OXFORD

ORIGINAL ARTICLE

Pyramid-Shape Crossings and Intercrossing Fibers Are Key Elements for Construction of the Neural Network in the Superficial White Matter of the Human Cerebrum

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Abstract

Structural analysis of the superficial white matter is prerequisite for the understanding of highly integrated functions of the human cerebral cortex. However, the principal components, U-fibers, have been regarded as simple wires to connect adjacent gyri (inter-gyral U-fibers) but have never been thought as indispensable elements of anatomical structures to construct the cortical network. Here, we reported such novel structures made of U-fibers. Seven human cerebral hemispheres were treated with Klingler's method and subjected to fiber dissection (FD). Additionally, tractography using diffusion spectrum imaging (DSI) was performed. Our FD and DSI tractography succeeded disclosing a new type of U-fibers that was hidden in and ran along the white matter ridge of a gyral convolution (intra-gyral U-fibers). They were distinct from inter-gyral U-fibers which paved sulcal floors. Both intra- and inter-gyral U-fibers converged from various directions into junctional areas of white matter ridges, organizing novel anatomical structures, "pyramid-shape crossings". U-fibers to form pyramid-shape crossings also render routes for communication between crossings. There were 97 (mean, range 73–148) pyramid-shape crossings per lateral cortical surface. They are key structures to construct the neural network for intricate communications throughout the entire cerebrum. They can be new anatomical landmarks, too, for the segmentation of the cerebral cortex.

Key words: anatomical network, Fiber dissection, human cerebrum, pyramid-shape crossing, U-fibers

Introduction

Fiber dissection (FD) is the most classical research method for analyses of intricate pathways in the human brain. This approach was first proposed by Nicholas Steno in 1665 (Clarke and O'Malley 1968). A few centuries after Steno's suggestion, the era of FD and histological sectioning methods began. Most of the long association fasciculi were found in this period (Clarke and O'Malley 1968; Schmahmann and Pandya 2006; Catani et al. 2012). In contrast to the long association fiber system in the deep white matter (DWM), observations of short arcuate fibers and their system were very limited (Meynert 1885; Dejerine and Dejerine-Klumpke 1895; Forkel et al. 2015). They might have been less attractive for the object of research since they are located in the superficial white matter (SWM) and their functions to interconnect adjacent gyri are easily assumed.

With recent outstanding advances of neuroimaging techniques, such as functional magnetic resonance imaging (fMRI), diffusion-weighted tensor image (DTI), diffusion spectrum imaging (DSI), cortico-cortical evoked potential, and direct electrical stimulation of the brain during an awake surgery, brain structural networks weaved with long and short fibers are well understood (Duffau 2015; Guevara et al. 2017; Maier-Hein et al. 2017; Yamao et al. 2017). Additionally, techniques such as resting-state fMRI enabled the visualization of functional connectivity (Vassal et al. 2017; Bramati et al. 2019).

There are advantages and limitations in both FD and neuroimaging techniques, including the DTI and DSI methods (Dell'Acqua and Catani 2012). For example, although the DTI technique is the most widely used method in clinical settings, it enables the observation of white matter only indirectly through the movement of water molecules. Also, tracking axon fibers in the cortex and the white/gray matter border is difficult with the present MRI techniques because the diffusion anisotropy in these areas is often too low for modeling the local orientations (Huettel et al. 2009; Reveley et al. 2015). FD is a classical method but has an advantage of providing real-world information; hence, FD is often used as a reliable method to validate the results of MRI observation.

Our FD and DSI study reported here novel brain structures, tridimensional pyramid-shape crossings, in the SWM of the human cerebrum. They may be available for recording the addresses of the origins, courses, terminations, and branching locations of medium- and long-range fasciculi. Since adjacent pyramid-shape crossings are mutually connected with connector bundles of U-fibers, they formed networks throughout the SWM. They are direct anatomical evidence for explaining cerebral connectivity among cortical areas.

Materials and Methods

Fiber Dissection

We used seven human cerebral hemispheres donated to the Kanazawa University in compliance with the Japanese Laws of Dissection Cadaver Preservation (1949) and Body Donation (1983). This research was approved by the Committee of Medical Ethics of Kanazawa University (Nos. 2348 and 2957). Only macroscopically normal hemispheres were used for dissection. The age, sex, and cause of death of donors are listed in Table 1.

The body was fixed with 4.4% formalin (formaldehyde: water 4.4 vol%) by injection into the femoral artery, and blood was drained from the femoral and internal jugular veins (Klingler 1935; Latini et al. 2015). One week later, the brain was isolated and kept in 10% formalin at room temperature for at least 3 months. The brain was washed in running tap water for 24 h, wrapped in a vinyl bag, and placed in a freezer at a temperature of −5 to −10 °C for 8 to 10 days. The frozen brain was thawed at room temperature. This treatment of freezing and thawing (Klingler's method) was repeated at least twice before use. FD was performed by two of the authors: HS, who has been engaged in FD for more than 40 years, and XL, who began dissection 3 years ago.

Peeling fibers with tweezers is currently the only reliable technique for fiber tracking. Tweezers for electron microscopy, which have ultrathin tips, were used for fine peeling. Isolation and peeling of minute fiber bundles and confirmation of tiny tracks were performed under a stereomicroscope with ×8–40 magnification (SZX7, Olympus). Photographs were taken with a camera (D90, Nikon) attached to a photographic stage SL MPS-II (Sugiura Laboratory Inc.). Needles attached by spherical or disk heads were used as landmarks and offered support for tissues. Needle heads were 4 or 1 mm in diameter and could be used as scales as well.

Neuroimaging Study

The DSI Studio (http://dsi-studio.labsolver.org) was used to identify the tractography of representative short association fibers (SAFs). The diffusion MRI data of 1065 subjects from the Human Connectome Project (HCP) were used to construct the HCP-1065 atlas (1-mm template) utilized in this study. The diffusion data were reconstructed in the Montreal Neurological Institute space using the HCP subject-specific volumetric transformations. To maximize the detection of the studied tracts, the following tracking parameters were applied: an angular threshold of 60 degree, a step size of 0 mm (random), a smoothing value of 1.0, and a length constraint of 5.0–50.0 mm. All fibers terminating outside the trajectory of the involved tracts were deleted. For the reconstruction of SAFs, regions of interest (ROIs) were placed on the positions where pyramid-crossings have located during the precedent FD study.

Results

Fiber Dissection

First Stage of FD: The Superficial Layer
The lateral wall of the cerebrum was covered with many cortical convolutions, or gyri, which had heterogeneous width and length and joined one another (Fig. 1A: case 6). There were three types of gyral junctions (Fig. 1A): 1) three-way junction on a few gyri (cyan circles); 2) wide plate-like junction on several gyri (yellow circles); and 3) three-way junction on a gyrus and its small branch, or a bridging gyrus (red circles). Bridging gyri were often very small and situated deeply in sulci so that their existence was clearly discerned only after decortication. The plate-like junction usually had a shallow sulcus or dimple in the surface (Fig. 1A, star).

Decortication of the gray matter was performed along the major gyri in the temporal, occipital, parietal, and frontal lobes. This procedure revealed white matter ridges that underpinned corresponding gyral convolutions and their junctions. The white matter ridges were ~15 mm or more in height with a maximum of 35 mm from the white matter floor of the sulcus. The white matter ridges were principally made of upright bars of U-fibers, and the white matter floors of sulci were paved with curves

Table 1 Each donor's information

No. of cerebral hemisphere	Age	Sex	Right/left	Cause of death
1	82	M	L	Tuberculosis
2	58	M	L	Cancer
3	93	M	L	Sepsis
4	74	M	R	Cancer
5	95*	F	R	Renal failure
6	95*	F	L	Renal failure
7	82	M	R	Cancer

Note: M, male; F, female; L, left; R, right. The mean age of six donors was 80.7 years
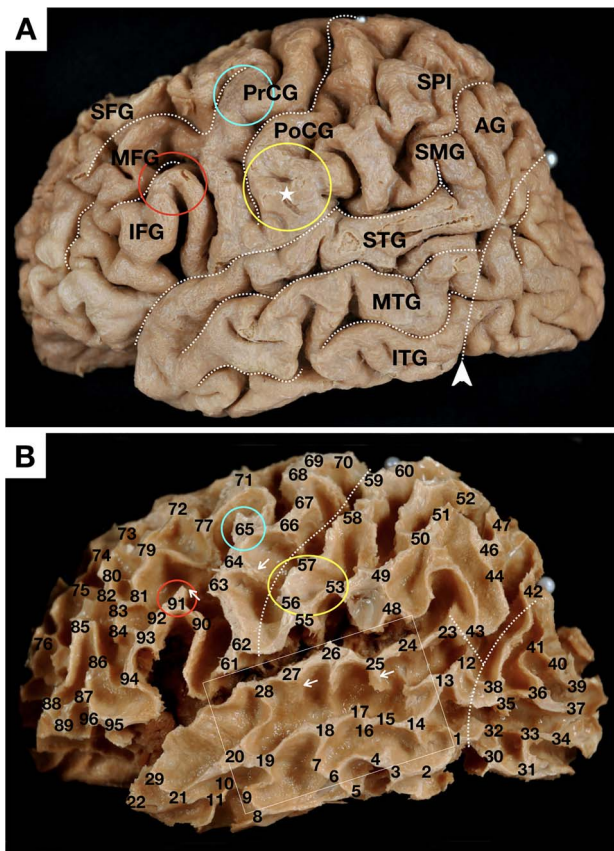*Bilateral cerebral hemispheres were observed in donors with asterisks

Figure 1. Decortication (case 6) revealed 96 crossings in the lateral surface. The left lateral surface of the human cerebrum is shown (A). The cyan circle indicates a three-way gyral junction. The yellow circle indicates a wide plate-like junction with a dimple sulcus (star) on several gyri. The red circle is a junction on a gyrus and its small extension, or bridging gyrus. The anterior white ball indicates the central sulcus, the posterior white ball indicates the parieto-occipital sulcus, and an arrowhead indicates the preoccipital notch. Decortication revealed white matter ridges correspondent to gyri and gyral junctions (B). Three types of ridge junctions, as described in A, are clearly observed in the subcortical level: three-way junction (cyan), plate-like junction (yellow), and small ridge junction (red). The large square indicates the area shown in Figure 2. IFG, inferior frontal gyrus; ITG, inferior temporal gyrus; SPI, superior parietal lobule; stippled lines, sulcus.

of U-fibers. White matter ridges were the upright border of sulcal white matter floors to divide the floor into polygonal and round sulcal motifs. To observe the white matter ridges and their junctions more clearly, tips of the ridges were cut and lowered to

10–15 mm from the white matter sulcal floors. We defined the white matter above the sulcal floor as the SWM, whereas that deeper than the sulcal floor as the DWM. White matter ridges did not always stand rectangular to the surface of the DWM; hence, the relative positions between adjacent ridges changed with decreases in their height, while the morphological correspondence of white matter ridges to gyri was stable (Fig. 1B). We kept two rules for numbering the junctions of white matter ridges or crossings. First, the numbering is sequential within a lobe for convenience of the later statistical analysis. Second, it should be sequential in the same gyrus, especially in the frontal and temporal gyri. The numbering order in the occipital and parietal lobes is somewhat arbitrary, although junctions in the postcentral (and precentral) white matter ridge are serially numbered. Three types of gyral junctions were correspondent to three types of white matter ridge junctions. Small ridges of bridging gyri were often overlooked when they were situated deeply in the sulcal floor (Fig. 1B, white arrows).

Microdissection of Novel U-fibers and Tridimensional Pyramid-Shape Crossings

Microdissection in the junctional area of white matter ridges reveals how U-fibers contribute to the conformation of novel anatomical fiber structures. The junctional area 18 in the middle temporal ridge was a convergence point of four white matter ridges extended from the junctional areas 19, 16/17, 7, and 26. Many U-fibers started from these junctional areas, radiated to pave sulcal floors, and arrived at the junctional area 18, making the maximum thickness of the ridges approximately 3 mm (Fig. 2A, black half rings). Peeling of these U-fibers made the white matter ridge attenuated to 1 mm or less (Fig. 2B,C, black half rings), although there were also many U-fibers to pave sulcal floors without positional relation to the junctional area. Then, a careful dissection of the thin ridges between the areas 18–16/17 and 18–19 disclosed, first, the bundles of U-fibers in the white matter ridge (Fig. 2D, black arrows), and second, a tridimensional pyramid-shape crossing conformed in the junctional area 18. The bundles of U-fibers are new and distinct from the classical type of U-fibers in two features (Fig. 2D, black arrows); first, they are buried in and run along the white matter ridges (intra-gyral U-fibers), while the classical U-fibers pave the sulcal floor; second, the intra-gyral U-fibers, if there were, combine consistently two adjacent pyramid-shape crossings, while the classical U-fibers connect adjacent gyri (inter-gyral U-fibers) indifferent to their positional relations to the pyramid-shape crossings (Fig. 2D, white arrows).
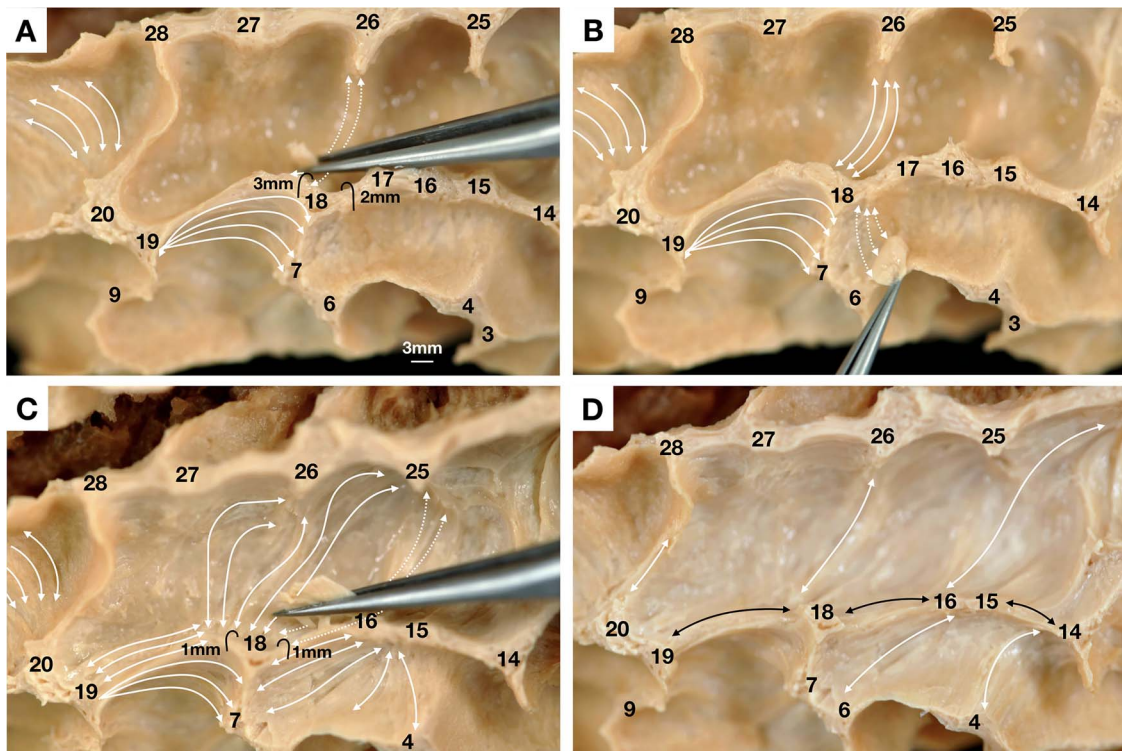
Figure 2. Excavation of two categories of U-fibers. Solid arrows indicate U-fibers. Stippled lines indicate U-fibers being peeled. The junctional area of white matter ridges in the MTG 18 was 3-mm thick at maximum (*A*, black half rings) but became attenuated by peels of U-fibers (*B*). When the ridges near the MTG18 became thin, 1 mm or less (*C*, black half rings), intra-gyral U-fibers between the areas 18–19, 18–16/17 and intergyral U-fibers 18-7 and 18-26 were easily exposed and tridimensional crossings of U-fibers 18 is excavated. This dissection revealed that there are three categories of U-fibers in terms of pyramid-shape crossings: the intra-gyral U-fibers to connect adjacent crossings in the same ridge (*D*, black arrows), the inter-gyral U-fibers to connect crossings between adjacent ridges (*D*, white arrows), and the U-fibers which form the sulcal floor and white matter ridge but do not connect two crossings. To disclose tridimensional pyramid-shape crossings, excavation of both intra- and inter-gyral U-fibers is mandatory.

Consequently, three categories of U-fibers were disclosed in our FD: the intra-gyral U-fibers to connect the adjacent pyramid-shape crossings in the white matter ridge of the same gyrus, the inter-gyral U-fibers to connect pyramid-shape crossings between the two ridges of adjacent gyri, and the U-fibers which form the white matter sulcal floor and ridges but do not connect two pyramid-shape crossings.

Since the "pyramid-shape crossing" is defined as a point of U-fiber convergence extended from various directions, excavation of both the intra- and inter-gyral U-fibers is mandatory to disclose tridimensional pyramid-shape crossings. In the temporal lobe, where three temporal ridges ran in parallel, the intra- and inter-gyral U-fibers formed square grid networks. In other part of the lateral hemispheric surface, U-fibers rarely formed such a regular networks except for the area demarked by the pre- and postcentral gyri.

Many pyramid-shape crossings have radiated the U-fibers to pave the first superficial layer of the sulcal floor, and the fibers were arrived at a second group of neighboring crossings. The second group of crossings was radiated the new U-fibers to pave the second sulcal floor, and the fibers were arrived at a third group of neighboring crossings. Naturally, not all U-fibers ran from one crossing to adjacent crossings, but these exchanges of U-fibers between adjacent crossings were a general construction plan for the sulcal floor; the sulcal floor usually had at least a few U-fiber layers and the deeper layer tended to contain longer fibers.

It should be noted that U-fibers are one-unit superficial arcuate fibers spanning over two pyramid-shape crossings in the SWM. Thus, medium-range association fibers are defined as those spanning over three or more crossings. Long-range association fibers are not critically defined from the middle-range fibers, but they may extend over several pyramid-shape crossings. As a general rule, association fibers were short in the SWM and became long in the DWM, interposing medium-range fibers in the transient depth.

Fine Structure of Pyramid-Shape Crossings

To understand the structure of pyramid-shape crossings more clearly, we added FD results of another lateral cerebral hemisphere (Fig. 3A: case 7) and provided a high-resolution inset of the temporal lobe (Fig. 3B) attached by the explanatory Japanese ink hand-drawing (Fig. 3C). There were 84 white matter ridge junctions which may contain the equal number of pyramid-shape crossings in the lateral surface. In the temporal lobe, the middle temporal white matter ridge had several pyramid-shape crossings. The adjacent crossings were connected with intra-gyral U-fibers. They were also connected with pyramid-shape crossings in the superior and inferior temporal white ridges through numerous inter-gyral U-fibers. The intra- and inter-gyral U-fibers extended sloping fibers higher to converge into a point in the middle temporal white matter and formed a tridimensional pyramid-shape crossing. Occasionally, bundles
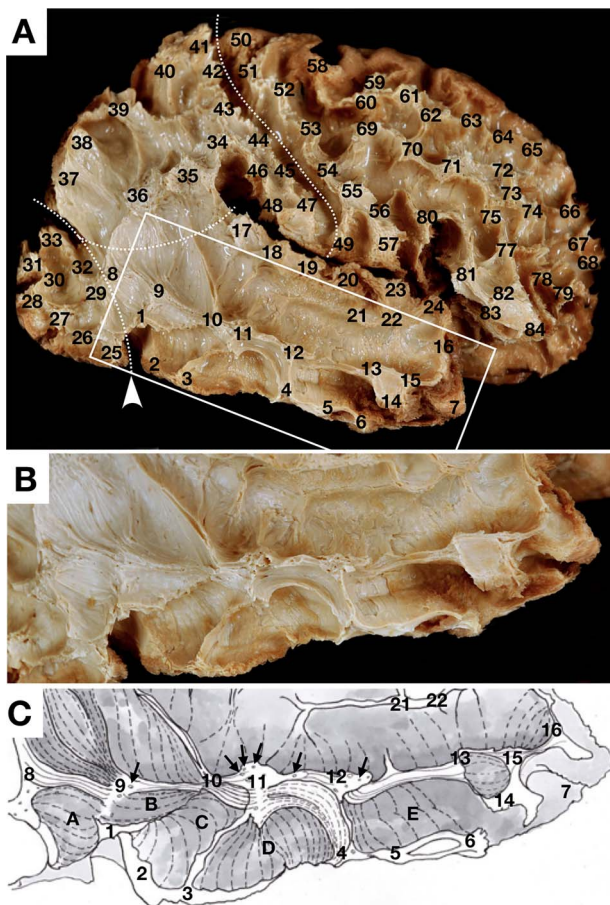
Figure 3. FD of another cerebral hemisphere (case 7). There are 84 junctional areas of white matter ridges (A). The close-up photograph of the middle temporal white matter ridge (B) was inset to show highly resolved intra- and inter-gyral U-fibers with the explanatory Japanese ink hand-drawing (C). The inter-gyral U-fibers are indicated by broken lines and the zone occupied by them is colored gray. They pave the sulcal floor and delineate various shapes of sulcal motifs (A–E). The intra-gyral U-fibers are drawn with thin solid lines in the middle temporal ridge (uncolored/white). They connect adjacent pyramid-shape crossings in the crossings 8–13. The crossings 1–6 are in the inferior temporal white matter ridge and the crossings 21 and 22 are in the superior temporal ridge. Note that pyramid-shape crossings 8–13 are sloped higher due to the convergence of intra- and inter-gyral U-fibers. Note also that some of the inter-gyral U-fibers connecting the crossings 11 and 4 pass via the crossing 12. On such occasions, critical discrimination of intra-gyral U-fibers from inter-gyral U-fibers become difficult; namely, the inter-gyral U-fibers may contain intra-gyral U-fibers to connect the crossing 11 and 12. Opening of perivascular canals (black arrows) is a common feature to identify pyramid-shape crossings.

of inter-gyral U-fibers are appeared to contain intra-gyral fibers. Opening of many perivascular spaces is a useful morphological feature to identify the crossings.

Second Stage of FD: The Middle Layer
With advance of FD, some crossings have diminished in size and disappeared, while adjacent crossings coalesced in their roots to make a larger crossing, as observed in areas 50/45, 49/48, and 53 (Fig. 4A: case 6). Thus, crossing-to-crossing connections became more clearly recognized in this stage than in the previous dissection stage. Therefore, this is the final stage of our FD to confirm the anatomical positions of pyramid-shape crossings and their numbers in each lobe. In the temporal operculum, the
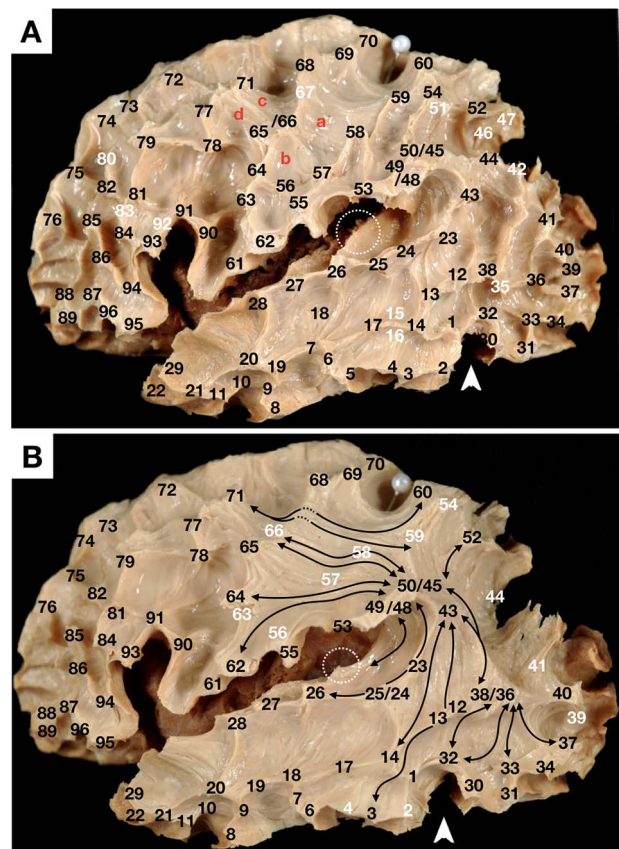
Figure 4. Crossing-to-crossing tracking of medium- and long-range fibers. (A) As white matter ridges became 10 mm or less in height, some crossings are diminished in size and disappeared (white numbers). In the temporal operculum, the anterior and posterior transverse temporal gyri (Heschl's gyri) conjoined to form a crossing (stippled circle). Sulci in the lateral cerebral surface formed round, oval, or polygonal concavities or sulcal motifs, in this stage of dissection. The four red letters a, b, c, and d are four sulcal motifs which look like four petals of poppy flowers (Catani et al. 2012). The crossing 65/66 corresponds to the receptacle of the petals to where the stalk of the poppy flower attaches. (B) In the first step, the anterior, lateral, and posterior streams were excavated. The anterior stream started at the posterior surface of the TTG, which had a TTG crossing (stippled circle). Some fibers originated near the TTG (or superior temporal gyrus [STG]) 26 crossing, passed along the STG 25–23 crossings and intermingled with fibers extending from the MTG 13, 14, and 17 before reaching the SMG 50/45 crossing.

anterior and posterior transverse temporal gyri (Heschl's gyri) conjoined and formed a cone-shaped crossing (Fig. 4A, stippled circle). An important observation in this stage of dissection was the appearance of sulcal motifs covering the entire lateral surface of the cerebral hemisphere. They were round, oval, or polygonal concavities of sulci bordered by pyramid-shape crossings and bundles of intra-gyral U-fibers. For example, the crossings 65/66 in the precentral gyrus (PrCG) were in the center of four sulcal motifs: a, b, c, and d, which other authors (Catani et al. 2012) called four petals of poppy flowers (Fig. 4A, red letters).

Number of Pyramid-Shape Crossings
All seven cerebral hemispheres were examined with a similar procedure (Table 2). The mean number of pyramid-shape crossings per lateral surface was 97 (range: 73–142). There were 36 (range: 26–48) in the frontal lobe, 21 (range: 13–32) in the parietal

Table 2 Distribution of pyramid-shape crossings in the lateral surface of the cerebrum

No. of cerebral hemisphere	Number of crossings in each lobe				
	Frontal	Parietal	Occipital	Temporal	Total
1	48	32	23	39	142
2	30	21	14	17	82
3	26	21	14	14	75
4	43	27	24	31	125
5	29	13	9	22	73
6	36	19	12	29	96
7	36	15	9	24	84
Mean	36	21	15	25	97
Range	26–48	13–32	9–24	14–39	73–142

lobe, 15 (range: 9–24) in the occipital lobe, and 25 (range: 14–39) in the temporal lobe. The numbers were variable according to the heterogeneous stages of dissection in different brain areas. Based on the results of the lateral surface, the total number of pyramid-shape crossings per cerebral hemisphere was estimated to be 150–200. The number of crossings increased due to bridging gyri that were newly found in the deep sulcal floor. However, the total number decreased rapidly during the excavation of long association fibers, because tracking of one long association fibers in the DWM inevitably required the removal of many pyramid-shape crossings in the SWM. The number of pyramid-shape crossings approached zero when the SWM was completely removed.

During the excavation of pyramid-shape crossings, we have realized longer association fibers started from (or terminated to) the crossings and tended passing preferentially underneath the crossings (Fig. 4B). For example, the pyramid-shape crossings 23–28 were in the superior temporal white matter ridge, which signified the presence of a long bundle of fibers, or the middle longitudinal fasciculus (MdLF), underneath them. Similarly, the inferior longitudinal fasciculus (ILF) was present underneath the pyramid-shape crossings 12–21 of the middle temporal white matter ridge. This means that, first, the location of pyramid-shape crossings is available for addressing the origins, courses, terminations, and branching locations of long association fasciculi; second, long association fibers can be excavated nonempirically by tracking from one crossing to the next. Crossing-to-crossing FD for the excavation of long- and medium-range association fibers began when white matter ridges and pyramid-shape crossings appeared about 5 mm or less in height from the sulcal floor (Fig. 4B). Since a considerable number of medium-range fibers originated from the temporal and occipital lobes stop in the parietal lobe, the crossing-to-crossing FD was applied in two steps: the first application to crossings in the temporo-parietal area and the second to the parieto-frontal area. Excavated association fibers are schematically shown with arrows.

In this stage, there were three streams of fibers (Fig. 4B): anterior, lateral, and posterior. The anterior stream started from the posterior surface of the transverse temporal gyrus (TTG), which had TTG crossings. The lateral stream originated principally from the middle temporal gyrus (MTGs) 12–14 and terminated in the supramarginal gyrus (SMG) 50/45 and 43. The posterior stream came from the crossing 38/36, located at the posterior end of the MTG, or motor temporal area. These three streams were not independent as they exchanged fibers mutually in their borders and entered into the conjoined large pyramid-shape crossing in the inferior parietal lobule.

Third Stage of FD: The Deep Layer
Continuation of the long anterior, lateral, and posterior streamlines to the precentral crossings became clearly trackable after removing the angular gyrus (AG) 43, SMG 50/45, and SMG 49/48 crossings, suggesting that they passed underneath these crossings (Fig. 5A). They presented beautiful streamlines of the classical superior longitudinal fasciculus (SLF). The anterior stream constitutes a principal part of the arcuate fasciculus (AF) and formed an infra-opercular bundle that ran along the superior peri-insular sulcus. The bundle was very thin and several millimeters wide, ran deep to the U-fibers of the insula and superficial to the cortico-putaminal fibers, and sent some fibers to both the operculum and insula. The anterior stream was clearly separated from the lateral stream, or a supra-opercular bundle, by the intervening parietal and frontal opercula. However, once the opercula were removed, the anterior and lateral streams became indistinguishable from each other, even if the landmark remained.

The lateral and posterior streams provided the streamlines of the SLF that spread over nearly half of the PrCG. The posterior stream appeared continuous with superior frontal crossings, but a turbulence of streamlines was present in the lower limb area of the PrCG (Fig. 5B, upper circle). The lateral stream also had a turbulence of fibers in the doorknob area; hence, streamlines to the middle frontal crossings were not smooth (Fig. 5B, lower circle).

Neuroimaging Study

Our DSI method revealed three seed ROIs in the MTGs that were connected with another three ROIs set in the adjacent gyrus through the bundles of U-fibers (Fig. 6A). They are classical inter-gyral U-fibers to connect the adjacent gyri. The DSI method also revealed the presence of U-fibers to connect two adjacent seed ROIs in the MTG (intra-gyral U-fibers) (Fig. 6B, see also Supplementary Videos 1 and 2). Similarly, we found the same structure in pre- and postcentral gyri in frontal gyri, as well as temporal gyri (Supplementary Figure 1 and Supplementary Videos 3 and 4).

For an immediate understanding of fiber organization in the pyramid-shape crossing, we presented an FD hand-drawing and DSI figures (Fig. 7). Most of pyramid-shape crossings can be observed on superficial layer, after removing cortical layer (Fig. 7Aa). The crossing in the hand-drawing was composed
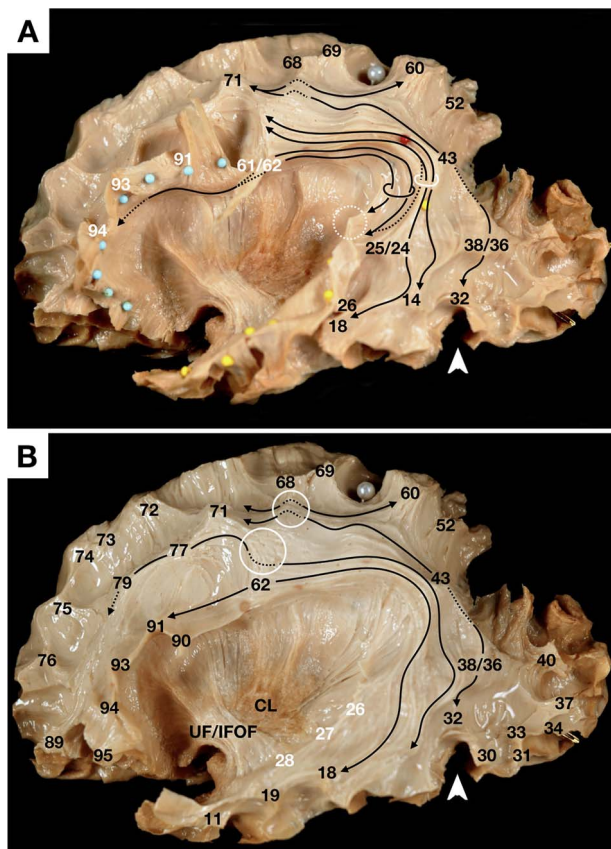
Figure 5. Continuation of the anterior, lateral, and posterior streams. (A) By removing crossings AG 43, SMG 50/45, and SMG 49/48, continuation of three streams to the precentral gyrus became clear (arrows). Medium- and long-range association fibers presented arched streamlines of the classical SLF. The anterior stream (black half ring) that constitutes the principal part of the AF formed an infra-opercular fasciculus that ran along the superior peri-insular sulcus. The lateral stream (white half ring) is a supra-opercular fasciculus that arrived at the precentral crossings from the doorknob to face areas. Some anterior stream-lines migrated into the lateral stream (stippled line); thus, the border between the anterior and lateral streams became unclear. (B) Medium- and long-range association fibers in the frontal lobe are shown. The posterior stream appeared continuous with superior frontal crossings, but a small turbulence of streamlines was present between the precentral and superior frontal crossings (stippled line of arrows in the upper circle). The lateral stream appeared continuous with the middle frontal crossings but had also turbulence between the precentral and middle frontal crossings (stippled line of an arrow in the lower circle).

of the intra-gyral fibers (solid lines) in the middle temporal white matter ridge and inter-gyral fibers (broken lines) which connected the crossing with the superior and inferior temporal white matter ridges (Fig. 7Ab). The crossing in the DSI was made of the intra-gyral U-fibers in the MTG and inter-gyral fibers extended from the superior temporal gyrus (STG) (Fig. 7B, Supplementary Video 5). These figures implied that both crossing figures are identical in the U-fiber organization.

Discussion

As far as we know, U-fibers have been regarded as simple wires to connect adjacent gyri but have never been evaluated as components of such special structures like tridimensional pyramid-shape crossings. Also, U-fibers have been thought classically as a homogeneous group of fibers to pave the sulcal floor and to form gyral ridges, but we excavated U-fibers that were buried in and
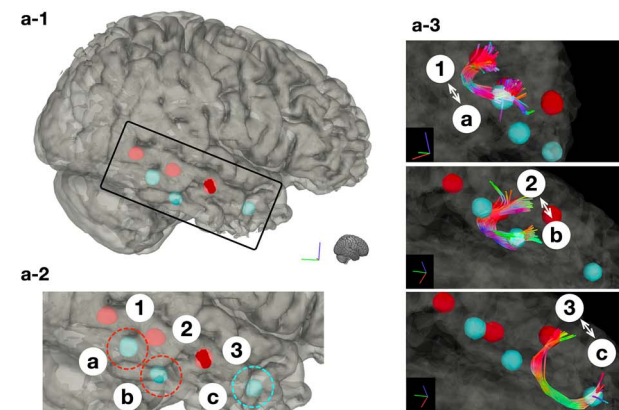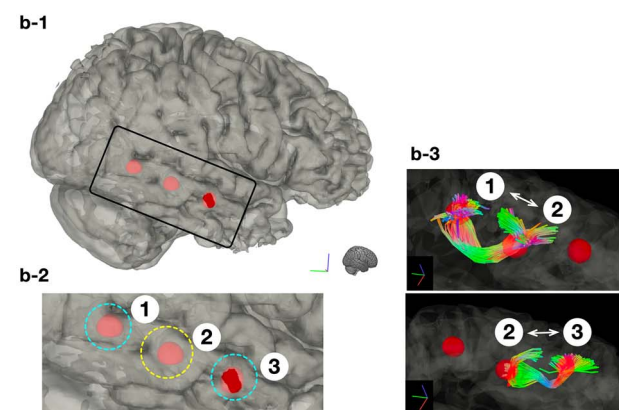

Figure 6. DSI of classical (inter-gyral) U-fibers and new (intra-gyral) U-fibers. (A) Three seed ROIs (red balls 1, 2, and 3) were set in the areas of gyral junction in the MTG and another three ROIs (light blue balls a, b, and c) were placed in the areas of gyral junction in the ITG (a-1 and a-2). DSI study revealed three bundles of inter-gyral U-fibers between ROIs 1 to a (a-3, upper column of d-2), 2 to b (a-3, middle column), and 3 to c (a-3, lower column). (B) The same three ROIs (red balls 1, 2 and 3) were set in the MTG (b-1 and b-2). DSI tractography revealed two intra-gyral U-fibers between 1 to 2 and 2 to 3 (b-3). Note that the inter-gyral bundles (A) and intra-gyral bundles (B) share the same ROIs (ROIs 1, 2, and 3), and each ROIs make a part of a tridimensional structure, namely pyramid-shape crossing. Pivoting of the two bundles of U-fibers around the axis should form part of a pyramid-shape crossing, supporting the presence in FD. Dotted circles indicate three-way junction (cyan), plate-like junction (yellow), and small ridge junction (red).

ran along the white matter ridge of one gyrus. The newly found U-fibers, in cooperation with classical U-fibers, participated in the conformation of pyramid-shape crossings and contributed, too, as connector fibers between the adjacent pyramid-shape crossings. The pyramid-shape crossings and connector fibers are comparable to anatomical addresses and roadmaps in the cerebrum. They weave the network covering the entire SWM of the cerebrum.

The Reason Why Anatomical Existence of "Pyramid-Shape Crossings" Has Been Unnoticed

The temporoparietal intersection area has been reported as a critical neural crossroad of seven pathways: SLF, MdLF, ILF, AF, inferior fronto-occipital fasciculus (IFOF), optic radiation, and tapetum (Martino et al. 2013). The temporoparietal intersection

A. Illustration of short association fibers
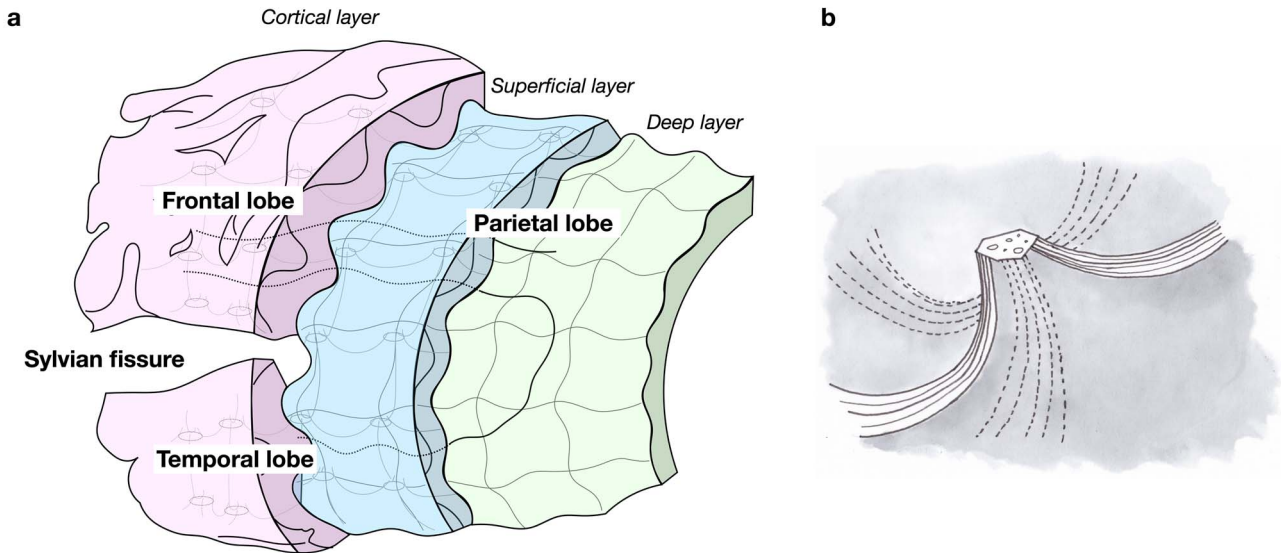
B. A typical image of pyramidal shape crossing
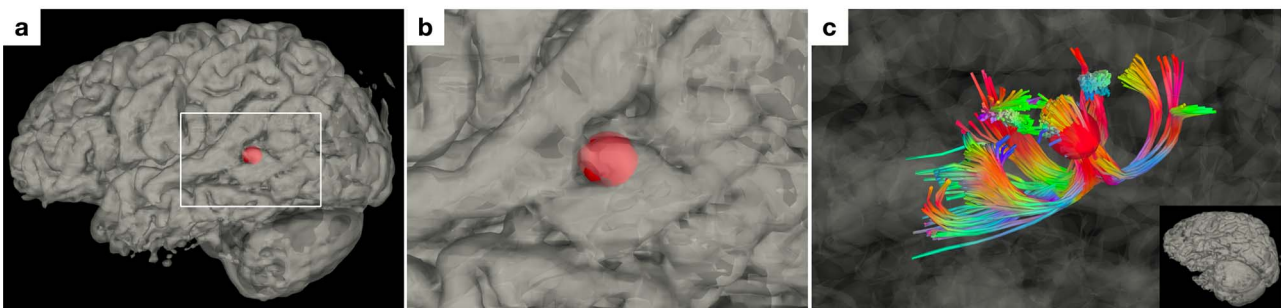
Figure 7. Visualization of pyramid-shape crossings in FD and DSI. U-fibers in the SWM and middle- and long-association fibers (stippled lines) situated in the DWM are shown (*Aa*). Wavy lines in the cortex express gyrus-sulcus undulations, while wavy lines in the white matter signify the cortex-subcortical border. When the SWM was removed, pyramid-shape crossings were no longer present. Medium- and long-range association fibers connect between two remote crossings, passing underneath more than three pyramid-shaped crossings. The hand-drawing of pyramid-shape crossing was obtained from the posterior part of the middle temporal white matter ridge in case 6 (*Ab*). The crossing was made of convergence of intra-gyral U-fibers (solid lines) in the MTG and inter-gyral U-fibers (broken lines) extended from the superior and inferior temporal white matter ridges. The gray color indicates the area paved with inter-gyral U-fibers. There are several openings of perivascular spaces in the crossing (*Ab*). When an ROI was placed on the area of small ridge junction in the posterior part of the MTG (*Ba* and *b*; *Bb* is zoom of white square in *Ba*), DSI tractography revealed both intra- and inter-gyrus U-fibers converging pyramid shape crossing (*Bc*). Both the FD and DSI crossings are identical in terms of inter- and intra-gyral U-fiber organization.

is a new anatomical name but is a conceptual substitute for the classical sagittal stratum to which FD has been applied most frequently. It consists of only long fiber layers in the variable depths of DWM from the superficially situated longitudinal fasciculi to the deepest optic radiation. The present study located 10 or more pyramid-shape crossings in the SWM of this area, and some of them were large and occupied anatomical "hub" positions. However, their presence has never been noticed in the history of FD for more than 350 years. An important point is that dissectors of this area might have devoted to excavate these long fibers in the DWM and scarcely paid attentions to structures in the SWM. Since FD advances from the superficial to deep layers of the brain, long association fibers appear only after the removal of the SWM (Shah et al. 2019). Structures such as pyramid-shape crossings in the SWM might have been removed without notice in the early stage of FD. Notably, when FD structures of previously published reports were carefully

re-examined, remnants of pyramid-shape crossings could be observed in some studies (Maldonado et al. 2013; Shah et al. 2019).

U-fibers

Those U-fibers organize tridimensional pyramid-shape crossings, which is the main observations in the current study. Traditionally, U-fibers are known to connect adjacent gyri, and they cover the superficial layer of the brain (Oishi et al. 2008; Zhang et al. 2018; Shah et al. 2019). In diffusion MRI study (Guevara et al. 2017), nearly 100 bundles of U-fibers were found in both hemispheres. They were classified in accord with the lengths of U-fibers, and bundles of very short fibers (10–12 mm long) and short fibers (13–52 mm long) were found mostly in the primary area (Bajada et al. 2019). For example, a neuroimaging study between the precentral and postcentral gyri

revealed a strong connectivity between them via short U-fibers (Magro et al. 2012). Thus, the U-fibers to connect adjacent gyri, or inter-gyral U-fibers, are commonly accepted. However, the presence of U-fibers to connect two pyramid-shape crossings in the same gyrus, or intra-gyral U-fibers, has not been recorded yet. This may be well understood, since the presence of pyramid-shape crossings per se has never been described until now, nor could anyone imagine the presence of U-fibers to interconnect two pyramid-shape crossings in the same gyrus. The intra-gyral U-fibers are indispensable fiber elements for the construction of pyramid-shape crossings and crossing-to-crossing connections. Successful excavation of the pyramid-shape crossings was led by such U-fibers.

An exquisite work has been proposed by Catani and co-workers (2012). They employed postmortem dissection and diffusion-weighted MRI data using a high angular resolution diffusion imaging optimized for spherical deconvolution. Their MRI presented sulcal motifs made of U-fibers in the frontal and parietal lobes. They exemplified a structure of the doorknob area in the PrCG as poppy flower; namely, four petals of sulcal motifs in the PrCG, postcentral gyrus (PoCG), superior frontal gyrus (SFG), and middle frontal gyrus (MFG) were supported by a long fiber stalk composed of ascending thalamo-cortical fibers and descending cortico-striatal, cortico-pontine, and cortico-spinal fibers. The four sulcal motifs, a, b, c, and d (Fig. 4A, red letters), correspond to their four petals. Our pyramid-shape crossing 65/66 (Fig. 4A) corresponds to the receptacle of four poppy flower petals to which the MRI stalk attaches. Although Catani et al. (2012) did not mention to the total number of poppy flowers in the lateral cortical hemisphere, our estimation based on FD results suggests that there are approximately 100. They are independent from one another, but they are exchanging neuronal talks among adjacent flowers through their petals.

Cortical Segmentation Based on Pyramid-Shape Crossings

Pyramid-shape crossings are primarily made of convergence of U-fibers. However, long association, commissural, and projection fibers are also thought to converge to or spread from the crossings. The connectome consists of two descriptors to define the network architecture: neural elements or nodes and neural connections or edges (Sporns et al. 2005; Wig et al. 2011). Usually, the descriptors are divided at three distinct levels of neural organization: the microscale, represented by neurons and synapses at the micrometer level; the macroscale, represented by segments of gyri and their neuronal pathways and indicated at the centimeter level; and the mesoscale, represented by minicolumns and their connection patterns comprising at the millimeter level (Sporns et al. 2005). Thus, our FD networks are compatible with connectomes at the macroscale level. The pyramid-shape crossings and bundles of U-fibers are white matter structures that are not comparable with the neuron-node and synapse-edge. However, pyramid-shape crossings are white matter structures, which connect directly with gyral junctions, suggesting that they are substitutes for the border where distinctive gyri, or nodes, cross one another.

Since the number of pyramid-shape crossings was about 100 in the lateral cerebral surface, we estimate the total number per cerebral hemisphere would be 150–200, including those crossings in the medial and inferior surfaces. The Brodmann's mapping has no more than 50 cortical areas (Ono et al. 1990;

Nieuwenhuys et al. 2008). At present, six long association fasciculus, SLF, MdLF, ILF, AF, IFOF, and cingulate fasciculus, are known in the human cerebral hemisphere. If we divide the SLF into three independent subdivisions, SLF1, 2, and 3, and include other fasciculi such as the vertical fasciculus, the number of conventional areas/divisions is obviously insufficient for the description of origins, courses, terminations, and branching points of the fasciculi. Actually, the connectome estimates the number of cortical segments will be up to a few hundred per cerebrum (Sporns et al. 2005; Zalesky et al. 2010). Our estimation, 150–200 pyramid-shape crossings per hemisphere, is compatible with the connectomic number and might offers evidence to guide further neuroimaging studies.

Possible Role of Pyramid-Shape Crossing in Brain Plasticity

The technique of resting-state fMRI enables the visualization of functional connectivity (Vassal et al. 2017; Bramati et al. 2019). In graph theory, a functional network is constructed by nodes each of which has specific brain function and edges which connect between neighboring nodes (Chiang and Haneef 2014). We speculate the functional connectivity that can be explained based on the presence of anatomical network structures.

Another possibility is that inter- and intra-gyral U-fibers might play a role in functional reorganization. Recent findings suggest that the shift of functional localization, or cortical plasticity, takes place from the original locus to its peripheral loci with the progression of brain tumors and the stages of ischemic expansion. For instance, we sometimes encounter patients with brain tumors whose function is relatively normal even though the brain tumor is invading a functional brain locus, such as a motor or language area (Kristo et al. 2015; Saito et al. 2016; Magill et al. 2017). Also, recent studies using fMRI or intra-operative direct electrical stimulation revealed that the brain function of patients with tumor can shift to another place from an original position (Jang et al. 2002; Kawashima et al. 2013; Hayashi et al. 2014). Other resting-state fMRI studies found that functional connectivity could change according to functional recovery by increasing connections to neighboring gyri via U-shaped fibers or strengthening connections to the contralateral cerebral-hemisphere (Vassal et al. 2017; Bramati et al. 2019). However, the transformation of structural subcortical networks following a functional transformation of the cortical brain area is not well known. Here, we speculate that inter- and intra-gyral connectivity, especially connectivity via pyramid-shape crossings, might be a necessary structure for reorganization or unmasking. Further study will be required to reveal the functional role of pyramid-shape crossings at the functional and molecular level.

Limitation

We should mention the age deviation of donors, the average age 80.7 (58–95, range), as the first limitation. Aging is one of the possible factors to influence upon anatomical changes of the SWM and number of pyramid-shape crossings in the SWM, but how and to what extent aging influences must await further investigations. Second, the number of pyramid-shape crossings varied with a wide range (73–142) in seven cerebral hemispheres. This wide range may largely due to the individuality of donors, but also due to the heterogeneous degrees of FD progress among the four lobes and areas at the stage of the counting. However,

not a few pyramid-shape crossings were consistently found in some gyri without regard to dissectors (HS and XL) in this study. The more extensive survey is needed to resolve this problem. The current study reported the existence of pyramid-shape crossings in the SWM, but the meaning of their anatomical locations and functional roles remain unexplained. Moreover, we used MRI tractography to support the existence of pyramid-shape crossings in FD, but neuroimaging studies are mandatory for further anatomical validation of pyramid-shape crossings and for their application to functional analyses of the brain network system.

Conclusions

We introduced novel SWM structures, pyramid-shape crossings, and connector bundles. They are obviously key anatomical structures for the construction of the subcortical network that may exert for intricate mutual communications in the human cerebrum. The pyramid-shape crossings are present in correspondent to gyral junctions; therefore, they can be new landmarks for the segmentation of the cortex in neuroimaging studies.

Supplementary Material

Supplementary material is available at *Cerebral Cortex* online.

Author Contributions

Author contributions to the study and manuscript preparation include the following. Conception and design: M.N. and H.S. Acquisition of data: H.S. and X.L. Analysis and interpretation of data: H.S., X.L., R.N., and M.K. Drafting article: M.N., H.S., and R.N. Critically revising the article: all authors. Reviewed final version of the manuscript and approved it for submission: all authors. Study supervision: M.N.

Funding

Conflict of Interest

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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