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Functional connectivity hubs in the human brain

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Abstract

Brain networks appear to have few and well localized regions with high functional connectivity density (hubs) for fast integration of neural processing, and their dysfunction could contribute to neuropsychiatric diseases. However the variability in the distribution of these brain hubs is unknown due in part to the overwhelming computational demands associated to their localization. Recently we developed a fast algorithm to map the *local* functional connectivity density (*lFCD*). Here we extend our method to map the *global* density (*gFCD*) taking advantage of parallel computing. We mapped the *gFCD* in the brain of 1031 subjects from the 1000 Functional Connectomes project and show that the strongest hubs are located in regions of the default mode network (DMN) and in sensory cortices, whereas subcortical regions exhibited the weakest hubs. The strongest hubs were consistently located in ventral precuneus/cingulate gyrus (previously identified by other analytical methods including *lFCD*) and in primary visual cortex (BA 17/18), which highlights their centrality to resting connectivity networks. In contrast and after rescaling, hubs in prefrontal regions had lower *gFCD* than *lFCD*, which suggests that their local functional connectivity (as opposed to long-range connectivity) prevails in the resting state. The power scaling of the probability distribution of *gFCD* hubs (as for *lFCD*) was consistent across research centers further corroborating the “scale-free” topology of brain networks. Within and between-subject variability for *gFCD* were twice than that for *lFCD* (20% vs. 12% and 84% vs. 34%, respectively) suggesting that *gFCD* is more sensitive to individual differences in functional connectivity.

INTRODUCTION

The network architecture of the brain may include infrequent “hubs” (network nodes with high functional connectivity) and abundant weakly connected nodes (Bullmore and Sporns, 2009), which is in agreement with the structure of the small-world (Watts and Strogatz, 1998) and scale-free (Barabasi and Albert, 1999) networks. Studies based on graph theory have documented the scale-free and small-world topologies of the functional connectivity at microscopic level with optical imaging (Bonifazi et al., 2009) and at macroscopic level with magnetic resonance imaging data (Achard et al., 2006; Beu et al., 2009; Buckner et al., 2009; van den Heuvel et al., 2008) as well as with magneto and electroencephalography (Stam, 2004; Stam and de Bruin, 2004). However, a better knowledge on the spatial localization and strength of these hubs is essential because its distribution is targeted by neuropsychiatric disorders (Bassett et al., 2008; Buckner et al., 2009; He et al., 2008;

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Leistedt et al., 2009; Supekar et al., 2008; Supekar et al., 2009) and could be influenced by aging and genetic background (Biswal et al., 2010; Kelly et al., 2007; Van Dijk et al., 2010; Wolfson et al., 2009).

Whereas hub distribution and strength is expected to vary across individuals, its variability in the normal population is largely unknown due to the computational demands of voxelwise hub-detection algorithms. To overcome this limitation we developed functional connectivity density mapping (FCDM), a voxelwise technique to detect hubs with high *local* functional connectivity density (*l*FCD) that is 1000 faster than traditional graph theory methods, and studied the distribution of the *local* functional connectivity hubs (Tomasi and Volkow, 2010). Our study quantified the variability of the *l*FCD in 979 subjects from a public image database (Biswal et al., 2010) and showed that the posterior cingulate/ventral precuneus is the main *l*FCD-hub in the human brain. However, a limitation of FCDM was that it does not allow to detect long-range connectivity hubs (Buckner, 2010). Because of the intense computational demands associated with voxelwise graph theory measures in our prior paper we were able to compute *global* functional connectivity density (*g*FCD) in 34 subjects only.

Here, we used parallel computing to determine the location and variability of the *g*FCD-hubs using “resting state” MRI datasets corresponding to 1031 subjects from a large public database (Biswal et al., 2010) (http://www.nitrc.org/projects/fcon_1000/). We hypothesized that the location of the *g*FCD-hubs would be similar to that of the *l*FCD-hubs in posterior cortical regions but that it would differ in subcortical regions. We also hypothesized that the *g*FCD would have greater intersubject variability than the *l*FCD (Tomasi and Volkow, 2010).

METHODS

Subjects

Functional scans corresponding to 1031 healthy subjects from 21 research sites of the “1000 Functional Connectomes” Project (http://www.nitrc.org/projects/fcon_1000/) were included in the study (Table 1). Other datasets were not included because the data was either not available (pending verification of IRB status) or did not meet the imaging acquisition criteria ($3s \geq TR$, full brain coverage, time points > 100 , spatial resolution better than 4-mm). Note that one dataset (Baltimore) was not included in the analysis because did not demonstrate *g*FCD-hubs in any brain region. We excluded datasets with partial head coverage ($N = 44$) and incomplete time series acquisition ($N = 3$).

Image preprocessing

The statistical parametric mapping package SPM2 (Wellcome Trust Centre for Neuroimaging, London, UK) was used for image realignment and spatial normalization to the stereotactic space of the Montreal Neurological Institute using a 12-parameters affine transformation with medium regularization, 16-nonlinear iterations, voxel size of $3 \times 3 \times 3$ mm³. Other preprocessing steps were carried out using IDL (ITT Visual Information Solutions, Boulder, CO). The time-varying realignment parameters (3 translations and 3 rotations) were used within a multilinear regression approach to minimize motion related fluctuations in the MRI signals (Tomasi and Volkow, 2010). The global signal intensity was normalized across time points. Band-pass temporal filtering (0.01–0.10 Hz) was used to remove magnetic field drifts of the scanner (Foerster et al., 2005) and minimize physiologic noise of high frequency components (Cordes et al., 2001). Note that a signal-to-noise threshold of 50 was used to remove voxels showing severe susceptibility-related signal-loss artifacts, which are usually located in the vicinities of the sinus cavity and temporal bones; this threshold was chosen to remove only a small fraction of voxels (~1%) that show the

unwanted effects. Image time series reflecting the preprocessing steps were saved in hard drive for subsequent analyses.

Global FCD

The preprocessed image time series underwent Pearson linear correlations to evaluate the strength of the gFCD in the brain. Two voxels were considered connected if their correlation coefficient $r > 0.6$; we selected this arbitrary correlation threshold to be consistent with the threshold used for the calculation of the fFCD. The gFCD at a given voxel x_0 was computed as the number of functional connections, $k(x_0)$, between x_0 and all other ($v = 57713$) voxels in the brain. A parallel algorithm was developed in C-language to speed up the calculation of the gFCD by taking advantage of multiprocessor computer architectures. A workstation with two Intel® Xeon® X5680 processors (12MB L3 Cache, 64-bit, 3.33 GHz, 24 processing threads total) running Windows 7 was used to compute the gFCD maps for each subject. In average, the parallel algorithm required only five minutes to complete the gFCD calculation per subject. These gFCD-maps that reflect the total number of functional connections per voxel were then spatially smoothed (8-mm) to minimize the differences in the functional anatomy of the brain across subjects.

Local FCD

The preprocessed image time series also underwent Functional Connectivity Density Mapping (Tomasi and Volkow, 2010) to compute the strength of the fFCD. Specifically, the local $k(x_0)$ was determined through Pearson correlations between time-varying signals at x_0 and those from its closest neighbors (x_M ; $M = \{j\}$) using an arbitrary threshold $r > 0.6$. As an example let's consider x_j , a voxel that is adjacent to a voxel that belong the list of neighbors of x_0 (x_N ; $N = \{i\}$; i.e. voxels that are linked by a continuous path of functionally connected voxels). If the Pearson linear correlation factor, $r_{0j} > 0.6$, x_j is added to the list (x_{N+1}); if $r_{0j} < 0.6$ the list of neighbors remains unchanged. Then the calculation is repeated for the next voxel that is adjacent to the voxels that belong to the list of neighbors. The searching algorithm computes the fFCD as $k(x_0)$ (the number of elements in the local functional connectivity cluster) when no new neighbors can be added to the list of neighbors of x_0 . Then the calculation is initiated for a different x_0 . Whereas this calculation is performed for all voxels ($v = 57713$), the necessary correlations to compute a map of the fFCD is usually reduced by a large factor (~ 1000). This "growing" algorithm was developed in IDL (Tomasi and Volkow, 2010). The fFCD-maps were spatially smoothed (8-mm).

Principal component and statistical analyses

Principal component analysis (PCA) was used to analyze the variability of gFCD within- and between-subjects. Specifically, gFCD maps with zero empirical mean were calculated and the principal components were computed in IDL using the covariance of the data. Group analyses were performed with t-tests using the general lineal model in SPM2. Clusters with $p_{corr} < 0.05$, corrected for multiple comparisons using a family-wise error (FWE) threshold, were considered significant for group analysis in SPM.

Variability of global FCD patterns across research sites

In order to evaluate the variability of the gFCD patterns across research centers, average maps of the relative gFCD across subjects, k_i , were computed for each i -site. Deviations from the mean relative gFCD pattern, $\mu(x, y, z) = (1/v)\sum_i k_i(x, y, z)$, were accessed in IDL with the aid of the chi-square distribution:

$$\chi_{(v)}^2 = (1/v) \sum_{xyz} [(k_i(x, y, z) - \mu(x, y, z))/\sigma(x, y, z)]^2,$$

where $\sigma(x, y, z)$ is the standard deviation map of the gFCD across research sites. Assuming a Gaussian sample, $k_i(x, y, z)$ -patterns with $\chi^2_{(v)} > 3.84$ ($P < 0.05$) were considered significantly different to $\mu(x, y, z)$; those with $\chi^2_{(v)} > 9.00$ ($P < 0.003$; > 3 standard deviations from μ) were considered outliers.

Region-of-interest (ROI) analyses

Isotropic cubic masks containing 27 imaging voxels (0.73 ml) were defined at the centers of relevant functional connectivity hubs (Table 2) to extract the average strength of the gFCD signal from individual gFCD maps. The average and standard deviation values of the gFCD within these ROIs were computed for each subject using a custom program written in IDL. The ROI measures were used to assess the significance of the gFCD using non parametric statistics, as well as the variability of the gFCD across and within subjects and the statistical significance of gender effects on gFCD. Nonparametric statistics (Kolmogorov-Smirnov and Mann-Whitney-Wilcoxon tests) that do not make assumptions on probability distributions were carried out in Statview (SAS Institute Inc, Cary, NC).

Retest-reliability

The 3 sessions of the New York test-retest (NYU_TRT) dataset were used to evaluate the reliability of gFCD. Specifically, for each subject the average gFCD- measures k^m_{ij} corresponding to the $1 \leq m \leq M$ ROIs in Table 2, $1 \leq i \leq I = 25$ subjects, and $1 \leq j \leq J = 3$ sessions were used to compute the within-subjects variance: $V_w = \sum_j (k^m_{i\bullet} - k^m_{i\bullet j})^2 / (J - 1)$, as well as the between-subjects variance,

$$V_B = \sum_i (k^m_{\bullet\bullet} - k^m_{i\bullet})^2 / (I - 1),$$

for each ROI, where $k^m_{i\bullet}$, $k^m_{i\bullet j}$, and $k^m_{\bullet\bullet}$ are averages of k^m across sessions, subjects, and both sessions and subjects for each ROI, respectively. The relative variability of gFCD within-subjects was computed as:

$$\Delta_w = 100 \times \sum_m (V_w^{1/2} / k^m_{\bullet\bullet}) / M,$$

$$\Delta_B = 100 \times \sum_m (V_B^{1/2} / k^m_{\bullet\bullet}) / M.$$

Intraclass correlation

Intraclass correlation (ICC) was also computed as an additional index of test-retest reliability (Shrout and Fleiss, 1979). Specifically, for each ROI the gFCD measures were merged into 25×3 gFCD-matrices. One-way random average ICC measures were computed according to (Shrout and Fleiss, 1979):

$$ICC(3, 1) = (S_B - S_W) / (S_B + 2S_W),$$

where S_B and S_W are the between- and within-subjects sum of squares. For this purpose we used the matlab IPN toolbox for Test-Retest Reliability Analysis (<http://www.mathworks.com/matlabcentral/fileexchange/22122-ipn-tools-for-test-retest-reliability-analysis>). Note that ICC (1, 3) coefficients range from 0 (no reliability) to 1 (perfect reliability).

RESULTS

Hub-variability

The distribution of gFCD-hubs varied significantly across datasets from different research centers (Fig 1). Whereas all datasets showed prominent gFCD-hubs in occipital (BA 17/18) and/or ventral-medial and posterior parietal (BA 31 and 39) cortices, the availability of intense hubs (defined as 40% of the maximum gFCD independently for each dataset) in other brain regions was less consistent. Intense gFCD-hubs were found in auditory cortex (11 datasets), dorsal parietal cortex (14 datasets), insula (14 datasets), and ventral frontal cortex (12 datasets). The cerebellar vermis also reached high gFCD values for some of the datasets (6 datasets). Principal component analysis (PCA) allowed us to determine the average inter-subject variability of the gFCD for all datasets (Table 1). The average variability of gFCD across datasets was $48 \pm 16\%$ (mean $\pm$ SD). The first and second principal components (PC#1 and PC#2) of the gFCD accounted for $48 \pm 16\%$ and $15 \pm 6\%$ of the variability, respectively, and 7 ± 7 principal components were needed to account for 80% of the variance.

Average distribution of global FCD-hubs

Grand mean scaling was implemented to minimize the effect of differences in acquisition parameters and instruments across research centers (Tomasi and Volkow, 2010). Specifically, the use of a single scaling factor for each dataset, $1/k_0$, reflecting the mean gFCD across subjects and voxels in the brain, k_0 , allowed us to normalize the distribution of the gFCD. Voxelwise Gaussian fits of the gFCD-probability distribution across subjects confirmed the normal distribution of the rescaled gFCD for all voxels, which encouraged the use of parametric tests to assess the statistical significance of gFCD differences. The gFCD was statistically significant in the whole brain ($P_{FWE} < 0.05$, familywise error corrected for multiple comparisons; t-test; Fig 2). The average distribution of the gFCD in the human brain across 1031 subjects showed prominent gFCD-hubs (Fig 3; red) that were bilaterally distributed in visual cortical areas (V1, V2, and V3) and posterior ventral-medial parietal cortices (Table 2). Other cortical areas (auditory, dorsal parietal, insula, and ventral frontal cortex; Fig 3, green) also showed intense gFCD-hubs. Conversely, the availability of gFCD-hubs in subcortical brain regions was minimal (Fig 3, blue). The analysis of the variability across research sites indicate that for two of the research sites (MIT and Ontario) the average gFCD pattern was significantly different than the mean pattern ($\chi^2_{(v)} > 4.52$; $P < 0.033$; Table 1). However, none of the research sites could be identified clearly as outlier (3 standard deviations from the mean pattern). The gFCD-patterns obtained with 3T MRI scanners resembled the mean pattern better than those obtained at other field strengths ($\chi^2_{(v)} = 2.3 \pm 1.1$ versus 3.6 ± 0.9 ; $P = 0.05$, t-test). The gFCD patterns collected at 3-Tesla resembled the mean pattern better for shorter than for longer timeseries (Fig 4A) and for larger than for smaller samples (Fig 4B).

Test-retest reliability

The New York University test-retest reliability (NYU_TRT) dataset (Shehzad et al., 2009) was used to evaluate the reproducibility of the gFCD. Using uncorrected threshold levels ($P < 0.005$, 100 voxels), significant gFCD-differences between groups were noticed in cuneus, auditory cortices and ventral precuneus/posterior cingulate ($P_{corr} < 0.001$, cluster level corrected for multiple comparisons). Nonparametric tests (Mann-Whitney) on ROI measurements confirmed the statistical significance of between-subjects differences in gFCD in these regions ($P < 0.05$). Similarly, within-subjects differences were found in striatum, frontal cortex and cerebellum using SPM ($P_{corr} < 0.001$) and the nonparametric Wilcoxon signed rank test ($P < 0.0001$). However, the minimal amplitude of the gFCD in these regions suggests that instability in the gFCD measure could have been underestimated

and the statistical significance, computed on the basis of random field theory, could have been overestimated (Hayasaka and Nichols, 2003). Using ROI analyses we verified that in average across the ROIs listed in Table 2 the variability of the gFCD data between subjects was $84 \pm 23\%$ and within subjects was $20 \pm 11\%$. In average across ROIs, the intraclass correlation coefficient across all three sessions was $ICC(1, 3) = 0.55$ (Table 2). This reflects higher between- than within-subjects gFCD-variability and is a common measure of reliability in resting-state functional connectivity studies (Zuo et al., 2010).

Gender effects

Gender, aging, and potential differences in resting conditions (e.g. eyes opened/closed, awake/sleep, etc) could partially explain the variability of the gFCD across datasets. Factor analysis was performed on datasets with a narrow age range (18–30 years; Beijing, Cambridge, Leiden, Oulu, and Saint Louis; 336 females and 225 males) to evaluate gender effects within an age constrained group. This analysis revealed significant gender effects on gFCD that were distributed bilaterally in the brain. Specifically, the factors of PC#2 were higher and those of PC#4 lower for women than for men ($P_c < 0.01$, Bonferroni corrected for multiple comparisons, t-test). The pattern of PC#2 overlapped DMN regions (ventral precuneus, ventral frontal cortex, and angular gyrus); the pattern of PC#4 included visual, motor, and somatosensory cortices, middle cingulum, orbitofrontal cortex, frontal and parahippocampal gyri, cerebellum, thalamus, caudate/putamen, and hypothalamus. Follow up SPM t-tests assessing the statistical significance of gender effects on gFCD revealed that females had up to 45% higher gFCD than males, bilaterally, in DMN regions (ventral precuneus, angular gyrus, ventral frontal cortex; $P_{FWE} < 0.001$; Fig 5A). Nonparametric statistical testing on ROI measures confirmed the gender effects on gFCD in these regions ($P < 0.001$; Kolmogorov-Smirnov and Mann–Whitney–Wilcoxon tests; Fig 5B).

Global versus local hubs

In order to assess differences between local and global measures of functional connectivity hubs, the rescaled gFCD was contrasted against the rescaled lFCD we documented previously (Tomasi and Volkow, 2010) using voxelwise SPM (Fig 6A). The rescaled gFCD was higher than the rescaled lFCD, bilaterally, in posterior parietal (BAs 3, 5, 7, 37, and 40), occipital (BAs 17–19), temporal (BAs 21 and 22) and frontal (BA 10) cortices ($P_{FWE} < 0.001$; paired t-test; Fig 6B, red-yellow). The rescaled gFCD was lower than the rescaled lFCD, bilaterally, in dorsolateral prefrontal (BAs 6, 8–9, 11, 44–48), inferior parietal (BA 40), temporal (BA 20), and limbic (BA, 23–24, and 32) cortices, as well as subcortical regions (putamen, caudate, thalamus, midbrain, and cerebellum) ($P_{FWE} < 0.001$; paired t-test; Fig 6B, blue-green). Nonparametric testing confirmed the statistical significance of differences between global and local ROI-measures of FCD in these regions ($P < 0.001$; Wilcoxon signed-rank test). Overall, increased gFCD was associated with increased lFCD across voxels in the brain ($R = 0.83$, Pearson linear correlation; Fig 6C).

Probability distributions

The probability distributions of the gFCD and lFCD, $P(k/k_0)$, were computed as the ratio between the number of voxels with k/k_0 functional connections, $n(k/k_0)$, and the total number of voxels in the brain, n_0 . Figure 6D shows that the availability of lFCD- and gFCD-hubs decreases with k/k_0 following a power scaling $P(k/k_0) = (k/k_0)^{-\gamma}$. The scaling factor, γ , was significantly lower for gFCD-hubs than for lFCD-hubs ($P < 0.0001$, $F(56) = 315$, test of coincidence of slopes and intercepts; comparison of two linear regressions), indicating that gFCD hubs were more frequent than lFCD hubs.

DISCUSSION

Here we used parallel computing to map the gFCD-hubs of the brain without a priori hypotheses in a large sample (1031 subjects from the 1000 Functional Connectomes Project) using unprecedented spatial resolution (3-mm isotropic). The most prominent gFCD-hubs were located in the posterior cingulate/precuneus and in the primary visual cortex (V1, V2, and V3; Fig 7). Though prominent hubs were also detected in other sensory cortices, other regions in the DMN and in cerebellum, their location and strength was highly variable across subjects. Indeed test-retest datasets demonstrated that these patterns had high between-subject variability (~ 80%) whereas within-subject variability was much lower (~20%). Gender is one of the factors that contributed to within-subject variability, with females showing 45% higher gFCD than males in DMN regions.

Spatial distribution of gFCD

The present study is the first to document that in resting conditions one of the two most prominent functional connectivity hubs is located in the primary visual cortex. The other hub, located in posterior cingulate/ventral-precuneus, had been identified previously by us and others (Tomasi and Volkow, 2010). Our previous study documented fFCD-hubs in BA 18 (cuneus) which were much less prominent than the gFCD-hubs in this region and that major hubs are functionally connected to largely independent networks (Tomasi and Volkow, 2011a). In addition gFCD-hubs were also located in other DMN regions (angular gyrus, and the ventral frontal cortex) and other visual areas (lateral), as well as auditory and somatosensory regions. Previous studies that used voxelwise data-driven approaches also documented functional hubs in ventral precuneus and frontal cortex and visual areas (Buckner et al., 2009; Cole et al., 2010; Guye et al., 2010; Tomasi and Volkow, 2010; van den Heuvel et al., 2008) and magneto encephalography studies have indentified the visual cortex (Hari et al., 1997) as one of the sources for alpha rhythms. Interestingly resting functional connectivity studies reported increased connectivity in the visual cortex with eyes closed when compared with eyes open (McAvoy et al., 2008), which is consistent with the potential location of the source of alpha rhythms, waves that are typically present when a subject is awake with closed eyes (Jann et al., 2009). Our findings are also consistent with the association of cortical hubs and primary sensory cortices in the infant brain (Fransson et al., 2011) and with the hierarchical modularity of brain networks (Meunier et al., 2009). The brain regions with the highest gFCD have been shown to exhibit some of the highest resting metabolic rates in humans (Langbaum et al., 2009; Raichle and Gusnard, 2002) (including visual cortex) and to have up to 2.5 times higher neuronal density than other cortical regions (Changeux, 1997), which is also consistent with their role as prominent functional hubs.

Variability

This study is the first to assess the variability of the global functional connectivity hubs across research institutions as well as between and within subjects. Principal component analyses demonstrated a large variability of gFCD patterns (~ 50%) that might reflect differences in resting conditions, demographic variables, acquisition techniques and instruments. In particular the influence of resting conditions in the inter-subject variability is highlighted by the greater variability in sensory regions that are sensitive to study conditions such as visual (eyes closed or open) and auditory cortices (scanner noise, music) as well as regions affected by the level of alertness (precuneus). Moreover the amplitude of the BOLD signal fluctuations in these regions (including visual and auditory cortices and retrosplenial cortex) has been shown to change significantly between the eyes closed versus the eyes open conditions (McAvoy et al., 2008). Note also that gender affected variability (see below). The high between-subjects variability of the gFCD (80%) encourages further studies to assess its significance to brain function, behavior and psychopathology (Cohen et al., 2009). In

contrast, the lower within- than between-subject variability of the gFCD patterns (only 20%) advocates for their reproducibility but also suggests that gFCD-hubs are dynamic and may be sensitive to the state of the subjects (alertness, fatigue, excitement). Indeed the functional connectivity strength of long-range connections have been shown to decrease more than that of short-range connections in the transition from wakefulness into slow wave sleep (Spoormaker et al., 2010), and the long-range functional coupling between the DMN and the attention networks is decreased by sleep deprivation (Sämann et al., 2010). Overall, these results are consistent with the reproducibility of graph theory metrics in functional connectivity networks (Telesford et al., 2010). However, visual inspection of Fig 1 reveals institution-dependent gFCD differences that cannot be plausibly attributed to the state of the subjects. In fact, here we document that the gFCD patterns varied with magnetic field strength, sequence parameters, and sample size. Thus standardization of study techniques, instrumentation and conditions could further improve reproducibility in resting-state functional connectivity studies and should be encouraged for future studies that aim to integrate large resting functional connectivity datasets.

Gender effects

The PC#2 factor of the gFCD was 45% higher for females than for males and this gender difference encompassed DMN regions. Regions from the DMN have the highest metabolic rate at rest (Langbaum et al., 2009) and are deactivated during cognitive tasks (Tomasi et al., 2006). Our findings suggesting that the strength of the gFCD hubs in the DMN is higher for females than for males is consistent with the gender effects on lFCD (Tomasi and Volkow, 2011b) and with previous studies based on “seed-voxel” correlation analyses (Biswal et al., 2010). Higher brain connectivity in females was also reported by diffusion magnetic resonance imaging (MRI) tractography studies that showed greater overall anatomical connectivity in the female than in the male brain (Gong et al., 2009). However, our study failed to identify the females lower functional connectivity in occipital, parietal, temporal, and prefrontal cortices (Biswal et al., 2010). The higher gFCD in females than males would predict higher energy consumption and cerebral blood flow (CBF) at rest in females, which is consistent with the higher CBF per unit weight of brain (Gur et al., 1982) and higher baseline brain glucose metabolism in females than in males reported by brain imaging studies (Baxter et al., 1987). The females higher connectivity in DMN regions, which show metabolic decrements in Alzheimer’s disease (AD) (Small et al., 2000), could help explain why female AD-patients show greater impairments than male patients for the equivalent reduction in cerebral metabolic rate (Pernecky et al., 2007).

Global hubs versus local hubs

The computing-demanding gFCD and ultra-fast lFCD calculations are complementary. The former captures the total number of functional connections of every voxel but does not distinguish between short-range and long-range connections. The later captures the number of short-range connections but does not account for long-range functional connections (Tomasi and Volkow, 2010). The variability of gFCD (within and between) subjects was significantly higher than that of lFCD. The direct comparison of the rescaled lFCD and gFCD allowed us to identify the DMN (associated with consciousness and “self” processing) as well as visual, auditory, and somatosensory areas as regions with a higher proportion of long-range connections than short-range connections. Since sensory input is central to survival, these findings argue that the posterior cortex includes the most prominent long-range hubs in order to support fast communication of sensory information to the rest of the brain. Conversely, the direct comparison of global and local measures of functional connectivity suggested a higher proportion of short-range than long-range connections in prefrontal and parietal areas that are activated by cognitive tasks and in subcortical brain regions. The relatively lower global to local functional connectivity in the prefrontal region

that are central to executive control was unexpected since these regions are not only highly locally interconnected but also have widespread neuroanatomical connections with all sensory neocortical and motor systems and with subcortical structures enabling integration of a diverse range of information (Miller, 2000). Thus the relatively lower global than local connectivity of the prefrontal cortex is likely to reflect the resting condition; we predict that during task conditions the prefrontal cortex (perhaps also parietal cortices) will reveal a greater density of global connectivity (driven by increases in long-range functional connections when performing a task). Indeed, enhanced connectivity of the prefrontal cortex with the premotor cortex was reported during cognitive processing (Abe et al., 2007). The influence of the resting state condition on gFCD could also explain why the hub with the highest connectivity density was located within the DMN, which is most active at rest. The lower gFCD in subcortical limbic regions (caudate, putamen, globus pallidus, hypothalamus, amygdala, midbrain) and in cerebellum, suggests that in resting conditions these regions are segregated, perhaps performing specialized processing with less/slow access to cortical resources. Fewer well connected subcortical and limbic regions, when compared with cortical regions, have also been reported with “seed-voxel” correlation approaches both during waking and sleep conditions (Achard et al., 2006; Spoormaker et al., 2010).

Power scaling

The probability distribution of the gFCD demonstrated the power scaling of the scale-free networks (Barabasi and Albert, 1999), as reported by previous studies on functional connectivity (van den Heuvel et al., 2008) and by our own studies on lFCD-hubs (Tomasi and Volkow, 2010). However, the power scaling, γ , of the lFCD-hubs was significantly higher than that of the gFCD-hubs, which was expected since the gFCD measure includes the lFCD, and suggests that long-range functional connectivity hubs are essential to the highly distributed architecture of brain networks.

Study limitations

Some of the variability of the gFCD patterns might reflect differences in the quality of EPI data, subject motion, or could reflect anxiety-related differences in respiratory volume per unit time (RVT) which causes BOLD modulation patterns (Birn et al., 2008) that share some similarities with the gFCD patterns reported in the present study. We were unable to control for this effect because RVT data was not available to us. Ideally, future resting-state studies on gFCD should control for differences RVT. The global signal is another potential source of variability in functional connectivity because a large fraction of the signal fluctuations are shared throughout the brain (Fox et al., 2009). Global signal fluctuations were removed during preprocessing steps in the present study in order to minimize their contribution to the variability of the gFCD patterns. However, recent studies suggest that much of the global signal reflects neuronal activity (Schölvinck et al., 2010). Thus the removal of global signal fluctuation could have decreased the amplitude of the gFCD in this study.

Conclusion

Here we used parallel computing and high-resolution graph theory to evaluate the availability of global functional connectivity hubs in the brain of 1031 subjects. Significant variability was found between subjects (80%) and gender effects accounted a significant fraction of the variability (45%). Posterior brain regions (most prominent and consistent in visual cortex and precuneus/posterior cingulate) associated with consciousness and sensory processing exhibited powerful long-range hubs. The prefrontal and subcortical regions predominantly exhibited short-range hubs in resting conditions. Findings encourage the use of graph theory approaches on multicenter imaging datasets to explore brain connectivity in neuropsychiatric populations.

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Research Highlights

- The study maps brain functional connectivity hubs in 1031 subjects.
- The default mode network and sensory cortices include prominent hubs for long-range functional connectivity; subcortical regions include short-range connectivity hubs.
- The variability of the global hubs was higher than that of the local hubs.
- Gender effects accounted for 45% of the variability of the global functional connectivity density.

Fig 1. Variability of the gFCD

Spatial distribution of the gFCD superimposed on middle sagittal (left panel) and axial (right panel) MRI plane for each dataset (white labels).

Fig 2. Statistical parametric mapping of the rescaled gFCD
Surface rendering showing the high statistical significance of the functional connectivity hubs in the brain. Statistical significance threshold: $P < 0.05$, corrected for multiple comparisons (FWE). Sample: all 1031 subjects included.

Fig 3. Average gFCD distribution

Spatial distribution of the gFCD superimposed on axial MRI views of the human brain (radiological convention). These maps reflect the average number of functional connections per voxel across 1031 subjects from 21 research sites around the world. White labels indicate the axial distance to the origin of the stereotactic space (Montreal Neurological Institute).

Fig 4. Variability across institutions

Chi-square per degree of freedoms of the gFCD patterns as a function of EPI time frames (A) and sample size (B) for research sites that used 3-Tesla MRI instruments.

Fig 5. Gender effects of gFCD

(A) Spatial distribution of the rescaled gFCD for females and males, as well as the statistical gender differences ($P_{\text{corr}} < 0.001$; SPM two-sample t-test) rendered on a brain surface template. (B) Nonparametric analyses of ROI measurements confirming the SPM findings on gender effects on gFCD (* $P < 0.001$, Kolmogorov-Smirnov and Mann-Whitney-Wilcoxon tests). Sample: 336 females and 225 males (Beijing, Cambridge, Leiden, Oulu, and Saint Louis datasets; age range: 18–30 years).

Fig 6. Global versus local FCD

Spatial distribution of the rescaled gFCD and lFCD distributions (A) and statistical maps showing significant differences between them ($P_{\text{corr}} < 0.001$; paired t-test; color bars reflect t-scores) (B) superimposed on surface and axial views of the human brain. (C) Scatter plot showing the linear correlation of gFCD and lFCD across voxels. (D) Probability distributions showing the power scaling of the global and local FCD in the brain. Sample: 1031 subjects.

Fig 7. gFCD-hubs in the visual cortex

[Top panels] Surface rendering (INFLATED) highlighting the visual areas as depicted in the Visuotopic Human.Colin.R Atlas of the Computerized Anatomical Reconstruction and Editing Toolkit (Caret; Washington University School of Medicine). [Bottom panels] Spatial distribution of the rescaled gFCD showing the location of prominent global hubs in visual areas. Sample: all 1031 subjects included.

Demographic data and imaging parameters. Principal component analysis was used to assess the variability of the global FCD-hubs.

Table 1

Dataset	Subjects	Age [years]	B [T]	Tp	TR [s]	Variability [%]	PC#1 [%]	χ^2_v	γ
Ann Arbor A	20M/3F	15–41	3	295	1.0	32	63	3.22	3.1
Bangor	20M/0F	19–38	3	265	2.0	63	34	2.44	4.1
Beijing	76M/122F	18–26	3	225	2.0	56	40	1.31	3.1
Berlin	13M/13F	23–44	3	195	2.3	52	38	1.33	3.5
Cambridge	75M/123F	18–30	3	119	3.0	69	31	1.44	4.2
Cleveland	11M/20F	24–60	3	127	2.8	39	55	1.69	3.6
Dallas	12M/12F	20–71	3	115	2.0	24	68	2.78	2.7
ICBM	17M/25F	19–85	3	128	2.0	35	57	0.79	2.6
Leiden	23M/8F	20–27	3	215	2.2	47	51	2.58	3.7
Leipzig	16M/21F	20–42	3	195	2.3	63	31	1.09	4.5
MIT	17M/18F	20–32	3	145	2.0	51	47	5.60	2.7
Newark	9M/10F	21–39	3	135	2.0	61	31	2.22	3.4
New York A	40M/19F	20–49	3	192	2.0	50	43	2.47	2.7
New York B	8M/12F	18–46	3	175	2.0	52	45	2.87	2.5
NYU_TRT	10M/15F	22–49	3	197	2.0	34	70	1.61	3.0
Ontario	11 subj.	N/A	4	105	3.0	69	33	4.52	3.0
Orangeburg	15M/5F	20–55	1.5	165	2.0	62	36	3.33	2.5
Oulu	37M/65F	20–23	1.5	245	1.8	60	32	2.98	3.4
Oxford	12M/10F	20–35	3	175	2.0	20	80	2.79	2.3
Palo Alto	2M/15F	22–46	3	235	2.0	67	28	2.75	4.3
Queensland	11M/7F	21–34	3	190	2.1	25	71	2.10	2.7
Saint Louis	14M/17F	21–29	3	127	2.5	34	58	1.26	2.7
Taipei A	13 subj.	N/A	3	295	2.0	22	72	3.70	2.5
Taipei B	8 subj.	N/A	3	175	2.0	55	36	1.75	7.2

B: magnetic field strength; tp: number of time points in the image time series; TR: repetition time; PC#1: variance of the first principal component; χ^2_v chi-square per degree of freedom corresponding to the average gFCD; γ average scaling factor

Table 2

MINI-coordinates of prominent *g*FCD-hubs in the human brain

Brain Region	BA	X [mm]	Y [mm]	Z [mm]	<i>g</i> FCD [k/k ₀]	ICC(1,3)
Cuneus	18	0	-81	18	9.7	0.60
Precuneus	31	0	-60	33	9.5	0.65
Middle Occipital Gyrus	18	-24	-87	27	7.7	0.63
Angular Gyrus	39	-45	-69	36	6.2	0.70
Anterior Cingulate	32	0	54	-3	5.6	0.44
Medial Frontal Gyrus	9	0	57	15	5.2	0.51
Angular Gyrus	39	51	-63	30	5.2	0.47
Supramarginal Gyrus	40	60	-33	36	4.6	0.56
Postcentral Gyrus	40	60	-21	15	4.4	0.44
Transverse Temporal Gyrus	41	-57	-21	12	4.2	0.44
Inferior Parietal Lobule	40	45	-42	54	4.0	0.63