significant partial coherence and PDC values in the frequency domain by comparing them with the postmortem values (Fig. 4). Assuming Gaussian distribution, the frequency components were grouped into two discrete frequency bands, one frequency population <0.15 Hz and the other between 0.2 and 0.4 Hz. Two bands were similarly identified in the partial coherence and PDC values. We further illustrated significant connectivity with simplified diagrams according to the analysis methods (Fig. 5). The connectivity network based on cross-correlation were drawn with meaningful pairwise connections (Fig. 5A). Partial coherence analysis revealed a connectivity pattern that is slightly interhemispherically asymmetric yet similar to that described in the cross-correlation matrix (Fig 5B). In the connectivity diagrams derived from the low-frequency component group (<0.15 Hz), more connections (i.e., arising from and passing to a brain region) were present in the left hemisphere than in the right hemisphere for both the partial coherence and PDC methods (Fig. 5B and C). Overall reduction in connection strength and percentage frequency width was also apparent for high-frequency (0.2–0.4 Hz) partial coherence and PDC results (Fig. 5C) compared with low-frequency connections (<0.15 Hz). However, highly robust connectivity was still predominant throughout the sensorimotor system. Compared with the low-frequency PDC results, several new interhemispheric connections appeared in the
high-frequency range (i.e., a causal link from the CPu in the right hemisphere to the S1 region in the left hemisphere and bidirectional links between the left TA and right S1). In addition, the high-frequency PDC diagram also indicates the emergence of solely unidirectional intrahemispheric connections from M1/M2 and S1 (e.g., M1/M2 → CPu in the left hemisphere and S1 → CPu in the both hemisphere) as well as unidirectional interhemispheric connections from right S1 to left M1/M2 and from left TA to right M1/M2. In particular, absence of the strong interhemispheric bidirectional connections is notable for the high-frequency PDC connectivity unlike those observed in the low-frequency data in M1/M2, CPu, and TA regions. Irrespective of frequency, the PDC analysis yielded strong unidirectional connections from M1/M2 to TA.

We simulated PDC analysis using hypothetical network models to understand the influence of physiological and MRI system noise on the accuracy of PDC analyses. The results of computer simulation are shown in Fig. 6. First, we successfully reproduced the results previously reported by Baccalá and Sameshima (2001; Fig. 6A, left). Hypothetical directional connections from area 1 to areas 2, 3, and 4 were demonstrated in the diagonally asymmetric pattern of the PDC value matrix (from source to target), whereas bidirectional connection between areas 4 and 5 was reflected by the near symmetry of the PDC matrix (Fig. 6A, right). Inclusion of white noise larger than the actual white noise from the MR images yielded no change in either the significance threshold or the PDC value matrix. However, with the addition of interactively patterned directional noise (Fig. 6B), a markedly elevated significance threshold was observed throughout the entire frequency range. Moreover, this type of noise also transformed the frequency-dependent PDC values, significantly altering the effective significance threshold. Compared with the case with white noise only, the addition of these patterned noises caused false-negative PDC values to appear in certain connection (i.e., source 1 → target 4 between 0.4 and 0.5 Hz in Fig. 6B) and prompted the production of traceable false-positive PDC values (i.e., source 1 → target 5, source 5 → target 2, and source 5 → target 3).

DISCUSSION

We used the PDC analysis method to investigate the causal connectivity networks in the rat sensorimotor system. The goals of this work were to 1) characterize the frequency components of directional links in the association of anatomically distinct brain regions, and 2) evaluate how noise sources affect the PDC analysis for improved understanding of the neural system in vivo. We selected eight ROIs from sensorimotor-related regions to examine the causal networks (Fig. 1). The functional associations of these areas have been well-documented in other prior functional connectivity studies (Pawela et al. 2008; Zhao et al. 2008). In agreement with these earlier studies, our cross-correlations and coherence network maps reproduced tightly coupled intra- and interhemispheric links (Fig. 3). Similarly, the directional connectivity map obtained from the PDC analysis revealed significant information flows (Fig. 3C). In particular, as shown in Fig. 5C, we constructed connectivity maps of causal networks based on the frequency bandwidth, strength, and directionality of both intra- and interhemispheric connections using the experimentally determined PDC significance threshold of each frequency component connecting these areas.

To validate the significance of the calculated neural networks, we opted to distinguish experimentally the in vivo neurohemodynamic signals from the noise source. Although limited to non-fMRI studies, previous investigations using PDC method have predominantly used computational simulations to determine meaningful PDC values (Baccalá and Sameshima 2001; de Brito et al. 2010; Sameshima and Baccalá 1999; Schelter et al. 2009). However, simulations alone cannot account for all possible factors that may intervene with in vivo evaluation (e.g., periodic physiological noise and/or MRI system noise in this study). We assumed that the data from both postmortem and/or phantom have only noise-oriented connectivity except true neural connectivity. Hence, we performed both postmortem (with and without mechanical ventilation) and phantom studies to understand the effects of both respiration and the MRI system. Our data showed that no significant peaks are formed throughout the entire power spectra in either postmortem brains or in the phantom, whereas strong low-frequency peaks were manifest in the in vivo time courses (Fig. 2). Cross-correlation coefficients in the postmortem brains were small but appreciable (−0.03) in all possible ROI pairs (Fig. 3A), revealing the very low level of the noise contribution. Notably, no obvious spectra were detected as a result of periodic mechanical ventilation (40–50 strokes per minute). Although unrelated to neurohemodynamic activity, respiration exerts a direct influence on the MRI signal contrast by way of susceptibility-induced changes in the magnetic field, with a frequency much slower than the heart rate (~250 beats per minute). This respiratory frequency is similar to the MRI sampling frequency (60 images per minute) and likely has a greater influence on the fMRI power spectrum than any other physiological fluctuations, considering the property of frequency aliasing (Biswal et al. 1997; Chuang and Chen 2001; Majeed et al. 2009). However, in our experiment, the mechanical ventilation failed to alter either spectra or outcomes of the network analyses. Similarity between the phantom and postmortem brain data further demonstrates the limited effects of the baseline MRI signal characteristics (i.e., relaxation time constants T1 and T2 and signal-to-noise ratio), strongly suggesting that the inherent system noise (e.g., scanner vibration) has a dominant influence on the connectivity analysis. Considering the complete loss of neural activity in the postmortem brain and the limited contribution from periodic physiological fluctuations such as respiration, we justified the use of the PDC values measured postmortem as reference measures (Fig. 3). However, in our experiment, the mechanical ventilation failed to alter either...
spectra or outcomes of the network analyses, and thus the null hypothesis testing for the absence of correlation between the respiration and PDC noise level cannot be rejected. As for human studies, despite the difference in respiration frequency characteristics and acquisition conditions, the high field strength (i.e., 9.4 T) used in the current study precludes the possibility of significant respiration-related noise contribution in the human resting-state fMRI, which is usually conducted in relatively low magnetic fields.

As a result of frequency-domain analyses, we identified two main frequency bands using occurrence histograms (Fig. 4). Interestingly, despite the absence of high-frequency peaks in the in vivo power spectrum (Fig. 2), the histograms clearly revealed the statistically significant high-frequency components in both partial coherence and PDC values. This finding experimentally shows that distinctive peaks in the power spectrum may not be required to support the statistically significant coherence among MRI time series. For both the partial coherence and PDC methods, constructed neural networks were highly dependent on the frequency band (Fig. 5, B and C), in which both similar and different connections were present across the frequency bands. Such apparent dependence on frequency suggests that the neural connection between a pair of brain regions uses a specific frequency route and also that a single connection may use multiple frequency bands for transporting resting-state signals. In particular, as demonstrated by the PDC analysis, variations in the causality among different brain regions and its frequency dependence may reflect diverse multiple signal sources within a resting-state connection, which lead us to posit further that a specific combination of causality and frequency represents the functionally unique resting-state signal. Bidirectional connections defined in the PDC results for physically close regions (i.e., M1/M2, CPu, and TA) are likely caused by interactions between the neural dendrites. A major anatomical connection such as the corpus callosum is likely responsible for driving similarly strong interhemispheric, bidirectional links between all bilaterally homologous cortical and subcortical regions. However, we cannot rule out the possibility that common neural inputs interactively affect the entire sensorimotor system via afferent thalamic pathway. On the other hand, it is notable that these interhemispheric connections delineated by PDC prevail mostly in the low-frequency band. Despite the high connection strength and wide frequency distribution of the significant PDC values (Figs. 3C and 5C), these interhemispheric, bidirectional connectivities between bilaterally homologous regions (i.e., M1/M2, CPu, and TA) are not supported by the high-frequency components. An exception is the S1 connection, which is present in both the low- and high-frequency bands. On the contrary, when identified by partial coherence, these connections between bilaterally homologous regions are nearly invariable and not dependent on the frequency. Such difference between partial coherence and PDC might be derived from the filtration of indirect pathways by the PDC analysis. Since the PDC method have an advantage in distinguishing direct from indirect pathways (Baccalá and Sameshima 2001; Yamashita et al. 2005), it would be suggested that underlying characteristics of the causal connectivity can be revealed by the comparison between the partial coherence and PDC values. As results, we infer that these interhemispheric connections between the bilaterally homologous regions are indirect in the high-frequency band.

We observed significant unidirectional connections from the cortex (M1/M2) to the thalamus (TA) and from the cortex (S1) to the basal ganglia (CPu); to the best of our knowledge, these connections have not been previously reported in resting-state time series. Among these unidirectional connections we observed, most notable was corticothalamic connectivity, which exhibited a robust unidirectional causal connection from the motor cortex to the thalamus in the PDC connectivity map (Figs. 3C and 5C). However, results from previous resting-state connectivity studies do not favor strong signal connections between the thalamus and cortex. Irrespective of causality, a number of resting-state studies have reported thalamocortical connectivity with only a weak correlation value (Majeed et al. 2009; Pawela et al. 2008; Salvador et al. 2005; Schwarz et al. 2009); in other studies, this connectivity was undetected altogether (Fukunaga et al. 2006; Moosmann et al. 2003; Zhao et al. 2008). Anatomically, substantial bidirectional paths, consisting of both afferent and efferent links, connect the thalamus and cortex (Killackey and Sherman 2003). In addition to relaying afferent inputs from the sensory cortex to the cerebral cortex, a major portion of the thalamic circuitry is massively innervated by fibers arising from the cortex itself. This corticothalamic projection is known to supply the major source of excitatory synapses on thalamic neurons; corticothalamic synapses largely exceed the number of afferent synapses, supporting the observed unidirectional and dominant influence from the cortex. On the other hand, our previous study showed that both the thalamus and the S1 hemodynamically responded during electrical forelimb stimulation (Kim et al. 2005, 2006), in which the thalamic fMRI activation was not as robust as the responses detected in S1. This observation recapitulates the well-documented role of the thalamus for input relay in the somatosensory pathway to the cortex via an afferent path. The PDC data, on the other hand, hint at the functional influence of cortical activity on the thalamus, which has often been neglected despite the obvious anatomical presence. Based on the unidirectional PDC results, we suggest that cortical activity dominates overall inputs to the thalamus during rest, particularly without the sensorimotor stimulation.

We posit that various sources of noise cause inaccurate estimation of the PDC value, thereby leading to incorrect interpretation of the connectivity network. Therefore, understanding the nature of noise and effective significance level of PDC values is highly important to improve the measurement accuracy. First, using model simulation, we demonstrated that the inclusion of simple white noise does not affect either the significance threshold (Fig. 6A) or the PDC analysis result. In addition to nondiscriminatory white noise, we investigated the
Fig. 5. Connectivity diagrams derived from cross-correlation ($r$ (A) and partial coherence (B) and PDC (C) analyses for the entire frequency and low- and high frequency groups. For cross-correlation, the line thickness illustrates correlation coefficients ($r$ threshold = 0.35). For partial coherence and PDC diagrams, the line thickness scaled with the connection strength (i.e., difference between in vivo and postmortem values), whereas the opacity divides the percentage frequency width (frequency width of significant values $\div$ total frequency range 0.01–0.5) into 3 groups (black $\geq$ 10% > dark gray $\geq$ 5% > light gray).
effects of randomly patterned interactive signals on the simulated resting-state fMRI data using the general form of PDC already described in the previous studies (Baccalá and Sameshima 2001; Schelter et al. 2006). Although hypothetical, these types of spatiotemporal noises, which may be derived from nonneural physiological fluctuations and inconsistent MRI signals (e.g., cardiac cycle, scanner vibration, etc.), well-mixed in the true neurohemodynamic signals as aliased forms are likely the most detrimental factor for accurate determination of the causative events. As described in Fig. 6B, a causal network composed of signals with noise cannot be completely distinguished from the network consisting of noise only even when the calculated PDC significance threshold is applied. However, more importantly, the results also demonstrated that application of an assumed or indirect PDC threshold that is lower than the true threshold may result in a high number of false-positive, frequency-dependent connections and therefore alter the interpretation. These simulation results further emphasize the importance of understanding the effects of systematic noise before determining meaningful PDC values. In addition, model-based errors can be also considered as significant factors in the accuracy of PDC analysis. For instance, both increasing the number of ROIs and selecting the inadequate VAR model order $p$ are known to increase estimation inaccuracy (Sato et al. 2009; Schelter et al. 2006). Interestingly, despite the relatively low sampling rate and length (compared with electroencephalogram), neither the model order nor number of ROIs was much influential in this work, in which the tested VAR model order ranged between 4 and 10. Thus it is important to specify the measurement criteria and significance threshold in each study condition to delineate accurately the PDC-based connectivity. As the effects of physiological noises appear negligible, we suggest that the MRI system causes the patterned noises, the predominant source of error in the PDC analysis of resting-state fMRI time series.

Recently, Smith et al. (2011) demonstrated rather poor performance of the PDC method when directly applied to analyze simulated fMRI data, and others also suggested that VAR-based method based on the temporal precedence might not be the most appropriate approach to delineate functional connectivity without considering generative model or supertemporal resolution (Friston 2011; Roebroeck et al. 2011). Despite these drawbacks, we posit that performance of the PDC method can be significantly improved with determination of proper threshold values. The current study shows that PDC connectivity network patterns derived from the comparison between in vivo and postmortem conditions are highly similar to those acquired from cross-correlation and partial coherence. Moreover, clusters of two frequency bands were also observed in statistically significant connections identified by both partial coherence and PDC methods. Although direct verification is desirable, the results indirectly imply that the proposed PDC method is appropriate to investigate causal networks based on resting-state fMRI signals.

In summary, to assess frequency distribution of the causal connectivity in the rat sensorimotor system, we calculated the statistically significant PDC values by comparing data acquired from in vivo and postmortem conditions. Additionally, signal simulations were performed in conjunction with in vivo, postmortem, and phantom studies to address the influence of physiological and system noise on the accuracy of PDC values. The major findings of this study are: 1) significant PDC values are present not only at low-frequency range (<0.15 Hz), but also at high frequency (0.2–0.4 Hz) in rat; 2) the significance threshold for each in vivo PDC value is frequency-dependent and can be obtained from postmortem imaging; and 3) there is a significant causal influence from the motor cortex to the thalamus during resting state. Despite some limitations, we believe that the frequency dependence of the causal network will contribute to unravel the signaling process in the brains at rest. Moreover, application of proper significance threshold and dissociation of the multiple signal sources discussed in this report will improve the efficacy of the PDC method and enhance its potential as an analysis tool for isolating and assessing neural connectivity.

APPENDIX

Cross-Correlation

Cross-correlation is a measure of similarity of two time series. We used cross-correlation analysis between average time courses of voxels in ROIs. The correlation coefficient ($r$) was calculated using the formula

$$ r = \frac{\sum [x(i) - \bar{x}] [y(i) - \bar{y}] \sqrt{\sum [x(i) - \bar{x}]^2 \sum [y(i) - \bar{y}]^2}} $$

where $x(i)$ and $y(i)$ represent the $i$th time point from two time courses, and $\bar{x}$ and $\bar{y}$ represent the means.

---

References...

---

**Fig. 6.** Simulation results of the PDC values using an assumed network with the inclusion of white noise ($A$) and hypothetical systematic noise ($B$). The structure of the model and estimated casual flows by PDC were shown as matrices. The red lines represent averaged PDC values of signals $s_1(t)$ and $s_2(t)$ at the fixed frequency, whereas black lines denote PDC values of 0-mean uncorrelated white process $w_i(t)$ in set 1 and order $p_i(t)$ in set 2.
Partial Coherence

Partial coherence measures the linear time-invariant relationship in frequency domain. Partial coherence $\text{Coh}_{xy}(\lambda)$ between two time series, $x$ and $y$, at frequency $\lambda$ given common input $k$ is defined as

$$\text{Coh}_{xy}(\lambda) = \frac{|R_{xy}(\lambda) - R_{xk}(\lambda)R_{yk}(\lambda)|^2}{1 - |R_{xk}(\lambda)|^2 |1 - |R_{yk}(\lambda)|^2|}$$

(A2)

where $R_{xy}(\lambda)$ is the complex coherency of $x$ and $y$.

The partial coherence $\text{Coh}_{xy}(\lambda)$ can be used to determine whether the relationship between two time series is the consequence of a common input, $k$, or an estimate of the amount of additional improvement for predicting $y$ from $x$ given $k$. Partial coherence ranges between 0 and 1, where 0 indicates no linear relationship between $x$ and $y$, and 1 indicates that $x$ is closely related to $y$ at frequency $\lambda$.

PDC

PDC is a full multivariate spectral measure, based on VAR modeling, to determine the direct relationships among given pairs of time series. This method, introduced as a frequency-domain alternative to Granger causality, describes the influence between time series $x_i(t)$ and $x_j(t)$ by estimating predictability whether the knowledge of past values of $x_i(t)$ reduces the prediction error for the present value of $x_j(t)$.

In this study, we used a generalized PDC (GPDC) approach; an advantage of this approach is its interpretability as a measure of the strength of connectivity (Baccalá et al. 2007; Sato et al. 2009). The advantage of GPDC lies in its interpretability as a measure of connectivity strengths between neural structures. Thus zero GPDC can be interpreted as the absence of connectivity at a given frequency, and high GPDC indicates strong causal relationship from source to target.

If

$$x(t) = [x_1(t), x_2(t), \ldots, x_n(t)]$$

is a stationary $n$-dimensional time series with mean 0 at time $t$, then a VAR model of order $p$ [$\text{VAR}(p)$] for $x$ is represented by

$$x(t) = \sum_{j=1}^{n} a(r)x(t - r) + e(t)$$

(A3)

where $a(r)$ are the $n \times n$ coefficient matrix of the model $p$, and $e(t)$ is a multivariate Gaussian white noise process. The coefficient $a_{ij}(r)$ describes how the present values of $x_i$ linearly depend on the past values of the components $x_j$. Thus $x_i(t)$ is said to Granger-cause $x_j$ if the coefficient $a_{ij}(r)$ is non-zero with respect to the full process $x$; the values of $x_i$ can be predicted by observing the past $r$th time point values of $x_j$ and said to “$x_i$ is Granger-caused by $x_j$.”

To provide a frequency-domain description, we let

$$A_{ij}(\lambda) = \delta_{ij} - \sum_{r=1}^{p} a_{ij}(r)e^{-2\pi nr\sqrt{-1}}$$

(A4)

denote the Fourier transform of the coefficient series $a_{ij}(r)$ where $\delta_{ij} = 1$ if $i = j$ and 0 otherwise. Then the GPDC $\Pi_{i \rightarrow j}(\lambda)$ for a $\text{VAR}(p)$ process is defined as

$$\Pi_{i \rightarrow j}(\lambda) = \frac{\left|A_{ij}(\lambda)\right|^2}{\delta_{ij}}$$

(AS)

From this definition, $\Pi_{i \rightarrow j}(\lambda)$ vanishes for all frequencies $\lambda$ when all coefficients $a_{ij}(r)$ are 0 if $x_i$ is not Granger-caused by $x_j$ given the other variables. This suggests that the GPDC $\Pi_{i \rightarrow j}(\lambda)$ can provide a measure for the direct linear influence of $x_i$ on $x_j$ at frequency $\lambda$.

The model $p$ used in our study was chosen to meet Akaike’s information criterion (AIC; Akaike 1974; Ding et al. 2000).

GRANTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (nos. 2011-0028826 and 2011-0030094) and by Korea Basic Science Institute Grant T3222K.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


J Neurophysiol • doi:10.1152/jn.00332.2012 • www.jn.org


