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Supplementary Material for

The Geometric Structure of the Brain Fiber Pathways

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The Geometric Structure of the Cerebral Pathways

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Materials and Methods

1. Diffusion MRI. Diffusion spectrum MRI (DSI) scans were acquired ex vivo in perfusion-fixed whole brain specimens of rhesus, owl, and marmoset monkeys and the pro-simian galago, and in normal human subjects, as previously described²¹, in compliance with institutional requirements for animal tissue and IRB requirements including informed consent for human studies. All DSI acquired data for 515 diffusion sample vector q-values comprising a cubic lattice contained within a sphere $q \leq q_{max}$. Specimens were imaged at 4.7 Tesla with isotropic resolution of $400\mu m - 500\mu m$ depending on brain size, with maximum gradient $G_{max} = 380 \text{ mT m}^{-1}$ and diffusion sensitivity $b_{max} = 40 \text{ ms } \mu m^2$. Human subjects were imaged in vivo at 3 Tesla with isotropic spatial resolution of 2.6 mm, 515 diffusion samples with peak gradient intensity $G_{max} = 60 \text{ mT m}^{-1}$ and maximum sensitivity $b_{max} = 8 \text{ ms } \mu m^2$. Diffusion orientation density functions were reconstructed, orientation maxima identified, and paths computed with streamline tractography and visualized using MGH TrackVis software²¹.

2. Immunofluorescence microscopy. A block of fixed tissue from the rhesus occipital lobe was cut into frozen sections $75\mu m$ thick, within the sagittal stratum, parallel to its oblique plane. Sections were processed free-floating in Mouse Monoclonal [SMI-312] to Neurofilament (abcam ab24574, 1:1000) for 3 days, then with Alexa Fluor 488 goat antimouse IgG₁ (Invitrogen). Confocal images were collected at 1 µm intervals along the z-

axis with a Plan-Apochromat 20x/0.8 objective on a Zeiss LSM 710 confocal system (Carl Zeiss Microimaging). The stack of 54 images was reconstructed using ImageJ (National Institutes of Health, version 1.43u).

Supporting Materials

S1. Three-dimensional structure of midline cerebral pathways in the rhesus monkey. In rhesus frontal lobe (seen from above), paths through regions at three depths have parallel crossings (A), indicating that the near orthogonal structure of pathways is not limited to a particular plane but exists throughout a 3D volume. Here the pathways are longitudinal and transverse, being elements of SLF1 and of the corpus callosum.

Crossing paths of rhesus midline system form a closed 2D sheet (B). Four seed regions are selected. The paths of each (shown as perpendicular single-color pairs) intersect as a grid to form a single closed sheet. In each case, the pair of paths crosses every other pair not in one but in two locations, demonstrating the geometrically exceptional co-planar character of these pathways. Two closed rectangles of paths are indicated: turquoise and orange (arrows), magenta and yellow (arrowheads).



S2. Sheet structure. The documented sheet structure is highly exceptional geometrically and cannot reasonably be explained as a technical artifact, but is best explained as an authentic component of brain structure.

In three dimensions, two smooth families of curves can have 2D surfaces in common only if they are inter-related in a particular way. By the Frobenius theorem due to Deahna and Clebsch*^{28,28}, such surfaces can exist only if a specific function - the normal component of the Lie bracket of the streamlines, representing their mutual twist – is equal to zero at every point in the region in question. Because this function *a priori* can have any value, the observation that it is effectively zero everywhere is highly exceptional. This condition is equivalent to the requirement that quadrilaterals formed of these two fields of curves close in 3D so as to define 2D surfaces rather than forming open spirals in 3D.

In contrast to the observed data, the typical condition of crossing paths in 3D is illustrated in Figure S2 (i.e. any case in which the normal component of the Lie bracket of the streamlines is not zero throughout the region). This Figure illustrates one slice in a 3D structure homogeneous along the *z*-axis, known as a "contact structure"*²⁷. Here, two fields of orthogonal streamlines were represented by red and green; the green line-field is perfectly horizontal (left-right) everywhere, while the red line-field changes pitch uniformly from left to right^{*}. Each square represents these two directions at each location. We see it is impossible to smoothly connect these infinitesimal planes into a

^{*} The two direction fields, green and red, have $\{x, y, z\}$ -components: $V_{green} = \{1, 0, 0\}$; $V_{red} = \{0, cos(px), sin(px)\}$, where p = 0.06 radian/column is the helix-pitch of the red field, and the $\{x, y, z\}$ -components correspond to the horizontal, depth, and vertical axes in the graphic, resp.

continuous 2D surface (i.e. a continuous 2D surface whose tangent planes these are) of finite extent. The obstacle is that if one tries to build such a surface around a closed loop, local directions force it into an open spiral in 3D rather than a closed quadrilateral (Fig. S2 bright red and green lines). This occurs throughout the space, and so no 2D surfaces exist to which both these direction fields are tangent. This figure also illustrates that the case in which paths close and tangent surfaces exist, as observed in the brain, is the exceptional case. Note too that as these direction fields vary smoothly with position and are always orthogonal, neither smoothness nor orthogonality creates false sheet structure.



The present methods of image acquisition and reconstruction have no known bias to favor the observed long-range correlations, i.e. the closure of fiber path loops over large areas and their formation of large-scale 2D surfaces. This follows from the fact that these imaging processes are purely local operations. Specifically, DSI acquisition and reconstruction are single-voxel operations, and the present tractography operates on only single or adjacent voxels. Consequently, these processes have no obvious mechanisms to create long-range spatial correlations including path closure and sheet structure. The closure of path-loops is a universal feature of the present data, seen every present study in all, brain regions, species, orientations, and conditions of regional curvature. Thus it appears inescapable that sheet organization is a true characteristic of cerebral white matter.

A negative control is presented in DSI tractography of the rat heart, where crossing paths in the heart left ventricular myocardium are known not to form macroscopic sheets³⁰ Fig S2B (stereo pair, the heart is viewed along the ventricular axis and toward the apex; the septum is at bottom.) DSI was acquired in fixed specimens with 515 samples, $b_{max} = 8 ms \mu m^{-2}$ and isotropic spatial resolution 300 μ m. Paths were reconstructed corresponding to myocardial fibers (defined the principal direction of diffusion) and myocardial inter-cellular lamellae), and the neighborhood of a selected voxel (green) constructed comprising both fiber and lamellar pathways (the fibers. yellow, a twisting ramp; the lamella, red, a conical disk). These paths were approximately perpendicular at every location. Their mutual intersections did not form any 2D sheet, but rather twisted apart. This example illustrated with diffusion MRI the expected generic case, that distinct path-systems in 3D are non-co-planar. Further, near-orthogonal crossing did not create a false appearance of sheet structure. In contrast to the fibers of the brain, this case did not satisfy the Frobenius condition; this was established quantitatively below.



S2B. Path neighborhood in rat left ventricular myocardium (stereo pair), comprised circumferential fibers (yellow) and radial directions of principal slip (red). Here, as a negative control, the present methods demonstrated that these two sets of paths twisted apart and had no 2D sheet in common.

To quantify sheet structure, consider a path neighborhood such as that of Fig. 1C, defined as the set of all paths cross two seed-paths that in turn cross in a base-point. Such a neighborhood is a 2D sheet if and only if every pair of non-parallel paths within it cross. Thus, the gaps between non-parallel paths of this neighborhood reflect sheet structure. To quantify this, note that the seed paths meet at the base-point with a gap of zero, and hence, gaps between non-parallel paths should be a linear function of the distance of each path to the base-point. Note any two non-parallel paths, together with segments of seed paths, form a possibly open quadrilateral with a potential gap between the two non-seed paths. Thus the size of this gap should scale in proportion to the area of

this quadrilateral. This construct is a finite counterpart to the Lie bracket of the tangent vector fields of the paths.

In a path neighborhood of 2 seed paths, for all pairs of non-parallel paths we computed scatter-plots of their path defect as a function of the area of their quadrilateral to the base point, shown in Figs. 1D for the neighborhoods the rhesus brain and rat heart of Figs. 1C and S1C. A plot of fiber crossing angles is given in S2D. In the heart, the closure gaps increase linearly with area, which indicated incompatibility with sheet structure. By linear regression, this data had slope $r \approx 0.63\pm0.01$ (95% confidence interval) which signifies that the two families of paths part with a solid angle of 0.63 ± 0.001 st $\approx (36^{\circ})^2$. In the brain, the observed closure gaps approached the resolution limit, and the regressed separation angle between the two path families was measured to be 0.055 ± 0.003 st $\approx (3^{\circ})^2$ whose non-zero mean in part reflected the positive Riccian distribution of absolute distances. Thus, crossing pathways in the brain showed little or no growth of closure defects as a function of enclosed area, as predicted of sheet structure.



S2C. Scatter density plots of path quadrilateral area vs. closure gap (horizontal "x.y", vertical "[Lx,Ly]", with units = 0.1 voxel) in DSI of the rat left ventricular myocardium (left; the neighborhood of Fig S1C comprising circumferential fiber paths and radial slip paths) and of the rhesus brain midline system (right; the path neighborhood Fig 1C comprising callosum and SLF1). The path closure gap in the rat heart increased linearly with enclosed area, indicating the absence of sheet structure, whereas in the rhesus brain the closure gap was small and relatively independent of the enclosed area, indicating the sheet structure. The upper and lower branches of the cardiac scatter plot correspond to sub-epicardial and sub-endocardial myocardium.



S2D. Distributions of crossing angles of grid structure in three regions of the rhesus brain – callsom, sagittal stratum, and central sulcus; plot x-axis crossing angle in degrees; y-axis fraction of total crossings per degree. Crossing angle densities were peaked at 90° in neighborhoods within deep white matter, with variation of $\pm 20^{\circ}$ (full width at half maximum). In the gyral white matter of the central sulcus, this distribution was less sharply peaked and broadened to $\pm 30^{\circ}$. This may reflect limitation of measurement accuracy in this region due to the need for higher spatial resolution with high curvature, or shearing of the local grid structure concomitant with plastic deformation in this region during development.

S3. Supporting analysis of path grid structure in the rhesus monkey. Grid structure of the rhesus temporal lobe adjacent to the superior temporal sulcus (STS) is presented in (A), left lateral view. Here, grid structure was aligned with the gyral axis. The long association pathways (medial-dorsal longitudinal fasciculus MdLF and inferior longitudinal fasciculus ILF; blue) coincided with the long axes of the two temporal gyri (the superior and inferior temporal gyri). Crossing at right angles to these longitudinal paths and coinciding with the short axes were transverse association paths including short paths (red) and long commissural paths of the splenium of the corpus callosum (pink).

Grid structure of the ventral frontal lobe seen in an axial view from above is shown in (B). The neighborhood of a callosal path (red) in the rhesus frontal lobe included the subcallosal bundle of Muratoff (MB) that passes longitudinally over the dome of the caudate nucleus, SLF3, and the fornix (FX; blue). These longitudinal pathways all were locally parallel and were all perpendicular to the transverse callosal fibers. They showed that the major association pathways of the rhesus frontal lobe constituted local condensations of a single system longitudinal fiber system. This neighborhood also included dorso-ventral projection pathways (PP; green) perpendicular to both the callosal and to the longitudinal paths.



S4. Coherent grid structure at different depths in rhesus frontal lobe, left lateral view. Parallel grid structure of fiber pathways is shown in rhesus frontal lobe in a sequence of parallel sheets from medial to lateral. Beginning medially (A and then B), parallel sheets of approximately orthogonal pathways encompassed the longitudinal pathways SLF1, SLF2, and the cingulum bundle (CB)). More laterally (C and then D), SLF3 appeared as a parallel longitudinal component of deep white matter. Paths beneath the central sulcus (CeS) include both longitudinal paths that were components of or parallel to SLF2 and SLF3 (blue), and transverse paths belonging to the callosum or parallel to them (red, orange, yellow). These more lateral layers showed the continuity of biaxial structure: the paths of the deep white matter pathways are continuous with the grid structure of the gyri including the subcortical white matter of the central sulcus and pre- and post-central gyri. Note that the gyral paths (best seen in D) were oblique to the long- and short- axes of the sulcus, following helical paths relative to the cylindrical shape of the sulcus, while still adhering to biaxial grid structure. (Note specifically in Figs. SD that that the orientation of transverse paths (orange) is near vertical, while that of the central sulcus topography is tilted clockwise, from upper right to lower left). This contrasted with the gyri of the temporal lobe in Fig. S3A.



S5. Path turning between two principal axes; rhesus monkey frontal lobe (anterior view). A bifurcating path is identified in a tracer study inset right (Schmahmann and Pandya⁵ with permission), and a similar bifurcation is shown with DSI tractography. Proceeding from the superior frontal gyrus, a cord of fibers divides into two branches: a dorso-ventral branch of projection pathways (PP; green in the DSI) and a branch of association fibers that make an abrupt turn laterally of almost 90°, denoted SB (for "subcortical bundle", light green and gold in the DSI). The adjacent parallel callosal paths (red) confirm their transverse orientation. Components of SLF2 (blue) are truncated in the DSI for clarity.



S6. Coherent grid structure of the pathways of the owl monkey cerebral hemisphere, left lateral view. Four path neighborhoods are shown: the corpus callosal / cingulum bundle grid (CC/CB) of the prefrontal midline, the supra-Sylvian grid (SuSy) of the lateral frontal lobe, the occipital grid and sagittal stratum (OC, SS), and the anterior commissure / fornix grid (AC/FX) of the medial temporal lobe. For better visualization of large-scale structure, paths in each neighborhood are colored white in spatially periodic bands. This picture covers a majority of the owl monkey cerebral hemisphere. We see here that the grid motif predominates at all observed locations; is spatially continuous within path neighborhoods and brain regions; and is continuous with the principal longitudinal and transverse axes of the brain from the smallest resolved scale to the scale of the cerebral hemisphere.



S7. Dorso-ventral structure of the cerebral grid in vivo in the human (A) and exvivo in the human (B) and the rhesus monkey (C), frontal lobe viewed posteriorly. These studies show the near-orthogonal crossing of short and long dorso-ventral projection pathways (PP; green) with transverse paths of the corpus callosum (CC; orange, red). Note that the nearly orthogonal relationship between these crossing paths maintained as they rise and rotate toward the medial surface of the hemisphere (cingulate gyrus, etc.), producing the observed fanning configuration. Nearby longitudinal paths are also seen. The human ex vivo study (B) used MRI DSI methodology identical to that of the animal tissue as previously described^{20,21}, with isotropic spatial resolution of 750µm. This tissue was prepared by immersion fixation.

Homologous structure in the rhesus monkey (C; stereo pair) includes crossings of the dorso-ventral cortico-spinal projection pathway (PP; green), the longitudinal SLF1-2 (blue) and the transverse corpus callosum (cc; orange, yellow, light green). Note that the pairs of these paths cross as nearly orthogonal grids in three curved mutually perpendicular planes.



S8. Validation with alternate diffusion encoding. To test the stability of the present findings of brain structure relative to the specifics of the diffusion MRI, we obtained MRI in the rhesus brain with "circular encoding" of diffusion²⁸, a diffusion contrast mechanism physically and mathematically quite different than the q-space encoding of DSI and related methods. Using the analysis of paths described above, grid structure was found in rhesus brain with circular diffusion encoding to be in agreement with the conventional DSI studies, which indicated the relative independence of the present findings from experimental technique.

Circular diffusion contrast is that produced by a multi-pulse encoding, a sequence of gradients that traverse a closed 2D planar loop in 3D phase (k-) space. This encoding retains the signals of spins whose diffusion is restricted to the single 3D direction perpendicular to the 2D plane of the gradient trajectory and attenuates signal of spins with any component of mobility parallel to this plane. This encoding is then repeated for an ensemble of planes sampling the possible directions in 3D, and fiber orientations are identified as directions of high signal. Thus, this method maps fiber orientation under a different physical principal than conventional q-space diffusion imaging – simultaneous hindrance of diffusion in 2-dimensions - and reconstructs fiber orientations with different mathematics - identifying the fiber orientation distribution function (ODF) directly with the angular variation of the diffusion data, and without Fourier, Funk-Radon, or other integral transform^{*}.

^{*}Fiber orientation MRI is based on the isomorphism of Grassmann manifolds of k-planes in n-dimensional space G(n,k) with their perpendicular (n-k)-planes: $G(n,k) \Leftrightarrow G(n,n-k)$, specifically, $G(3,1) \Leftrightarrow G(3,2)$ between lines and planes in 3D. In traditional diffusion imaging (DSI, etc.), this isomorphism is used first by diffusion encoding from the distribution of fibers to the distribution of signal in q-space $G(3,1) \Rightarrow G(3,2)$, and then reversed by an integral transform (3DFT, Radon, etc.) $G(3,2) \Rightarrow G(3,1)$. In circular encodings, these two functions are combined within the physics of the circular encoding $G(3,1) \Rightarrow G(3,1)$.

In the present experiment, we obtained "DSI with circular encoding" in which each conventional DSI q-encode was replaced by a circular encode in the perpendicular plane, scaled to its b-value. In each excitation, the gradient pulse sequence components in the 2D plane are represented by time series

$$x' = \{1, 1, -1, -1\}$$
$$y' = \{2, -2, -2, 2\}$$

with $\{x', y'\}$ orthogonal coordinates within the plane, giving a rhomboid trajectory. This trajectory has equal b-sensitivities in its x' and y' components to suppress in-plane orientation bias.

DSI with circular encoding was acquired for a fixed rhesus monkey brain specimen, with 600 μ m isotropic resolution, with circular diffusion encodes. The circular encodes were an ensemble of 515 measurements, each one corresponding to (scaled and normal to) a vector in the 3D cubic lattice of 5 units in radius (i.e., identical vectors as the DSI sampling ensemble) with b_{max} = 3.10⁴ s cm⁻² (where b = $\int |q|^2$ dt, q = $\int \alpha q d\tau$, G the gradient, α the ¹H gyromagnetic ratio). The 3D array of data at each voxel was converted into an orientation density function by radial summation, streamline tractography was computed as above, and neighborhood analysis was performed to identify path structure.

Results are shown in Fig, S8. Here, neighborhoods of the cingulate and central sulci demonstrated sheet- and grid- structure in agreement with the conventional DSI. Specifically, the midline system demonstrated transverse callosal and longitudinal SLF1 pathways that crossed so as to form sheets as in Fig. 1, whereas that of the central sulcus comprised long and association pathways of transverse and longitudinal orientations, slightly oblique to the gyral axis, as in Fig 3. This concordance supported the relative

independence of present observations of path anatomy and grid structure from the precise structure of the diffusion propagator, provided it preserves the symmetries of fibers and their diffusion contrast. It also suggests independence of image resolution and signal-to-noise within the ranges tested, a factor of 3 in volume resolution (DSI at 400 μ vs. circular contrast at 650 μ), and a factor of 2 in signal-to-noise ratio (DSI 150:1 at b=0 vs. circular 75:1).



Fig. S8. Rhesus monkey high angular resolution diffusion MRI with circular diffusion contrast obtained grid structure matching conventional DSI, here shown in two path neighborhoods; that of the midline, comprising callosal and SLF1 and cingulum pathways, and that of the central sulcus.

S9. Confirmation in the brainstem. To test the present methodology in a simpler anatomic setting, an analysis was performed of the structure of the brainstem. In Fig. S9, neighborhood analyses of small regions within the rhesus monkey brainstem show complete three-dimensional grid structure in all three principal axes. The neighborhood of a single voxel consists of parallel paths in the three mutually orthogonal axes, which cross pair-wise to form grid structures that are well-defined and mutually orthogonal 2D sheets.



Fig. S9. Brainstem pathways in rhesus monkey form a complete three-dimensional grid and curvilinear coordinate system, view anterior lower left. Neighborhood of a small midline region in the rhesus monkey medulla consists of three nearly orthogonal families of paths in three axes identical or continuously related to the principal axes: rostro-caudal, turquoise; transverse, red; and dorso-ventral, dark blue. These paths cross in three nearly orthogonal planes: curved coronal planes (red and turquoise), tilted para-sagittal planes

(blue and turquoise), and axial planes (red and blue). Insets right; silver stain (BlackGold) microscopy (20x) of a para-sagittal slice in the rhesus medulla shows three mutually orthogonal fiber components matching the DSI: transverse (red), axial (green), and dorso-ventral (blue).