High-Resolution Functional Connectivity Density: Hub Locations, Sensitivity, Specificity, Reproducibility, and Reliability

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Abstract
Brain regions with high connectivity have high metabolic cost and their disruption is associated with neuropsychiatric disorders. Prior neuroimaging studies have identified at the group-level local functional connectivity density (lFCD) hubs, network nodes with high degree of connectivity with neighboring regions, in occipito-parietal cortices. However, the individual patterns and the precision for the location of the hubs were limited by the restricted spatiotemporal resolution of the magnetic resonance imaging (MRI) measures collected at rest. In this work, we show that MRI datasets with higher spatiotemporal resolution (2-mm isotropic; 0.72 s), collected under the Human Connectome Project (HCP), provide a significantly higher precision for hub localization and for the first time reveal lFCD patterns with gray matter (GM) specificity >96% and sensitivity >75%. High temporal resolution allowed effective 0.01–0.08 Hz band-pass filtering, significantly reducing spurious lFCD effects in white matter. These high spatiotemporal resolution lFCD measures had high reliability [intraclass correlation, ICC(3,1) > 0.6] but lower reproducibility (>67%) than the low spatiotemporal resolution equivalents. GM sensitivity and specificity benchmarks showed the robustness of lFCD to changes in model parameter and preprocessing steps. Mapping individual’s brain hubs with high sensitivity, specificity, and reproducibility supports the use of lFCD as a biomarker for clinical applications in neuropsychiatric disorders.

Key words: aging, FCDM, global signal regression, motion, multiband, physiologic noise, resting state

Introduction
Functional connectivity density mapping (FCDM) is a powerful graph theory tool for exploring the topology of human brain function using magnetic resonance imaging (MRI) datasets collected at rest and task states (Tomasi and Volkow 2010; Tomasi, Wang, Wang, et al. 2014). FCDM quantifies local degree, the size of the local network cluster functionally connected to a brain network node, which measures the local centrality of every voxel in the human brain connectome. In contrast to seed-voxel correlation analysis (Biswal et al. 1995), data-driven FCDM is ideal for exploratory analyses because it does not require a priori hypotheses. Due to extremely high computational demand, it is almost impossible to perform voxelwise analysis using other graph theory metrics such as global degree, clustering, and path length, yet ultrafast FCDM quantifies the strength of the local functional connectivity hubs (network nodes with high connectivity to nearby brain regions) at 3-mm isotropic resolution in just a few minutes/subject (Tomasi and Volkow 2010). These characteristics make FCDM optimal for data mining of large image repositories of “resting-state” functional connectivity datasets such as the “1000 Functional Connectome Project” (Biswal et al. 2010) and the attention deficit hyperactivity disorder (ADHD)-200 (Tomasi and Volkow 2012a).

Using FCDM, we have demonstrated the distribution of local (lFCD) and global (gFCD) hubs with high functional connectivity...
density (i.e., degree; Tomasi and Volkow 2011a, 2011b) and the functional asymmetry of the human brain (Tomasi and Volkow 2011d), as well as the effects of task performance (Tomasi, Wang, Wang, et al. 2014), gender (Tomasi and Volkow 2011c), and aging (Tomasi and Volkow 2012b) on IFCD and gFCD, and the metabolic cost of functional connectivity (Tomasi, Wang, et al. 2014). Recent studies using FCDM have demonstrated modulatory effects of catechol-O-methyltransferase and dopamine D2 receptors (Tian et al. 2013) and the influence of the oxytocin receptor gene (Wang, Qin, et al. 2013) on the strength of the functional connectivity hubs. Independent laboratories (including ours) have shown disrupted IFCD and gFCD in ADHD (Tomasi and Volkow 2012a), traumatic axonal injury (Caeyenberghs et al. 2014), non-epileptic seizures (Ding et al. 2014), schizophrenia (Tomasi and Volkow 2014; Zhuo et al. 2014; Liu et al. 2015), congenital blindness (Qin et al. 2015), and cocaine addiction (Konova et al. 2015). IFCD and gFCD hubs have also been shown to be sensitive to stimulants (Konova et al. 2015), fluid reasoning capacity (Lang et al. 2015), and brain development (Tomasi and Volkow 2014).

Whereas functional connectivity metrics promise to have a major impact on neuroscience research, several limitations still prevent their translation as clinical biomarkers in neurology and psychiatry. For instance, at 3-mm isotropic resolution (standard native spatial resolution in fMRI studies), the functional connectivity metrics include significant contamination from blood vessels and have suboptimal tissue specificity (Menon 2002; Turner 2002). More importantly, functional connectivity metrics from data collected with low temporal resolution (1.5–3.0 s) include significant aliasing artifacts of physiological origin (Birn et al. 2006). Thus, similar to other functional connectivity metrics, FCDM would benefit significantly from greater accuracy in spatial localization and faster image acquisition.

The HCP (https://db.humanconnectome.org/; Van Essen et al. 2012) recently released a large fMRI database including “resting-state” functional connectivity datasets with unprecedented spatiotemporal resolution (2-mm isotropic; 0.72 s) that allow mapping the distribution of functional connectivity hubs at the individual level (Cohen et al. 2008) with high spatial specificity and reduced physiologic noise contamination. We hypothesized that spontaneous fluctuations in the resting state are driven by neuronal populations (Logothetis et al. 2001; Logothetis 2002; Shmuel and Leopold 2008; Lei et al. 2014; Li et al. 2014; Siero et al. 2014), and we predicted that the better spatiotemporal resolution of the data would enable us to observe these fluctuations only within the gray matter (GM). Therefore, the higher spatial resolution and faster sampling rate of the HCP datasets would lead to sharper IFCD patterns with greater precision in spatial localization at the individual and group levels and with reduced physiologic noise artifacts.

Here, we assess for the first time the distribution of IFCD hubs with high spatiotemporal resolution at the individual and group levels in 40 healthy adults using the HCP-Q1 dataset. Aiming to identify the brain location of functional hubs with unprecedented spatial resolution, we assess GM sensitivity and specificity, reproducibility and test–retest reliability, as well as the effects of MRI acquisition (i.e., LR vs. RL phase encodings), and specific IFCD parameters (i.e., correlation threshold) and preprocessing steps (i.e., motion covariates, 0.08 Hz low-pass filtering, and global signal normalization) on the strength of the IFCD patterns. We hypothesized that higher spatiotemporal resolution would enable precise identification of hub locations within occipitoparietal and primary sensory cortices, which are the regions that house the most prominent hubs in the human brain (Tomasi and Volkow 2010).

Materials and Methods

Subjects

Data were drawn from the publicly available repository of the WU-Minn HCP (http://www.humanconnectome.org/). The scanning protocol was approved by Washington University in the St. Louis’s Human Research Protection Office (HRPO), IRB# 201204036. No experimental activity with any involvement of human subjects took place at the author’s institutions.

The 40 participants (age: 31 ± 3 years; 31 females) of the WU-Minn HCP-Q1 data release included in this study provided written informed consent and were scanned on a 32-channel 3.0-T Siemens Skyra unit according to procedures approved by the IRB at Washington University. Resting-state functional images were acquired while the participant relaxed with eyes open using a gradient-echo-planar imaging (EPI) sequence with multiband factor 8, time repetition (TR) 720 ms, time echo 33.1 ms, flip angle 52°, 104 × 90 matrix size, 72 slices, 2 mm isotropic voxels, and 1200 time points (Smith et al. 2013; Ugurbil et al. 2013). Scans were repeated twice using different phase-encoding directions (RL and RL) in each of the 2 sessions (REST1 and REST2). To capitalize on the high-quality data offered by the HCP, we used the “minimal preprocessing” datasets (hp2000_clean.nii files), which include gradient distortion correction, rigid body realignment, field map processing, spatial normalization to the stereotactic space of the Montreal Neurological Institute (MNI), high-pass filtering (1/2000 Hz frequency cutoff; Glasser et al. 2013) and independent component analysis (ICA)-based denoising (Salimi-Khorshidi et al. 2014), and brain masking. Thus, 160 “resting-state” time series image with 902 629 2-mm isotropic voxels and 1200 time points (4.3 Gb memory space) collected over 864 s were used in this study. In addition, we used the HCP’s gray and white matter parcellations (guided by “wmparc” and “ROIs” files) of each subject’s brain structural scans, to create a GM template. This template was used to assess the GM specificity of the IFCD.

IFCD Pipelines

The interactive data language (IDL, ITT Visual Information Solutions, Boulder, CO, USA) and a workstation with 2 Intel® Xeon® X5680 processors and 48 Gb random access memory were used in subsequent FCDM-processing steps (Fig. 1). Seven IFCD pipelines were implemented. Pipeline 1 included “scrubbing” to remove image time points that could be severely contaminated with motion. Specifically, framewise displacements, FDs, were computed for every time point from head translations and rotations, using a radius of r = 50 mm to convert angle rotations to displacements. The mean FD was not different for the REST1 (0.17 ± 0.05 mm; mean ± standard deviation) and REST2 (0.18 ± 0.05 mm) sessions or for the LR (0.17 ± 0.05 mm) and RL (0.18 ± 0.05 mm) phase-encoding directions (P > 0.24; paired t-test). Scrubbing was implemented in IDL to remove image time points with temporal derivative of the root mean square variance of the signal over voxels, DVARS >0.5% and FD >0.5 mm (Power et al. 2012). The number of time points removed per time series by scrubbing was not significantly different for REST1 (0.5 ± 1.1; mean ± SD) and REST2 (0.8 ± 1.1) or for LR (0.5 ± 1.2) and RL (0.8 ± 1.1; P > 0.12, t-test).

Pipeline 1 also included multilinear regression to minimize motion-related fluctuations in the MRI signals (Tomasi and Volkow 2010), 3D global signal intensity normalization across time points to minimize global fluctuations, and standard 0.08 Hz low-pass filtering to remove magnetic field drifts and minimize physiologic noise of high frequency components. Note that
these standard fCD preprocessing steps partially overlap with those used by the HCP (ICA-based denoising) to remove unwanted noise sources (Salimi-Khorshidi et al. 2014).

In addition, pipeline 1 assessed the fCD at every voxel in the brain, computed as the number of elements in the local functional connectivity cluster, using a “growing” algorithm written in IDL (Tomasi and Volkow 2010). The Pearson correlation was used to assess the strength of the functional connectivity, \( R_{ij} \), between voxels \( i \) and \( j \) in the brain, and a correlation threshold, \( R_{th} > 0.3 \), was selected to ensure significant correlations between time-varying signal fluctuations are corrected at \( P_{FWE} < 0.05 \). A voxel \( x_0 \) was added to the list of voxels functionally connected with \( x_0 \) only if it was adjacent to a voxel that was linked to \( x_0 \) by a continuous path of functionally connected voxels and \( R_{th} > 0.3 \). This calculation was repeated for all brain voxels that were adjacent to those that belonged to the list of voxel functionally connected to \( x_0 \) in an iterative manner until no new voxels could be added to the list. Spatial smoothing was not used to preserve the high spatial resolution of the resting-state functional dataset.

![Figure 1. FCD processing pipelines. Seven FCD maps (see text) were computed for each subject, session, and phase-encoding direction. A total of 960 FCD maps covering the whole brain (white matter and cerebrospinal fluid regions were not masked out to assess the strength of the fCD in these regions) with 2-mm isotropic resolution and 91 \( \times \) 109 \( \times \) 91 voxels were computed using 160 HCP datasets with “minimal preprocessing” (Glasser et al. 2013) from the Q1 release. Smoothing was not used to preserve the high spatial resolution of the resting-state functional dataset.](https://academic.oup.com/cercor/article-abstract/26/7/3249/1746193)

Reproducibility, Sensitivity, and Specificity Indices

The “reproducibility” of the fCD hubs in the brain across resting-state sessions was quantified across voxels for each subject or across subjects for each voxel as:

\[
\text{Reproducibility} = 1 - \frac{1}{N} \sum_{i}^{N} \frac{\text{abs}(\text{fCD}_{i}^{\text{REST1}} - \text{fCD}_{i}^{\text{REST2}})}{\text{fCD}_{i}^{\text{REST1}} + \text{fCD}_{i}^{\text{REST2}}}.
\]

The gray and white matter parcellations provided with the Q1 release of the HCP dataset were used to compute 2 additional indices assessing tissue-specific differences in fCD across image voxels for each subject or across subjects for each image voxel:

\[
\text{Sensitivity} = \frac{N_{GM}}{N_{\text{tissue}}} \sum_{i}^{N_{\text{tissue}}} \frac{\text{fCD}_{i}}{\sum_{j}^{N_{\text{brain}}} \text{fCD}_{j}} \text{ if } \sum_{j}^{N_{\text{brain}}} \frac{1}{\text{fCD}_{j}} \geq \sum_{i}^{N_{\text{tissue}}} \frac{1}{\text{fCD}_{i}}
\]

gauges the proportion of fCD within the tissue of interests (i.e., cortical and subcortical GM and white matter), normalized by...
tissue volume to that of GM. Note that brain counts, \( \sum_{i}^{N} (\text{brain})_i \), must be larger than tissue counts, \( \sum_{i}^{N} (\text{tissue})_i \), in order to assess the true positive rate of FCD in the tissue. This criterion is naturally satisfied across voxels for each individual (gray matter volume > brain volume) but is not warranted across subjects for each image voxel because normalized brain anatomy in the MNI space is highly similar across subjects. Thus for subcortical structures and cerebellum, and major sulci and gyri sensitivity could not be determined.

\[
\text{Specificity} = \frac{\sum_{i}^{N} (\text{WM})_i}{\sum_{i}^{N} (\text{WM})_i},
\]

\[
\epsilon^t = \begin{cases} 1 & \text{if } \text{FCD}_i \leq \frac{1}{N} \sum_{k=1}^{N} (\text{FCD})_k \rightarrow (\text{FCD})_k \leq \epsilon^t \text{FCD} \\ 0 & \text{if } \text{FCD}_i > \frac{1}{N} \sum_{k=1}^{N} (\text{FCD})_k \rightarrow (\text{FCD})_k > \epsilon^t \text{FCD} \end{cases}
\]

a true negative rate test, gauges the proportion of white matter measures with lower FCD than the whole-brain average. Specificity could not be determined across subjects for voxels that were either classified as GM or had lower FCD than the whole-brain average in all subjects.

### Reliability

The test-retest reliability of the FCD patterns was evaluated for each imaging voxel using two-way mixed single-measures intra-class correlation (Shrout and Fleiss 1979)

\[
\text{ICC}(3,1) = \frac{\text{BMS} - \text{EMS}}{\text{BMS} + (k-1)\text{EMS}}.
\]

Specifically, ICC(3,1) was mapped in the brain in terms of between-subjects (BMS) and residuals mean-square (EMS) values computed for each voxel using the IPN matlab toolbox (http://www.mathworks.com/matlabcentral/fileexchange/22122-ipn-tools-for-test-retest-reliability-analysis) and the FCD maps corresponding to REST1 and REST2 sessions (k = 2). Note that ICC(3, 1) coefficients range from 0 (no reliability) to 1 (perfect reliability).

### Physiologic Noise

The HCP datasets included physiologic data (cardiac and respiratory) corresponding to the resting-state functional scans, which were collected with 2.5 ms temporal resolution. The fast Fourier transform was used to assess the amplitude of the different frequencies of the cardiac and respiratory signals in the 0- to 200-Hz bandwidth of the frequency domain representation. In addition, the physiologic data were downsampled to the 0.72-s temporal resolution of the multiband acquisition using the trigger signal embedded in the physiologic data file, which render frequency domain representations of the cardiac and respiratory signals in the 0.0- to 0.7-Hz bandwidth. The power amplitudes in the low-frequency bandwidth, relative to the full power of the Fourier spectra, were computed for each scan.

### Statistical Methods

A full factorial design was used to compare FCD between sessions (REST1 vs. REST2) and phase encoding directions (LR vs. RL) and to compare FCD differences associated with specific FCD preprocessing steps (i.e., multilinear motion regression, low-pass filtering, and global signal normalization). The statistic-al parametric mapping package (SPM8) was used for this purpose. Statistical significance was set by \( P_{\text{FWE}} < 0.05 \), corrected for multiple comparisons at the cluster level with the random field theory and a family-wise error correction with a cluster-forming threshold of \( P < 0.001 \) and a minimum cluster size of 625 voxels (5 mL).

### Results

#### FCD Patterns

For all resting-state scans, the individual’s FCD patterns predominantly followed the shape of cortical GM, and had minimal overlap with white matter and cerebrospinal fluid (Fig. 2A). These individual’s cortical FCD patterns were highly reproducible across sessions and phase-encoding conditions. Figure 2 demonstrates the reproducibility of the FCD patterns from pipeline 1...
(see Materials and Methods) across sessions and phase-encoding conditions for 2 randomly selected subjects. In most cortical regions, the average fCD across subjects was stronger than the whole-brain mean fCD. Inferior ventral, orbitofrontal, and insular cortices and subcortical regions showed attenuated fCD, perhaps reflecting the lower sensitivity of the 32-channels coil used to collect the data in these regions (Anteraper et al. 2013). The remarkable GM specificity of the fCD in cortical regions across all subjects was used as one of the benchmark criteria to assess the effect of image preprocessing steps on fCD.

Reproducibility

The fCD patterns computed with global signal normalization and correlation threshold $R > 0.3$ had reproducibility of 70±4% across sessions. The lack of global signal normalization improved the sensitivity and specificity (Fig. 2B,C) indices but reduced fCD reproducibility to 67±5% ($P < 0.002$; paired t-test, $df = 79$). fCD patterns (without global normalization) computed with $R > 0.6$ had higher reproducibility (88±4%) and those with $R > 0.15$ had lower reproducibility (59±6%) than at $R > 0.3$. The reproducibility of the fCD patterns did not differ between LR and RL runs. The reproducibility index was maximal in white matter and minimal in the visual cortex (Fig. 3). Voxelwise analysis contrasting REST1 and REST2 did not show statistically significant fCD differences between sessions in any brain region. To assess the impact of higher spatiotemporal resolution, we compared the reproducibility of fCD maps for high versus low spatiotemporal resolution (downsampled) datasets, which were computed with $R > 0.3$ and without global signal normalization (pipeline 4 vs. 7). The reproducibility index was significantly lower for datasets with high spatiotemporal resolution (67±5%; mean ± SD) than for those with low spatiotemporal resolution (82±3%; $P < 10^{-26}$, paired t-test).

Sensitivity

Across subjects, the “sensitivity” index (i.e., the proportion of fCD within a tissue of interest; see Materials and Methods) was higher for cortical gray matter than for white matter and subcortical GM (including cerebellum; Fig. 2B). This differential pattern was highly significant across sessions and reproducible across sessions and phase-encoding conditions ($P < 10^{-18}$; paired t-test, $df = 39$; Supplementary Fig. 1). For cortical GM, the sensitivity index reached a maximal value (85±3%; mean ± SD) for fCD maps computed without global signal normalization and using a correlation threshold $R > 0.3$ (Fig. 2B). Decreasing or increasing the correlation threshold from $R > 0.3$ to $>0.15$ or $>0.6$ reduced the sensitivity index to 80±3% or 76±8% ($P < 10^{-10}$; paired t-test, $df = 39$) for cortical GM. The lack of 0.08 Hz low-pass filtering significantly reduced the sensitivity index in cortical regions to 55±5% ($P < 10^{-17}$). Multilinear motion regression did not have a significant effect on the sensitivity index ($P > 0.4$), suggesting that ICA-based denoising properly corrects for motion artifacts in fCD.

The cortical GM sensitivity index was significantly higher for datasets with high spatiotemporal resolution (85±3%; mean ± SD) than for those with low spatiotemporal resolution (65±9%; $P < 10^{-18}$, paired t-test). Figure 3 shows that the sensitivity index was maximal in cortical regions, minimal in white matter, and null in the ventricles. Note that sensitivity could not be assessed for major sulci and gyri, thalamus, caudate putamen, amygdala, hippocampus, midbrain, and cerebellar GM, due to the absence of white matter in these regions in all subjects.

Figure 3. Reproducibility and GM sensitivity and specificity of fCD patterns computed across subjects superimposed on axial view of the GM template. Sensitivity and specificity could not be computed for major sulci and gyri, thalamus, caudate putamen, amygdala, hippocampus, midbrain, brainstem, and cerebellar GM, due to the absence of white matter in these regions in all subjects.
Specificity

The specificity of the iFCD patterns for cortical GM was higher than 93.6% across threshold and global normalization conditions, sessions, and phase-encoding directions ("specificity index" = 96.3 ± 1.7; mean ± SD; Fig. 2C). Global normalization improved the specificity index (P < 0.04, paired t-test, df = 39). The correlation threshold used in the computation of iFCD did not have a significant impact on the specificity index, in different sessions or phase-encoding directions. The specificity index was significantly higher for datasets with high spatiotemporal resolution (97 ± 1%; mean ± SD) than for those with low spatiotemporal resolution (92 ± 7%; P = 0.0001, paired t-test; pipeline 4 vs. pipeline 7). The specificity index was maximal in white matter regions, minimal in cortical regions, and null in the ventricles (Fig. 3). Similar to sensitivity, specificity could not be assessed for major sulci and gyri, thalamus, caudate putamen, amygdala, hippocampus, midbrain, brainstem, and cerebellar GM, due to the absence of white matter in these regions in all subjects.

Strength

Figure 4 highlights the distribution of the iFCD in the human brain, which is consistent with our previous reports at lower spatiotemporal resolution (Tomasi and Volkow 2010, 2011b). Specifically, in cuneus, precuneus, and primary visual cortices, the iFCD was 3 times or higher than the whole brain average. Within this large region, the cuneus and the precuneus, encompassing posterior ventral regions of the parieto-occipital fissure, exhibited maximal iFCD. The angular gyrus, posterior cingulum, and the posterior regions of the default-mode network also demonstrated prominent iFCD with 6 times or higher strength than the whole brain average. Other regions with high iFCD included precentral gyrus, anterior cingulum, as well as primary auditory, somatosensory, and motor cortices.

Hubs

The local maxima of the average iFCD highlight the precise location of the hubs (i.e., nodes with high local connectivity) in the human brain (Fig. 5). The strongest hubs (iFCD > 5.0) were densely located in a posterior occipito-parietal network, which included cuneus, middle and superior occipital, supramarginal and angular gyri, and inferior parietal cortex (BAs 2, 7, 18, 19, 39, and 40; Supplementary Table 1). The probability of iFCD local maxima within a 1-mL cubic volume, P, was used to assess the reproducibility of hub locations across subjects. The locations and intensity of the
hubs varied across subjects (see Supplementary Fig. 2), but were highly reproducible within the 1-mL cubic search volume ($P > 0.8$, Supplementary Table 1). Similarly, the locations and intensity of the IFCD hubs were highly reproducible across sessions and phase-encoding directions (Fig. 6). The linear associations between GM sensitivity and specificity, reproducibility, and strength of the IFCD hubs were not statistically significant ($P > 0.07$, Pearson correlation).

Global Signal Normalization
Global signal normalization did not alter the IFCD in most cortical regions, but caused significant increases in IFCD in white matter, ventral temporal cortex, basal ganglia, thalamus, and brainstem and in the lateral ventricles (Fig. 7B).

Phase-Encoding Direction
The IFCD was sensitive to the phase-encoding direction of the multiband EPI acquisition, particularly in brain regions affected by magnetic field gradients induced by magnetic susceptibility differences at air-tissue interfaces such as the sinus cavity. In contrast to LR phase encoding, RL phase encoding increased IFCD in right orbitofrontal gray and white matter and left ventral temporal white matter and decreased it in the contralateral regions (Fig. 7C).

Physiologic Noise
When sampled at TR = 0.72 s, physiological (cardiac and respiratory) signals are aliased into the 0- to 0.7-Hz bandwidth and lack of proper low-pass filtering could spuriously increase MRI signal fluctuations (see Supplementary Fig. 3). The IFCD maps corresponding to datasets processed without low-pass filtering had lower strength in cortical regions and higher strength in white matter, ventral temporal cortex, basal ganglia, thalamus, brainstem, and the lateral ventricles than those corresponding to datasets processed with low-pass filtering (Fig. 7D).

Reliability
Intraclass correlation analyses of test–retest datasets demonstrated the high reliability (ICC(3,1) > 0.6) of the IFCD in cortical regions (Fig. 7E). IFCD measures with global signal normalization showed increased reliability in cortical regions, compared with those without global signal normalization (see Supplementary Fig. 4). Test–retest reliability was higher for $R > 0.3$ than for $R > 0.15$. The high correlation threshold ($R > 0.6$) had poor reliability.

Discussion
Here, we show for the first time patterns of IFCD with high spatio-temporal resolution that can reliably identify the precise location of functional connectivity hubs in each individual. Specifically, we quantified the sensitivity and specificity of IFCD for GM as well as the reproducibility and reliability of the IFCD patterns while assessing effects of image preprocessing steps and IFCD model parameters. Based on these analyses, optimal sensitivity and specificity were obtained for the threshold $R > 0.3$, 0.01–0.08 Hz frequency bandwidth, and without global normalization.

Figure 6. IFCD hubs: Reproducibility. Local maxima of the average IFCD across subjects, detected with probability $P > 0.5$ within a 1-mL cubic volume, for each session (REST1 and REST2) and phase-encoding direction (LR and RL), superimposed on axial (top row) views of a GM template of the human brain. The colored pattern indicates the strength of the IFCD hubs in a logarithmic scale.
The FCD is a graph theory metric that measures the degree of the local cluster functionally connected to a given network node. Using MRI data (Achard et al. 2006; van den Heuvel et al. 2008; Beu et al. 2009; Buckner et al. 2009; Tomasi and Volkow 2010, 2011b), magnetoencephalography and electroencephalography (Stam 2004), and optical imaging (Bonifazi et al. 2009), others and we...
have demonstrated that brain network architecture is based on few hubs (regions with high degree) and numerous nodes with low degree (Bullmore and Sporns 2009). This distribution of the nodes is also in agreement with the structure of the small-world (Watts and Strogatz 1998) and scale-free (Barabasi and Albert 1999) networks. The hubs of the human brain are energy demanding (Tomasí, Wang, et al. 2013) and prone to malfunction in neuropsychiatric disorders of metabolic origin (Bassett et al. 2008; He et al. 2008; Buckner et al. 2009; Supekar et al. 2009; Crossley et al. 2014). Even in normal subjects, the strength of the functional connectivity hubs is influenced by aging (Tomasí and Volkow 2012b) and genetic background (Kelly et al. 2007; Biswal et al. 2010; Van Dijk et al. 2010; Wang, Qin, et al. 2012). This work demonstrates for the first time the precise localization of the functional connectivity hubs in the brain, as defined by IFCD local maxima. Using high spatiotemporal resolution fCDM, we demonstrate that cortical hubs are densely located in occipito-parietal cortices, which are known to be disrupted in patients with schizophrenia (van den Heuvel et al. 2013), autism spectrum disorders (ASDs) (Blanken et al. 2015), and Alzheimer’s disease (Sheline and Raichle 2013). At lower spatiotemporal resolution (~3-mm isotropic; ~2 s), we have quantified the variability across subjects in the position of the 2 more prominent hubs as 4.4 ± 2.7 and 6.7 ± 4.1 mm, respectively, which are located in posterior and inferior parietal cortices (Tomasí and Volkow 2010). However, in our previous study, the variability in the spatial location of other hubs was larger (~11 mm). In the present work, we identify the precise locations of 120 cortical hubs that can be detected across subjects with probability higher than 80% within a 1-mL cubic volume (i.e., within 5 mm from MNI coordinates in Supplementary Table 1). The locations of these cortical hubs were highly reproducible across subjects and sessions.

This is also the first study to quantify sensitivity, specificity, and reproducibility of functional connectivity measures in GM tissue, which is where neuronal activity gives rise to oscillations in the blood oxygenation level-dependent MRI signals (Logothetis et al. 2001; Lu et al. 2016; Carandini et al. 2015). The fCDM patterns were highly reproducible within and across subjects and exhibited high GM specificity such that they accurately delineated the shape of cortical gyri and sulci for each individual subject. Reproducibility ranged from 59 ± 6%, for the lowest correlation threshold, to 88 ± 4%, for the highest correlation threshold, and the fraction of IFCD (sensitivity index) in GM reached a maximum of 85 ± 3% for the medium correlation threshold. In this study, the reliability of the IFCD was high in cortical regions (ICC(3,1) = 0.65 ± 0.14; mean ± SD) and moderate in basal ganglia and cerebellum (ICC(3,1) < 0.40 ± 0.20). Previous studies have reported low to moderate reliability (0.15 < ICC(3,1) < 0.6) of functional connectivity measures (Zuo et al. 2010; Braun et al. 2012; Guo et al. 2012; Yang et al. 2012; Birn et al. 2013; Wang, Jiao, et al. 2013), in part due to intrinsic limitations in the spatiotemporal resolution of the MRI data. The present work shows that MRI acquisition protocols with high spatial and temporal resolutions, as those used under the HCP (Van Essen et al. 2012; Ugurbil et al. 2013), can provide a quantum leap in sensitivity, specificity, reproducibility, and reliability of the cortical IFCD hubs at the individual- and group-level analyses. Precise identification of hubs at the individual level is needed in order to develop biomarkers that assess variability of these hubs through an individual’s brain developmental trajectory and in disease, and to assess the effects of therapeutic interventions.

The predominance of IFCD in occipito-parietal cortices at the group level is consistent with findings from our previous studies at lower spatiotemporal resolution (Tomasí and Volkow 2010, 2011b). The strength of the IFCD hubs in cuneus and primary visual cortex was 10 times or higher than the whole brain average. The cuneus and visual cortex have higher neuronal density than other cortical regions (Changeux 1997) and show high glucose metabolic rates (Raichle and Guanerd 2002) that are associated with their functional connectivity (Tomasí, Wang, et al. 2013).

Many sources of fMRI signal variability confound the detection of spontaneous fluctuations in resting-state functional connectivity studies. The data used in this work were cleaned by the HCP using FIX (“FMRIB’s ICA-based X-noiseiﬁer”), an ICA-based automatic noise detection algorithm that can minimize the effect of various types of noise sources (Salimi-Khorshidi et al. 2014). Here, we assessed residual effects of global signal normalization, low-pass ﬁltering, and motion regression, preprocessing steps that overlap with those implemented in FIX. We show that the GM localization of the IFCD patterns was signiﬁcantly improved by 0.08 Hz low-pass ﬁltering. These improvements are consistent with the prediction that proper low-pass ﬁltering attenuates the spurious effect of physiologic noise (cardiac and respiratory ﬂuctuations) on resting-state functional connectivity patterns (Birn et al. 2006, 2008). Whereas previous resting-state fMRI studies at lower temporal resolutions (TR > 1 s) were unable to effectively filter out physiologic noise, the higher temporal resolution (TR = 0.72 s) enabled by the HCP’s multiband EPI acquisition provided the necessary bandwidth to ﬁlter out spurious signal ﬂuctuations of physiologic origin. However, the IFCD in orbitofrontal and ventral temporal cortices was sensitive to the multiband phase-encoding scheme, which might be a concern for studies targeting these brain region such as in ADHD, obsessive compulsive and addiction disorders, and for those on impulsivity and reward processing.

We also studied the effect of global signal normalization on the strength of IFCD in GM, as well as the speciﬁcity, reproducibility, and reliability of the IFCD patterns. Global signal normalization increased the sensitivity, reproducibility, and reliability of the IFCD in cortical GM, but spuriously increased IFCD in white matter and subcortical regions, which might confound results in these regions (Wong et al. 2012). Techniques such as global signal regression/normalization are proposed to control for scanner instabilities in resting-state functional connectivity. However, global signal ﬂuctuations could reﬂect true electrophysiological activity (Schölvinck et al. 2010) and global normalization may lead to side effects such as creating spurious anticorrelations (Murphy et al. 2009; Chai et al. 2012; Saad et al. 2012).

Overall, this work demonstrates the robustness of FCDM at high spatiotemporal resolution. The high sensitivity, speciﬁcity, and reliability make high-resolution IFCD ideal for the development of imaging biomarkers for neurological and psychiatric disorders and for studying normal variability in human brain development and aging. Finally, the established reproducibility and robustness of the high spatiotemporal resolution IFCD metric offer promise as a potential biomarker for the evaluation of therapeutic interventions at the individual level.

Supplementary material

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

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References
