Morphological covariance in anatomical MRI scans can identify discrete neural pathways in the brain and their disturbances in persons with neuropsychiatric disorders

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Abstract

We hypothesize that coordinated functional activity within discrete neural circuits induces morphological organization and plasticity within those circuits. Identifying regions of morphological covariance that are independent of morphological covariance in other regions therefore may therefore allow us to identify discrete neural systems within the brain. Comparing the magnitude of these variations in individuals who have psychiatric disorders with the magnitude of variations in healthy controls may allow us to identify aberrant neural pathways in psychiatric illnesses.

We measured surface morphological features by applying nonlinear, high-dimensional warping algorithms to manually defined brain regions. We transferred those measures onto the surface of a unit sphere via conformal mapping and then used spherical wavelets and their scaling coefficients to simplify the data structure representing these surface morphological features of each brain region. We used principal component analysis (PCA) to calculate covariation in these morphological measures, as represented by their scaling coefficients, across several brain regions. We then assessed whether brain subregions that covaried in morphology, as identified by large eigenvalues in the PCA, identified specific neural pathways of the brain. To do so, we spatially registered the subnuclei for each eigenvector into the coordinate space of a Diffusion Tensor Imaging dataset; we used these subnuclei as seed regions to track and compare fiber pathways with known fiber pathways identified in neuroanatomical atlases.

We applied these procedures to anatomical MRI data in a cohort of 82 healthy participants (42 children, 18 males, age 10.5 ± 2.43 years; 40 adults, 22 males, age 32.42 ± 10.7 years) and 107 participants with Tourette’s Syndrome (TS) (71 children, 59 males, age 11.19 ± 2.2 years; 36 adults, 21 males, age 37.34 ± 10.9 years). We evaluated the construct validity of the identified covariation in morphology using DTI data from a different set of 20 healthy adults (10 males, mean age 29.7 ± 7.7 years). The PCA identified portions of structures that covaried across the brain, the eigenvalues measuring the magnitude of the covariation in morphology along the respective eigenvectors. Our results showed that the eigenvectors, and the DTI fibers tracked from their associated brain regions, corresponded with known neural pathways in the brain. In addition, the eigenvectors that captured morphological covariation across regions, and the principal components along those eigenvectors, identified neural pathways with aberrant morphological features associated with TS. These findings suggest that covariations in brain morphology can identify aberrant neural pathways in specific neuropsychiatric disorders.

Introduction

Morphological variability in brain regions and subregions ultimately derives, at least in part, from the number, density, axonal branching, dendritic arborization, or synaptic connectivity of neurons. Variability in the size and numbers of glial cells and blood vessels feeding those regions, and even the amount of extracellular space, likely also contribute to variance in brain morphology. We presume that organizational influences during development, and activity-dependent plasticity in maturity, will generate correlations in the morphological characteristics of neurons within discrete brain circuits and fiber pathways. We also presume that the correlations between neurons will contribute to correlations in anatomical measures of brain regions that comprise those neural circuits and fiber pathways. In other words, morphological measures will be
intercorrelated across nodes of each circuit and within the white matter that interconnects those nodes.

Although autoradiographic tracing is the gold-standard method for identifying neural pathways (Launer et al., 2011; Ou and Davatzikos, 2009), this is strictly a postmortem technique and cannot track fiber pathways in vivo. Diffusion Tensor Imaging (DTI) provides the unique opportunity to track fiber pathways in vivo (Bammer et al., 2002; Dotson et al., 2009a; Wang et al., 2010) by quantifying the spatial diffusion of water molecules, thereby allowing inferences about the local properties of brain tissue, especially the integrity and coherence of fiber pathways within white matter (Basser, 1995; Basser and Jones, 2002; Basser et al., 1994; Mori and van Zijl, 2002; Mori and Zhang, 2006; Ou and Davatzikos, 2009; Pierpaoli and Basser, 1996; Pierpaoli et al., 1996; Thambisetty et al., 2012; Zacharaki et al., 2011; Zhang and Davatzikos, 2011). Disturbances in DTI measures within white matter fiber pathways have been associated with various disease processes (Batmanghelich et al., 2012; Casanova et al., 2011; Davatzikos et al., 2008, 2011; Filipovych and Davatzikos, 2011; Filipovych et al., 2011; Haris et al., 2011; Koutsouleris et al., 2012; Kubicki et al., 2002; Lim and Helpern, 2002).

DTI, however, has several prominent limitations when used to study neural pathways in health and illness: (1) Voxel-based DTI measures, such as fractional anisotropy (FA), mean diffusivity (MD), and coherence, typically identify only a few clusters of voxels as deviant within the entire brain. Although atlases can be used to map these voxels to known fiber bundles, significant effects of interest at only a few voxels generally do not constitute sufficient evidence to implicate an entire neural pathway, or even a specific portion of a pathway, in that effect, such as a disease process. (2) The biological determinants of diffusion measures are still unknown and can derive from such diverse tissue features as neuronal density, degree of myelination, axonal diameter, intracellular organelles, or extracellular water. Furthermore, noise in the DTI data may contribute to statistically significant findings that are not biologically valid. (3) Voxel-wise analyses of DTI data may not uniquely associate a specific neural pathway with a given effect of interest because several fiber pathways are typically in close spatial proximity to the identified voxels, and those voxels cannot clearly identify any of the pathways as generating the effect of interest. (4) The cumulative influence of noise (Dotson et al., 2009a) when tracking fiber pathways can cause trajectories to jump to adjacent pathways (Zacharaki et al., 2009), leading to errors in reconstructing fiber trajectories (Batmanghelich et al., 2009; Zacharaki et al., 2009). These limitations likely contribute to the conflicting findings in DTI studies of neuropsychiatric disorders (Hamm et al., 2010; Schwartz et al., 2010; Zhang and Davatzikos, 2010).

Recent anatomical MR studies have used novel assessments of covariation in measures of either cortical thickness (He et al., 2008; Lerch et al., 2006) or local deformation fields (Kim et al., 2012) to infer the strength of connectivity between brain regions. One study, for example, mapped the strength of correlations in cortical thickness across the cerebrum to infer patterns of connectivity and found that those patterns corresponded well with patterns of connectivity obtained using DTI-based fiber tracking (Lerch et al., 2008). Those correlation maps using cortical thickness measures, however, could not identify any of the many important pathways to subcortical nuclei that contribute to the pathogenesis of neuropsychiatric illnesses. Another study used nonlinear warpings to spatially normalize T1-weighted images across participants, identified the determinant of the Jacobian matrix from those warpings as an index of local deformation and volume, and then showed that the correlations among the Jacobian determinants, as an index of connectivity, were greater within a manually delineated region of white matter (WM) than between those WM regions (Kim et al., 2012). The biological relevance of the Jacobian matrix is unclear, however, making the biological validity of the reported pattern of connectivity across WM regions in that study difficult to assess.

To overcome the limitations of these previous approaches to inferring connectivity within anatomical MRI datasets, we use spherical wavelet analyses to assess the correlations in morphological features across the cerebral surface and surfaces of subcortical nuclei. We also use information from autoradiographic studies of postmortem tissue and from in vivo DTI-based fiber tracking to constrain interpretation of these patterns of morphological covariation. Because it is axiomatic that the symptoms of specific neuropsychiatric illnesses derive from functional disturbances in specific neural pathways, we also assess whether morphological covariation is also associated with a specific neuropsychiatric illness.

Methods

Overview

We applied nonlinear, high-dimensional warping algorithms to manually defined brain regions — the cerebral hemispheres, hippocampus, amygdala, thalamus, putamen, caudate, and globus pallidus — in order to quantify morphological variation across the surfaces of those regions in each of our study participants. As a single regional surface typically consists of several thousand points, the maps of morphological measures across these surfaces constituted a high-dimensional feature space. To reduce the dimensionality of this feature space, we transferred the measures of individual variability onto the surface of a unit sphere via conformal mapping. We used spherical wavelets and their scaling coefficients to simplify the data structure representing this individual variability in morphological features of the surface of each brain region. We used spherical wavelet analysis instead of spherical harmonics analysis (SPHARM) (Müller, 1966) because wavelets have finite nonzero support and therefore encode unique variations in our morphological measures across non-overlapping subregions of each brain region. We used wavelets at their lowest spatial resolution of 12 scaling coefficients to encode the variation in our morphological measures because this reduced the dimensions of the feature space. We then used linear regression to adjust the scaling coefficients for the effects of age and sex, normalizing the adjusted coefficients to have zero mean and unit standard deviation. These normalized coefficients for all brain regions were concatenated to form a single feature vector for each participant.

We then used principal component analysis (PCA) to quantify the covariation in morphological measures, represented by these scaling coefficients, across brain regions. The PCA eigenvectors identified portions of these structures that covaried across regions, and the associated eigenvalues measured the magnitude of that covariation. These methods, though admittedly highly abstract and mathematically complex, permitted us to quantify precisely covariation on surface morphology across several brain regions simultaneously. We hypothesized that brain subregions with covarying morphological measures, identified by eigenvectors with large eigenvalues, would identify specific neural pathways of the brain that differ significantly between 82 healthy participants and 107 participants who had the neuropsychiatric disorder, Tourette Syndrome (TS). We assessed the reproducibility of our patterns of morphological covariation across brain regions by performing these analyses independently in the healthy and TS groups, and then assessing visually in the two groups their patterns of covariation across the brain and the principal components for their imaging measures.

We assessed the face validity of the implicated neural pathways by comparing visually the maps of covariances with atlases of known white matter pathways in the brain. We assessed their construct validity by normalizing the principal components into the coordinate space of a DTI dataset from 20 healthy individuals and then using as seeds for fiber tracking the regions where PCA identified significant morphological covariation in their eigenvectors. We expected that DTI-based fiber tracking would identify fibers interconnecting these covarying subregions and that the fibers from various subregions would have overlapping trajectories (i.e., they would pass through the same voxels of the brain). The use of DTI data to support the construct validity of our measures of morphological covariation provides the strongest support for the
hypothosis that brain regions with covarying morphology can identify unique neural pathways in the brain. We evaluated the reproducibility of the fiber tracts by assessing whether the covariations replicated across participants in two independent datasets. Finally, we assessed the discriminant validity of the neural pathways by statistically comparing principal components across the healthy and TS groups to determine whether these procedures are able to identify morphological features of neural pathways that are aberrant in a specific neuropsychiatric disorder. Each of these methods for assessing validity individually provides incremental evidence to support the hypothesis that covariation in surface morphology identifies unique neural circuits in the brain, but when combined they provide compelling evidence in its support.

**MR pulse sequences**

**Anatomical MRI**

T1-weighted MR images were acquired using a sagittal spoiled gradient recall sequence (TR = 24 ms, TE = 5 ms, 45° flip, frequency encoding S/L, no wrap, 256 × 192 matrix, FOV = 30 cm, 2 acquisitions, slice thickness = 1.2 mm, 124 contiguous slices). This sequence was selected to provide quality images of the brain with superior signal-to-noise (SNR) and contrast-to-noise (CNR) ratios in high-resolution images having nearly isotropic voxels (1.171 × 1.171 × 1.2 mm). We discarded all anatomical MR images with any visible motion artifact leading to blurring of the boundaries between brain tissue and/or Gibbs artifact and delineated brain regions only in images with clearly defined tissue boundaries.

**Diffusion tensor imaging**

DTI data were acquired in an axial oblique orientation parallel to the AC–PC line using a single-shot echoplanar DTI imaging sequence, with TR = 15700 ms, TE = 74 ms, FOV = 24 cm, Flip = 90°, acquisition matrix = 128 × 128 (acceleration factor = 2), zero-padded to 256 × 256, slices = 60, slices thickness = 2.5 mm. We acquired 3 baseline images with b = 0 s/mm², and 25 diffusion weighted images at b = 1000 s/mm² with diffusion gradients applied in 25 directions, sampling 3D space uniformly (Jones et al., 1999).

**Participants**

We acquired anatomical MRI data in 82 healthy participants (42 children, 18 males, age 10.5 ± 2.43 years; 40 adults, 22 males, age 32.42 ± 10.7 years) and 107 participants with Tourette's Syndrome (TS) (71 children, 59 males, age 11.19 ± 2.2 years; 36 adults, 21 males, age 37.34 ± 10.9 years). In addition, we acquired DTI data from a different set of 20 healthy adults (10 males, mean age 29.7 ± 10.9 years). In addition, we acquired DTI data from a different set of 20 healthy adults (10 males, mean age 29.7 ± 10.9 years). In addition, we acquired DTI data from a different set of 20 healthy adults (10 males, mean age 29.7 ± 10.9 years). In addition, we acquired DTI data from a different set of 20 healthy adults (10 males, mean age 29.7 ± 10.9 years).

**Preprocessing and delineating brain regions**

The brains were first pre-processed using automated algorithms in widely used, freely available software packages, including FreeSurfer (Dale et al., 1999). This included the removal of inhomogeneities in image intensities using low-order polynomials that modeled variations in intensity (Sled et al., 1998), isolation of brain from non-brain tissue, and segmentation of gray and white matter. Images were flipped randomly in the left–right direction to ensure no operator bias in the definitions of the hemispheres and brain regions. Connecting dura was removed and errors in gray–white matter segmentation were corrected manually by experts in neuroanatomy within each slice in the sagittal, coronal, and axial views. The amygdala, hippocampus, thalamus, caudate, putamen, and globus pallidus were manually delineated using previously published algorithms (Kates et al., 1997; Peterson et al., 1993; Watson et al., 1992). The intraclass correlation coefficients (ICCs) for all of these procedures were > 0.98.

**Surface morphometry**

We applied previously developed methods (Bansal et al., 2005) to quantify precisely local variations in the surface morphology features of each brain region. We used a rigid body similarity transformation to first coregister each region to the corresponding region in a template brain so that mutual information was maximized across the images (Wells et al., 1995). We then applied a high-dimensional warping algorithm (Christensen et al.) to nonlinearly deform each region to match precisely the gray scale characteristics of the corresponding template region. Each region was thus warped to the exact same size and shape as the template region, permitting the identification of corresponding points on the surfaces of each region with the surfaces of corresponding template. The high-dimensional warping was then reversed, bringing each region back into the rigid body registration with the template region while retaining at each point the labels identifying the point correspondences with the template surface. We then used the established point correspondences to calculate Euclidean distances between corresponding points across the surfaces of each region with its template. The distances were labeled positive for protrusions and negative for indentations in the brain surface compared to the template surface. Although the distances were not constrained to be normal to the surfaces, the distance differed only slightly from normal because the high-dimensional warping algorithms establish correspondences between the nearest points, so that those distances are small and nearly normal. The set of signed Euclidean distances in our participants represented a smooth random field on the surface of the template region (Bansal et al., 2007), where we next used conformal mapping to model variation in morphological measures across the surface of a brain region (Bansal et al., 2007).

**Conformal mapping**

We used conformal mapping to transfer brain measures from the template surface onto a unit sphere. To accomplish this, we used a Marching Cubes algorithm to first approximate the surface of each region with a triangular mesh (Lorensen and Cline, 1987). We then used a 2-step procedure to conformally map the vertices of this mesh to the vertices of a unit sphere (Angenent et al., 1999): (1) We mapped the template surface to a plane while preserving the angles between lines on the surface and the mapped lines points on the plane; (2) we then used stereographic projection to map the points on the plane to points on the unit sphere. Finally, we used this conformal mapping to transfer brain measures from the template surface to the surface of the unit sphere. Although conformal mapping preserves angles between straight lines, it may introduce metric and area distortions between the mapped surfaces. These metric distortions may differ for differing amounts of rotations of one surface, thereby possibly affecting the covariations in surface morphology. We therefore assessed the effects of rotations of one surface by visual inspection of the eigenvalues and the magnitudes of the eigenvalues.

**Spherical waves**

We applied a spherical wavelet analysis to transform brain measures on the unit sphere into scaling coefficients at decreasing scales. A wavelet transform analyzes a function at decreasing spatial resolutions by identifying a basis wavelet and scaling functions that have local support in both space and frequency (Daubechies, 2004). A wavelet transform uses
dyadic translations and dilations of the basis wavelet and the scaling functions to generate scaling coefficients such that the function is represented accurately by a small subset of the coefficients. The scaling and wavelet functions are selected such that a scaling function \( \phi_{L,j} \), and a wavelet \( \psi_{L,j} \) at a resolution \( j \) and at index \( k \in K(j) \) (where \( K(j) \) is an index set at a resolution \( j \)) is a linear combination of the scaling functions \( \phi_{L,j} \) at the higher resolution \( j + 1 \). If \( L_2(S^2, d\sigma) \) is the space of all scalar functions with finite energy over the sphere \( S^2 \), multi-resolution analysis generates a sequence of closed subspaces \( V_j \subset L_2, \forall j \geq 0 \), such that: (1) \( V_j \subset V_{j+1} \), (2) \( \bigcup_{j \geq 0} V_j \) is dense in \( L_2 \), and (3) the set of scaling functions \( \phi_{L,j} \) is a Riesz basis of \( V_j \).

Lifting schemes are used to extend biorthogonal wavelet transformation to scalar functions defined on a sphere (Schröder and Sweldens, 1995; Sweldens, 1994). A lifting scheme modifies the scaling and wavelet functions to build smoother biorthogonal wavelets that have finite support. The spherical wavelet transform in a wavelet analysis mode begins at the finest spatial resolution and calculates coefficients until the coarsest spatial resolution is reached. At each resolution, unlifted wavelet coefficients are calculated and then lifted. Conversely, the inverse transform in a wavelet synthesis mode begins at the coarsest resolution first inverses the lifting and then calculates coefficients for the scaling function until its finest resolution is reached. We used a linear lifted wavelet (i.e., an interpolating transformation) to calculate the scaling coefficients for brain measures located at each of the two nearest neighboring points on the surface of the sphere. Because scaling functions have local support, scaling coefficients encode local variations in morphological measures. Therefore, using these scaling coefficients as our feature vectors represents the pattern of local variations in brain measures for each participant.

**Principal component analysis**

We used principal component analysis (PCA) to analyze our feature vectors, which comprised scaling coefficients for all brain regions in all of our participants, in order to identify brain subregions for which surface morphological measures covary and that presumably are components of large-scale neural networks in the brain. PCA linearly transforms a set \( x_i; i = 1, \ldots, m \) of vectors from \( m \) participants, where \( x_i = [x_{i1}, \ldots, x_{iT}] \) is a \( p \)-dimensional vector of scaling coefficients \( x_{it} \) for the \( i \)th participant, and then it calculates a set \( z_i = [z_{i1}, \ldots, z_{ip}] ; i = 1, \ldots, m \) of \( p \)-dimensional vectors of principal components for each of the \( m \) participants such that the principal components \( z_{it} \) and \( z_{iu} \), for \( s \neq t; s, t = 1, \ldots, p \), are uncorrelated (Xu et al., 2007). In our analyses, we selected \( p = 12 \), the lowest spatial resolution for our scaling coefficients to ensure smallest dimensionality of the feature space. PCA applied singular vector decomposition (SVD) to the covariance matrix \( \Sigma \) of our scaling coefficients to calculate a set \( E_s, s = 1, \ldots, p \) of orthogonal eigenvectors and a set \( \lambda_s, s = 1, \ldots, p \) of eigenvalues corresponding to each eigenvector, in which each eigenvalue \( \lambda_s \) is the variance of the \( s \)th principal component \( z_{it} ; i = 1, \ldots, m \) of all \( m \) participants. A principal component \( z_{it} \) is obtained by projecting (i.e., by calculating the inner product of) the vector \( x_t \) of scaling coefficients for the \( i \)th participant on the \( s \)th eigenvector \( E_s \) in feature space. Usually only a few principal components account for the majority of variance across all scaling coefficients, and the eigenvectors corresponding to the principal components with the largest variances represent subnuclei that strongly covary across brain regions. To ensure that the eigenvectors were not unduly influenced by covariances only in larger brain regions, we applied PCA to the correlation matrix rather than directly to surface morphological measures or their scaling coefficients when calculating the eigenvectors and eigenvalues in our sample of participants.

**Controlling for confounding factors**

We used linear regression to adjust scaling coefficients for age and sex of the participants. We also assessed the effects of age and sex on these scaling coefficients by visually comparing eigenvectors that were generated with and without correcting for age and sex.

**Visualizing eigenvectors**

Each eigenvector that PCA generates encodes the simultaneous variation in morphological measures across multiple brain regions. Along a single eigenvector in feature space, one scaling coefficient may increase, another may decrease, and yet another may remain constant. Regardless of this behavior of individual coefficients along the eigenvector, scaling coefficients (and therefore also the surface morphological features that they represent) that simultaneously vary along an eigenvector presumably identify variations in specific large-scale networks in the brain. In representing eigenvectors and therefore their associated neural networks, we encode scaling coefficients that increase along one direction of the eigenvectors with the colors red and yellow, coefficients that decrease with violet and blue, and those that are relatively constant with green. The color coding of red and yellow versus violet and blue is merely relative, however, because the change in coefficients is reversed along the other direction of the eigenvector, and so the color scheme could be reversed arbitrarily without changing interpretation of the maps. The coding is intended only to allow easy visualization of the simultaneous increases or decreases in morphological measures across the various brain regions in our analyses. In addition, variance is greater along some eigenvectors than along others, and therefore we scale their color codings for each eigenvalue as its ratio to the largest eigenvalue.

**Tracking fibers**

We implemented the deterministic “Fiber Assignment by Continuous Tracking” (FACT) algorithm (Thambisetty et al., 2012; Zipunnikov et al., 2011) to track fiber bundles in the brain. DTI data assign a principal direction (i.e., the direction of maximal diffusion of water) to each voxel in imaging space. The principal direction (PD) is assumed to be the direction of a fiber bundle within that voxel. Fiber tracking began at identified “seed regions”, which, in our dataset was a region of interest (ROI) within each manually defined region of the brain where scaling coefficients either increased or decreased significantly along the specific eigenvector. FACT began at a voxel in the center of the ROI, moved a small distance along the PD, used trilinear interpolation of the PDs to estimate a new direction for the fiber bundle at neighboring voxels, and then moved again a small distance along the new direction. This procedure was then repeated at the new voxel. Fiber tracking terminated at the new location if either the FA there was <0.2 or if the angle between the current direction and the new direction was >75°. All voxels along the tracked pathway were concatenated to define a fiber bundle that passed through the original voxel in the ROI. Using DT image, we assessed connectivity between brain regions with covarying morphology using voxelwise maps of fractional anisotropy (FA) and fiber count. We generated a voxelwise map of fiber count by first tracing all fibers from each region with covarying morphology for an eigenvector and then adding the number of fiber tracts that passed through each voxel in the brain.

**Labeling from white matter atlases**

We used two white matter atlases (Ou and Davatzikos, 2009) to label pathways traced in our DTI data from 20 healthy participants: (1) An ICBM-DTI atlas that manually delineated 50 regions across white matter in the diffusion tensor map averaged across 81 healthy participants (42 males, mean age 39 years, age range 18–59 years); (2) Another atlas of 20 white matter fiber tracts that were identified by first performing deterministic tractography in DTI data independently for each of 28 healthy participants (17 males, mean age 29 years), and then generating a map of the probability that each voxel belonged to a
specific fiber tract or not (Ou and Davatzikos, 2009). When using this latter probabilistic atlas, we assigned a voxel to the tract that had the highest probability value there. We used an affine transformation that maximized mutual information across images to spatially normalize the two into the coordinate space of our template brain.

Statistical validations

We assessed in 4 ways the validity of our claim that correlated variations in morphological measures along eigenvectors would be associated with underlying neural pathways. We assessed whether the covariations (1) replicated across participants in two independent datasets (reproducibility), (2) were visually consistent with previously identified neural pathways in the brain (face validity), and (3) were interconnected by fiber pathways traced in our DTI data (construct validity), and (4) whether the magnitude of variation along specific eigenvectors differed significantly between our healthy and TS participants (discriminant validity).

Reproducibility

We assessed whether the patterns of correlated variations in measures replicated across participants in two cohorts: (1) one comprising 42 healthy children and 40 healthy adults, and (2) another comprising 71 children and 36 adults with TS. The replication of patterns of variation was assessed qualitatively by visually comparing the color maps for the eigenvectors calculated independently in the two groups of participants, and quantitatively by correlating the principal components across groups of (1) 42 healthy children and 71 children with TS, and (2) 40 healthy adults and 36 TS adults. We calculated the principal components using the eigenvectors identified empirically for our claim that each eigenvector would be associated with specific neural pathways in the brain.

Face validity

We assessed face validity by comparing visually the color-coded maps of eigenvectors with the neural pathways identified in a standard atlas as connecting the regions that morphologically covaried (Launer et al., 2011; Lian and Davatzikos, 2011).

Construct validity

We evaluated the construct validity of the putative neural pathways that we identified by tracking fibers from subregions where significant eigenvectors were located, but spatially normalized into the coordinate space of DTI data from 20 healthy participants. The DTI data of all 20 participants were also spatially normalized independently to the coordinate space of the template brain.

First, the DTI data of each participant were rigidly coregistered to their anatomical MR image. Then the anatomical MR image of each participant was similarity-transformed to match a template brain by maximizing mutual information (Viola and Wells, 1995), and then they were warped to the template using a high-dimensional nonlinear deformation field (Christensen et al., 1994). We applied a technique for Procrustean estimation (Xu et al., 2008) to the deformation field to reorient diffusion tensors in each participant brain in the coordinate space of the template brain, and we used a geodesic analysis of symmetric spaces to average the DTI data across all 20 participants while ensuring that the averaged tensors would be positive definite (Fletcher and Joshi, 2004).

We then identified voxels that would be used as seeds for DTI fiber tracking. These were voxels where covariation in surface morphological features was identified. For each eigenvector calculated using the anatomical MRI data, we identified empirically the voxels where changes in the eigenvalue along the eigenvector produced only small changes in the identified voxels for morphological covariation, indicating that those voxels were stable and relatively robust to errors in estimating the eigenvector. The threshold of 75% was established empirically by visually assessing changes in the identified voxels for varying values of thresholds (fig. 1). We determined the threshold to be >75%, which provided the most stable identification of the voxels where morphology covaried such that small variations around this threshold produced only little change in the identified voxels. These identified voxels were coregistered and nonlinearly warped to the corresponding regions of the template brain and then used as seed regions for fiber tracking. We considered brain regions associated with a specific eigenvector to be connected if fibers tracked from those regions traversed the same voxels in the brain, indicating that the regions were either connected directly or indirectly. We used the two white matter atlases (Ou and Davatzikos, 2009) to label those voxels as belonging to specific fiber pathways, thereby allowing us to assess the validity for our claim that each eigenvector would be associated with specific neural pathways in the brain.

Discriminant validity

We assessed whether the specific neural pathways that we identified differed significantly between the healthy and TS groups by comparing principal components across groups of (1) 42 healthy children and 71 children with TS, and (2) 40 healthy adults and 36 TS adults. We calculated the principal components using the eigenvectors identified for our entire cohort of healthy and TS participants. The null hypothesis was that the principal components along eigenvectors, and therefore their underlying neural pathways, would not differ between the two groups. We tested this hypothesis by comparing groups on the average principal component with an unpaired t-statistic.

Patterns of morphological covariation in TS and healthy participants

Neural pathways connecting the left and the right hemispheres

In our healthy participants we applied PCA to the scaling coefficients from local variations in the surfaces of brain regions in each hemisphere to identify putative neural pathways connecting the left and the right
hemispheres. We also applied PCA to the scaling coefficients that were adjusted for the effects of age and sex. We repeated these same procedures in the 71 children with TS. We visually compared the maps of eigenvectors across these two groups to assess the reproducibility of our findings. We quantitatively evaluated the reproducibility of eigenvectors, and therefore of the putative neural pathways that the eigenvectors represent, by correlating the principal components for each region across the two cohorts. Finally, we combined the imaging data of these two groups of children with the data of 40 healthy adults and 36 adults with TS, calculated scaling coefficients adjusted for age and sex, and applied PCA to calculate the eigenvectors in these 189 participants. We used a 75% threshold on each of these eigenvectors to delineate ROIs that covaried morphologically with one another, and then we used these ROIs as seeds to track fiber pathways in our DTI dataset from 20 healthy participants. We labeled the tracked fiber bundles using the two anatomical atlases for white matter.

Neural pathways connecting the left and the right hemispheres

In our 82 healthy participants, 71 children with TS, and 36 adults with TS, we generated scaling coefficients for seven brain regions in the left hemisphere: the amygdala, hippocampus, thalamus, caudate, globus pallidus, putamen, and hemisphere. These scaling coefficients were adjusted for age and sex effects. We used PCA to calculate eigenvectors for those adjusted coefficients. We plotted the eigenvectors generated independently for (1) healthy participants only, and (2) healthy participants and participants with TS combined. Our findings showed that the two sets of eigenvectors matched each other well, and therefore we used the eigenvectors from the entire cohort of participants for fiber tracking. In addition, we compared principal components along eigenvectors calculated using our entire cohort to identify the neural pathways that differed significantly between (1) healthy children and children with TS, and (2) healthy adults and adults with TS, thereby allowing us to identify aberrancies in putative neural pathways in persons with TS. We analyzed the principal components of the children and adults separately because prior brain imaging studies have shown conclusively that morphological features of the brain in children with TS differ significantly with those in adults (Plessen et al., 2009).

Results

Morphological covariation and the neural pathways connecting surfaces of the cerebral hemispheres

The eigenvectors calculated from scaling coefficients for the two cerebral hemispheres matched one another visually across diagnostic groups (Fig. 2). Findings were similar whether or not we adjusted the scaling coefficients for age and sex. We demonstrated large covariations in: (1) medial and lateral surfaces of the brain (Fig. 2, Top Row), representing covariation across the left–right axis of the brain. We presume this covariation derived from the neural pathways that project through the corpus callosum to connect homologous regions across the two hemispheres; (2) Dorsal convexity and ventral brain surfaces (Fig. 2, Middle Row), representing covariation in the dorsal–ventral vertical axis of the brain. We presume this covariation derived projection fields of corticospinal fiber pathways (Lian and Davatzikos, 2011); and (3) Precentral and postcentral gyri, superior frontal gyrus, and posterior parietal surfaces (Fig. 2, Bottom Row), representing a positive correlation within the anterior–posterior axis (superior frontal gyrus and parietal regions) that was anti-correlated with measures in sensorimotor regions. We suggest that this covariation may have derived from morphological anti-correlation between sensorimotor and higher-order cognitive regions.

The eigenvectors generated from the 71 children with TS visually matched well those generated from the healthy participants (Fig. 2, Right Column). In addition, the Pearson’s correlation coefficients of their PCs were 0.7 (P-value < 0.00001) for the PCs along the first 16 eigenvectors, which represented more than 88% of the variance in the data from the TS children. Statistically significant correlations confirmed the replication of the eigenvectors and presumably in the associated neural pathways for the two groups of participants. Because eigenvectors replicated across these two groups, we used the combined data of the healthy participants together with data from the children and adults with TS to identify fiber tracts from morphological covariation of the cerebral hemispheres, and to define the ROIs used as seed regions for DTI-based fiber tracking in the 20 different healthy participants. The two DTI atlases normalized into the coordinate space of the tracked fibers allowed us to label the fiber tracts and to identify the white matter regions associated with each eigenvector.

The white matter fibers tracked from the eigenvector accounting for the largest portion of variance (about 23% of the total variance) consisted mostly of fibers passing through the corpus callosum (Fig. 3, Top Row). Fibers for the eigenvector accounting for the second largest portion of variance (about 20% of the total variance) consisted mostly of fibers in the corticospinal tract, which connects the brain stem to the brain’s dorsal convexity (Fig. 3, Bottom Panel). The maps therefore supported our contention that morphological covariations in brain surfaces are associated with specific fiber pathways (Fig. 4). We tracked fibers for these two eigenvectors because they accounted for the largest covariation in surface morphological measures. Eigenvectors with smaller eigenvalues are susceptible to noise in the imaging data and to errors in spatial normalization, and therefore may not identify specific neural pathways reliably in the brain.

Neural pathways connecting subcortical and cortical brain regions within a hemisphere

The eigenvectors calculated using the morphological data from 7 brain regions in our 82 healthy participants visually matched those calculated using the combined data for 82 healthy children and adult participants, 71 children with TS, and 36 adults with TS (Fig. 5). The eigenvector with the largest variance identified morphological covariation across the medial aspect of the amygdala, the head of the hippocampus, the tail of the caudate nucleus, the dorsal surfaces of the thalamus, putamen, and globus pallidus, and the medial surface of the cerebral hemispheres (Fig. 5, Top Left). The eigenvector with the next largest variance showed that the anterior, lateral, and ventral surfaces of the caudate nucleus covared with the corresponding surfaces of the putamen (Fig. 5, Top Middle). Visual inspection of the eigenvectors and the magnitude of the eigenvalues, however, showed that metric and area distortions had minimal, if any, effects on the eigenvectors and therefore on the subregions identified as covarying in morphological features (Fig. 6).

The fibers tracked independently from ROIs defined by the eigenvector accounting for the largest variance in our surface measures (immediately above) identified neural pathways connecting the ROIs within the amygdala and hippocampal regions of the brain. Fibers tracked separately from these ROIs passed through the same voxels in the brain, indicating that those subregions were indeed anatomically interconnected by white matter fiber tracts. Fibers identified from the first eigenvector were located primarily within the limbic system (Fig. 7), and therefore we conclude that this eigenvector was associated with a specific and distinct neural pathway in the brain.

DTI atlases that were normalized into the coordinate space of the fiber tracts labeled the tracked fibers from the first eigenvector as comprising the corticospinal tract, anterior thalamic radiation, inferior longitudinal fasciculus, and inferior fronto-occipital fasciculus (Fig. 8). In addition to these major neural pathways, however, several smaller fiber bundles were tracked to regions for which morphological features did not covary with those of the ROIs. To assess how the major neural pathways differed from these smaller fiber tracts, we calculated fractional anisotropy (FA) along the tracked fibers and generated a voxel-wise map of fiber counts across the entire brain (Fig. 9). The FA values and fiber count data were similar along both the major and smaller
The principal components (PCs) of the TS and healthy children differed significantly along many eigenvectors, including (1) the eigenvector (PCs in healthy children = −0.72 ± 1.97, TS = 0.6 ± 1.15, P-value = 3.53 × 10^{-6}) representing in the TS children a simultaneous decrease in surface measures of the anterior hippocampus and the ventro- and dorso-medial thalamus; (2) the eigenvector (PCs in healthy = 1.29 ± 1.16, TS = 0.43 ± 1.17, P-value = 3.79 × 10^{-5}) representing in the TS group a simultaneous increase in surface measures of the ventral-posterior amygdala and decreases in the posterior caudate nucleus, the parietal lobe, and the dorsal-anterior and lateral putamen; (3) the eigenvector (PCs in healthy = −0.75 ± 1.07, TS = −0.03 ± 0.9, P-value = 2.66 × 10^{-3}) representing in the TS group a simultaneous decrease in morphological measures across the ventral globus pallidus and the dorsal- and ventral-anterior hippocampi; and (4) the eigenvector (PCs in healthy = −1.59 ± 1.06, TS = −0.68 ± 1.02, P-value = 3.29 × 10^{-6}) representing simultaneous decreases in the TS group across the dorsal-anterior amygdala, the dorsal-posterior globus pallidus, and the lateral and medial cerebral hemispheres.

These simultaneous differences in morphological covariation across brain regions thereby implicate in children with TS disturbances in the fiber pathways connecting anterior hippocampus to dorso-medial thalami, ventro-posterior amygdala to posterior caudate and dorso-anterior and lateral putamen, ventral globus pallidus to dorsal and ventro-anterior hippocampus, and dorso-posterior amygdala to dorso-posterior globus pallidus. In contrast, the PCs of adults with TS and healthy adults differed significantly only along the eigenvector (PCs in healthy = 0.23 ± 1.38, TS = −0.45 ± 1.6, P-value = 0.005) representing in the TS adults simultaneous increases in morphological measures of the anterior hippocampus and the ventral- and dorsal-medial thalami, group differences in morphological measures that were opposite in direction from those in children with TS compared with healthy children. Opposing findings in adults and children with TS relative to control values are a nearly ubiquitous finding in prior imaging studies of TS and likely represent failed neuroplastic compensation in adults with TS (Plessen et al., 2009).

Fig. 2. Visualizing three eigenvectors calculated from variations in surface features across the two hemispheres in our cohort of 82 healthy children and adults and 71 children with Tourette Syndrome (TS). The eigenvectors were calculated by applying a principal component analysis to the correlation matrix of the scaling coefficients that we derived from a spherical wavelet analysis of surface morphological measures of the cerebrum. Each row shows one of the 3 largest of the set of 24 eigenvectors. Each eigenvector is color-coded to show subregions in the two hemispheres whose surface features covary for a small change along the direction of that eigenvector. Regions with similar variations in surface features are coded with similar colors: If red (purple) encodes regions with increases in feature values, then purple (red) encodes regions with decreases in feature values; green encodes regions without any change in feature values for that eigenvector. The color scheme used to code the relative change in feature values is shown at the bottom of the figure. These spatial patterns of colors, and therefore of morphological covariation across the two hemispheres, can be used to infer the presence of neural pathways associated with each eigenvector. The 3 eigenvectors shown encode the spatial pattern of covariations in morphological features in the healthy participants without adjusting for age and sex (left column), eigenvectors for healthy participants when adjusted for age and sex (middle column), and eigenvectors calculated for the cohort of 71 children with TS adjusted for age and sex (right column). These maps show that the eigenvectors calculated from scaling coefficients that were unadjusted for age and sex were the same as those that were adjusted, and they were the same as the eigenvectors calculated for TS children.
Discussion

We hypothesized that correlated variations in morphological measures across brain regions would identify discrete neural pathways in the brain. To test this hypothesis, we first calculated morphological measures across the surfaces of brain regions using high-order, nonlinear deformations to identify corresponding points on the surfaces of those regions across individuals. Distances from each of those points in each participant from the corresponding points in the same region of the template brain quantified local individual variation in the morphological features of each region. We then conformally mapped those distances for each region onto the surface of a unit sphere representing that region. On the surface of the unit sphere for each region, we used a spherical wavelet transform to generate scaling coefficients that captured morphological variation for that region in each participant at 12 progressively decreasing spatial resolutions. We applied PCA to those 12 scaling coefficients for each region to identify the portions of each region that covaried morphologically with portions of other regions across the brain. Each PCA eigenvector identified subregions where morphological measures covaried across the brain. The principal components quantified the magnitude of covariation, and the associated eigenvalue quantified the variance explained by the principal components along its eigenvector.

We rigorously assessed the validity of the putative networks of morphological covariation that we identified. First, we demonstrated the excellent reproducibility of the eigenvectors and their associations with specific neural pathways by showing the qualitative and quantitative similarities in the eigenvectors calculated independently in two groups of participants. Then we evaluated their face validity by identifying in a white matter atlas the afferent and efferent projections to the subnuclei that each eigenvector identified. Next, we assessed their construct validity by tracking fibers in DTI data of healthy individuals from seed regions in subnuclei that were identified by each eigenvector. We used two standard atlases to identify the tracked fibers and confirm that these subregions were components of known major neural pathways in the brain. Because neural pathways in the atlases were not identified using histological methods for fiber tracking, however, the validity of the neural pathways in those atlases and, by extension, the pathways identified in our study, are limited by the reliance of the atlases on in vivo DTI data to identify those pathways. Finally, we demonstrated the discriminant validity of the covariance structure we identified by detecting significant differences between healthy and TS participants in the magnitudes of morphological covariation within their brains. These latter findings suggested that measures of morphological covariation can identify neural pathways that differ significantly between healthy participants and persons with a specific neuropsychiatric disorder.

We also compared the principal components for covariation in morphological measures across brain regions identified in a cohort of healthy participants and in a group of participants with TS. This comparison allowed us to identify neural pathways that are aberrant in persons with TS. These included fiber pathways connecting the anterior hippocampus to dorso-medial thalamus, ventro-posterior amygdala to posterior caudate and dorso-anterior and lateral putamen, ventral globus pallidus to dorsal and ventro-anterior hippocampus, and dorso-anterior amygdala to dorso-posterior globus pallidus. A better understanding of the cellular and molecular processes along these pathways will improve our understanding of the pathogenesis of TS.
The neural pathways that we identified in this study all comprise long-range fiber projections. We identified only long-range neural pathways because we used from a spherical wavelet analysis only the 12 scaling coefficients at the lowest spatial resolution. Congruently, scaling coefficients at a higher spatial resolution could identify neural pathways at a correspondingly higher spatial resolution in the brain. At higher spatial resolutions, however, the number of coefficients increases rapidly, thereby increasing the dimensionality of the feature space. High-dimensional spaces require data from an exponentially increasing number of participants to delineate neural pathways robustly. Thus, although our methods can theoretically identify neural pathways at higher spatial resolutions, they were limited by the number of participants for whom we had the imaging data.

The accuracy of our measures of the covariance in surface morphology was also limited by inevitable errors in the morphological measures incurred during image processing, including small errors in coregistering the images, defining individual brain regions, calculating measures of brain morphology, mapping of morphological measures onto a unit sphere, calculating the spherical wavelet coefficients, and applying principal component analyses to the wavelet coefficients. The finding identified by Eigenvector 3 in the TS children, for example, indicated that regions along the mesial aspect of the right hemisphere covaried with morphologies of the lateral and anterior regions of the brain (Fig. 2). This finding gives the impression of potential artifact, presumably generated from either errors in image processing or as a spurious, false-positive statistical finding. Despite this possibility, we note that this finding was replicated, albeit in a smaller spatial extent, in our independent sample of healthy children, suggesting that it may be valid. Regardless, analyses of morphological covariation would undoubtedly benefit from inclusion of a larger number of participants to help reduce the likelihood of false positive and false negative findings.

Although we hypothesized a priori that the presence of activity-dependent organization and plasticity would allow us to detect morphological covariation across cerebral surfaces, we note that the presence of significant morphological covariation does not necessarily prove that activity-dependent organization and plasticity are the sole or even primary determinants of that covariation. Morphological features could covary across brain regions for numerous other reasons. For example, we showed that surface morphological features of the two hemispheres covaried along the anteroposterior (AP), dorsoventral (DV), and left–right (LR) axes of the brain. This morphological covariation conceivably could have derived from overall differences in brain volumes, rather than from any circuit-specific effects. We regard this possibility as highly unlikely, however, because we first coregistered each participant’s brain to the template brain using a similarity transformation that adjusted for these overall scaling effects. More plausible is the possibility that correlated gene expression that is independent of functional co-activation could have driven morphological covariation along these three axes. For example, the graded signaling of morphogens during early embryogenesis is known to produce a differential expression of genes and transcription factors that in turn arrange characteristic cell types in a reproducible, three-dimensional spatial pattern along these three orthogonal axes (Stiles, 2008; Stiles and Jernigan, 2010; Technau, 2008). We note that these morphogen gradients can also guide axonal pathfinding (Charron and Tessier-Lavigne, 2005) and therefore may also influence regional connectivity. Thus individual variations in the morphogens that drive brain development along these three axes could produce the observed covariations in morphological features across the cerebral hemispheres by virtue of either their regional effects on morphological brain development or their organizational effects on anatomical connectivity.

Finally, DTI tractography provided compelling confirmatory evidence that subregions identified through our assessment of morphological covariation do indeed belong to discrete brain circuits, which we presumed was the consequence of activity-dependent plasticity. Nevertheless, variation in the anatomical features of the white matter tracts within those circuits, rather than activity-dependent plasticity, could have produced the morphological covariation that we were measuring.
Fig. 5. Visualization of the six eigenvectors that accounted for the greatest portion of variation in surface measures across seven brain regions. These eigenvectors were calculated for 7 regions (the amygdala, hippocampus, caudate, globus pallidus, putamen, thalamus, and hemisphere) in the left hemisphere in a cohort of 82 healthy participants, 71 children with TS, and 36 adults with TS. We applied PCA to scaling coefficients, adjusted for age and sex, to calculate the eigenvectors. Visually and quantitatively, these eigenvectors matched those calculated using the imaging data for 82 healthy participants only. The color coding used to display the variations in surface measures is the same as that used in Fig. 1. AMY = amygdala; CN = Caudate Nucleus; GP = Globus Pallidus; HEM = Hemisphere; HC = Hippocampus; PUT = Putamen; TH = Thalamus.

Fig. 6. Variations in the Surface morphology for the eigenvector with the largest variance. We applied PCA to scaling coefficients for the measures of surface morphology conformally mapped onto a unit sphere. Because a conformal map only preserves angles between straight lines, a rotated conformal map could alter the scaling coefficients calculated from that map, and therefore it could also alter the eigenvectors and eigenvalues from the PCA applied to those scaling coefficients. To assess the effects of rotation in the conformal map onto the calculated eigenvectors and eigenvalue, we rotated the conformal map for a selected value (20° along the X-axis, 20° along the Y-axis, and 20° along Z-axis). Left Column: Color encoding of the eigenvector, calculated without rotating the conformal map. Right Column: Color encoding of the eigenvector, calculated with rotation of the conformal map. The maps show that the two eigenvectors identified the same regions of the brain as covarying in their morphological features to a similar extent. Although the magnitude of covariation differed in some regions, especially in the posterior caudate nucleus and mesial wall of the cerebral hemisphere, these differences were small. Therefore, rotating the conformal map had only a minimal effect on the estimation of covariation in surface morphological features across brain regions. AMY = amygdala; CN = Caudate Nucleus; GP = Globus Pallidus; HEM = Hemisphere; HC = Hippocampus; PUT = Putamen; TH = Thalamus.
Fig. 7. Fibers tracked from seed regions defined by the eigenvector for morphological covariation. This eigenvector (the same as shown in the first row and first column of Fig. 4) accounted for the largest portion of variance in surface measures of the seven brain regions in the left hemisphere for 82 healthy controls, 71 children with TS, and 36 adults with TS. The eigenvector map was normalized into the coordinate space of the template brain for diffusion tensor imaging (DTI) data and was used to define the seed regions to track fibers in the DTI data for the average brain (Top Row) and the template brain (Second Row). The average DTI brain was calculated by averaging the DTI data of 20 healthy individuals normalized into the coordinate space of the template brain. The tracked fibers were color encoded, with green denoting fibers along the anterior to posterior direction, red denoting fibers from left to right, and blue denoting fibers along the superior to inferior direction in the brain. The tracked fibers were displayed on images showing the ROIs in gray scale. Visually, the fibers tracked in the template brain were very similar to the fibers tracked in the average brain. Bottom Seven Rows: The fibers tracked from the ROIs defined by each brain region were displayed to assess the contribution of the fibers from a specific brain region to the neural pathway connecting the brain regions with covarying morphology. The tracked fibers from each ROI shown in gray passed through the same voxels of the brain, demonstrating that the subregions with covarying morphology were interconnected by DTI-identified fiber pathways. AMY = amygdala; CN = Caudate Nucleus; GP = Globus Pallidus; HEM = Hemisphere; HC = Hippocampus; PUT = Putamen; TH = Thalamus.
on cerebral surfaces. For example, variations in either the number of neurons, their dendritic arborization, or their myelin content within each circuit could have produced systematic variations across individuals in the morphological features of the brain regions that those white tracts interconnected. Moreover, these same features of white matter could vary systematically across a disease group compared with healthy control participants, thereby producing the group differences in morphological variation that we observed. Thus, anatomical MRI cannot identify the biological processes that drive the development and maintenance of neural circuits; nevertheless, we have provided extensive evidence that morphological covariation can identify valid neural pathways in the brain.

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**Fig. 8.** White matter regions and neural pathways associated with fibers tracked from seven brain regions in the left hemisphere for our cohort of 82 healthy participants, 71 children with TS and 36 adults with TS. The fiber pathways were tracked in the DTI data for the template brain (Fig. 5) from ROIs defined by the eigenvector that accounted for the largest portion of variance in our measures of morphological covariation (Fig. 4, first row and first column). The WM regions and the neural pathways associated with the tracked fibers are shown in axial sections from the most inferior to most superior region of the brain. For each slice, we show (1) WM regions, (2) fibers tracked in the atlas, and (3) fibers tracked in the template brain from the ROIs defined by the eigenvector. Manual WM Atlas: Inferior slice: anterior corona radiata, posterior thalamic radiation. Middle slice: anterior corona radiata, anterior and posterior limb of the internal capsule. Superior slice: anterior, superior, and posterior corona radiata. DTI Atlas: Inferior slice: anterior thalamic radiation, corticospinal tract, inferior fronto-occipital fasciculus, and superior longitudinal fasciculus. Middle slice: anterior thalamic radiation, corticospinal tract, inferior fronto-occipital fasciculus, and inferior longitudinal fasciculus. Superior slice: anterior thalamic radiation, corticospinal tract, inferior fronto-occipital fasciculus, and inferior longitudinal fasciculus. aCR = Anterior Corona Radiata; pTR = Posterior Thalamic Radiation; aIC = Anterior Internal Capsule; pIC = Posterior Internal Capsule; pCR = Posterior Corona Radiata; aTR = Anterior Thalamic Radiation; CS = Corticospinal tract; iFOF = Inferior Fronto-occipital Fasciculus; sLF = Superior Longitudinal Fasciculus.

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**Fig. 9.** Voxelwise map of fiber counts across the entire brain. We generated a voxel-wise map of fiber count by first tracking all fibers across the brain from each seed ROI in the left hemisphere and then summing in each voxel the number of fibers tracked through it. Top Left: Voxel-wise map of fiber counts across the entire brain, with a range of 1 to 350 fibers in a voxel tracked from the ROIs defined in the 7 brain regions. The gray scale value of a voxel linearly maps the intensity to the fiber count data. Top Right: The map of FA values for the template brain. Bottom Row: Fibers tracked from each of the 7 brain regions in the left hemisphere. These maps show that fibers tracked from each of the region in the left hemisphere identify a major neural pathway. The fiber counts were slightly greater in the right hemisphere (about 40 to 50) than in the left hemisphere (35 to 45). These counts show that each region in the left hemisphere is connected to a brain region in the right hemisphere. However, within deep white matter the diameters of the fiber tracts in the right hemisphere are on average much smaller (about 2.5 mm) compared to those in the left hemisphere (about 5 mm) and therefore likely accounts for the differing fiber counts across hemispheres. Moreover, FA values were about the same in fiber tracts in both the left and the right hemispheres. Therefore, the FA and fiber count data cannot be used as a DTI-based measure to assess the strength of connectivity between brain regions. Instead, the diameter of the tracked fiber may be a better indicator of the strength of connectivity between brain regions. Our analyses showed that brain regions with covarying morphology were interconnected with neural pathways of larger diameter and that brain regions interconnected with smaller diameter pathways did not covary in morphology. We attribute this lack of covariations to the fact that the smaller diameter pathway interconnected spatially small brain regions and our analyses were limited to detect large regions with covarying morphology.