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DIRECT INSIDE-OUT OBSERVATION OF SUPERFICIAL WHITE MATTER FASCICULI IN THE HUMAN BRAIN

^{a,b}Guillaume Dannhoff*; ^bAlex Morichon*; ^bMykyta Smirnov, PhD; ^bLaurent Barantin, PhD;

^{b,c}Christophe Destrieux, MD, PhD; ^bIgor Lima Maldonado, MD, PhD

^a Centre Hospitalier Régional Universitaire de Strasbourg, Strasbourg, France

^b Inserm U1253, iBrain, Université de Tours, Tours, France

^c CHRU de Tours, Tours, France

* These authors contributed equally to this work

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Corresponding author

Igor Lima Maldonado, M.D., Ph.D.

UMR 1253, iBrain, Université de Tours, Inserm, Tours, France

10 Boulevard Tonnellé - 37032 Tours, France

Tel: (00-33) 2 47 36 61 37; Fax (00 33) 2 47 36 62 07

limamaldonado@univ-tours.fr

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ABSTRACT

Background: Recent methodological advances in the study of the cerebral white matter have left short association fibers relatively underexplored due to their compact and juxtacortical nature, which represent significant challenges for both post-mortem post-cortex removal dissection and magnetic resonance-based diffusion imaging. **Objective:** To introduce a novel inside-out post-mortem fiber dissection technique to assess short association fiber anatomy.

Methods: Six cerebral specimens were obtained from a body donation program and underwent fixation in formalin. Following two freezing and thawing cycles, a standardized protocol involving peeling fibers from deep structures towards the cortex was developed.

Results: The inside-out technique effectively exposed the superficial white matter. The procedure revealed distinguishable intergyral fibers, demonstrating their dissectability and enabling the identification of their orientation. The assessment of layer thickness was possible through direct observation and ex vivo morphological magnetic resonance imaging.

Conclusion: The inside-out fiber technique effectively demonstrates intergyral association fibers in the post-mortem human brain. It adds to the neuroscience armamentarium, overcoming methodological obstacles and offering an anatomical substrate essential for neural circuit modeling and the evaluation of neuroimaging congruence.

KEYWORDS (MeSH terms): superficial white matter; cerebral cortex; connectome; brain mapping ; u-fibers; white matter; neuroanatomy; connectivity

IMPACT STATEMENT: The inside-out fiber dissection technique enables a totally new perception of cerebral connectivity as the observer navigates inside the parenchyma and looks toward the cerebral surface with the subcortical white matter and the cortical mantle in place. This approach has proven very effective for exposing intergyral association fibers, which have shown to be much more distinguishable from an inner perspective. It gave rise to unprecedented images of the human superficial white matter and allowed, for the first time, direct observation of this vast mantle of fascicles on entire cerebral hemisphere aspects.

INTRODUCTION

Although historically under-evaluated, white matter (WM) anatomical explorations have been the subject of recent research focus due to methodological advances and the development of the connectionist model of brain function(Thiebaut De Schotten and Forkel, 2022). Previous literature has described large WM structures in detail(Dejerine, Joseph Jules, 1895; Forkel et al., 2015); however, accurate descriptions of the superficial layers remain limited(Meynert, 1888a; Sachs, 1893).

A closer examination of the literature allows us to appreciate the evolving trajectory of knowledge in this field, marked by changing priorities over time. The study of cerebral white matter extends over more than three centuries, but it witnessed a transformative period in the late 19th and early 20th century with seminal contributions such as those of Theodor Meynert, Joseph Jules Dejerine, and Heinrich Sachs. Notably, Meynert's profound influence lies in his pioneering work categorizing the cerebral cortex into distinct but interconnected areas, laying the foundation for our future endeavor in understanding white matter connectivity and the spatial organization of neural networks (Meynert, 1885). Additionally, Dejerine's research using serial sectioning significantly expanded our understanding of cerebral white matter and its crucial role in neurological function (Dejerine, 1895). Sachs's meticulous studies were dedicated to elucidating the intricate connectivity between different brain regions (Sachs, 1893). However, much of the 20th century saw white matter research recede into the background as gray matter, neurotransmitters, and synaptic connections garnered more attention. This era of relative neglect was, to a great extent, a consequence of methodological constraints.

With the introduction of Klingler's technique in the 1950s(Klingler, 1956), fiber dissection became the method of choice for the *post-mortem* anatomical demonstration of macroscopic fasciculi in the human brain. Optical and electronic microscopy investigations have confirmed that freezing-thawing cycles spread fiber bundles, as method first suggested by Ludwig and Klingler(Zemmoura et al., 2016). These cycles cause water volumes to increase due to crystallization, increasing the space between fibers without disrupting the fibers themselves, which renders the fibers macroscopically discernible and easier to dissect. As this technique is not influenced by diffusion artifacts, it has often been used as a source of independent results to discuss results obtained through tractography(Catani et al., 2012a; Fernández-Miranda et al., 2008; Magro et al., 2014).

However, both classical fiber dissection and imaging techniques are associated with significant difficulties when addressing superficial WM (SWM). In fiber dissection, the compact character of superficial fibers impairs their distinguishability and dissectability, resulting in the removal of "petal-like" packets of fibers that are associated with a significant loss of anatomical information. The subcortical location of these delicate structures, combined with the relative lack of interest in them compared to the deeper fasciculi, often results in their sacrifice during the early dissection stages. Diffusion imaging has also faced important challenges, related mainly to the proximity to the cortex and a lesser fractional anisotropy.

Despite these challenges, these fibers are acknowledged as clinically relevant and important. SWM abnormalities have been associated with numerous neuropsychiatric conditions, such as bipolar disorder, schizophrenia, and autism spectrum disorders(d'Albis et al., 2018; Hong et al., 2019; Ji et al., 2019; Lu et al., 2021; Zhang et al., 2018). Knowledge gaps regarding SWM anatomy hinder the comprehension of its three-dimensional organization, which is also an essential component for the development of surgical approaches to the cerebral parenchyma.

To address the aforementioned methodological difficulties, we explored new approaches to SWM fiber dissection. In this paper, we present a novel inside-out fiber dissection technique and how it can be used to assess short-association fiber anatomy in the human brain.

METHODS

Association fiber terminology

In early anatomical works, short association fibers (SAFs), especially intergyral fibers, were often referred to as 'arcuate fibers,' while in many dissection and MRI tractography studies, they have been referred to as 'U fibers.' To avoid oversimplifying the complex morphology of these fibers, we have chosen to employ the more inclusive term 'SAF'. As described by Schmahmann and Pandya in 2006, autoradiography portrays association fibers arranged in strata of increasing length as one delves deeper from the cortical surface into the gyral white matter. For the purpose of this study and to ensure a coherent presentation of our findings, we have established precise definitions and classifications for association fibers. Based on the number of gyri they bypass, we classified association fibers into the following categories: i) intergyral short association fibers (SAFs) connecting adjacent gyri, ii) intragyral SAFs connecting regions within the same gyrus, iii) neighboring association fibers (Schmahmann and Pandya, 2006) bypassing one gyrus, and iv) long association fibers bypassing two or more gyri.

Sample

In total, six human cerebral hemispheres (three left and three right) were obtained from our institution's body donation program. These hemispheres originated from a donor pool with an average age of 73 ± 10.5 years (mean \pm SD), with a male-to-female ratio of approximately 51/49, and no evident or documented neurological pathology. Specific information regarding the sex or age of each individual hemisphere was not available. Before death, all participants provided written consent to become body donors and for their bodies to be used for educational or research purposes. The study was approved by the University of Tours' body donation program board and performed in accordance with the relevant guidelines and local regulations.

Specimen preparation

Specimen preparation was adapted from the method originally described by Klingler (Klingler, 1956). To avoid decay, we performed a carotid sheath dissection of the neck and catheterized both common carotid arteries with atraumatic Nelaton catheters within 48 hours of death. Over an hour, 1.5 L of 10% formalin (Formaldehyde 4% w/v buffered at pH 6.9 RS, Carlo Erba Reagents, Cornaredo, Italy) was injected using a peristaltic pump and a "Y" connector, a technique used in other studies of our research team that has demonstrated efficacy in preparing specimens for fiber dissection (Maldonado et al., 2012, 2013, 2021). The following day, brains were extracted and immersed in a 10% formalin solution while being suspended via a thread around the basilar artery to limit shape distortion, which is commonly caused through contact with a container. Formalin fixation lasted two months at ambient temperature. Following this, we 1) split the brains into two hemispheres and detached the brainstems and cerebellums, 2) removed the leptomeninges and superficial vessels, 3) froze the hemispheres at -23°C for two weeks, and 4) slowly thawed the hemispheres at $+5^{\circ}\text{C}$ for five days. This freeze-thaw cycle

was repeated twice to optimize the structural effects of water crystallization and future dissection.

Preparatory arrangements, tools, and preliminary dissection

All researchers were trained in the dissection protocol before data acquisition. The sulci-gyral configuration of each cerebral hemisphere was studied before dissection to identify cortical landmarks for optimal navigation and to assess individual anatomical variations based on the reference atlases (Destrieux et al., 2017; Ono et al., 1990).

We used moistened delicate wooden spatulas, blunt dissectors, and microsurgical tools for dissection. We avoided sharp instruments to prevent artifactual sculpting and the formation of spurious tracts. We mobilized fragile and delicate fibers using small fragments of water-soaked cotton. To study SAFs of the superolateral aspect of the cerebral hemisphere, specimens were prepared using a classical dissection (superficial to deep) method, starting from the medial aspect until the thalamic radiations were exposed. Similarly, to study SAFs of the medial aspect, the specimens were prepared by exposing the corona radiata and internal capsule from a lateral view.

Inside-out technique for SWM assessment

Starting with the deep WM structures, the inside-out fiber dissection was performed toward the superficial WM layers via progressive peeling and removal of projection, commissural, and long association fibers until the SAFs were exposed (Fig. 1). In specimens used to characterize SAF arrangement, a fiber-by-fiber dissection was performed under magnification. Visualization of the inner aspect of the cortical mantle marked the end of dissection. Several elements were then assessed: the quality of the dissection plane in relation to deeper, longer fasciculi, visibility of SWM fibers, and visibility of fiber crossings. In specimens used to study SWM layer thickness, visualization of the inner aspect of the intergyral fiber layer marked the end of the dissection, following which no further peeling was performed.

Stereoscopic anaglyph red-cyan images construction and lateral hemispheric 3D photogrammetry model

We used a digital single-lens reflex Pentax K20D camera (Hoya Corp., Tokyo, Japan) equipped with a ring flash (Pentax AF160FC), a 50 mm macro lens (Pentax SMC P-D FA 1:2.8 50mm) for whole-hemisphere photographs, and a 100 mm macro lens (SMC P-D FA 1:2.8 100mm) for close-ups. The capture device was attached to a solid slider enabling horizontal translation from left to right and vice versa. Specimens were placed on a black background, with a sensor-specimen distance of 55 cm and 70 cm for the 50 and 100 mm lenses, respectively. These distances enabled a field of view small enough to frame the specimen alone in adequate quality but large enough to freely move the device on the transverse axis. The different dissection stages for each specimen were photographed twice, as the camera was moved transversely to create a parallax effect (Lechanoine et al., 2019). Transverse distances of 2.5 cm and 3 cm were chosen for the 50 and 100 mm lenses, respectively. Specimens were kept perfectly still between shots.

The physical background was replaced with a uniform black background (RGB value = 5, 5, 5) using Adobe Photoshop (Adobe Corp., San Jose, USA). The RGB channels were adjusted

so that the right images were displayed in tones of cyan and the left images in tones of red. The two photographs were then superimposed and aligned along the transverse axis for three-dimensional (3D) rendering.

In the pursuit of a comprehensible depiction of the inside-out fiber dissection technique, a virtual 3D model of the dissected lateral aspect with its SWM exposed was obtained using the Scaniverse free photogrammetry application (Niantic Inc., San Francisco, USA). Scaniverse is a user-friendly tool that facilitates the conversion of real-world objects into detailed 3D models. To capture the necessary data, we utilized the application on a mobile device, specifically an iPad Pro 6E (Apple Corp., Cupertino, USA). The process involved capturing a series of high-resolution photographs from multiple angles, covering the dissected lateral aspect and its exposed subcortical white matter. Scaniverse then processed this image data, combining it together to construct a 3D representation. To enhance accessibility, we chose to export our results in the .fbx format as texturized 3D models. The .fbx format enjoys broad compatibility with various 3D viewing softwares. To facilitate display in the absence of dedicated viewers, we also integrated these interactive "click-and-move" objects into PowerPoint presentation files. An illustrative example of a 3D model resulting from this acquisition technique is shown in Figure 2.

Magnetic resonance imaging process for SWM thickness assessment

To evaluate the capability of the inside-out technique to enable the thickness of the SAF intergyral layer to be assessed, serial cross-sections of selected sulci were performed. Additionally, one dissected lateral aspect underwent *ex vivo* morphological magnetic resonance imaging (MRI). Before MRI, the specimen was placed in a vacuum chamber (-400 mbar) filled with water for one day to remove residual bubbles. MRIs were performed using a 3T scanner (Prisma, Siemens, VE11E software version) and a 20-channel head/neck receiving coil. The specimen was placed in a dedicated 3D-printed box to enable it to be positioned in the center of the coil. The box was filled with distilled water for loading. The isotropic 3D T2-weighted image (SPACE) of the entire specimen used several parameters: TR/TE 1070/138ms, in-plane resolution 500x500 μ m, and slice thickness 500 μ m.

RESULTS

As dissection progressed from the deep to the SWM, it enabled clear visualization of long association, projection, and commissural fasciculi and their terminations in the corresponding gyri. This exposure also enabled appreciation of the colocalization of their terminations when converging on the same gyrus. Partial preservation of deep fasciculi during intermediate stages of dissection allowed the study of WM stratification. At the end of the peeling procedure, the isolation of the intergyral fiber layer enabled the assessment of its thickness and fiber orientations.

Neighboring fibers were the first to be seen in the SWM, visualized as evident links between non-adjacent gyri. After peeling neighboring fibers, a compact layer of intergyral SAFs with variable thicknesses was visible. This layer was adjacent to the cortex, except when secondary or tertiary gyri were present on the sulcal floor. SWM isolation from the deeper and longer tracts was successfully performed atraumatically due to a clear dissection plane. The adherence of the internal aspect of the SWM to the deeper long fasciculi was lower than that of its external aspect adherence to the cortical gray matter, particularly at the depth of sulci.

Inside-out exposure of the SWM

From medial to lateral (four hemispheres), several structures were identified and successively removed: the thalamus and its radiations, the internal capsule, and the lenticular nucleus. At this point, the external capsule, the medial aspect of the anterior, and the middle segment of the inferior fronto-occipital and uncinate fasciculi were exposed. More posteriorly, the corona radiata was exposed, as well as the optic radiations and inferior longitudinal fasciculus in the sagittal stratum (Fig. 3). Peeling of these structures was followed by the removal of the claustrum, arcuate fasciculus, and superior longitudinal fasciculus, exposing the SWM of the superolateral aspect of the cerebral hemisphere (Fig. 2 and 5). Please also see the online material for a stereoscopic video and “click-and-rotate” photogrammetric 3D model. Illustrative models of the intermediate stages of dissection are also available for download via our institution's platform (<https://utbox.univ-tours.fr/s/aa4msXX34L2XcAN>).

From lateral to medial (two hemispheres), the inside-out procedure began with the resection of thalamic radiations and the thalamus. Opening of the lateral ventricle resulted from the ablation of its later wall structures, such as the optic radiations, tapetum, caudate nucleus, and ependyma (Fig. 5). Hippocampal formation was identified on the medial wall of the temporal horn and removed. The cingulum was temporarily left in place to preserve the global hemispheric structure as the specimen became progressively thinner. The cingulum was removed during the last dissection steps to enable full access to the middle aspect subcortical SWM (Fig. 6).

One minor difference that did not affect the feasibility of the technique was the relative fragility of specimens during the final stages of dissection of the medial aspect. This was due to the reduced volume and increased mobility of the remaining tissues after the removal of the ventricular wall and corpus callosum, compared to the dissection of the lateral surface.

The dissection plane between SAFs and longer fibers was evident in all dissected specimens. Therefore, the inside-out technique successfully highlighted the anatomical relationships of superficial and deep WM structures (Fig. 7). Prompt identification of long fasciculi was enabled due to their morphological characteristics and the ability to trace the origins and terminations of their fibers.

Access to the study of SWM thickness and composition

The clear distinction of deeper fasciculi enabled SWM preservation where necessary. The dissection plane between the deeper, longer fasciculi and the intergyral fibers remained notably clear. This precision in the dissection plane played a crucial role in the inside-out fiber dissection technique. Isolation of the intergyral fiber layer enabled direct observation of its thickness on transverse sections and the assessment of variations along the same sulcus. These observations were possible due to the increased contrast between the cortex and WM generated by the Klingler preparation (Fig. 8). This distinction was also evident in ex vivo T2 imaging.

The inside-out fiber dissection technique was feasible and efficient for studying the composition of the sulcal SWM (Fig. 9) in all specimens. SAFs appeared initially as a lightly striated layer. A fiber-by-fiber peeling procedure, which was performed without significant difficulty using fine forceps, revealed the characteristic arcuate shape of intergyral fibers.

Different layers of intergyral fibers, with varied orientations among layers, became visible. A greater degree of dissectability of individual fibers was observed in this layer than that usually obtained by crossing the cortical mantle.

The result of applying the inside-out procedure for the study of intergyral fibers is illustrated in Fig. 10, which provides an image of a right inferior frontal sulcus. Longer and more oblique fibers crossing the sulci were typically more present in the inner layers of the SWM than the outer layers, which were richer in shorter transverse fibers. Fiber reinforcements forming minor bundles were observed when submerged gyri connected sulcal banks.

DISCUSSION

The SWM comprises neighboring fibers and SAFs and is of great importance within the human connectome. However, the characterization of these compact and delicate fibers has been limited, primarily due to methodological difficulties. To overcome this methodological challenge, we developed an inside-out fiber dissection protocol in which exposure of superficial bundles began at their deeper boundaries. The method was feasible and efficient in isolating SAFs from their surrounding environment, which were more dissectible and distinguishable than typically found using classic transcortical protocols. The inside-out technique demonstrated numerous advantages for 3D analysis, providing quality dissection beyond the limits of the conventional approach. This method opens a new research avenue by enabling direct observation and detailed characterization of SAFs in the human brain.

Precise anatomical characterization of SAFs is the cornerstone of future brain connectomics research in fundamental and applied neurosciences. SWM has been implicated in various pathological processes. Several studies have demonstrated the association between the modification of fractional anisotropy and mean diffusivity in the SWM (which suggests a loss of WM microstructure integrity) and neuropsychiatric disorders, such as bipolar disorder, schizophrenia, and autism spectrum disorders (d'Albis et al., 2018; Hong et al., 2019; Ji et al., 2019; Lu et al., 2021; Zhang et al., 2010). Neurodegenerative diseases and other neurological disorders, such as multiple sclerosis or epilepsy, are also thought to likely involve the SWM in their pathophysiological processes (Bakshi et al., 2001; Buyukturkoglu et al., 2022; Miki et al., 1998; Nazeri et al., 2013).

Understanding how different WM elements are spatially arranged in a given region is of great importance in neurosurgery. The number of anatomical studies combined with the increased attention on this topic in the neurosurgical community has led neurosurgeons to tailor their approach regarding WM tracts that may be affected by surgical intervention. “Zero-footprint” microanatomical works have focused on identifying regions of low eloquent WM fiber concentration that make ideal surgical corridors associated with fewer functional consequences (Monroy-Sosa et al., 2019). However, superficial fibers are not typically considered for surgical planning as the anatomical data is insufficient. Therefore, thorough understanding of superficial connectivity would enable the development of less invasive transparenchymal approaches (Latini and Ryttefors, 2015).

SAF anatomy has often been oversimplified in the literature, being defined as U-shaped fibers linking orthogonally adjacent gyri in a homogeneous layer. The term “U fibers” is reductionist as it does not reflect the complexity associated with the anatomical organization of different entities. The present study represents a significant step towards the development of adequate study methodologies that will help refine the current state of knowledge in structural connectivity.

Works proposing systematization and a dedicated nomenclature of SAFs remain scarce. Although labeling based on the overlying sulcus has been proposed in seminal studies (Dejerine and Dejerine-Klumpke, 1895), most works have referred to SAFs in a generic way. In a fiber dissection study focused on the superficial WM, Shinohara et al. (2020) highlighted that the 3D organization of these pathways is far more complicated than initially assumed (Shinohara et al., 2020). The authors described how intergyral and intragyral SAFs combine in the SWM and how convergence sites involve pyramidal-shape crossings that are likely key elements in the superficial connectivity network. The inside-out perspective highlights funnel-shaped regions of SAF convergence, mirroring those previously described by Shinohara. This observation in specimens with preserved cortex underscores the correlation between pyramidal-shaped crossings and sulcogyral anatomy.

Various methods have been proposed to study WM anatomy, and each has its advantages and limitations. Radioisotope tracing with autoradiography has been considered the gold standard. Its efficacy was first demonstrated in determining Rhesus monkey white matter anatomy, which was considered groundbreaking work at the time (Schmahmann and Pandya, 2006). This method, which requires animal sacrifice, is not applicable to humans. Furthermore, its results are only partially translatable to human anatomy (Thiebaut de Schotten et al., 2012). However, autoradiography remains the method that has contributed most to the understanding of the complex arrangement of SAFs and how bundle terminations cohabit within a cerebral gyrus. Schmahmann and Pandya (2006) demonstrated a general arrangement of the gyral WM in Rhesus monkeys, in which SAFs are more superficial. Though this concept has not been specifically studied in humans, multiple dissection studies suggest a similar organization (Dejerine, Joseph Jules, 1895; Vergani et al., 2014; Forkel et al., 2015).

While there is some partial overlap in the results when examining the relative stratification of the subcortical white matter (SWM), the inside-out technique in human specimens has proven highly effective in revealing the structural heterogeneity of the gyral white matter in the cerebrum, even within the same gyrus. This was evident in variations in the thickness of the intergyral fiber layer, the presence of thickenings associated with submerged secondary gyri, and regional differences in the proportions of various types of association fibers. We believe that the inside-out technique holds significant methodological potential for investigating the regional specifics of SWM in the human brain.

Tractography based on diffusion-weighted imaging is the only non-invasive technique enabling *in vivo* depiction of fiber tracts. This has led to the establishment of WM atlases for humans (Catani and Thiebaut de Schotten, 2012; Guevara et al., 2017) and the proposition of a complex, 3D model of SWM arrangement within the frontal lobe, termed the “poppy flower” arrangement (Catani et al., 2012b). However, tractography faces challenges in complex regions and those in proximity to the cerebral cortex (Jones, 2003; Jones and Cercignani, 2010; Le Bihan et al., 2006; Thomas et al., 2014).

Diffusion-weighted imaging studies that screened the entire SWM have suggested the existence of a high number of identifiable bundles, as many as 50 per hemisphere (Schüz and Miller, 2002; Zhang et al., 2010b; Guevara et al., 2017; Román et al., 2017; Shastin et al., 2022). The anatomy of these bundles can be debated, as SAFs form a continuous layer along the sulcal length that varies in thickness and fiber orientation. In this context, tractographic bundles may correspond to focal thickenings within a continuous sheath of SAF paving the sulcal floors, rather than discrete, separated bundles.

Possible avenues for future research include assessing emerging innovative imaging techniques capable of acquiring high-resolution volumetric datasets, such as polarized light imaging (David et al., 2019) and optical coherence tomography (Wang et al., 2014). Although

these methods are designed to study small tissue samples(Axer et al., 2011; Wang et al., 2014), and are not suitable for the study of an entire hemisphere, they could help identify disentangling fibers within complex zones where diffusion imaging struggles(Assayag et al., 2013; Magnain et al., 2014).

Following the renewed interest in WM dissection, many adaptations of the standard Klingler method have been proposed. These involve modifications of the fixation process(Latini et al., 2015) and dissection steps(Martino et al., 2011) or complex protocols incorporating several methods such as those used to integrate WM vasculature(Smirnov et al., 2023). Interestingly, during careful examination of figures from previous studies that investigated microsurgical or topographic anatomy, small areas of SWM exposed from similar inner perspectives can be identified(Demirtaş et al., 2022; Ture et al., 1999). Nevertheless, no study to date has proposed using this approach to characterize SAFs. Instead, most fiber dissection works involve the early sacrifice of these fibers to focus on deeper association(Bernard et al., 2019), commissural(Çavdar et al., 2021; Shah et al., 2021), and projection(Maldonado et al., 2021b; Peltier et al., 2006) fasciculi.

In classical fiber dissection, the removal of the cerebral cortex exposes a relatively amorphous subcortical WM, rendering fiber-by-fiber dissection difficult or impossible. As the outermost SAFs are adherent to the cortex, they are easily disrupted. Deeper layers are more easily cleaved from each other, allowing progressive peeling. As a consequence, when the SWM is approached using the inside-out method, fiber identification is optimized, and fibers that are traditionally sacrificed or disregarded become the backbone of the procedure. Since the inside-out technique is not limited to SAFs, it can also be applied to long tracts, thereby enabling new and useful perspectives, particularly for the study of their prolongation into the cerebral gyri. To dissect both medial and superolateral SWM from the inside, a possible variant of this method includes parasagittal sectioning of the cerebral hemisphere. However, parasagittal sectioning has several limitations, as it disrupts important landmarks, as well as the integrity of a hemisphere's convex superolateral aspect. Moreover, the dissection would start in a homogeneous flat plane, less favorable to the accurate identification of white matter fasciculi.

The inside-out fiber dissection technique has limitations. Fiber dissection is inevitably operator-dependent, and dissections in which the final product is a thin, fragile specimen may be challenging. A useful strategy is to protect the opposite surface of the specimen, such as the cortical surface during an inside-out dissection, with a semi-rigid material. In our laboratory, customized platforms molded in paraffin have been introduced for this purpose (Smirnov et al., 2023). Among the method's strengths are its simplicity, the lack of required additional equipment, and the preservation of the SWM-cortex interface.

The technique holds substantial clinical implications. It has the potential to influence neurosurgery, as a deeper understanding of SWM connectivity can enhance surgical planning, thus minimizing damage to specific WM elements and reducing post-operative deficits. A better comprehension of SWM connectivity can also facilitate the development of tailored rehabilitation strategies, promoting personalized treatment plans. Additionally, the method contributes to the development of advanced diagnostic tools, enhancing the accuracy of neuroimaging interpretation and serving as a pathway for the development of a valuable reference for diffusion imaging validation studies. Furthermore, it opens up promising avenues for future brain connectomics research, shedding light on the complexity of superficial neural circuits.

CONCLUSION

The present study presents an innovative inside-out fiber dissection technique for assessing SAFs in post-mortem human brains. This technique was feasible and efficient when applied to the medial and lateral aspects of the telencephalon, enabling atraumatic exposition of SWM, examination of the thickness of the intergyral short association fiber layer, and dissection of intergyral fibers.

The inside-out fiber dissection technique opens new research avenues regarding the characterization of the SWM using fiber dissection. It completes the neuroscientist's armamentarium, circumventing a severe methodological obstacle and providing the anatomical substrate necessary for modeling neural circuits and performing validation studies of SWM diffusion imaging.

DECLARATIONS

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Declaration of competing interests

We declare no competing interests.

Authorship statement

Conceptualization: Igor Lima Maldonado, Mykyta Smirnov, Christophe Destrieux; methodology: all authors; data collection: all authors; formal analysis and investigation: all authors; writing—original draft preparation: Guillaume Dannhoff, Alex Morichon; writing, review, and editing: Mykyta Smirnov, Laurent Barantin, Christophe Destrieux, Igor Lima Maldonado; Funding acquisition: Christophe Destrieux; Supervision: Igor Lima Maldonado. All authors contributed additional writing and commentaries. All authors read and approved the final manuscript.

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FIGURE LEGENDS

Figure 1. A schematic drawing of the concepts involved in conventional “superficial to deep” and inside-out dissection techniques for superficial white matter. **A.** The conventional transcortical approach. The blue arrows indicate the medial aspect, and the red arrows indicate the lateral aspect of the cerebral hemisphere. The red dotted line running deep into the thalamic radiations and internal capsule represents the starting point of the inside-out procedure. **B1.** The inside-out approach in the medial hemispheric (inverted blue arrows) reaches the superficial white matter through successive peeling of white matter fibers. **B2.** The inside-out approach of the medial aspect of the cerebral hemisphere (inverted red arrows).

Figure 2. A 3D photogrammetry model of a right human hemisphere, which was dissected using the inside-out technique, displayed from different angles. A manipulable object (same specimen) is available in the accompanying .pptx file. **A.** Medial view of the lateral hemispheric aspect superficial white matter. **B.** A schematic annotated drawing of the specimen in the same viewpoint as **A.** **C.** Superior view of the dissected specimen, demonstrating how deep white matter was “carved out” to reach the superficial white matter. **D.** Lateral view of the lateral convexity, demonstrating preservation of the cortex. **E.** Three-quarter view of the 3D model for easier comprehension of the relation between the superficial white matter relation and the overlying cortex. *sfs = superior frontal sulcus, ifs = inferior frontal sulcus, pec = precentral sulcus, c = central sulcus, poc = postcentral sulcus, ip = intraparietal sulcus, sts = superior temporal sulcus, its = inferior temporal sulcus.*

Figure 3. A step-by-step inside-out dissection towards the lateral aspect of a right cerebral hemisphere, documented by 2D (left column) and 3D stereoscopic (right column) photography. The initial preparation for this dissection involved exposing the corona radiata and internal capsule from medial to lateral. The white matter was progressively peeled away, and anatomical structures were exposed and removed from their medial aspect, such as the internal capsule (**A**), the lenticular nucleus (**B**), the external capsule (**C**), the extreme capsule, and (**D**) the arcuate fasciculus. Please use 3D red-cyan glasses to view pictures in the right column. *cr = corona radiata, ic = internal capsule, cp = cerebral peduncle, ln = lenticular nucleus, ifof = inferior fronto-occipital fasciculus, ec = extreme capsule, uf = uncinate fasciculus, ilf = inferior longitudinal fasciculus, af = arcuate fasciculus.*

Figure 4. The exposure of the intergyral fiber layer of the lateral aspect of a right cerebral hemisphere using the inside-out fiber dissection technique. **A.** 2D photograph. **B.** Stereoscopic anaglyph. Please use 3D red-cyan glasses to view. *sfs = superior frontal sulcus, ifs = inferior frontal sulcus, prcs = precentral sulcus, pocs = postcentral sulcus, ips = intraparietal sulcus, ec = extreme capsule, sts = superior temporal sulcus.* See Online Material for a photogrammetric 3D model and stereoscopic video.

Figure 5. A step-by-step dissection towards the medial aspect of a right cerebral hemisphere using the inside-out technique, documented using 2D (left column) and 3D stereoscopic (right column) photography. The initial dissection preparation involved exposing the thalamic radiations and sagittal stratum from lateral to medial. The white matter was then progressively peeled away, and anatomical structures were exposed and removed, such as the thalamic and optical radiations (**A**), the caudate nucleus and tapetum (**B**), the cingulum dorsal callosal radiations, and (**C and D**) the hippocampus. Please use 3D red-cyan glasses to view pictures in the right column. *thr = thalamic radiations, cr = corona radiata, ac = anterior commissure, or = optic radiations, ss = stratum sagittale, cn = caudate nucleus, clr =*

callosal radiations, tp = tapetum, fh = frontal horn, sp = septum pellucidum, cc = corpus callosum, fx = fornix, at = atrium, oh = occipital horn, th = temporal horn, h = hippocampus, cg = cingulum.

Figure 6. The exposure of the intergyral fiber layer of the medial aspect of a right cerebral hemisphere using the inside-out fiber dissection technique. **A.** 2D photograph. **B.** Stereoscopic anaglyph (red-cyan). Please use 3D red-cyan glasses to view. *cing = cingulum, cols = collateral sulcus, pof = parieto-occipital fissure, its = inferior temporal sulcus, ots = occipito-temporal sulcus, cals = calcarine sulcus.*

Figure 7. An inside-out dissection highlighting anatomical relationships among white matter fasciculi. Please use 3D red-cyan glasses to view pictures in the right column. **A.** Overview of different fiber layers co-localized in the temporoparietal junction from a medial to lateral perspective. **B.** 3D (red-cyan) anaglyph. *af = arcuate fasciculus, cr = corona radiata, ec = extreme capsule, extc = external capsule, cl = claustrum.*

Figure 8. Direct observation and magnetic resonance imaging of the outermost layer of the superficial white matter following inside-out dissection demonstrating preserved intergyral fibers. Only secondary submerged gyri were kept intact, as they are even more superficial and can only be reached if first-order gyri are sacrificed. The increased contrast generated by the Klingler preparation enabled a clear distinction between the cortex and the superficial white matter using both direct inspection and ex vivo T2 imaging. **A.** A right cerebral hemisphere in which lateral surface intergyral fibers were isolated along with adjacent cerebral cortex. **B.** Parasagittal section (a T2-weighted acquisition) of the same specimen. In this imaging modality, the cortex appears hypertense in relation to the (darker) underlying SWM. **C.** Serial cross-sectioning (anterior to posterior) of the right superior temporal sulcus exposing the cerebral cortex and the subcortical layer of white matter containing intergyral fibers (anterior view). **D.** Serial coronal views of the superior temporal sulcus on T2 ex-vivo MRI. These are presented here according to the same modalities as macroscopic cross-sections. Red line: superior temporal sulcus.

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Supplementary 3D Photogrammetry model. A manipulable 3D photogrammetry model of a right human hemisphere, which was dissected using the inside-out technique. **A.** .fbx file **B.** .pptx file

Supplementary Stereoscopic Video. The exposure of the intergyral fiber layer of the lateral aspect of a right cerebral hemisphere using the inside-out fiber dissection technique. Please use 3D red-cyan glasses to view.

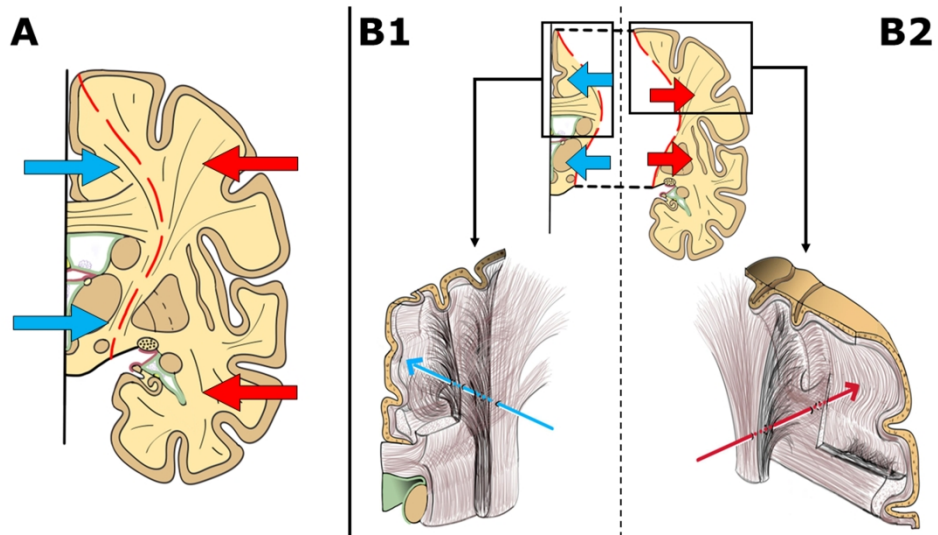


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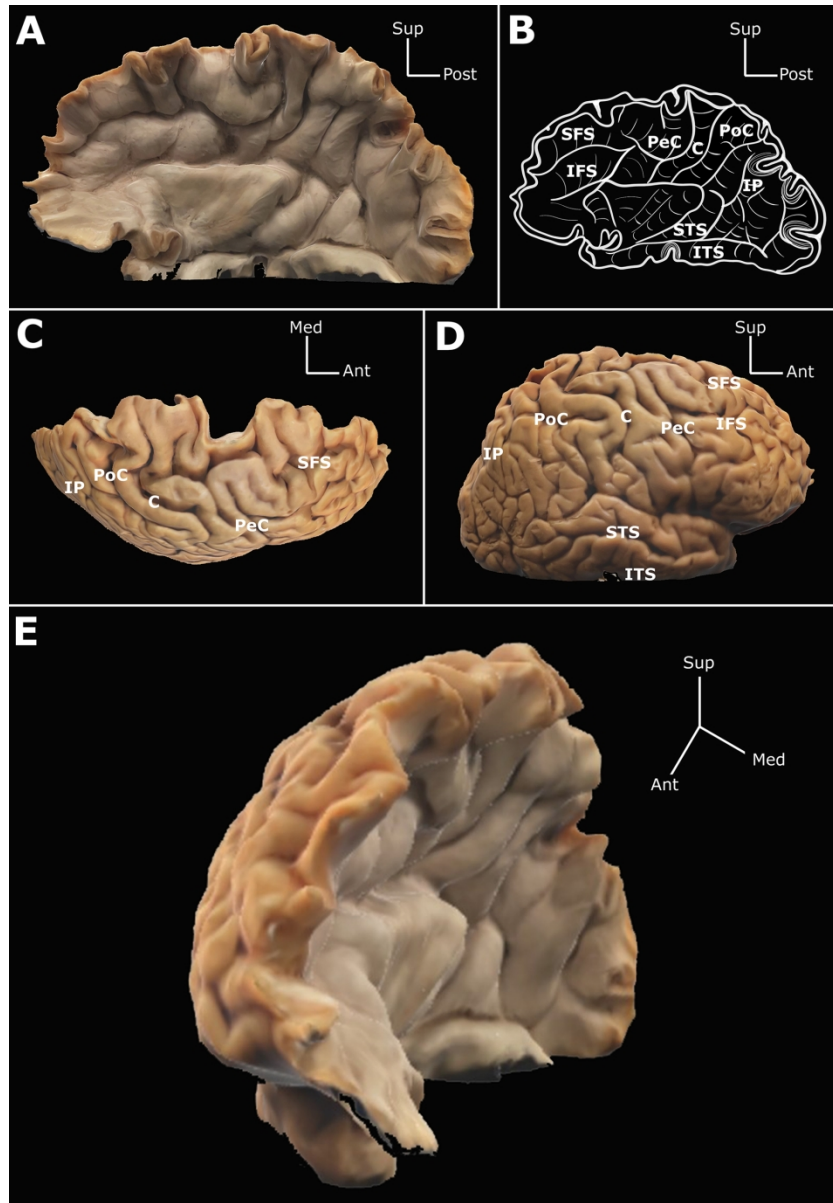


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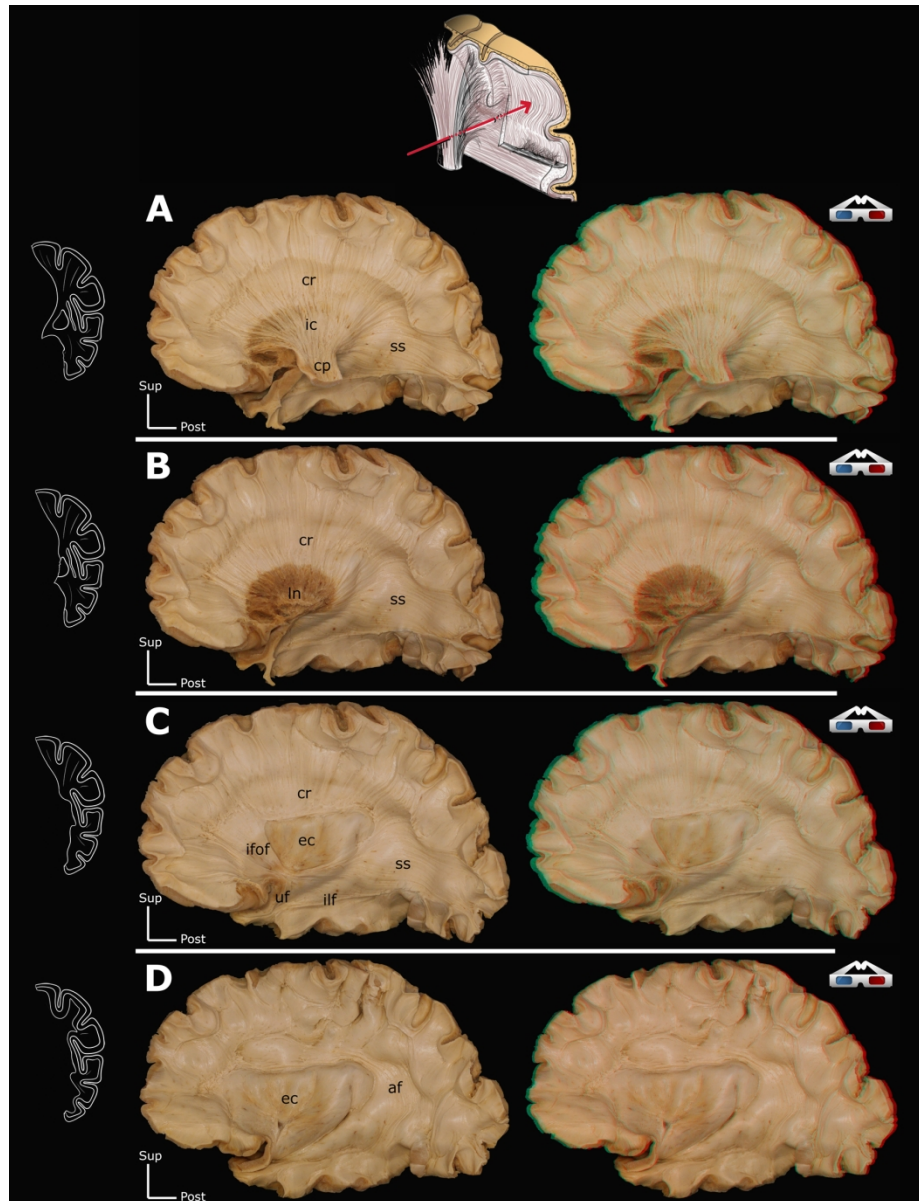


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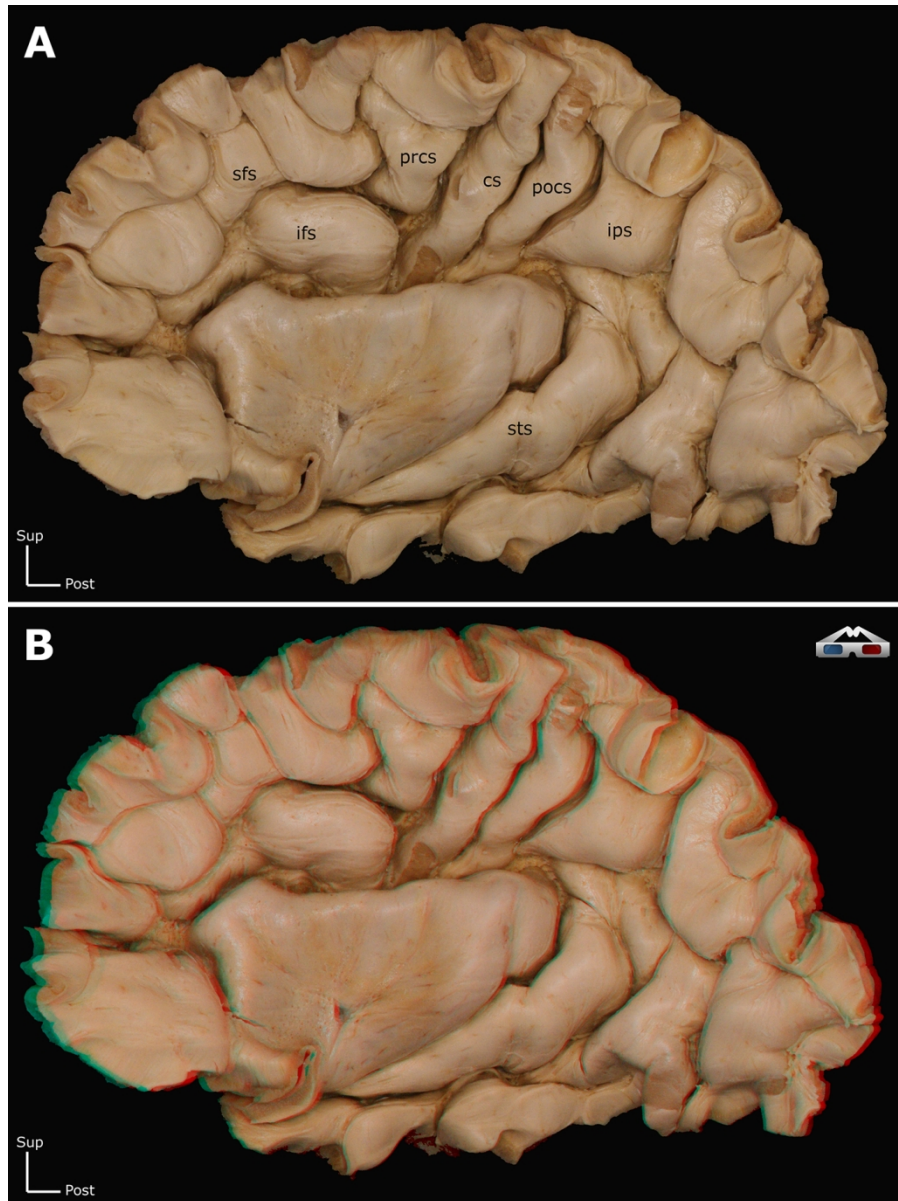


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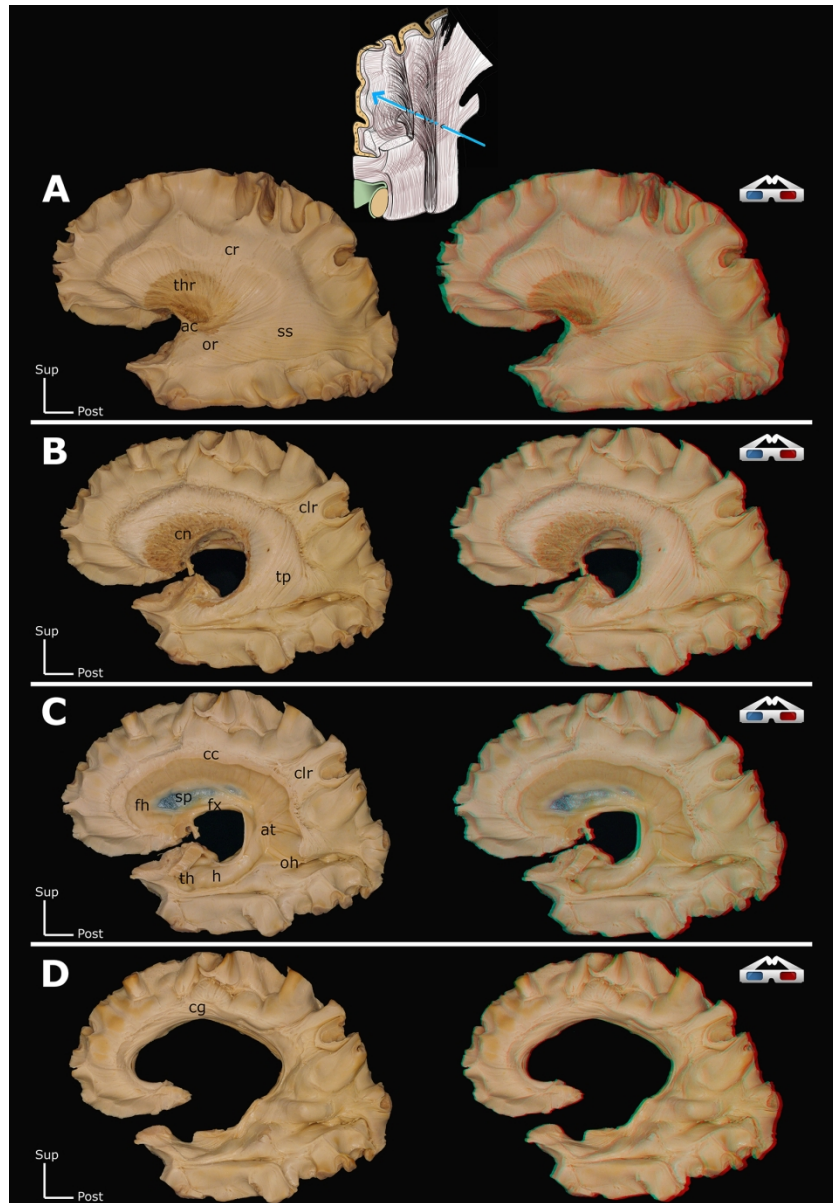


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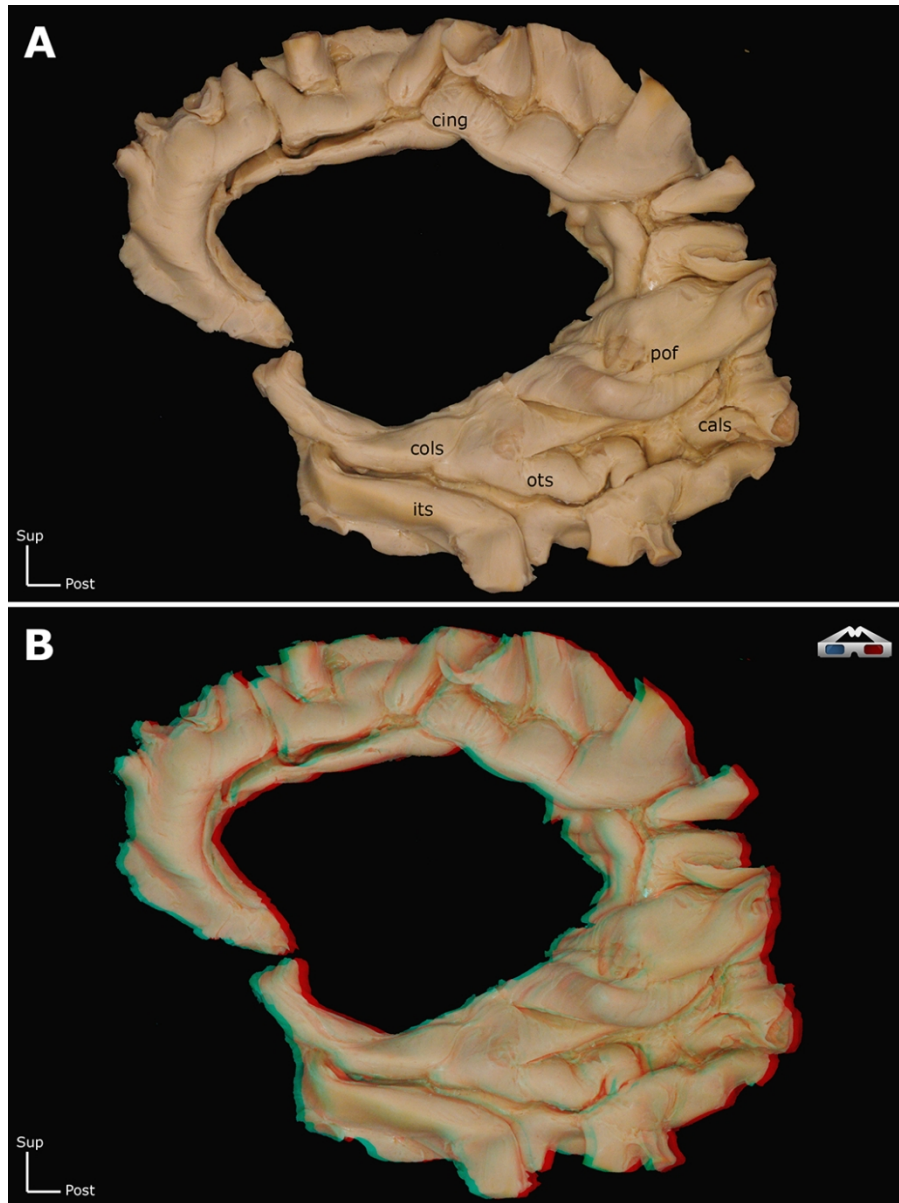


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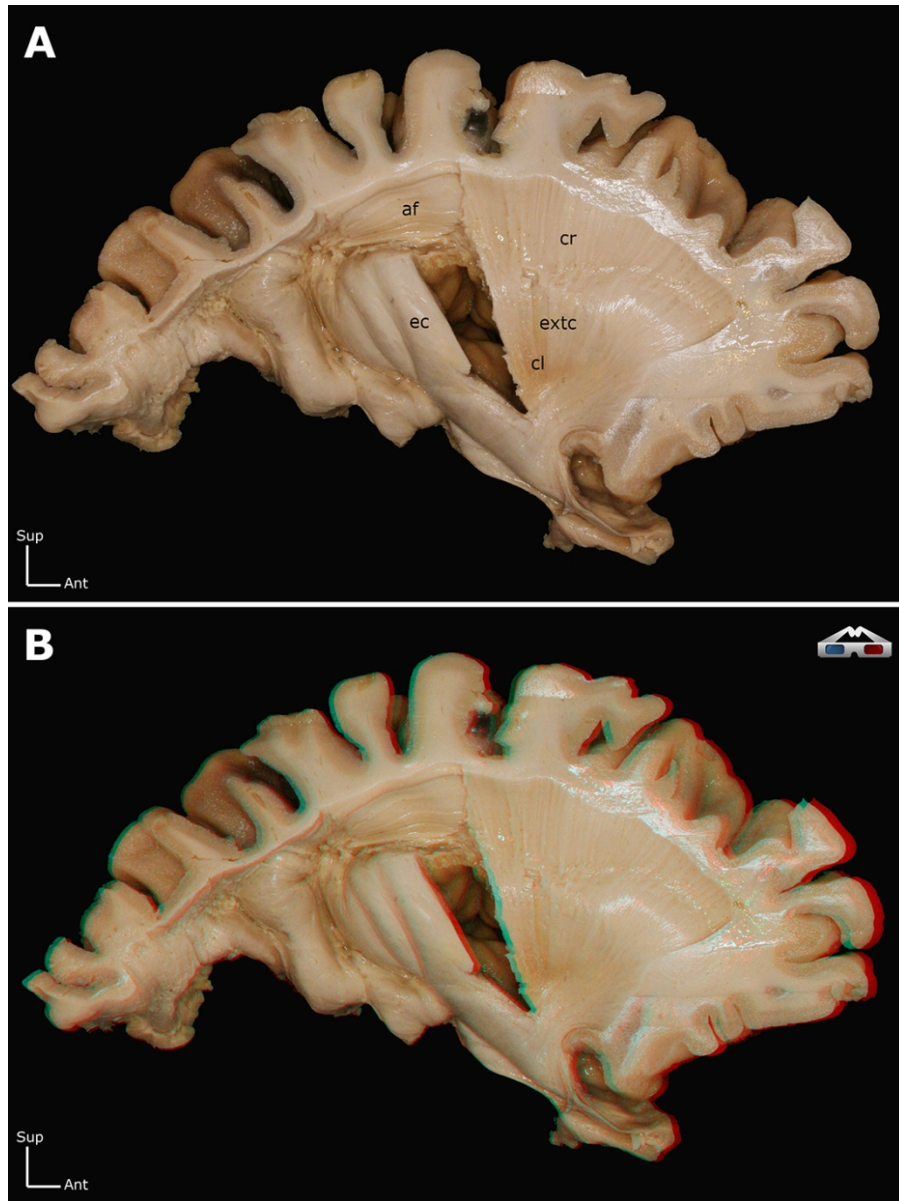


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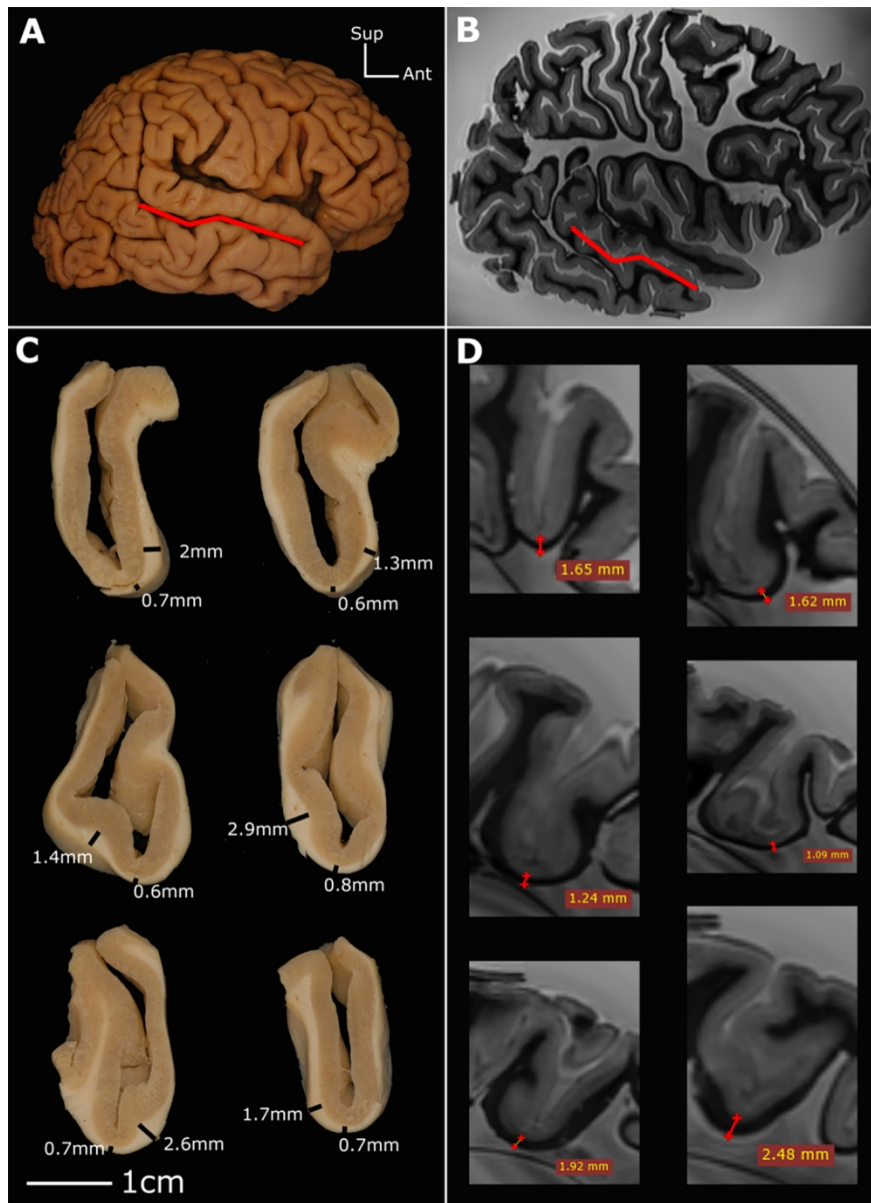


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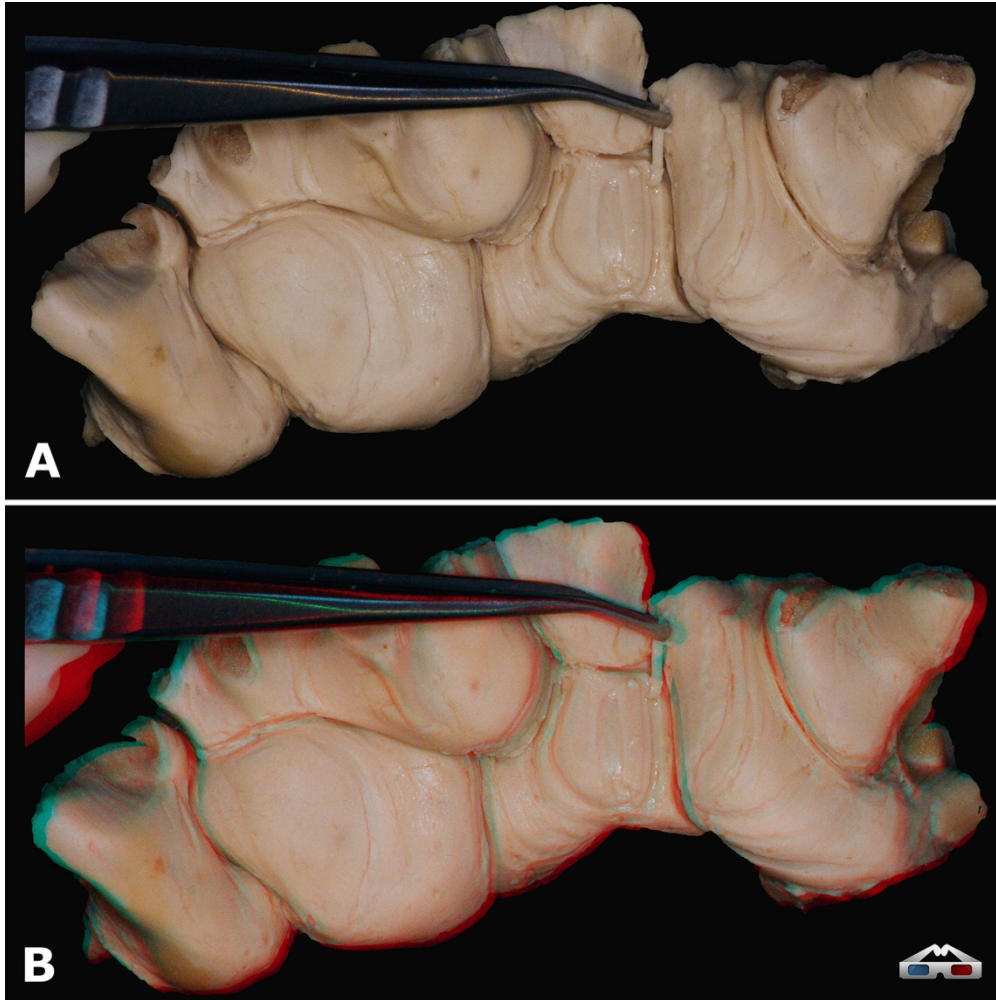


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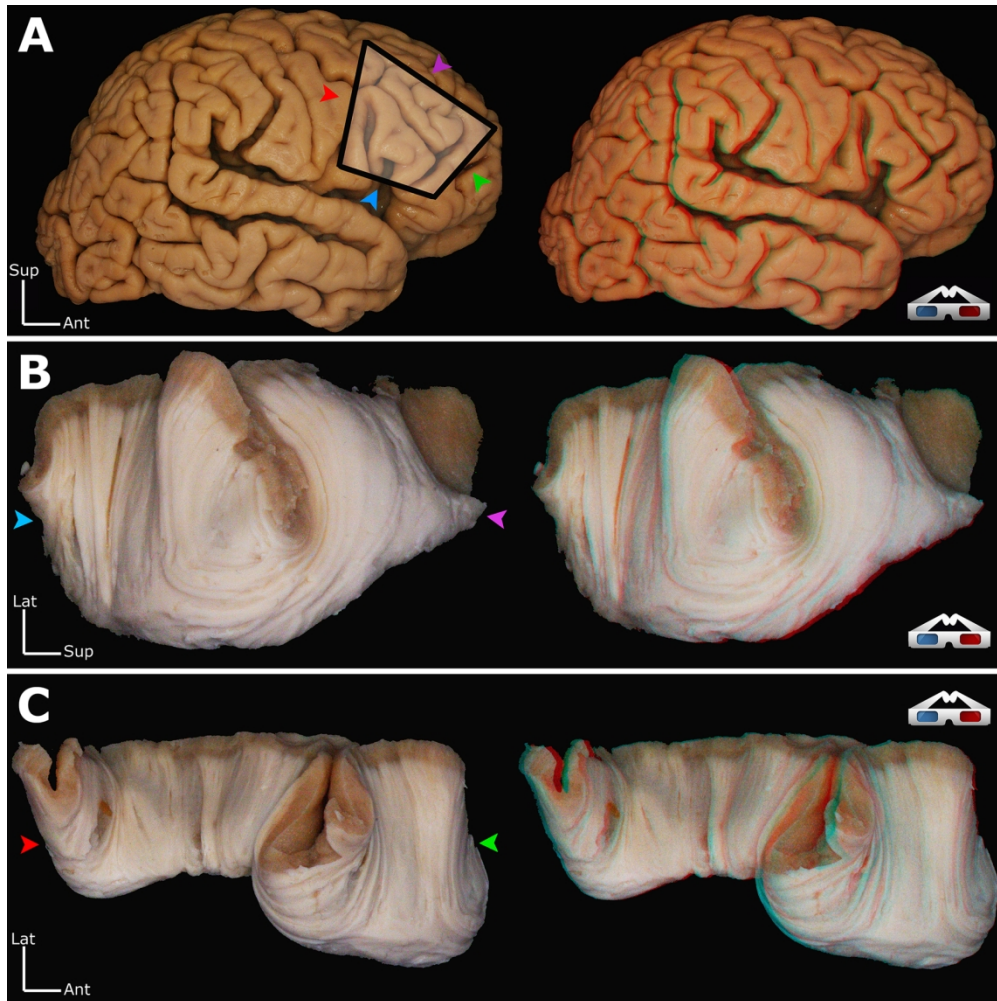


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