Brain Hemispheric Structural Efficiency and Interconnectivity Rightward Asymmetry in Human and Nonhuman Primates

Yasser Iturria-Medina1, Alejandro Pérez Fernández2,3, David M. Morris4,5, Erick J. Canales-Rodríguez6,7, Hamied A. Haroon1,4,5, Lorna García Pentón2, Mark Augath8, Lidice Galán García1, Nikos Logothetis4,8, Geoffrey J. M. Parker4,5 and Lester Melie-García1

1Neuroimaging Department, Cuban Neuroscience Center, CP 10 600, La Habana, Cuba, 2Laboratory of Cognitive Neuroscience, Universidad Diego Portales, 8370076 Santiago, Chile, 3Centro de Investigación y Desarrollo del Comercio Interior, CID-CI, CP 10 400, La Habana, Cuba, 4Imaging Science and Biomedical Engineering Research Group, School of Cancer and Imaging Sciences and, 5Physiology of Cognitive Processes Department, Max Planck Institute for Biological Cybernetics, 72076 Tübingen, Germany

Address correspondence to Yasser Iturria-Medina, Neuroimaging Department, Cuban Neuroscience Center, Avenida 25, Esquina 158, #15202, Apartado Postal 6648, Cubanacán, Playa, Habana 6 CP 10600, Cuba. Email: iturria.medina@gmail.com.

Evidence for interregional structural asymmetries has been previously reported for brain anatomic regions supporting well-described functional lateralization. Here, we aimed to investigate whether the two brain hemispheres demonstrate dissimilar general structural attributes implying different principles on information flow management. Common left hemisphere/right hemisphere structural network properties are estimated and compared for right-handed healthy human subjects and a nonhuman primate, by means of 3 different diffusion-weighted magnetic resonance imaging fiber tractography algorithms and a graph theory framework. In both the human and the nonhuman primate, the data support the conclusion that, in terms of the graph framework, the right hemisphere is significantly more efficient and interconnected than the left hemisphere, whereas the left hemisphere presents more central or indispensable regions for the whole-brain structural network than the right hemisphere. From our point of view, in terms of functional principles, this pattern could be related with the fact that the left hemisphere has a leading role for highly demanding specific process, such as language and motor actions, which may require dedicated specialized networks, whereas the right hemisphere has a leading role for more general process, such as integration tasks, which may require a more general level of interconnection.

Keywords: brain structural network, diffusion-weighted MRI, efficiency, fiber tractography, hemispheric asymmetries, interconnectivity

Introduction

The interregional structural connectivity asymmetry for left-right brain hemispheres is an important topic in the study of the neural basis of brain functional asymmetries, contributing to our understanding of the factors that modulate cognitive specialization in the brain. The recent development of diffusion-weighted magnetic resonance imaging (DW-MRI), a noninvasive technique that quantifies water diffusion process, has allowed the acquisition of structural information about the intravoxel axon arrangement, making possible the noninvasive study of the brain anatomical circuitry (Mori et al. 1999; Koch et al. 2002; Parker et al. 2002; Behrens, Johansen-Berg, et al. 2003; Tuch et al. 2003; Hagmann, Kurant, et al. 2006; Iturria-Medina et al. 2007). In that context, structural asymmetries have been explored analyzing mainly the fractional anisotropy (FA), a measure of local fiber coherence (Kubicki et al. 2002; Gong et al. 2005; Powell et al. 2006; Clark et al. 2007; Rodrigo et al. 2007) and the number of, or existence of, connecting paths between specific regions (Parker et al. 2005; Hagmann, Cammoun, et al. 2006; Powell et al. 2006; Glasser and Rilling 2008), contributing to our understanding of cognitive lateralized process like language and motor control.

These previous interregional studies were focused on identifying which white matter regions and/or connections, corresponding to reported functional lateralization, are more coherent or stronger in one hemisphere than in the other. In other words, to match well-described functional lateralization with the specific white matter structural asymmetries supporting the function. However, in order to characterize brain structural asymmetry through white matter connections, it is necessary to recognize not only the specific interregional asymmetries in a pairwise manner, which could represent a considerable challenge due to the high number of possible connections, but also the broader structural network asymmetries between the hemispheres, in order to assess differences in how their anatomical substrates may be configured to facilitate the management and integration of information flow in a more general sense.

In this study, we aimed to investigate if, besides the specific regional asymmetries, both hemispheres demonstrate dissimilar general structural attributes implying different principles on the management of the information flow. Our analysis is based on a mathematical network framework (Watts and Strogatz 1998; Latora and Marchiori 2001; Onnela et al. 2005; Boccaletti et al. 2006; Costa et al. 2007), allowing us to explore hemispheric differences in terms of quantitative parameters that can be structurally and, by inference, functionally interpreted. Previous brain structural network analyses have used connectivity information obtained from nonhuman post-mortem studies (Sporns and Zwi 2004; Costa and Sporns 2005; Sporns 2006), statistical concurrent change analysis between brain areas in one or more morphological variables (He et al. 2007; Bassett et al. 2008; Chen et al. 2008; He et al. 2008), or DW-MRI techniques (Hagmann, Kurant, et al. 2006; Hagmann et al. 2008; Iturria-Medina et al. 2008; Gong et al. 2009) to investigate large-scale connection patterns of the brain, such as small-world attributes, efficiency, degree distribution, motif composition, and structural core properties. Here, using 3 different tractography algorithms and a graph theory framework, we attempt to estimate white matter interregional
axonal pathways and to infer left and right common anatomical network properties, obtaining global and local measures that allow us to evaluate for the first time structural network (dis)similarities between hemispheres. In particular, we focus on graph measures of efficiency and interconnectivity, for 11 right-handed healthy human subjects and a single macaque monkey. Additional analyses include the identification of those anatomic regions that are significantly more central or indispensable (in terms of their connections) for the whole-brain structural network than their homolog regions in the opposite hemisphere. Finally, some comments are made concerning the relationship between the obtained anatomical findings and some previously reported functional asymmetries, such as visual attention neglect.

Materials and Methods

Data Acquisition and Preprocessing

Dataset 1
High-angular resolution DW-MRI datasets were acquired in 11 right-handed healthy subjects (age range: 23–38 years). All subjects provided informed consent, and the procedures were approved by the Local Research Ethics Committee. Handedness was determined by documenting the dominant hand of the participants.

Data were acquired on a 3-T Philips Achieva scanner (Philips Medical Systems) in Manchester, UK, using an 8-element SENSE head coil. Diffusion-weighted imaging was performed using a dual phase encoded pulsed gradient spin echo protocol designed to allow correction of susceptibility-induced and eddy current-induced distortions (Embleton et al. 2006). Acquisition parameters used were time echo (TE) = 54 ms, time repetition (TR) = 11884 ms, G_s = 62 mT/m, half-scan factor = 0.679, 112 × 112 image matrix reconstructed to 128 × 128 using zero padding, reconstructed resolution = 1.875 × 1.875 mm^2, slice thickness = 2.1 mm, 60 contiguous slices, 61 noncollinear diffusion sensitization directions at b = 1200 s/mm^2 (λ = 29.8 ms and δ = 13.1 ms), 1 at δ = 0, and SENSE acceleration factor = 2.5. For each diffusion gradient direction, 2 separate volumes were obtained with opposite polarity, & space traversal in order to enable the distortion correction method.

T2-weighted images were also acquired and automatically parcellated into 90 gray matter structures (parcellation scheme 1) (Mazziotta et al. 1995) using the IBASPM toolbox (available at http://www.filion.uc.ac.uk/spm/ext/#IJ/BASP) (Alemán-Gómez et al. 2006). Additionally, in order to evaluate possible parcellation scheme effects on lateralization of global/local efficiency and interconnectivity network measures, the registered T2-weighted images were also parcellated into 71 gray matter structures (parcellation scheme 2) corresponding to the Jacob Atlas developed by the Montreal Neurological Institute (http://www.mni.mcgill.ca/). In order to consider the same left and right gray matter regions, the brain stem region was rejected from this parcellation scheme (for details please see Supplementary Table 4).

Dataset 2
A high-angular resolution DW-MRI dataset was acquired in a formalinfixed postmortem macaque (Macaca mulatta) brain using a Bruker BIOSPEC 4.7-T vertical bore scanner. The macaque brain was perfused, removed from the skull, and kept in 4% paraformaldehyde for 5 years prior to scanning. A 2D spin echo MRI sequence was implemented with TE = 79 ms, TR = 9 s, G_s = 47 mT/m, 104 × 94 imaging matrix, 58 contiguous slices, isotropic voxel resolution = 0.8 mm, 61 noncollinear diffusion sensitization directions at b = 4000 s/mm^2 (λ = 39 ms and δ = 31 ms), 7 at b = 0, and 4 averages. The total imaging time was ca. 64 h. To improve the signal-to-noise ratio in the diffusion-sensitized images, for the purposes of tractography, we applied 5 iterations of 2D anisotropic diffusion smoothing (rsb.info.nih.gov/iij/plugins/anisotropic-diffusion-2d.html) using ImageJ (rsb.info.nih.gov/iij/index.html).

Following the procedure described in Haroon et al. (2008), we took the cortical parcellation scheme LVE00a (Lewis and Van Essen 2000) available as part of the Caret 5.5 software (available at http://www.braintools.wustl.edu/caret) for the F99U1I rhesus macaque brain atlas. Then, using the Normalize tool in SPM5 (available at http://www.fil.ion.ucl.ac.uk/spm/software/spm5/), a nonlinear warping was applied to the F99U1I MRI brain volume to spatially match the brain volume of our dataset. Finally, the nonlinear warping transformation parameters obtained were applied to the LVE00a parcellation scheme (for a list of region labels see Supplementary Table 5). In addition, macaque cortex structural connections information derived by invasive tracer studies was extracted from Cocomac LVE00a database (cocomac.org/home.asp) (for details please see Fig. 3a).

Voxel-Region Axonal Connectivity Estimation
For each subject of datasets 1 and 2, 3 axonal connectivity values between each brain voxel and the surface of each considered gray matter region (voxel-region connectivity) were estimated using 3 fully automated fiber tractography algorithms as follows:

1. Probabilistic tractography from the seed regions was carried out according to Behrens, Woolrich, et al. (2003) using the FSL software package (available at http://www.fmrib.ox.ac.uk/fsl/). For each brain voxel, an index of connectivity, representing the number of generated paths that passed through it from the seed region, was assigned. Tracking parameters used were 5000 as number of generated paths from each seed point (suggested in the FSL software package as optimum to reach the convergence of the algorithm), 0.5 mm as step size, 500 mm as maximum trace length, and a curvature threshold of ±80°. The results we refer to this algorithm as "FSL."

2. Second, probabilistic tractography was performed using the PICo algorithm (Parker et al. 2003; Parker and Alexander 2005). Similarly to the FSL method, a connection probability value with each seed region was assigned to the brain voxels. Tracking parameters used were 5000 as number of generated paths from each seed point, 0.5 mm as step size, 500 mm as maximum trace length, and a curvature threshold over voxel of ±90°. Intervoxel probability density functions of fiber orientation were generated using a model-based residual bootstrap Q-ball approach (Haroon et al. 2009). In the text we refer to this algorithm as "PICo."

3. Finally, a graph-based tractography algorithm (Iturria-Medina et al. 2007) was used, assigning to each brain voxel a probability of connection with each considered seed region. A maximum of 500 mm trace length and a curvature threshold of ±90° were imposed as tracking parameters. The intravoxel white matter orientational distribution function maps were estimated using the Q-ball approach (Tuch 2004). In the text we refer to this algorithm as "GM."

Additionally, in order to evaluate the performance of the previous fiber tractography algorithms (FSL, PICo, and GM) in comparison with the more often used deterministic streamline tractography (SLT) algorithm (Mori et al. 1999), we used also the SLT approach to estimate axonal connectivity values between brain voxels and the surface of each considered gray matter region for the macaque monkey subject (dataset 2). Tracking parameters used for SLT algorithm were 0.5 mm as step size, 500 mm as maximum trace length, a curvature threshold over voxel of ±60°, and a FA threshold of 0.2. Seed points were selected as all brain voxels with an FA value greater than 0.2 (the so-called brute-force approach). Following the procedure described in Li et al. (2009), 2 gray matter regions were considered to be connected if the reconstructed fiber trajectories with 2 end points located in these 2 regions, respectively, were present, and a final connection measurement between these regions was defined as the number of connecting fiber trajectories relative to the number of voxels over the surface of both regions (equivalent to arc weight definition in eq. 1).

Network Construction
For each subject, whole-brain undirected weighted networks were created for each tracking algorithm used as follows: 1) A node was defined to represent each considered anatomic region, 2) An
undirected arc $a_{ij}$ between nodes $i$ and $j$ was established if a nonzero connectivity value was found to exist between the boundary voxels of regions $i$ and $j$, and 3) Arc weight $w(a_{ij})$ was defined as the connection measurement (the output of the tractography algorithm) between regions $i$ and $j$ (Iturria-Medina et al. 2007), estimated by counting the "effective" number of voxels over the surface of both regions and weighting each voxel by its voxel-region connectivity value with the opposite zone, relative to the total number of considered superficial voxels. Mathematically:

$$w(a_{ij}) = \frac{\sum_{\forall \epsilon \in N^{i} \cup N^{j}} \xi_{\epsilon} + \sum_{\forall \epsilon \in N^{i} \cup N^{j}} \xi_{\epsilon}}{|N^{i}| + |N^{j}|}$$

where the term $\xi_{\epsilon}$ denotes the axonal connectivity value of each node $\epsilon \in N^{i}$ ($N^{i}$ being the set of superficial nodes of region $i$) with the superficial nodes of region $j$ ($N^{j}$). Similarly, $\xi_{\epsilon}$ denotes the connectivity value of any node $\epsilon \in N^{i}$ with superficial nodes of region $i$, $|N^{i}|$ and $|N^{j}|$ are the number of elements (superficial nodes) of regions $i$ and $j$, respectively. Note that the first term of the numerator quantifies connections of the region $i$ with $j$, and the second term quantifies connections of the region $j$ with $i$.

Eliminating interhemispheric connections, each whole-brain network was segmented into left and right hemispheric networks, each one containing the same number of homolog regions. Graphically, each created undirected weighted network is a discrete set of points (nodes) representing anatomic regions and a set of nondirectional lines (arcs) representing connections between them, which the width of each line reflecting the corresponding arc weight.

**Graph Analysis**

**Efficiency**

In terms of the information flow, the global efficiency ($E_{glob}$) of a network $G$ reflects how efficiently information can be exchanged over $G$, considering a parallel system in which each node sends information concurrently along the network. It is defined as (Latora and Marchiori 2001):

$$E_{glob} = \frac{1}{n(n-1)} \sum_{\forall \epsilon \in G} \frac{1}{d_{\epsilon j}}$$

where $n$ is the number of nodes, and $d_{\epsilon j}$ is the geodesic length over all pairs of nodes. In the unweighted network context, the shortest path length $d_{\epsilon j}$ is defined as the number of arcs along the shortest path connecting nodes $i$ and $j$. In the case of weighted networks, the path with the minimum number of nodes is not necessarily the optimal $d_{\epsilon j}$ and is necessary to define a physical length associated to each arc (this should be a function of the characteristics of the hypothetical link among any nodes $i$ and $j$). In this work, we assumed that the physical length of an arc connecting nodes $i$ and $j$ is inversely proportional to the strength of the analyzed connection (Iturria-Medina et al. 2008), i.e., $d_{\epsilon j} = \frac{w_{\epsilon}}{w_{\epsilon}}$. Thus, the shortest path length $d_{\epsilon j}$ is finally computed as the smallest sum of the arc lengths throughout all the possible paths from node $i$ to node $j$. Note that for the particular case of unweighted graphs, $w_{\epsilon} = 1$ for all arcs and the geodesic lengths $d_{\epsilon j}$ reduces to the minimum number of arcs traversed to get from $i$ to $j$.

The local efficiency ($E_{loc}$) of $G$ is defined as the average efficiency of the local subgraphs (Latora and Marchiori 2001):

$$E_{loc} = \frac{1}{n} \sum_{\forall \epsilon \in G} E_{glob}(G_{\epsilon})$$

where $G_{\epsilon}$ is the subgraph of the first neighbors of node $i$. This measure has been used to reveal how much a system is fault tolerant, showing how efficient the communication is among the first neighbors of $i$ when $i$ is removed.

In a physiological sense, the global efficiency of a structural brain/hemispheric network reflects the potential parallel exchange of neural information between the involved anatomic regions (a high global efficiency value, i.e., $E_{glob} \approx 1$, may indicate a high parallel information transfer in the brain/hemispheric system, in which each element node could efficiently send information concurrently along the network). The local efficiency of a structural brain/hemispheric network reflects its potential tendency to present communities or clusters of anatomically and physiologically different regions that deal with common neural information (where regions connected to a same region tend also to link to each other). In addition, concurrent higher values of global and local efficiencies indicate a system with a high balance between local necessities (fault tolerance) and wide-scope interactions.

**Interconnectivity**

In order to obtain a measure of the total amount of connectivity in a weighted network $G$, here we define the interconnectivity ($Q_{comb}$) of $G$ as the sum of its arc weights. It reflects the amount of connectivity between all the considered anatomic regions.

**Betweenness Centrality**

Betweenness centrality is a widely used measure to identify the most connected nodes in a graph, which are associated to those nodes that act as bridges between the others (Freeman 1977; Bassett et al. 2006; D’Allesio et al. 2006; Honey et al. 2007). It is defined as the fraction of shortest paths between pairs of nodes that passes through a given node. Mathematically, for weighted networks, if $\sigma_{G}^{ij}$ is the number of paths from node $i$ to node $j$, and $\sigma_{ij}^{G}(l)$ is the number of these paths passing through node $l$ in the weighted betweenness centrality of $l$ is (D’Allesio et al. 2006)

$$b_{ij}^{G} = \sum_{k, j \in G} \frac{\sigma_{ij}^{G}(l)}{\sigma_{ij}^{G}}$$

**Laterazation Index**

In order to characterize asymmetry for a given network measure $X$ (global/local efficiency, interconnectivity, or betweenness centrality), a lateralization index (LI) was computed as:

$$LI(X) = 100 \times \frac{X(\text{Right}) - X(\text{Left})}{X(\text{Right}) + X(\text{Left})}$$

This procedure allows us to look at differences between the right and left hemispheres, incorporating the relative network value of $X$ over both hemispheres in one value. The index, ranging between 100 and 0, is positive when $X$ is more prominent over the right hemisphere and lower over the left hemisphere ($X(\text{Right}) < X(\text{Left})$) and negative when the opposite is the case ($X(\text{Right}) > X(\text{Left})$).

**Receiver Operating Characteristic Analysis**

Receiver operating characteristic (ROC) curves are useful for visualizing, organizing, and selecting classifiers based on their performance (Fawcett 2006). Here, in order to evaluate the performance of the FSL, PICo, and GM fiber tractography algorithms in comparison with the traditional SLT algorithm, ROC curves for each of these algorithms were constructed, based on the comparison of their connection results for the macaque monkey subject (dataset 2) and the reference connection pattern defined by macaque cortex structural connections information derived by invasive tracer studies (Fig. 3a).

First, because in this analysis we are not differentiating between left and right hemispheres due to connection information from invasive tracing studies not being hemisphere specific, connectivity matrix representing each fiber tractography algorithm was created assuming, as final connection value between any 2 regions $i$ and $j$, the mean of the connection values obtained by this algorithm on both hemispheres (eq. 1). Next, the ROC curve corresponding to a specific tractography algorithm was represented as the fraction of "true positives" (TPR) versus the fraction of "false positives" (FPR) of its connectivity matrix with regard the reference connection pattern (Fig. 3a) as a discrimination threshold is varied. That is, that a set of threshold values, varying from minimum to maximum value, were applied to the analyzed connectivity matrix and resulting binarized matrix versions were compared with
the reference connection pattern (Fig. 3a), allowing to calculate corresponding TPR and FPR values as:

\[
\begin{align*}
TPR &= \frac{TP}{TP + FN} \\
FPR &= \frac{FP}{FP + TN}
\end{align*}
\]

(6)

where TP, FN, FP, and TN are the number of true positives, false negatives, false positives, and true negatives, respectively. For a valid comparison, only those matrix cells where a direct (dis)connection by invasive tracer studies (values 0 and 1 on Fig. 3a, i.e., a total of 462 values) have been reported were considered. One point in ROC space is considered better than another (each point belonging to a different tracking algorithm) if it is to the northwest (TPR is higher, FPR is lower, or both).

Results

Anatomical connections between cortical and subcortical regions for 11 right-handed healthy human subjects (dataset 1) and a single macaque monkey (dataset 2) were estimated using 3 different fiber tractography algorithms (see Materials and Methods). From the obtained voxel-region connectivity maps (Fig. 1b), weighted networks were created for the whole brain (Fig. 1c), in which each node represents an anatomic brain region (90 for humans or 176 for macaque monkey), arcs connecting nodes correspond to white matter links, and arc weights correspond to the degree of evidence supporting the

Figure 1. Schematic representation of the connectivity estimation and network construction procedure; for an example, human subject and the first tractography algorithm. (a) Axial map representing intravoxel mean fiber orientation (dyadic vectors), overlaid on the FA image; the inset figure provides detail of the high fiber orientation coherence within the splenium of the corpus callosum. (b) Axial voxel-region connectivity maps corresponding to region 1 (precentral gyrus) and region 90 (inferior temporal gyrus), overlaid on the registered \(T_2\)-weighted image; voxels are color-coded according to whether the connectivity of each voxel is high (white) or low (black). (c) Whole-brain structural network derived as described in Materials and Methods. (d) Right and left hemispheric networks (RH and LH, respectively), obtained by rejection of callosal connections on the whole-brain structural network (viewed from below). In (c) and (d), points (nodes) represent anatomic regions, lines without arrow (arcs) correspond to connections between them, and line widths reflect the corresponding arc weights. Lines colors were assigned according to the spatial position of the nodes.
existence of a white matter connection between regions. Eliminating callosal connections, each whole-brain network was segmented into left and right hemispheric networks, each one containing the same number of homolog regions (i.e., 45 regions for humans and 88 regions for macaque monkey; Fig. 1d).

In summary, for each subject we obtained a whole-brain network, and corresponding left/right hemispheric networks, each one replicated for each of 3 different fiber tracking algorithms.

Efficiency and Interconnectivity Asymmetries

We evaluated structural asymmetries between left hemisphere and right hemisphere networks according to 3 network properties: global efficiency ($E_{\text{glob}}$, a measure of how efficiently information can be exchanged over a network), local efficiency ($E_{\text{loc}}$, the average global efficiency of the local subnetworks), and interconnectivity ($I_{\text{conn}}$, a measure of the total amount of interconnectivity). These measures were separately computed for each individual’s left and right hemispheric networks (see Materials and Methods).

In order to characterize the hemispheric asymmetry for global/local efficiency and interconnectivity, a LI was calculated for each of these 3 network measures, with a positive value meaning a lateralization to the right while a negative value indicates a lateralization to the left (see Materials and Methods). Interestingly, the obtained LI results showed a consistent lateralization trend toward the right hemisphere for the 3 efficiency and interconnectivity network measures (Fig. 2).

Before testing for a statistically significant lateralization for human subjects, we compared tracking algorithm effects on the obtained LI values using a Kruskal–Wallis test, testing the null hypothesis that all values for a same network measure ($E_{\text{glob}}, E_{\text{loc}},$ or $I_{\text{conn}}$) were drawn from the same distribution independently of the fiber tracking algorithm used. The nonsignificant $P$ values obtained ($P > 0.05$; Table 1) supported that LI values obtained were not statistically dependent on the tracking method used. Right lateralization was then tested using a sign test with the hypothesis that all obtained LI values for a same network measure come from a distribution whose median is zero. The small $P$ values obtained (all $P < 7 \times 10^{-5}$; Table 1) supported the hypothesis of a significant positive lateralization for the 3 measures, indicating that human right hemisphere networks are anatomically more efficient and interconnected than left hemisphere networks for right-handed subjects. The LI values obtained for $E_{\text{glob}}, E_{\text{loc}},$ and $I_{\text{conn}}$ in the macaque monkey were also positive for the 3 tractography algorithms (Table 1).

Regional Betweenness Centrality Asymmetries

We computed the betweenness centrality associated with each brain region considered in humans (dataset 1) and macaque monkey (dataset 2) with respect to the whole-brain anatomical network. This measure reflects the relative centrality or indispensability of each gray matter region within the entire brain anatomical network in terms of its connections, in which higher betweenness centrality values imply greater importance (see Materials and Methods). Then, in order to characterize centrality asymmetries between left and right homolog regions, a betweenness centrality LI was calculated (a positive value meaning a lateralization to the right while a negative value indicates a lateralization to the left).

Results in Dataset 1

Before testing for a statistically significant lateralization of the betweenness centrality parameter between human homolog regions, we compared tracking algorithm effects on the measured betweenness centrality LI values using a Kruskal–Wallis test (Table 2). We observed that 9 region pairs had betweenness centrality values that were dependent on method but that the remaining 36 region pairs did not ($P > 0.05$). In a first lateralization analysis, we considered only those 36 pairs
of regions whose LI values can be statistically assumed to be drawn from the same distribution for the 3 tracking algorithms. Lateralization was then tested using a sign test, with the hypothesis that all measured LI values come from a distribution whose median is zero.

Significant lateralization of betweenness centrality was found for 15 pairs of homolog regions ($P < 0.05$; Table 2). Leftward asymmetries (negative LI) were observed in 10 pairs of regions: precentral gyrus ($P = 0.0350$), middle orbital frontal ($P = 0.0214$), supplementary motor area ($P = 0.0427$), olfactory bulb ($P = 0.0045$), superior medial frontal gyrus ($P = 0.0095$), anterior cingulate gyrus ($P = 1.30 \times 10^{-7}$), middle cingulate gyrus ($P = 0.0045$), inferior occipital ($P = 0.0266$), paracentral lobule ($P = 0.0002$), and caudate ($P = 0.0013$). Significant rightward asymmetries (positive LI) were observed in 5 pairs of regions: hippocampus ($P = 0.0350$), cuneus ($P = 0.0013$), superior occipital ($P = 0.0350$), supramarginal ($P = 0.0013$), and angular ($P = 6.61 \times 10^{-5}$).

In order to account for a degree for the demonstrated differences in output from different tracking processes, we also assessed those pairs of regions whose LI values can be statistically assumed to be drawn from the same distribution for 2 of the 3 tracking algorithms. Eight additional pairs of homolog regions were included in the lateralization analysis when using this criterion. By means of a sign test, 6 of the additional region pairs were found to demonstrate significant lateralization ($P < 0.05$; Table 2). Leftward (negative LI) asymmetries were observed in posterior cingulate gyrus ($P = 0.0043$), inferior parietal ($P = 0.0071$), superior temporal ($P = 0.0015$), superior temporal pole ($P = 1.09 \times 10^{-7}$), and middle temporal pole ($P = 0.0008$),...
whereas only the middle occipital ($P = 0.0046$) was found to demonstrate rightward asymmetry.

In summary, a total of 21 pairs of human homolog regions were found lateralized in terms of their centrality or indispensability to the whole-brain structural network. In line with a previous cortical thickness study (Luders et al. 2006), we found a more pronounced leftward than rightward asymmetry (15 vs. 6 lateralized regions, respectively).

Results in Dataset 2

Tracking algorithm effects for the macaque monkey data were compared using a Kruskal–Wallis test across all considered region pairs. The high $P$ value obtained ($P = 0.8393$) supported the conclusion that LI values obtained were not statistically dependent on the tracking method used. Lateralization was then tested via a nonparametric permutation test (see Appendix).

Significant lateralization of betweenness centrality was found for 19 pairs of homolog regions ($P < 0.05$). Leftward asymmetry (negative LI) was observed in 11 regions: temporoparietal area ($P = 0.0156$), somatosensory area 2 ($P = 0.0156$), middle temporal area ($P = 0.0313$), ventral subdivisions of the anterior inferotemporal cortex area TE ($P = 0.0156$), dorsal prelunate area ($P = 0.0313$), subdivisions of area 8 ($P = 0.0156$), dorsal cingulate area 24 ($P = 0.0156$), subdivisions of cingulate area 24 ($P = 0.0156$), area 31 ($P = 0.0156$), area 14r ($P = 0.0156$), and medial area 10 ($P = 0.0313$). Significant rightward asymmetry (positive LI) was observed in 8 regions: visual area 3 ($P = 0.0156$), visual area V3A ($P = 0.0156$), area 13m ($P = 0.0156$), ventral visual area ($P = 0.0156$), 11m ($P = 0.0156$), medial intraparietal area ($P = 0.0313$), area 6M ($P = 0.0156$), and area 32 ($P = 0.0156$).

Fiber Tracking Algorithms Performance Evaluation

In order to evaluate the validity of the fiber tractography algorithms used in this paper (FSL, PICo, and GM) in comparison with the more often used deterministic SLT algorithm (Mori et al. 1999), we compared structural networks results obtained for the macaque monkey (dataset 2) with macaque cortex structural connections information derived by invasive tracer studies. Figure 3a presents cortical–cortical connection information extracted from Cocomac LVE00a database, corresponding to 63 brain cortical regions (see Materials and Methods, Data Acquisition and Preprocessing), whereas Figure 3b presents resultant ROC curves from comparison between previous cortical–cortical connection information and connectivity matrices estimated with FSL, PICo, GM, and SLT algorithms (see Materials and Methods, Receiver Operating Characteristic Analysis).

Although it is not possible to make a statistical comparison between the obtained ROC curves (due to the fact that they correspond to a single dataset), a clear prevalence of the 3 fiber tracking algorithms used in this study (FSL, PICo, and GM) can be seen over the performance of the traditional deterministic SLT algorithm, which is also numerically supported by the corresponding areas under the ROC curves: FSL (AUC = 0.78), PICo (AUC = 0.72), GM (AUC = 0.77), and SLT (AUC = 0.62).

Discussion

We performed a structural network analysis based on DW-MRI tractography techniques and graph theory to identify brain hemispheric anatomical asymmetries. In contrast with previous structural network studies (Hagmann, Kurant, et al. 2006; Hagmann et al. 2008; Iturria-Medina et al. 2008; Gong et al. 2009), here we...
emphasize the use of 3 tractography algorithms, making the results robust to choice of tracking algorithm, which is potentially a significant source of bias, and thus providing a valuable assessment of the relative detail of network information across these methods and, more importantly, providing a robust set of results with which to assess lateralization. We found significant differences between right and left hemispheric networks at a hemispheric level using the metrics $E_{glob}$, $E_{loc}$, and $I_{conn}$ indicating that the right hemisphere is, at the whole-hemisphere level, more efficient and interconnected. However, the subhemisphere regional asymmetries observed using the betweenness centrality measure indicate that the left hemisphere presents more central or indispensable regions for the whole-brain structural network than the right hemisphere. These findings were found to hold true across the human dataset and in the single macaque dataset, indicating that general organizational strategies are broadly similar between these species.

**Efficiency and Interconnectivity Hemispheric Asymmetries**

We explored general structural differences between both hemispheres via 3 network measures: global efficiency, local efficiency, and interconnectivity ($E_{glob}$, $E_{loc}$, and $I_{conn}$, respectively). A significant rightward asymmetry on both efficiencies and interconnectivity was found (Table 1 and Fig. 2). To our knowledge, this is the first report of brain structural asymmetry of these measures. This whole-hemisphere right-greater-than-left interconnectivity and efficiency are consistent with the reported significant rightward asymmetry in the white matter volume for right-handed healthy subjects (Barrick et al. 2005). Furthermore, the greater efficiency and stronger interconnectivity in the right hemisphere obtained for the macaque monkey suggest that these asymmetry characteristics could be shared among primates, even when caution is applied when considering that a single nonhuman primate case was tested. Our results raise a number of intriguing questions. For example, what are the functional requirements underlying the fact that one hemisphere is more efficient and interconnected than the other? Interestingly, our leftward regional asymmetry findings, as expressed using the betweenness centrality measure, indicate that left hemisphere presents more central or indispensable regions for the whole-brain structural network than the right hemisphere, which is in line with previous studies (Luders et al. 2006). Since the connectivity of a given region, and thus the measurement of betweenness centrality, is related to its cellular characteristics such as cell packing density, cell size, and number of cortical neurons (Costa 2005; Lerch et al. 2006), connectivity asymmetries of the cortex might be related to hemispheric-specific functional specializations, which based on the high correspondence between our structural results and well-known functional regional asymmetries seems to support the fact that the left hemisphere is “regionally” more specialized than the right hemisphere. When considered in combination with the whole-hemisphere efficiency and interconnectivity results, this induces us to think that a greater left hemisphere functional specialization could lead to its apparently “worse” general structural organization (less efficiency and interconnectivity at the whole-hemisphere scale) in comparison with the right hemisphere. In terms of functional principles, these patterns appear to support the fact that the left hemisphere has a leading role for highly demanding specific process, such as language and motor actions, which may require dedicated specialized networks, whereas the right hemisphere has a leading role for more general process, such as integration tasks, which may require a more general level of interconnection.

**Regional Betweenness Centrality Asymmetries, Correspondence with Previous Findings**

Previous studies reporting structural differences between homologs areas have been mainly based on specific cytoarchitectonic and morphologic features while our regional asymmetry analysis is based on the fiber connectivity pattern of each region in terms of its relative importance to the whole-brain structural network. Despite the interpretative differences between the measures and procedures employed in different studies, we found a considerable regional correspondence between previous reports, old observations of asymmetric regional specialization (based on the effects of brain lesions), and our asymmetry findings, supporting the hypothesis of a determinant relation between cytoarchitectonic/morphologic characteristics and interregional connectivity (Costa 2005; Lerch et al. 2006; Van Essen 1997).

**Dataset 1**

In correspondence with previous human studies, leftward asymmetries for the precentral gyrus and supplementary motor area, regions associated with motor control actions as hand preferences, have been well recognized for right-handed subjects (Amunts et al. 1996; Rogers et al. 2004; Dadda et al. 2006; Luders et al. 2006). The paracentral gyrus and cingulate gyrus leftward asymmetries found here are in line with similar cortical thickness results (Luders et al. 2006), although this cingulate gyrus asymmetry is in contrast to rightward asymmetries previously reported using volumetric and voxel-based measures (Paus et al. 1996; Watkins et al. 2001). However, significant left-greater-than-right FA values have previously been reported for the cingulum bundle (i.e., white matter fibers projecting from the cingulate gyrus to the entorhinal cortex), suggesting a more coherent fiber organization in the left hemisphere that is consistent with our findings (Gong et al. 2005; Clark et al. 2007). In addition, our observed leftward asymmetries for superior temporal, superior temporal pole, and middle temporal pole regions agree with many structural reports (Niznikiewicz et al. 2000; Good et al. 2001a; Watkins et al. 2001; Luders et al. 2006) and the documented left hemispheric dominance for language (Price 2000). Nevertheless, we did not find significant localized asymmetries related to the inferior frontal regions, thought to contain Broca’s region, which is also associated with dominant language process. Interestingly, a recent study (Keller et al. 2009) concluded that contrary to what has frequently been reported in literature, structural asymmetry of Broca’s area has not been reproducibly demonstrated. According to this previous study, if a structural asymmetry of Broca’s area does exist, it is variable and may differ from that of the functional asymmetry of language, which is more consistent. Additionally, it is known that the gyral location and extent of Broca’s area vary considerably between subjects (Amunts et al. 1999, 2004; Mazzotti et al. 2001; Juch et al. 2005; Lindenberg et al. 2007). Such variation has been demonstrated using cytoarchitectonic and functional imaging methods but cannot be determined.
from the purely anatomical parcellation of the cortex used in our study. We must therefore conclude that there is a possibility that a true underlying localized lateralization associated with Broca’s area has not been detected using our methodology.

Asymmetries of the caudate nucleus, a region found here with a leftward betweenness centrality asymmetry and thought to be involved in higher order motor control (Graybiel 2005) and learning and memory process (Packard and Knowlton 2002), have been reported for normal children (Filipek et al. 1997; Pueyo et al. 2000; Castellanos et al. 2001) and for children with attention-deficit hyperactivity disorder (Aylward et al. 1996; Sugama et al. 2000; Castellanos et al. 2001; Schrimsher et al. 2002). However, reports on caudate asymmetry have been inconsistent (e.g., in normal subjects a common finding is left-greater-than-right caudate volume although there are also reports of no asymmetry and even of rightward asymmetry; see Schrimsher et al. 2002). Recently, Tremols et al. (2008) argued that these inconsistencies can be explained according to different asymmetric properties of the head and body parts of the caudate, which connect into different fiber pathways. Unfortunately, our study was limited to the whole caudate region without making distinction between its head and body parts due to the nonspecificity of our gray matter parcellation scheme. Further caudate network analysis should focus on more precise parcellations in order to explore how caudate connectivity could be related to head and body volume asymmetries.

In the case of those homotopic regions found with a betweenness centrality rightward asymmetry, the finding of the hippocampus is consistent with the widely reported right-sided asymmetries for this region (Frie et al. 1995; Bigler et al. 1997; Bilir et al. 1998; Good et al. 2001b; Pegues et al. 2003). It is striking that all the other right-lateralized regions (i.e., cuneus, superior occipital, supramarginal, angular, and middle occipital) are related to visual processes, and there is evidence that right hemispheric areas play a dominant role in the implementation of visuo-spatial attention (Fink et al. 2000, 2001; Corbetta and Shulman 2002; Halligan et al. 2003). The specific case of a betweenness centrality rightward lateralization for angular and supramarginal gyrus (Brodmann areas 39 and 40, respectively) is an additionally interesting result because evidence exists suggesting that lesions to these regions on the right hemisphere cause visuo-spatial attentional neglect (Vallar 1998; Halligan et al. 2003; Husain and Rorden 2003), a neuropsychological syndrome characterized by an attentional bias. To our knowledge, this is the first structural indication of why the widely investigated phenomenon of visuo-attentional neglect is more commonly a consequence of right hemisphere damage.

**Dataset 2**

Literature about macaque monkey interhemispheric brain asymmetries is scarce. However, in correspondence with our results, consistent evidence concerning leftward volumetric asymmetry at the cytoarchitectural level of the temporoparietal area (Tpt) has been reported (Gannon et al. 2008). This region, a homologous area of the human planum temporale, is thought to be linked to the participation of the left temporal lobe in auditory and vocalization process, cognitive tasks that have being reported to present a leftward lateralization in the macaque monkey (Heffner HE and Heffner RS 1984; Poremba et al. 2004). In addition, we found a leftward asymmetry to the middle temporal and the ventral subdivisions of the anterior inferotemporal cortex area TE. These results reinforce the view that the macaque could provide a model system for studying the evolutionary development of concepts and language in humans (Gil-Da-Costa et al. 2004).

Interestingly, we also find corresponding interspecies structural asymmetries in the visual system (rightward asymmetry) and cingulate gyrus (leftward asymmetry), which to our knowledge have not been reported before. However, as the parcellation schemes that we used for human and nonhuman primate differed considerably, further analyses are necessary to identify with confidence other possible asymmetry equivalences and differences between species.

**Methodological Issues and Future Work**

Previous structural and functional studies have provided evidence on how the apparent topological organization of brain networks can be modified by the different parcellation strategies applied (Hagmann, Kurant, et al. 2006; Li et al. 2009; Wang et al. 2009). In principle, our results could be biased by the chosen gray matter parcellation scheme, a key element of the creation of brain anatomical networks due to its influence on the node definition process. Thus, in order to explore possible parcellation effects on our efficiency and interconnectivity findings, we repeated the human structural brain graph asymmetry analysis for another parcellation scheme with a different set of 70 regions, using 1 of the 3 tractography algorithms (GM), and the results indicated a consistent hemispheric rightward asymmetry on these measures (see Supplementary Figure 1 and Supplementary Table 4). However, it is important to consider that although the atlases that we used were carefully created taking into account relevant anatomical and functional details, in the future it might be more meaningful to use advanced integrative atlases based on finer cytoarchitecture, myeloarchitecture, and MRI procedures (Toga et al. 2006).

In contrast to the more often used deterministic tractography approach (Conturo et al. 1999; Mori et al. 1999; Basser et al. 2000), which typically can only progress when there is high certainty of fiber direction limiting their usefulness in defining pathways near gray matter, the 3 different tractography algorithms used in this study include in the tracking procedure the uncertainty of each fiber orientation (based on the high information contained in the acquired high-angular resolution DW-MRI datasets) and attempt to establish the spatially distributed degree of connection confidence across the whole brain to a given start point. However, in spite its advantages over the conventional deterministic tractography (Fig. 3b), these methods still present important limitations, like a decrease in probability of connection with distance and the difficulty to separate real from false connections. Thus, in the future it might be more meaningful to use statistical methodologies such as those recently introduced by Morris et al. (2008), which proposed to address the mentioned limitations of current probabilistic tractography algorithms using an objective method for determining significant connections based on statistical comparison with a null pattern of connection, which also has the desirable effect of reducing the effect of distance on extracted patterns of connection.

Another consideration for our study is the relatively small size of the used subject sample, especially with respect to the
macaque brain. Despite this, as previously mentioned, the use of 3 different tractography algorithms allowed us to replicate network measures, making the statistical analysis nonspecific to only one tracking algorithm’s results and thus improving the robustness of the study. However, future work should be addressed to analyzing a bigger number of subjects, taking into account also handedness and sex effects, not considered here in spite of their evidenced influence on structural and functional asymmetries.

**Supplementary Material**

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

**Funding**

Biotechnology and Biological Sciences Research Council of the United Kingdom (BB/E002226/1).

**Notes**

We are also grateful to the anonymous reviewers for their useful comments and suggestions. Author contributions: Y.I.-M. and A.P.F. conceived and designed the experiment. G.J.M.P., D.M.M., H.A.H., M.A., and N.L. acquired and preprocessed the MRI datasets. D.M.M., H.A.H., G.J.M.P., Y.I.-M., and N.L. implemented the PICo tractography algorithm used. Y.I.-M. and G.J.M.P. implemented the graph-based tractography algorithm and network analysis tools used. Y.I.-M. and A.P.F. analyzed the data and wrote the paper with input from all other authors. All authors contributed to constructive discussions regarding the interpretation of the results. **Conflict of Interest**: None declared.

**Appendix**

**Permutation Test Used for the Betweenness Centrality Lateralization Analysis of Dataset 2**

The analysis consisted of the following steps (Blair and Karniski 1993; Galan et al. 1997):

1. The null hypothesis ($H_0$) of zero mean for all the measured LI values is decomposed into the marginal hypotheses $H_{0r}$: $m_r = 0$, where $m_r$ is the mean of the LI values for a given brain region $r$.

2. Each marginal hypothesis $H_{0r}$ is tested by:

   a) Computing the $t$-statistic for the original LI values of the region $r$ (i.e., to compute the $t$ value corresponding to the hypothesis that the data in the vector LI come from a distribution with mean zero).

   b) Obtaining a larger number of permutation resample from the data (without replacement) and constructing the permutation distribution of the $t$-statistic (in each permutation the sign of at least one of the elements of the original LI vector was changed, being the complete permutation space $2^n$, where $n$ is the number of fiber tracking algorithms used).

   c) Finding the $P$ value of $H_{0r}$ by comparing the original $t$-statistic (obtained in step a) with the $t$-statistic permutation distribution (obtained in step b), specifically, to perform a 2-sided test (sign test) with the hypothesis that the data in the $t$-statistic permutation distribution come from a distribution whose median is the original $t$-statistic.

**References**


