The frontal longitudinal system as revealed through the fiber microdissection technique: structural evidence underpinning the direct connectivity of the prefrontal-premotor circuitry

***Spyridon Komaitis, MD, MSc,[1–3] Aristotelis V. Kalyvas, MD, MSc,[1–3] Georgios P. Skandalakis, BBA, MSc,[1,3] Evangelos Drosos, MD,[1,2] Evgenia Lani, MD,[1,3] Evangelia Liouta, MSc,[6] Faidon Liakos, MD,[1] Theodosis Kalamatianos, PhD,[6] Maria Piagkou, PhD,[3] John A. Emelifeonwu, MD,[4,5] George Stranjalis, MD, PhD,[1,2,6] and Christos Koutsarnakis, MD, MSc[1–3,5]**

[1]Athens Microneurosurgery Laboratory, National and Kapodistrian University of Athens School of Medicine, Athens; [2]Department of Neurosurgery, National and Kapodistrian University of Athens; [3]Department of Anatomy, National and Kapodistrian University of Athens School of Medicine, Athens, Greece; [4]Department of Clinical Neurosciences, Western General Hospital, Edinburgh; [5]Department of Clinical Neurosciences, Edinburgh Microneurosurgery Education Laboratory, Edinburgh, UK; and [6]Hellenic Center for Neurosurgical Research, "Petros Kokkalis," Athens, Greece

OBJECTIVE The purpose of this study was to investigate the morphology, connectivity, and correlative anatomy of the longitudinal group of fibers residing in the frontal area, which resemble the anterior extension of the superior longitudinal fasciculus (SLF) and were previously described as the frontal longitudinal system (FLS).

METHODS Fifteen normal adult formalin-fixed cerebral hemispheres collected from cadavers were studied using the Klingler microdissection technique. Lateral to medial dissections were performed in a stepwise fashion starting from the frontal area and extending to the temporoparietal regions.

RESULTS The FLS was consistently identified as a fiber pathway residing just under the superficial U-fibers of the middle frontal gyrus or middle frontal sulcus (when present) and extending as far as the frontal pole. The authors were able to record two different configurations: one consisting of two distinct, parallel, longitudinal fiber chains (13% of cases), and the other consisting of a single stem of fibers (87% of cases). The fiber chains' cortical terminations in the frontal and prefrontal area were also traced. More specifically, the FLS was always recorded to terminate in Brodmann areas 6, 46, 45, and 10 (premotor cortex, dorsolateral prefrontal cortex, pars triangularis, and frontal pole, respectively), whereas terminations in Brodmann areas 4 (primary motor cortex), 47 (pars orbitalis), and 9 were also encountered in some specimens. In relation to the SLF system, the FLS represented its anterior continuation in the majority of the hemispheres, whereas in a few cases it was recorded as a completely distinct tract. Interestingly, the FLS comprised shorter fibers that were recorded to interconnect exclusively frontal areas, thus exhibiting different fiber architecture when compared to the long fibers forming the SLF.

CONCLUSIONS The current study provides consistent, focused, and robust evidence on the morphology, architecture, and correlative anatomy of the FLS. This fiber system participates in the axonal connectivity of the prefrontal-premotor cortices and allegedly subserves cognitive-motor functions. Based in the SLF hypersegmentation concept that has been advocated by previous authors, the FLS should be approached as a distinct frontal segment within the superior longitudinal system.

https://thejns.org/doi/abs/10.3171/2019.6.JNS191224

KEYWORDS FLS; frontal longitudinal system; fiber dissection technique; prefrontal cortex; premotor cortex; anatomy

ABBREVIATIONS BA = Brodmann area; DLPFC = dorsolateral prefrontal cortex; DTI = diffusion tensor imaging; FAT = frontal aslant tract; FLS = frontal longitudinal system; SLF = superior longitudinal fasciculus.

SUBMITTED May 1, 2019. **ACCEPTED** June 13, 2019.

INCLUDE WHEN CITING Published online October 4, 2019; DOI: 10.3171/2019.6.JNS191224.

* S.K. and A.V.K. contributed equally to this study.

The hypothesis of an underlying connection between prefrontal and motor and premotor areas has been advocated by various theories that stem from studies in humans and nonhuman primates. These studies support the notion that specific loci within the premotor cortex—mainly confined in the ventral and rostrodorsal premotor cortices—are subject to higher-order control from prefrontal areas.[1,10,14,25] This circuitry allegedly participates in the cognitive aspect of motor behavior such as space perception and action understanding and imitation.[9,12,13,24,32,34,37] However, although there is a clear anatomical background regarding the connectivity of primary motor-premotor cortices with the supplementary motor area,[4,5] as well as caudal areas like the parietal, temporal, and occipital cortices,[3,28,29,43] the structural framework of the prefrontal-premotor circuitry remains vague.

To this end, in a recent anatomo-tractographic study titled "Short frontal lobe connections of the human brain," Catani and colleagues identified for the first time in the literature a group of fibers residing in the prefrontal area, which they named the frontal longitudinal system (FLS).[5] The authors, by applying the fiber dissection technique in one hemisphere and further augmenting their results with tractographic data, revealed that the FLS is a superficial fiber system found to travel within the middle frontal gyrus and to consist of a superior and an inferior frontal chain, radiating up to the frontal pole and essentially resembling an anterior extension of the superior longitudinal fasciculus (SLF). This novel finding has indeed offered an initial insight into the structure to function relationship between the precentral gyrus and prefrontal areas, but further anatomical evidence validating current knowledge and enhancing understanding of the regional axonal connectivity is currently lacking.

Hence, the purpose of this study was to offer a focused and definitive anatomical proof for the existence, topography, correlative anatomy, and connectivity of the FLS as an intrinsic frontal pathway, by implementing the Klingler white matter dissection technique. This method has been recently proven to be very sensitive and accurate in reflecting the cerebral fiber tract architecture and is therefore being currently used to validate structural data deriving from diffusion tensor imaging (DTI) tractographic studies. With this technique in our armamentarium we attempted to shed light on the subcortical stream subserving prefrontal-premotor cross-talking in the human brain.

Methods

Fifteen normal adult cadaveric hemispheres (7 left hemispheres and 8 right hemispheres) were dissected using the Klingler white matter microdissection technique.[16,20] All hemispheres were previously fixed in a 15% formalin solution for a minimum period of 3 months. The arachnoid membrane and vessels were meticulously removed before the freezing process and the sulcogyral anatomy was thoroughly recorded. Prior to dissection, the most prominent superficial landmarks including the premotor gyrus; the superior, middle, and inferior frontal gyri; the precentral, superior, inferior, and middle frontal sulci; and the pars opercularis, pars orbitalis, and pars triangularis were identified (Figs. 1A and 2A).

The FLS complex has been recorded by previous studies to reside in the area anterior to the precentral gyrus and to run parallel to the superior and inferior frontal sulci.[5] Therefore, stepwise lateral to medial dissections were used in all hemispheres (Figs. 1 and 2). Starting from the frontal lobe, the dissection process gradually extended in the entire frontoparietal lateral surface with the aim to demonstrate the relationship between the fibers belonging to the SLF and FLS complexes. During the initial steps of the procedure the cortex of areas that represent the anatomical equivalent of the motor and premotor areas[30,33]—i.e., the primary motor area (Brodmann area [BA] 4) and the ventral and dorsal premotor areas (BAs 6 and 44)—has been spared in order to offer a better perspective of the trajectory and termination pattern of the FLS. In 5 hemispheres, the dissection proceeded up to the level of the frontal aslant tract (FAT) in order to offer a better understanding of the topographic and correlative anatomy of the FLS fibers with regard to the deeper frontal fiber white matter pathways.

All dissections were performed using a Carl Zeiss OPMI microscope and surgical microdissectors, microscissors, and anatomical microforceps. During the study, multiple photographs were obtained from various angles and points of view, with the aim being to vividly illustrate the regional anatomy.

Results

Morphology, Fiber Allocation, and Connectivity of the FLS

The frontal longitudinal fiber system was identified and recorded in all studied hemispheres. In 80% of cases, where a prominent middle frontal sulcus was present, the FLS was encountered just under the most superficial U-fibers at the depth of the sulcus (Figs. 1 and 2). In the remaining 20% of cases, where the middle frontal sulcus was absent, the FLS was seen to lie just medial to the superficial U-fibers of the middle frontal gyrus. It was further found to consist of a superficial layer of fibers, which closely resembled the U-fibers and interconnected adjacent areas within the precentral, middle, superior, and inferior frontal gyri, and of a deeper layer of longer fibers connecting more remote areas in the premotor and prefrontal cortex (Fig. 3).

In addition, the fibers of the FLS were observed to travel in two discrete levels; i.e., a superior and an inferior level, therefore resembling a superior and inferior longitudinal chain of fibers (as described by Catani et al.). In 2 of 15 hemispheres the superior and inferior longitudinal chains represented two completely separate groups of fibers with a clear cleavage plane between them (Fig. 1C; also see Fig. 5A). In this case the superior chain was seen to run from the dorsal premotor cortex toward the middle part of the superior frontal gyrus (BA 9), whereas the inferior chain was seen to connect the ventral premotor cortex (that is, the ventral precentral gyrus and pars opercularis [BA 44]) with the pars triangularis (BA 45), pars orbitalis (BA 47), middle frontal gyrus (BA 46), and frontal pole. In the rest of the hemispheres the superior and inferior longitudinal

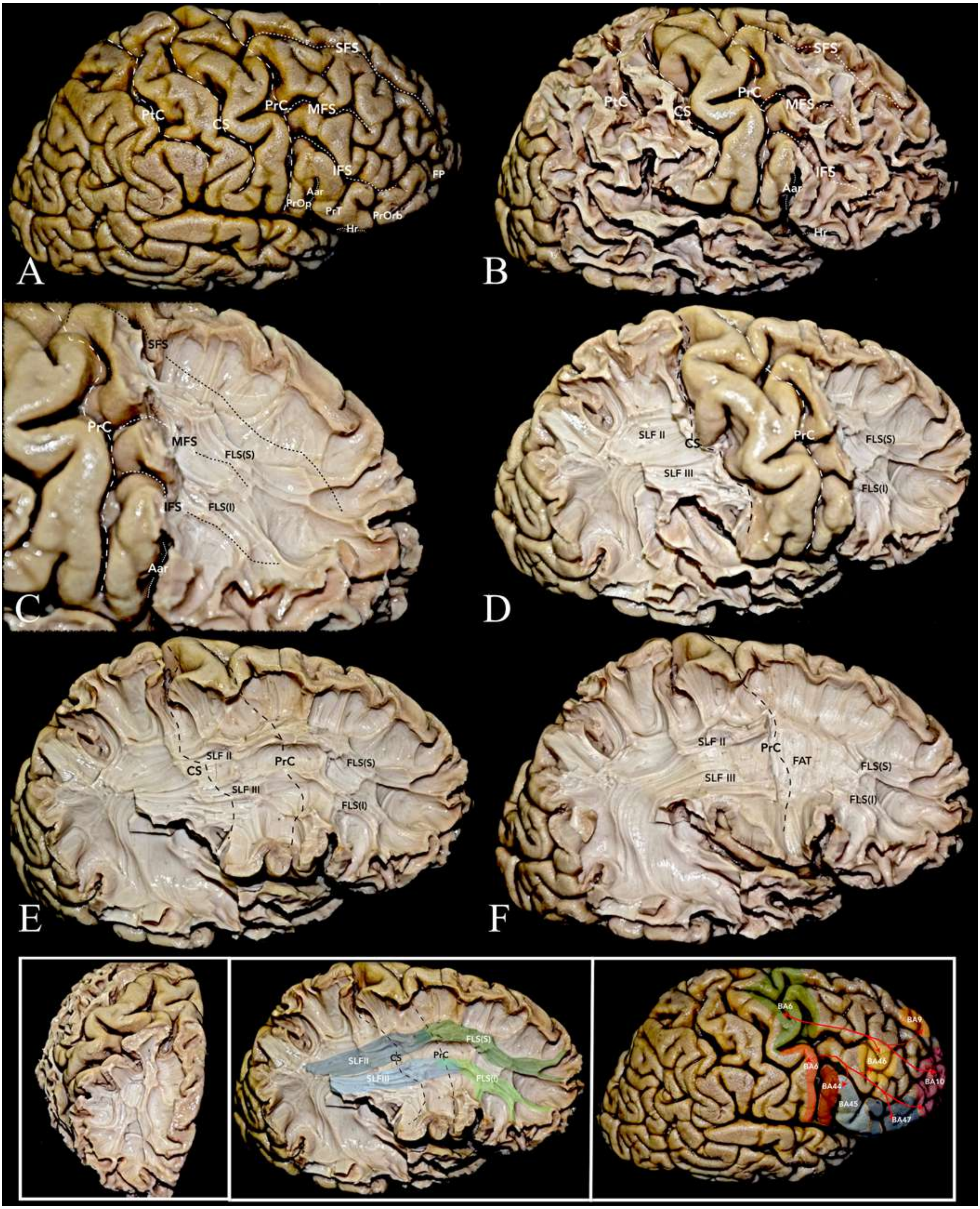

FIG. 1. Progressive dissection of a right hemisphere illustrating the structural morphology and correlative anatomy of the FLS. **A:** The lateral surface anatomy is illustrated. Note the presence and location of the middle frontal sulcus. **B:** After removing the cortical gray matter the superficial U-fibers become evident. The cortex of the area of the precentral gyrus and posterior frontal gyri (corresponding to the motor and premotor areas) has been spared. The contours of the main sulci are illustrated. **FIG. 1.** (*continued*)→

FIG. 1. C: A focused illustration of the frontal area after removing the U-fibers. The FLS is apparent as a superior and an inferior longitudinal system of fibers running parallel to the superior and inferior frontal sulci, respectively. Note that a clear plane between the two fiber systems is evident. **D:** Next step with the U-fibers removed at the temporoparietal areas and revealing the SLF complex; SLF II and SLF III tracts are illustrated. **E:** The cortex and U-fibers in the precentral/posterior-frontal area are removed, revealing the relationship of the fibers of the SLF and FLS. In this specimen, the two fiber tracts appear continuous, with the SLF II merging with the superior chain and the SLF III merging with the inferior chain of the FLS. Note that the fibers of SLF and FLS exhibit major terminations in the precentral area. We were not able to trace fibers of the FLS posterior to this level. **F:** The posterior part of the FLS has been removed, revealing the FAT interconnecting the superior and inferior frontal gyri. **Insets:** *Left*: Anterosuperior view of the FLS. *Center*: Relationship between the FLS and SLF. The SLF II and SLF III are highlighted in *dark blue* and *light blue*, respectively. The FLS(S) and FLS(I) are highlighted in *dark green* and *light green*, respectively. *Right*: Trajectory and connectivity of the FLS superimposed on the superficial anatomy. The different anatomofunctional areas are highlighted in different colors, as follows: *green*, dorsal premotor cortex (BA 6); *light red*, ventral premotor cortex (BA 6); *dark red*, ventral premotor cortex/pars opercularis (BA 44); *yellow*, dorsolateral prefrontal cortex (BA 46); *orange*, dorsolateral prefrontal cortex (BA 9); *light blue*, ventrolateral prefrontal cortex/pars triangularis (BA 45); *dark blue*, ventrolateral prefrontal cortex/pars orbitalis (BA 47); and *magenta*, rostrolateral prefrontal cortex/frontal pole (BA 10). (The anatomical boundaries of the premotor area have been defined according to findings of previous studies.[28,35]) Aar = anterior ascending ramus of the sylvian fissure; BA = Brodmann area; CS = central sulcus; FAT = frontal aslant tract; FLS(I) = frontal longitudinal system: inferior frontal longitudinal chain; FLS(S) = frontal longitudinal system: superior frontal longitudinal chain; FP = frontal pole; Hr = horizontal ramus of the sylvian fissure; IFS = inferior frontal sulcus; MFS = middle frontal sulcus; PrC = precentral sulcus; PrOp = pars opercularis; PrOrb = pars orbitalis; PrT = pars triangularis; PtC = postcentral sulcus; SFS = superior frontal sulcus; SLF II = superior longitudinal fasciculus II; SLF III = superior longitudinal fasciculus III. Figure is available in color online only.

chains were recorded to converge and form a single stem with no evident dissection plane between them (Figs. 2–4 and 5B–D).

The termination pattern of the FLS was relatively consistent (Figs. 1–4). Posteriorly, the fibers of the FLS were always observed to terminate to the ventral and dorsal premotor cortex. Terminations toward the precentral gyrus were also encountered in 67% of the cases. The dorsolateral prefrontal cortex (DLPFC, BA 46), pars triangularis (BA 45), and frontal pole (BA 10) received fibers in all of the studied hemispheres (100%), whereas in 47% of hemispheres the FLS also exhibited connections with the pars orbitalis (BA 47). Finally, fibers of the inferior frontal longitudinal chain were recorded to terminate toward BA 9 in 60% of the specimens. No variability or asymmetry between the right and left side concerning the morphology, topography, or termination pattern of the FLS was recorded.

Correlative Anatomy

Relationship Between the FLS and SLF

During the dissections, the FLS was seen to exhibit a variable relationship with the fibers of the SLF system (Figs. 1E, 2D, and 3C). This relationship was highly dependent on the termination pattern of the two fiber groups in the area of the posterior frontal lobe. In 20% of the studied hemispheres the SLF and FLS were recorded as two completely distinct tracts, with the former terminating in the area of the precentral gyrus anteriorly and the latter terminating in the posterior part of the superior, middle, and inferior frontal gyri posteriorly (Fig. 2D and right inset). In the majority (80%) of cases we macroscopically identified the FLS as the anterior extension of the SLF fiber system (Fig. 1E and center inset, Fig. 3C and lower inset). Specifically, the SLF III was observed to blend with the fibers of the inferior chain of the FLS in the area of the ventral precentral gyrus and pars opercularis, therefore exhibiting an anatomical continuity. The same applies for the middle component of the SLF complex, namely the SLF II, which was recorded to intermingle with fibers of the superior chain of the FLS in the area of the dorsal part of the precentral gyrus and posterior part of the middle and superior frontal gyri. Nonetheless, the FLS exhibits a different architecture compared to the SLF. First, the uppermost fibers recorded to belong to the FLS reside in a more superficial plane than the relevant fibers of the SLF. Second, the fibers of the FLS follow a significantly shorter trajectory compared to those of the SLF, in order to connect adjacent frontal areas. It has to be highlighted that we did not identify and isolate any of the FLS fibers beyond the level of the precentral gyrus.

Although the FLS displays a similar trajectory to the SLF, resembling its anterior continuation, it certainly exhibits significant morphological differences and therefore these tracts should be considered as two discrete anatomical entities.

Relationship of the FLS With the FAT

The FLS was documented to travel in a more superficial plane than that of the FAT. Therefore, removing the fibers of the FLS at the level of the precentral gyrus, pars orbitalis, and pars triangularis reveals the vertical fibers of the FAT seen to course from the posterior part of the superior frontal gyrus to the ventral part of the precentral gyrus and the posterior part of the inferior frontal gyrus, including the pars orbitalis and pars triangularis (Figs. 1F and 2E).

The FLS morphology, correlative anatomy, and cortical terminations are summarized in Table 1.

Discussion

The motor and premotor areas have been constantly in the epicenter of various anatomical and functional endeavors in both humans and nonhuman primates. Since the era of Penfield, Jasper, and Rasmussen, data stemming from microstimulation, cytoarchitectonic, neurophysiological, and functional studies have paved the way toward a better and more comprehensive understanding of the organization and connectivity of the motor and premotor circuitry. The initial conceptualization of these regions as discrete units underpinning the pure execution of motor actions has been overshadowed during recent decades. Various field researchers nowadays support the notion that specific motor and cognitive functions interact and share common anatomofunctional substrates.[6,23,34,37,38] Thus, the emerging

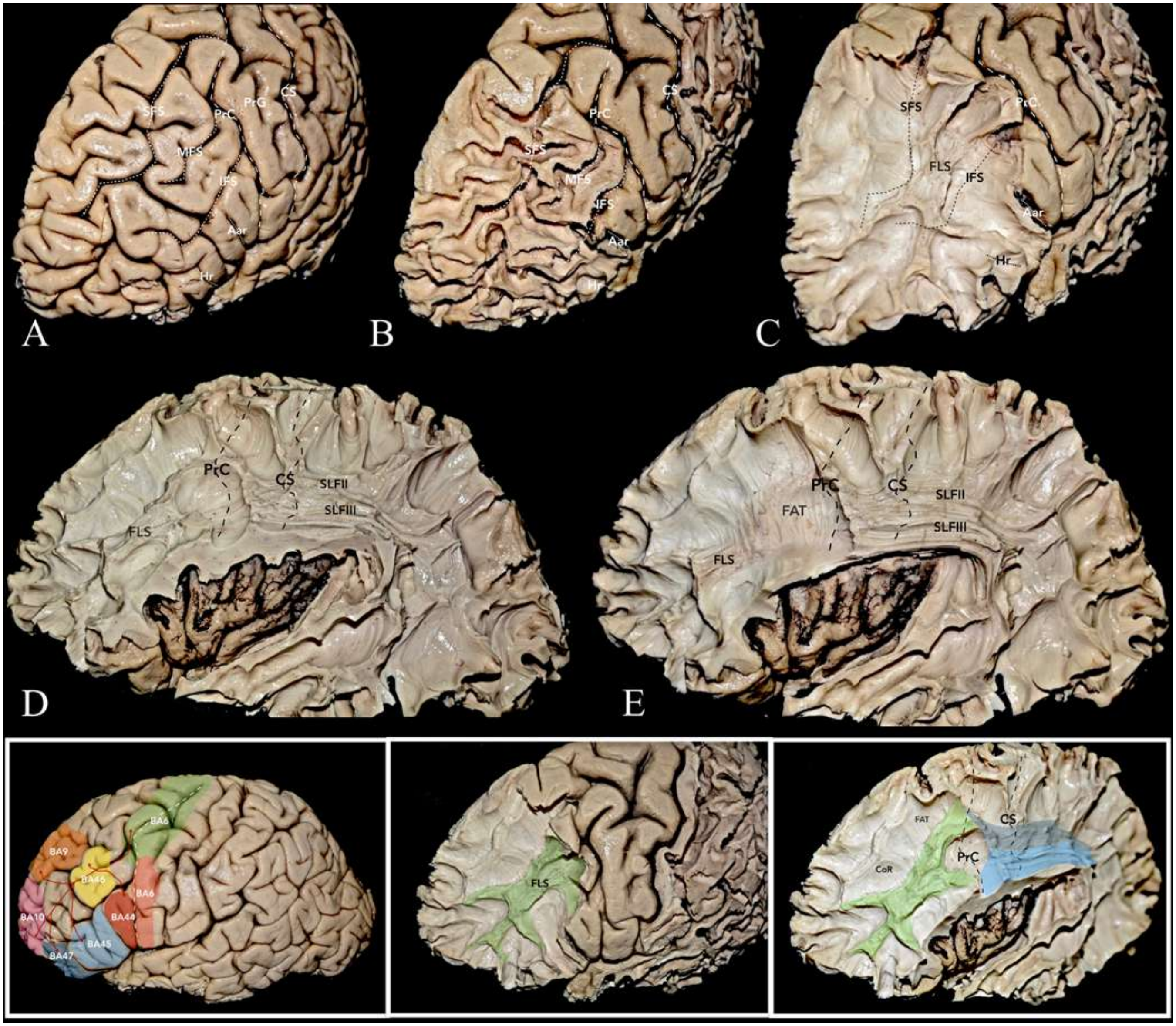

FIG. 2. Progressive dissection of a left hemisphere illustrating the morphology, correlative anatomy, and connectivity of the FLS. **A:** Anterosuperior view and surface anatomy of a left lateral cerebral surface. **B:** Superficial U-fibers and the contour of the main frontal sulci are illustrated. **C:** The FLS is revealed as a single stem of fibers traveling as far as the area of the frontal pole. The superior fibers of the FLS can be seen terminating toward the superior frontal gyrus, frontal pole, and posterior part of the middle frontal gyrus. The inferior fibers of the FLS terminate in the pars opercularis, pars triangularis, pars orbitalis, and frontal pole. **D:** Lateral view. The U-fibers of the lateral aspect of the hemisphere have been removed to show the relationship between the fibers of the FLS and SLF. In this case the SLF terminates abruptly in the area of the precentral gyrus. The superior fibers of the FLS terminate posteriorly in the same area, whereas the inferior fibers terminate in the pars opercularis. No anatomical continuity between the fibers of the two systems is documented in this specimen. **E:** After removing the posterior part of the FLS the fibers of the FAT—connecting the posterior parts of the superior frontal gyrus and inferior frontal gyrus —become evident. **Insets:** *Left*: Cortical terminations and trajectory of the FLS superimposed on the superficial anatomy. The different anatomofunctional areas are highlighted in different colors, as follows: *green*, dorsal premotor cortex (BA 6); *red*, ventral premotor cortex (BA 6); *dark red*, ventral premotor cortex/pars opercularis (BA 44); *yellow*, dorsolateral prefrontal cortex (BA 46); *orange*, dorsolateral prefrontal cortex (BA 9); *light blue*, ventrolateral prefrontal cortex/pars triangularis (BA 45); *dark blue*, ventrolateral prefrontal cortex/pars orbitalis (BA 47); and *magenta*, rostrolateral prefrontal cortex/frontal pole (BA 10). (The anatomical boundaries of the premotor area have been defined according to findings of previous studies.[28,35]) *Center*: Morphology of the FLS highlighted in *green*. *Right*: The correlative anatomy and termination plane of the FLS and SLF is illustrated. The SLF II and SLF III are highlighted in *dark blue* and *light blue*, respectively, whereas the FLS is highlighted in *green*. The level of the precentral and central gyri is also illustrated. Note the different termination plane of the two fiber tracts. Aar = anterior ascending ramus of the sylvian fissure; BA = Brodmann area; CoR = corona radiata; CS = central sulcus; FAT = frontal aslant tract; FLS = frontal longitudinal system; Hr = horizontal ramus of the sylvian fissure; IFS = inferior frontal sulcus; MFS = middle frontal sulcus; PrC = precentral sulcus; PrG = precentral gyrus; SFS = superior frontal sulcus; SLFII = superior longitudinal fasciculus II; SLFIII = superior longitudinal fasciculus III. Copyright Christos Koutsarnakis. Published with permission. Figure is available in color online only.

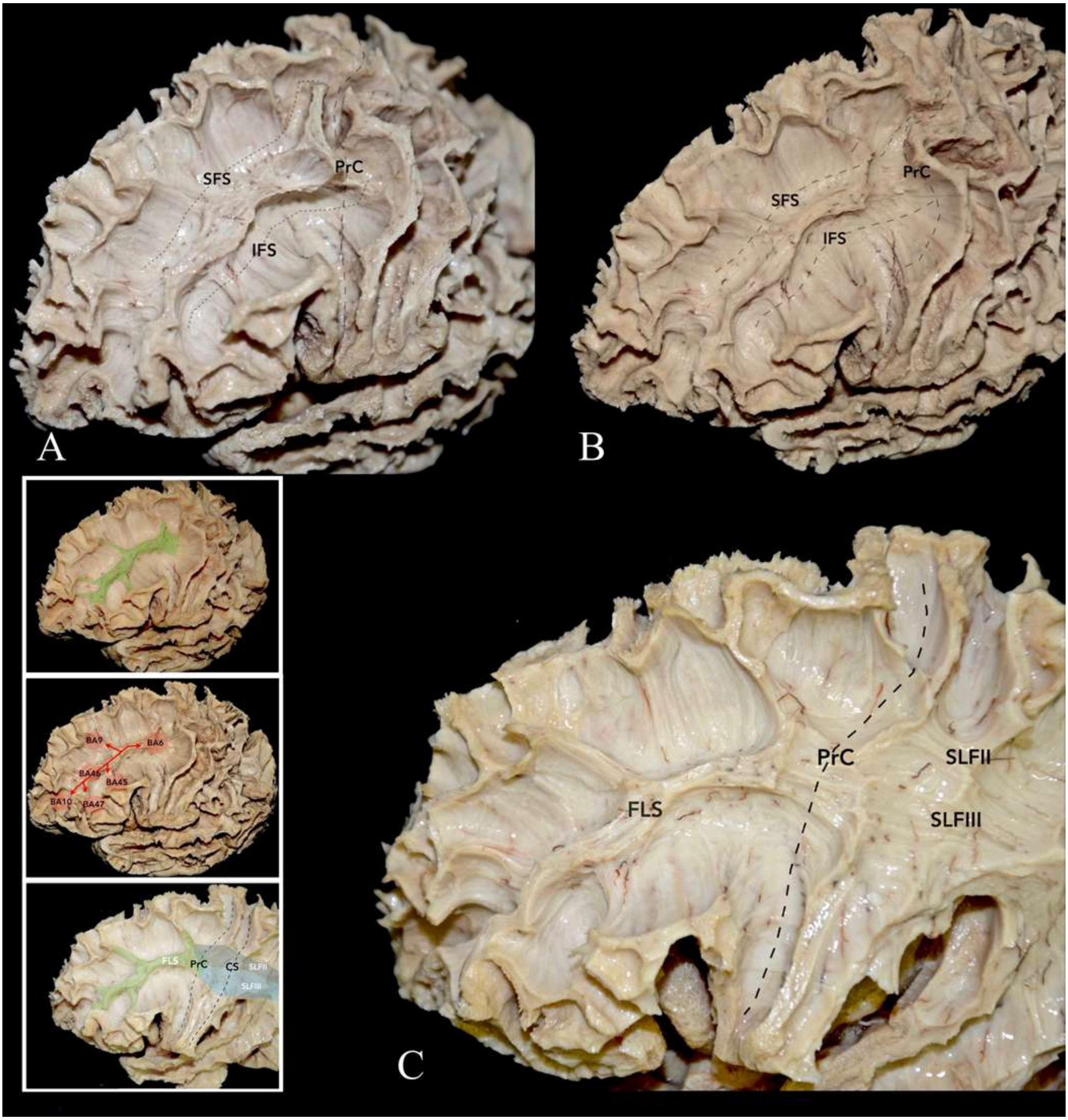

FIG. 3. Anterosuperior views of a dissected left hemisphere. Allocation of fibers within the FLS and relationship between the FLS and SLF fibers. **A:** After the most superficial U-fibers of the frontal area have been removed, the superficial fiber layer of the FLS is revealed. This layer blends with the deep U-fibers of the middle frontal gyrus to interconnect adjacent areas of the middle, superior, and inferior frontal gyri. **B:** Removing the superficial layer of the FLS reveals the deeper fibers contributing to this system. These fibers follow a longer trajectory compared to the fibers of the superficial layer and connect more remote areas in the frontal lobe. **C:** In the same specimen, the FLS appears as an anterior extension of the SLF system. **Insets:** *Upper:* Morphology of the FLS highlighted in *green. Center:* Connectivity and trajectory of the FLS. *Lower:* Relationship between the FLS and SLF fibers. The FLS is highlighted in *green*, whereas the SLF II and SLF III are highlighted in *dark blue* and *light blue*, respectively. In this case, these two fiber systems appear macroscopically continuous. BA = Brodmann area; CS = central sulcus; FLS = frontal longitudinal system; IFS = inferior frontal sulcus; PrC = precentral sulcus; SFS = superior frontal sulcus; SLFII = superior longitudinal fasciculus II; SLFIII = superior longitudinal fasciculus III. Copyright Christos Koutsarnakis. Published with permission. Figure is available in color online only.

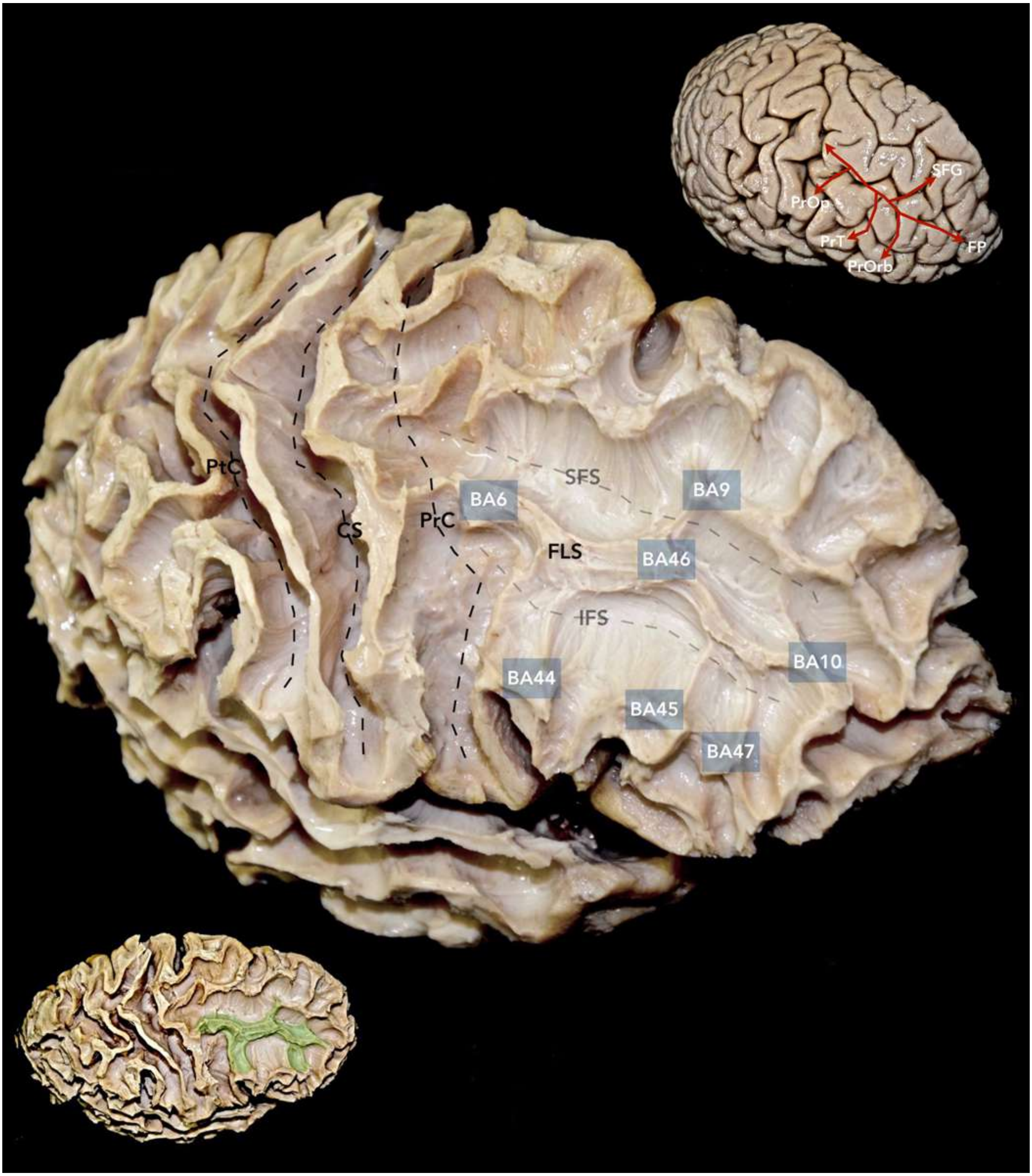

FIG. 4. Morphology and connectivity of the FLS. Anterosuperior view of a dissected right hemisphere. The FLS fibers are evident at the level of the middle frontal gyrus, traveling anteriorly as far as the frontal pole. The interconnected Brodmann areas are highlighted in *blue boxes*. **Insets:** *Upper right:* Trajectory and terminations of the FLS superimposed on the superficial anatomy. *Lower left:* Morphology of the FLS highlighted in *green*. BA = Brodmann area; CS = central sulcus; FLS = frontal longitudinal system; FP = frontal pole; IFS = inferior frontal sulcus; PrC = precentral sulcus; PrOp = pars opercularis; PrOrb = pars orbitalis; PrT = pars triangularis; PtC = postcentral sulcus; SFG = superior frontal gyrus; SFS = superior frontal sulcus. Copyright Christos Koutsarnakis. Published with permission. Figure is available in color online only.

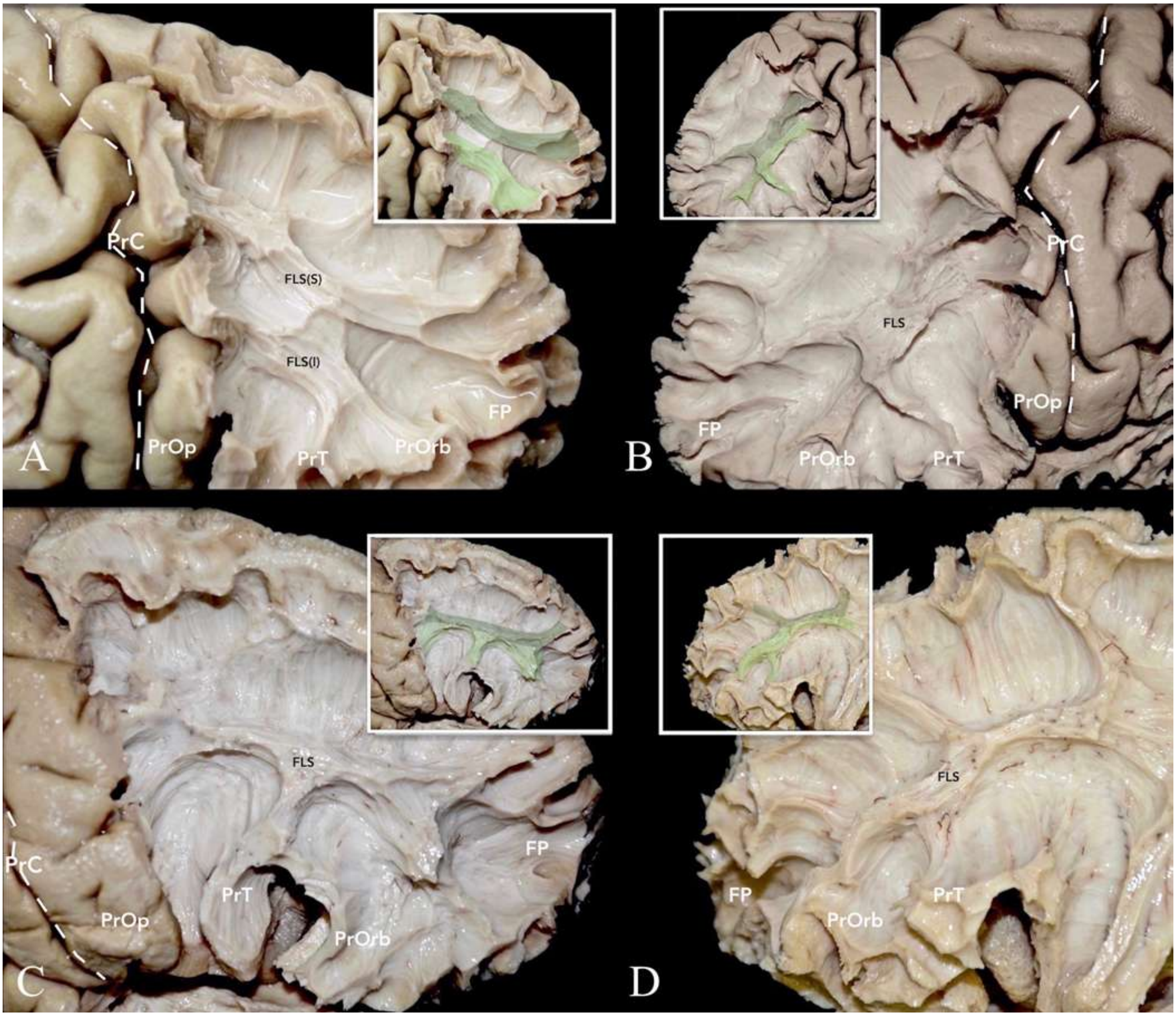

FIG. 5. Different morphological patterns of the FLS in two left and two right hemispheres. **A:** Right hemisphere. The FLS consists of two discrete fiber systems: a superior and an inferior frontal longitudinal chain. **Inset:** The superior and inferior frontal longitudinal fibers are highlighted in *dark green* and *light green*, respectively. **B:** Left hemisphere. The superior and inferior fibers of the FLS converge into a single stem with no clear cleavage plane between them. **Inset:** The superior and inferior fibers are highlighted in *dark green* and *light green*, respectively. **C:** Right hemisphere. Again, the fibers of the FLS form a single stem and travel as far as the frontal pole. **Inset:** The superior and inferior aspects of the FLS are highlighted again in *dark green* and *light green*, respectively. **D:** Left hemisphere. The fibers of the FLS form a single stem. **Inset:** Again the superior fibers are highlighted in *dark green*, whereas the inferior fibers are highlighted in *light green*. FLS(I) = frontal longitudinal system: inferior frontal longitudinal chain; FLS(S) = frontal longitudinal system: superior frontal longitudinal chain; FP = frontal pole; PrC = precentral sulcus; PrOp = pars opercularis; PrOrb = pars orbitalis; PrT = pars triangularis. Copyright Christos Koutsarnakis. Published with permission. Figure is available in color online only.

hypothesis, which strongly advocates that the premotor cortex acts as an intermediate hub between prefrontal and primary motor areas, has been gaining ground.[1,14,25] In this vein, the term "cognitive aspects of motor function" was recently coined to denote motor behaviors such as withholding and releasing of motor task responses; mental rotation; imagery; prediction of sequential motor, visual, and auditory patterns; and motor mimicking.[34]

Adding to this concept, previous studies have considered the rostral part of the premotor cortex—primarily the rostrodorsal premotor cortex—as an area believed to subserve mixed cognitive and motor functions.[12,39] The DLPFC in turn (including BAs 9 and 46), which is documented to mediate core cognitive high-order executive functions such as working memory, cognitive flexibility, and planning, has been documented to maintain a tight functional relationship with the premotor hub.[14] Studies in both nonhuman primates and humans support the hy-

TABLE 1. Anatomical characteristics of the FLS

Hemisphere & Laterality	MFS Present	Distinct FLS(S) & FLS(I) Present	Relationship to Superficial Anatomy	Cortical Terminations: Premotor Cortex: PCG (BAs 4 & 6)	Pst MFG &/or SFG (BA 6)	Pars Opercularis (BA 44)	Prefrontal Cortex: DLPFC (BA 46)	Pars Triangularis (BA 45)	Frontal Pole (BA 10)	Pars Orbitalis (BA 47)	DLPFC (BA 9)	Relationship to SLF
No. 1 (rt)	No	No	Just under MFG superficial U-fibers	Present	Present	Present	Present	Present	Present	Present	Not present	Continuous
No. 2 (lt)	Yes	No	Just under the depth of the MFS	Present	Present	Not present	Present	Present	Present	Not present	Present	Continuous
No. 3 (rt)	Yes	Yes	FLS(S): inf bank of the SFS; FLS(I): sup bank of the IFS	Not present	Present	Present	Present	Present	Present	Present	Present	Incontinuous
No. 4 (rt)	No	No	Just under MFG superficial U-fibers	Not present	Present	Present	Present	Present	Present	Present	Not present	Continuous
No. 5 (rt)	Yes	No	Just under the depth of the MFS	Present	Present	Present	Present	Present	Present	Not present	Present	Continuous
No. 6 (lt)	Yes	Yes	FLS(S): inf bank of the SFS; FLS(I): sup bank of the IFS	Present	Present	Present	Present	Present	Present	Present	Present	Continuous
No. 7 (lt)	Yes	No	Just under the depth of the MFS	Present	Present	Present	Present	Present	Present	Not present	Not present	Continuous
No. 8 (lt)	Yes	No	Just under the depth of the MFS	Present	Present	Present	Present	Present	Present	Not present	Not present	Continuous
No. 9 (rt)	Yes	No	Just under the depth of the MFS	Present	Present	Not present	Present	Present	Present	Not present	Not present	Continuous
No. 10 (lt)	Yes	No	Just under the depth of the MFS	Present	Present	Present	Present	Present	Present	Present	Present	Continuous
No. 11 (rt)	No	No	Just under MFG superficial U-fibers	Not present	Present	Present	Present	Present	Present	Present	Present	Continuous
No. 12 (lt)	Yes	No	Just under the depth of the MFS	Present	Present	Present	Present	Present	Present	Present	Present	Incontinuous
No. 13 (lt)	Yes	No	Just under the depth of the MFS	Not present	Present	Present	Present	Present	Present	Not present	Present	Continuous
No. 14 (rt)	Yes	No	Just under the depth of the MFS	Present	Present	Present	Present	Present	Present	Not present	Not present	Continuous
No. 15 (rt)	Yes	No	Just under the depth of the MFS	Not present	Present	Present	Present	Present	Present	Not present	Present	Continuous
Percentage				67%	100%	87%	100%	100%	100%	47%	60%	

BA = Brodmann area; DLPFC = dorsolateral prefrontal cortex; FLS(I) = FLS: inferior frontal longitudinal chain; FLS(S) = FLS: superior frontal longitudinal chain; IFS = inferior frontal sulcus; inf = inferior; MFG = middle frontal gyrus; MFS = middle frontal sulcus; PCG = precentral gyrus; Pst = posterior; SFG = superior frontal gyrus; SFS = superior frontal sulcus; sup = superior.

pothesis of an alleged circuitry serving the connectivity between the aforementioned areas.

However, little anatomical evidence exists to support this notion in the human cerebrum. In this context, recently Catani and colleagues focused on the short connections of the human frontal lobe and provided for the first time in the literature structural evidence supporting the presence of a fiber system that could potentially underpin a prefrontal-premotor circuitry. The authors revealed, through limited anatomical dissections in one cadaveric hemisphere further enhanced by a tailored tractographic protocol, a white matter pathway—which they named the frontal longitudinal system—consisting of short and long fibers traveling in the middle frontal gyrus and connecting the dorsolateral aspects of premotor and prefrontal regions.[5] According to them, the FLS comprised two segments, a superior and an inferior longitudinal chain of fibers, with the first running from the precentral gyrus to the middle and superior frontal gyri, and the latter from the precentral gyrus to the middle and inferior frontal gyri. They also observed that these fiber chains consistently converged toward the frontal pole.

This axonal connectivity pattern lends support to a prefrontal-premotor circuitry in the human brain. Nonetheless, since this first description there has been no additional structural evidence validating the existence of the FLS and further unveiling its morphology, topography, and inherent subcortical architecture. Our aim was therefore to address this gap and shed light on the exact anatomical characteristics of this tract by using the white matter fiber dissection technique as our main method of investigation. This resurgent anatomical procedure, which entails the so-called freeze-thaw preparation of cadaveric specimens, has been microscopically proven to preserve the structural integrity of the white matter axons, and therefore the results obtained are of high anatomical accuracy and reliability.[45] On the contrary, although the DTI tractographic method is a fast, noninvasive in vivo technique for exploring the cerebral white matter architecture, it is prone to anatomical inaccuracies, which can lead even to the reconstruction of so-called erroneous fiber tracts, mainly due to the "crossing," "kissing," and "bending" fiber effects and because of the differences observed in the postprocessing and acquisition parameters. As a consequence, DTI findings particularly regarding previously unrecognized or understudied white matter tracts have to be fully validated and confirmed by the more robust and anatomically accurate white matter dissection technique (see *Strengths and Limitations of the Study*).[22,40,41]

Indeed, by using the fiber microdissection technique we were able to consistently encounter a fiber system with the same gross anatomical characteristics as described by Catani and colleagues. According to our findings the FLS represents a system that comprises short superficial fibers connecting adjacent areas of the precentral, superior, middle, and inferior frontal gyri, and the frontal pole, as well as deeper fibers with longer configuration, which connect more distant loci of the same areas. The FLS was found to reside within the white matter of the middle frontal gyrus, running parallel to the superior and inferior frontal sulci as previously described. We could effectively distinguish two distinct fiber systems, representing the superior and inferior longitudinal chains, in only 13% of the studied hemispheres. In the majority of the dissected specimens the superior and inferior fibers of the FLS converged to form a single stem that was recorded to reach the area of the frontal pole.

In addition, the termination pattern of the FLS was meticulously studied and recorded. Interestingly, the most consistent cortical terminations of the FLS were those found toward the posterior part of the middle and superior frontal gyri, which anatomically correspond to the rostral part of the dorsal premotor cortex (BA 6), and those on the middle frontal gyrus as well as the anterior part of the superior frontal gyrus, corresponding to the DLPFC (BAs 46 and 9). This pattern of axonal connectivity is in line with data stemming from functional studies and documenting that the DLPFC and the rostral aspect of the dorsal premotor cortex hold a close functional relationship and represent the major hubs of the prefrontal-premotor crosstalking system.[12] The potential functional role of the areas implicated in the prefrontal-premotor circuitry possibly subserved by the FLS is summarized in Table 2.

During our dissections, special attention was given to the relationship between the frontal fibers assigned to the FLS and the fibers classically described as the SLF. The SLF has been recorded in the majority of previous DTI and fiber dissection studies to terminate in the area of the precentral gyrus (BAs 4 and 6) and the posterior part of the middle (BA 6) and inferior (BA 44) frontal gyri.[3,27,28,43] Terminations extending as far as the DLPFC (BAs 46 and 9) also have been described by some authors.[15,36,44] In a further step, Yagmurlu and colleagues identified and recorded possible terminations of the SLF II in the area of the frontal pole (BA 10) in 28% of the studied specimens.[44] Additionally, in the extended 2018 tractographic study titled "A connectomic atlas of the human cerebrum—Chapter 10: Tractographic description of the superior longitudinal fasciculus," Conner and colleagues advocate that the SLF complex extends as far as the posterior part of the middle and inferior frontal gyri as well as to a part of the DLPFC.[7] These authors further support the local functional and white matter connectivity between adjacent frontal areas including the premotor cortex, the dorsolateral and rostrolateral prefrontal cortices, and the frontal pole.[2]

We therefore aimed at defining the relationship between the SLF and the group of longitudinal fibers encountered in the frontal area, described by Catani and colleagues to compose the FLS. Our data support the notion that the FLS macroscopically resembles an anterior extension of the SLF fibers. Indeed, in the majority of the studied specimens we observed the two fiber pathways to exhibit structural continuity. The concept of hypersegmentation of the SLF has been advocated by previous authors.[27,29] In our view, the FLS should be treated as a separate segment within the superior longitudinal fiber system for two reasons. First, we recorded cases in which the FLS and SLF terminate in different areas and therefore constitute two distinct systems. Second, the FLS exhibits an intrinsic configuration and architecture that is different from that of the SLF, because its fibers seem to be confined and terminate exclusively in the area of the frontal lobe, in contrast

TABLE 2. Functional implications of prefrontal-premotor circuitry

	Cortical Terminations				
	Anatomical Area	BA	Functional Area	Functional Implications	References
Posterior terminations	Precentral gyrus	4 & 6	Primary motor cortex & caudal part of the dorsal & ventral premotor cortex	• Mediating voluntary movement • Controlling kinematic & dynamic parameters of movement • Executive aspect of motor action	6, 13, 38
	Posterior part of the MFG & SFG	6	Rostral part of the dorsal premotor cortex	• High-order motor planning • Motor imagery • Processing of information related to target location • Selection of suitable motor response • Switching between withholding & executing motor actions according to environmental cues • Control of motor impulse	1, 13, 23, 32, 33, 37, 38
	Posterior part of the IFG (pars opercularis)	44	Rostral part of the ventral premotor cortex*	• Processing of visuospatial features related to motion • Codes & modifies grasping & holding motor actions according to object characteristics • Hand movement organization & execution • Imagining & analyzing observed hand movements • Hand mental rotation • Action understanding & imitation	1, 24, 30, 33, 34, 37, 38
Anterior terminations	Middle third of the MFG	46	DLPFC	• Conversion of sensory signals into motor information • Motor planning in multiple-step tasks • Memorizing spatial locations or goals • Processing of visuospatial information • Integrates working memory into motor responses • Switching between withholding & executing motor actions according to environmental cues	14, 32, 37–39
	Middle third of the SFG	9			
	Pars orbitalis	47	Ventrolateral prefrontal cortex	• Right ventrolateral prefrontal cortex plays crucial role in motor inhibition • Stops or overrides motor responses	10, 24
	Pars triangularis	45			
	Frontal pole	10	Rostrolateral prefrontal cortex	• Organization of subtasks within a motor-behavioral sequence in the absence of external cues	9

BA = Brodmann area; DLPFC = dorsolateral prefrontal cortex; IFG = inferior frontal gyrus; MFG = middle frontal gyrus; SFG = superior frontal gyrus.

* The pars opercularis has been considered a part of the ventrolateral prefrontal cortex. However, modern studies deem this area to be homologous to area F5 of the nonhuman primate brain and therefore part of the ventral premotor cortex.[32]

to the fibers of the SLF complex. In any case, these fiber pathways possibly share a pivotal role in the connectivity of the motor and premotor areas, with the SLF allegedly subserving its caudal connectivity and the FLS its rostral connectivity.

To our knowledge this is the first focused and dedicated anatomical study that sheds light into the exact anatomical and morphological characteristics of this frontal fiber system. Our data could offer valuable insights into the connectivity of the premotor area and furthermore act as a sound anatomical basis for the tight functional relationship revealed between the prefrontal and premotor areas.

Strengths and Limitations of the Study

The fiber microdissection technique originally described by Klingler[16] has been revived and recruited during the last years as a valuable tool offering new insights in the field of white matter anatomy. This process includes the fixation of cerebral hemispheres in a formalin solution followed by a freeze-thaw process. The latter acts by interfering solely with the extracellular matrix, thus separating the axons without affecting the structural integrity of the white matter, as proven by recent studies.[45] Therefore, the results obtained by the Klingler microdissection technique can be considered extremely accurate and may offer significant contributions to the understanding of the infrastructure of the white matter pathways within the human cerebrum. Additionally, recent studies report that the fiber microdissection technique can provide safe conclusions concerning the cortical termination pattern of the white matter fiber tracts.[29,35] Due to the aforementioned reasons this technique represents the gold standard for challenging the validity of DTI studies. This also resonates with previous studies that we have conducted, offering robust anatomical details on the intrinsic and correlative anatomy of various fiber tracts.[17–19,21]

Conversely, the fiber dissection technique is a time-consuming, expensive, in vitro procedure offering lower spatial resolution when compared to histology and optical coherence tomography.[8,11,26,31,42] Additionally, the sensitivity of the procedure is significantly lower when studying crossing fiber tracts, because the dissection of one fiber tract may interfere with the structural integrity of the other.

Incorporating the fiber dissection technique into the study of the frontal longitudinal fibers has been proven to offer two significant benefits. First, the FLS is a fiber system traveling within the prefrontal white matter, an area where the percentage of crossing fibers is significantly lower when compared to other regions in which various tracts intermingle. This eliminates the possibility of erroneous results according to what has been previously mentioned. Second, one of the basic goals of this study was to investigate the spatial relationship of the fibers of the FLS and the SLF. These fibers share a common trajectory, do not cross at any point, and can therefore be steadily recognized and followed under the microscope by using the Klingler technique. This allows the operator to examine the intrinsic characteristics of the two systems by following their fibers and recording their distribution, length, and termination pattern.

Conclusions

Using a focused microdissection technique, we were able to consistently identify and record the FLS as a group of fibers that reside within the frontal area and run between the prefrontal and premotor cortices. We additionally managed to elucidate its exact anatomical characteristics including its morphology, correlative anatomy, and termination pattern, and readily differentiate it from adjacent groups of fibers such as the SLF. Our results provide sound structural evidence supporting the theory of a direct axonal connectivity between the prefrontal and premotor cortices, which are believed to underpin the cognitive aspect of motor behavior. Future studies are definitely needed in order to refine the FLS structure to function relationship and offer a more sophisticated concept of the circuitry conveying motor actions.

References

1. Abe M, Hanakawa T, Takayama Y, Kuroki C, Ogawa S, Fukuyama H: Functional coupling of human prefrontal and premotor areas during cognitive manipulation. **J Neurosci 27:**3429–3438, 2007
2. Baker CM, Burks JD, Briggs RG, Conner AK, Glenn CA, Morgan JP, et al: A connectomic atlas of the human cerebrum—Chapter 2: The lateral frontal lobe. **Oper Neurosurg (Hagerstown) 15 (Suppl 1):**S10–S74, 2018
3. Bernal B, Altman N: The connectivity of the superior longitudinal fasciculus: a tractography DTI study. **Magn Reson Imaging 28:**217–225, 2010
4. Bozkurt B, Yagmurlu K, Middlebrooks EH, Cayci Z, Cevik OM, Karadag A, et al: Fiber connections of the supplementary motor area revisited: methodology of fiber dissection, DTI, and three dimensional documentation. **J Vis Exp (123):**55681, 2017
5. Catani M, Dell'acqua F, Vergani F, Malik F, Hodge H, Roy P, et al: Short frontal lobe connections of the human brain. **Cortex 48:**273–291, 2012
6. Chouinard PA, Paus T: The primary motor and premotor areas of the human cerebral cortex. **Neuroscientist 12:**143–152, 2006
7. Conner AK, Briggs RG, Rahimi M, Sali G, Baker CM, Burks JD, et al: A connectomic atlas of the human cerebrum—Chapter 10: Tractographic description of the superior longitudinal fasciculus. **Oper Neurosurg (Hagerstown) 15 (Suppl 1):**S407–S422, 2018
8. Dammers J, Axer M, Grässel D, Palm C, Zilles K, Amunts K, et al: Signal enhancement in polarized light imaging by means of independent component analysis. **Neuroimage 49:**1241–1248, 2010
9. Desrochers TM, Chatham CH, Badre D: The necessity of rostrolateral prefrontal cortex for higher-level sequential behavior. **Neuron 87:**1357–1368, 2015
10. Duque J, Labruna L, Verset S, Olivier E, Ivry RB: Dissociating the role of prefrontal and premotor cortices in controlling inhibitory mechanisms during motor preparation. **J Neurosci 32:**806–816, 2012
11. Goergen CJ, Radhakrishnan H, Sakadžić S, Mandeville ET, Lo EH, Sosnovik DE, et al: Optical coherence tractography using intrinsic contrast. **Opt Lett 37:**3882–3884, 2012
12. Hanakawa T: Rostral premotor cortex as a gateway between motor and cognitive networks. **Neurosci Res 70:**144–154, 2011
13. Hardwick RM, Rottschy C, Miall RC, Eickhoff SB: A quantitative meta-analysis and review of motor learning in the human brain. **Neuroimage 67:**283–297, 2013
14. Hoshi E: Functional specialization within the dorsolateral prefrontal cortex: a review of anatomical and physiological

studies of non-human primates. **Neurosci Res 54:**73–84, 2006
15. Kamali A, Flanders AE, Brody J, Hunter JV, Hasan KM: Tracing superior longitudinal fasciculus connectivity in the human brain using high resolution diffusion tensor tractography. **Brain Struct Funct 219:**269–281, 2014
16. Klingler J: Erleichterung der makrokopischen Präparation des Gehirns durch den Gefrierprozess. **Schweiz Arch Neurol Psych 36:**247–256, 1935
17. Komaitis S, Skandalakis GP, Kalyvas AV, Drosos E, Lani E, Emelifeonwu J, et al: Dorsal component of the superior longitudinal fasciculus revisited: novel insights from a focused fiber dissection study. **J Neurosurg** [epub ahead of print March 1, 2019; DOI: 10.3171/2018.11.JNS182908]
18. Koutsarnakis C, Kalyvas AV, Komaitis S, Liakos F, Skandalakis GP, Anagnostopoulos C, et al: Defining the relationship of the optic radiation to the roof and floor of the ventricular atrium: a focused microanatomical study. **J Neurosurg 130:**1728–1739, 2019
19. Koutsarnakis C, Kalyvas AV, Skandalakis GP, Karavasilis E, Christidi F, Komaitis S, et al: Sledge runner fasciculus: anatomic architecture and tractographic morphology. **Brain Struct Funct 224:**1051–1066, 2019
20. Koutsarnakis C, Liakos F, Kalyvas AV, Sakas DE, Stranjalis G: A laboratory manual for stepwise cerebral white matter fiber dissection. **World Neurosurg 84:**483–493, 2015
21. Koutsarnakis C, Liakos F, Liouta E, Themistoklis K, Sakas D, Stranjalis G: The cerebral isthmus: fiber tract anatomy, functional significance, and surgical considerations. **J Neurosurg 124:**450–462, 2016
22. Le Bihan D, Poupon C, Amadon A, Lethimonnier F: Artifacts and pitfalls in diffusion MRI. **J Magn Reson Imaging 24:**478–488, 2006
23. Lee JH, van Donkelaar P: The human dorsal premotor cortex generates on-line error corrections during sensorimotor adaptation. **J Neurosci 26:**3330–3334, 2006
24. Levy BJ, Wagner AD: Cognitive control and right ventrolateral prefrontal cortex: reflexive reorienting, motor inhibition, and action updating. **Ann N Y Acad Sci 1224:**40–62, 2011
25. Lu MT, Preston JB, Strick PL: Interconnections between the prefrontal cortex and the premotor areas in the frontal lobe. **J Comp Neurol 341:**375–392, 1994
26. Magnain C, Augustinack JC, Reuter M, Wachinger C, Frosch MP, Ragan T, et al: Blockface histology with optical coherence tomography: a comparison with Nissl staining. **Neuroimage 84:**524–533, 2014
27. Makris N, Kennedy DN, McInerney S, Sorensen AG, Wang R, Caviness VS Jr, et al: Segmentation of subcomponents within the superior longitudinal fascicle in humans: a quantitative, in vivo, DT-MRI study. **Cereb Cortex 15:**854–869, 2005
28. Martino J, De Lucas EM: Subcortical anatomy of the lateral association fascicles of the brain: a review. **Clin Anat 27:**563–569, 2014
29. Martino J, De Witt Hamer PC, Berger MS, Lawton MT, Arnold CM, de Lucas EM, et al: Analysis of the subcomponents and cortical terminations of the perisylvian superior longitudinal fasciculus: a fiber dissection and DTI tractography study. **Brain Struct Funct 218:**105–121, 2013
30. Mayka MA, Corcos DM, Leurgans SE, Vaillancourt DE: Three-dimensional locations and boundaries of motor and premotor cortices as defined by functional brain imaging: a meta-analysis. **Neuroimage 31:**1453–1474, 2006
31. Palm C, Axer M, Gräßel D, Dammers J, Lindemeyer J, Zilles K, et al: Towards ultra-high resolution fibre tract mapping of the human brain – registration of polarised light images and reorientation of fibre vectors. **Front Hum Neurosci 4:**9, 2010
32. Pilacinski A, Wallscheid M, Lindner A: Human posterior parietal and dorsal premotor cortex encode the visual properties of an upcoming action. **PLoS One 13:**e0198051, 2018
33. Preuss TM, Stepniewska I, Kaas JH: Movement representation in the dorsal and ventral premotor areas of owl monkeys: a microstimulation study. **J Comp Neurol 371:**649–676, 1996
34. Rizzolatti G, Fogassi L, Gallese V: Motor and cognitive functions of the ventral premotor cortex. **Curr Opin Neurobiol 12:**149–154, 2002
35. Sarubbo S, De Benedictis A, Maldonado IL, Basso G, Duffau H: Frontal terminations for the inferior fronto-occipital fascicle: anatomical dissection, DTI study and functional considerations on a multi-component bundle. **Brain Struct Funct 218:**21–37, 2013
36. Schmahmann JD, Smith EE, Eichler FS, Filley CM: Cerebral white matter: neuroanatomy, clinical neurology, and neurobehavioral correlates. **Ann N Y Acad Sci 1142:**266–309, 2008
37. Schubotz RI, Anwander A, Knösche TR, von Cramon DY, Tittgemeyer M: Anatomical and functional parcellation of the human lateral premotor cortex. **Neuroimage 50:**396–408, 2010
38. Schubotz RI, von Cramon DY: Functional-anatomical concepts of human premotor cortex: evidence from fMRI and PET studies. **Neuroimage 20 (Suppl 1):**S120–S131, 2003
39. Simon SR, Meunier M, Piettre L, Berardi AM, Segebarth CM, Boussaoud D: Spatial attention and memory versus motor preparation: premotor cortex involvement as revealed by fMRI. **J Neurophysiol 88:**2047–2057, 2002
40. Thomas C, Ye FQ, Irfanoglu MO, Modi P, Saleem KS, Leopold DA, et al: Anatomical accuracy of brain connections derived from diffusion MRI tractography is inherently limited. **Proc Natl Acad Sci U S A 111:**16574–16579, 2014
41. Vos SB, Jones DK, Viergever MA, Leemans A: Partial volume effect as a hidden covariate in DTI analyses. **Neuroimage 55:**1566–1576, 2011
42. Wang H, Black AJ, Zhu J, Stigen TW, Al-Qaisi MK, Netoff TI, et al: Reconstructing micrometer-scale fiber pathways in the brain: multi-contrast optical coherence tomography based tractography. **Neuroimage 58:**984–992, 2011
43. Wang X, Pathak S, Stefaneanu L, Yeh FC, Li S, Fernandez-Miranda JC: Subcomponents and connectivity of the superior longitudinal fasciculus in the human brain. **Brain Struct Funct 221:**2075–2092, 2016
44. Yagmurlu K, Middlebrooks EH, Tanriover N, Rhoton AL Jr: Fiber tracts of the dorsal language stream in the human brain. **J Neurosurg 124:**1396–1405, 2016
45. Zemmoura I, Blanchard E, Raynal PI, Rousselot-Denis C, Destrieux C, Velut S: How Klingler's dissection permits exploration of brain structural connectivity? An electron microscopy study of human white matter. **Brain Struct Funct 221:**2477–2486, 2016

Disclosures

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

Author Contributions

Conception and design: Komaitis, Koutsarnakis. Acquisition of data: Komaitis, Koutsarnakis, Kalyvas, Skandalakis, Drosos, Lani. Analysis and interpretation of data: Komaitis, Koutsarnakis, Kalyvas, Skandalakis, Liouta, Liakos, Piagkou, Emelifeonwu. Drafting the article: Komaitis, Koutsarnakis, Kalamatianos. Critically revising the article: Koutsarnakis, Stranjalis. Reviewed submitted version of manuscript: Koutsarnakis, Komaitis. Approved the final version of the manuscript on behalf of all authors: Koutsarnakis. Study supervision: Koutsarnakis, Stranjalis.

Correspondence

Christos Koutsarnakis: Evangelismos Hospital, University of Athens, Athens, Greece. ckouts@hotmail.co.uk.