Multimodal Anatomy of the Human Forniceal Commissure

Kevin Akeret (✉ kevin.akeret@gmx.ch)

University and University Hospital of Zurich <https://orcid.org/0000-0002-5946-4999>

Stephanie Forkel

<https://orcid.org/0000-0003-0493-0283>

Raphael Buzzi

University Hospital Zurich and University of Zurich <https://orcid.org/0000-0002-9584-3590>

Flavio Vasella

University Hospital Zurich and University of Zurich

Irmgard Amrein

University of Zurich

Giovanni Colacicco

University of Zurich

Carlo Serra

University of Zurich

Niklaus Krayenbühl

University of Zurich

Article

Keywords: Fornix, Hippocampal commissure, Klingler dissection, Psalterium, Tractography

Posted Date: January 24th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1193017/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Communications Biology on July 25th, 2022. See the published version at <https://doi.org/10.1038/s42003-022-03692-3>.

Multimodal anatomy of the human forniceal commissure

Kevin Akeret¹, Stephanie J. Forkel^{2,3,4}, Raphael M. Buzzi⁵, Flavio Vasella¹, Irmgard Amrein^{6,7}, Giovanni Colacicco⁶, Carlo Serra¹, Niklaus Krayenbühl^{1,8}

1 Department of Neurosurgery, Clinical Neuroscience Center, University Hospital Zurich and University of Zurich, Zurich, Switzerland.

2 Groupe d'Imagerie Neurofonctionnelle, Institut des Maladies Neurodégénératives-UMR 5293, CNRS, CEA, University of Bordeaux, Bordeaux, France

3 Brain Connectivity and Behaviour Laboratory, Sorbonne Universities, Paris, France

4 Centre for Neuroimaging Sciences, Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK

5 Division of Internal Medicine, University Hospital Zurich and University of Zurich, Zurich, Switzerland.

6 Institute of Anatomy, University of Zurich, Zurich, Switzerland.

7 Department of Health Sciences and Technology, ETH, Zurich, Switzerland.

8 Division of Pediatric Neurosurgery, University Children's Hospital, Zurich, Switzerland.

Corresponding author:

Niklaus Krayenbühl

Division of Pediatric Neurosurgery and Department of Neurosurgery

University Children's Hospital and University Hospital of Zurich

Steinwiesstrasse 75

CH-8032 Zurich, Switzerland

niklaus.krayenbuehl@kispi.uzh.ch

Abstract

Anatomical studies investigating the forniceal commissure have mainly been performed in non-human primates. However, ambiguity surrounds the existence, morphology, and functionality of this structure in the human brain.

We investigated the morphology of the human forniceal commissure by including advanced in vivo tractography, ex vivo fiber dissection, and histology. A whole-brain tractography from 7T data of 180 participants was produced, each fornix dissected, and fiber percentage maps calculated. In nine human brains, we performed stepwise multidirectional Klingler dissection under the operating microscope. For histological analysis, multiplanar serial floating sections of six specimens were obtained.

Virtual fiber dissection did not identify interhemispheric connections between the crura fornicis. Klingler dissection demonstrated a thin soft-tissue membrane spanning between the left and right crus fornicis, but no commissural fibers. These findings were corroborated by the multiplanar histological analysis with myelin staining that visualized delicate cruciform fibers extending between the crura fornicis, which were enclosed by connective tissue.

The human forniceal commissure appears much more delicate than previously described and presented in anatomical textbooks. This finding is consistent with the phylogenetic trend of a striking reduction of the forniceal commissure in non-human primates compared to non-primate mammals and might have functional implications in clinical neurology.

Keywords

Fornix, Hippocampal commissure, Klingler dissection, Psalterium, Tractography

Abbreviations

ANTs, advanced normalization tools; GreedySyN, Greedy symmetric diffeomorphic normalisation; NHP, non-human primates.

Introduction

Interhemispheric white matter circuits transverse the hemispheric midline and connect homotopic brain regions. Amongst these interhemispheric connections, the corpus callosum is the most prominent neopallial commissure in the human brain. Phylogenetically older commissures common to all vertebrates – the anterior commissure, ventral hippocampal, and forniceal (dorsal hippocampal) commissure – demonstrate a disproportionate reduction in primates.^{1–5} While the anatomy and functions of the first two have been well described in the human brain,^{1,3–5} the existence, morphology and functionality of a forniceal commissure remains elusive.

The first anatomical descriptions of the forniceal commissure as a triangular subsplenial structure date back to macroscopic studies in monotremata and marsupialia in the late 19th century.^{6,7} The forniceal commissure was first referred to as (dorsal) psalterium or David's lyra, given its macroscopic similarity to the psalter, a zither-like instrument.^{6,7} Subsequent terms included the dorsal hippocampal commissure⁸ – in contrast to the ventral hippocampal commissure – the fornix transversus,⁸ forniceal commissure,⁹ or the interammonic commissure.¹⁰

The first demonstration of the forniceal commissure in non-human primates (NHP) using silver impregnation methods emphasized the relative paucity of these fibers compared to non-primate eutherian mammals.^{11–20} The striking reduction in its magnitude was confirmed during extensive investigations of the commissural connections of the hippocampal formation in macaques using histological tracer techniques.^{21–23} These tracer studies showed that the fibers of the forniceal commissure in NHP did not originate in the hippocampal formation proper but in parahippocampal areas: the periallocortical presubiculum and entorhinal cortex, as well as – albeit to a lesser extent – the isocortical and proisocortical areas of the posterior parahippocampal gyrus.^{21–23}

In 1993, Gloor et al. published a comprehensive histological description of the hippocampal commissural system in humans.² While there was no significant remnant of the ventral hippocampal commissure, the dorsal hippocampal commissure (i.e., forniceal commissure) was described as a voluminous structure with a midline thickness corresponding to approximately one-fourth of the splenium.² This description of the forniceal commissure in humans distinctly exceeded that in NHP and thus contradicts the overall phylogenetic trend. It also contradicted electrophysiological studies, that have implied the non-existence of a direct inter-hippocampal commissural connection in the human brain.^{24–26} To the best of our knowledge, the study of Gloor et al. constitutes the only histological study of the human

forniceal commissure to date.² More recent studies using ex vivo fiber dissection^{27–30} and in vivo tractography^{30–37} provided conflicting results regarding the existence and morphology of a forniceal commissure in the human brain.

The aim of this multimodal anatomical study was to investigate the morphology of the human forniceal commissure by including advanced in vivo tractography, multidirectional ex vivo fiber dissection, and multiplanar histological analysis.

Methods

This study was approved by the local ethical review board of the Canton of Zurich, Switzerland (KEK ZH 2016-00957).

Nomenclature

As outlined above, the nomenclature of the structure examined in this study is complex for historical reasons. We used the descriptive terms *psalterium* and *forniceal commissure*. The psalterium was defined as a triangular subsplenic membrane extending between the crura fornicis. The term forniceal commissure was reserved for interforniceal fiber tracts. We chose to adhere to this nomenclature as the term dorsal hippocampal commissure can be considered misleading given the absence of fibers originating from the hippocampus proper in NHP and the absence of an identifiable ventral hippocampal commissure in humans.

Imaging datasets

Structural connectome data were downloaded from <http://www.bcblab.com/BCB/Opendata.html>. This publicly available dataset was derived from the diffusion-weighted imaging dataset of 180 participants acquired at 7 Tesla by the Human Connectome Project Team (<http://www.humanconnectome.org/study/hcp-young-adult/>; WU-Minn Consortium; Principal investigators: David Van Essen and Kamil Ugurbil; 1U54MH091657).³⁸ The scanning parameters have been described previously.^{38,39} In brief, each diffusion-weighted imaging consisted of a total of 132 near-axial slices acquired with an acceleration factor of 3,⁴⁰ isotropic (1.05 mm³) resolution and coverage of the whole head with a TE of 71.2 ms and with a TR of 7000 ms. At each slice location, diffusion-weighted images were acquired with 65 uniformly distributed gradients in multiple Q-space shells⁴¹ and 6 images with no diffusion gradient applied. This acquisition was repeated four times with a b-value of 1000 and 2000 s/mm² in pairs with anterior-to-posterior and posterior-to-anterior phase-encoding directions. The default Human Connectome Project preprocessing pipeline (v3.19.0)⁴² was applied to the data.⁴³ The susceptibility-induced off-resonance field was estimated from pairs of images with diffusion gradient applied with distortions going in opposite directions⁴⁴ and corrected for the whole diffusion-weighted dataset using TOPUP.⁴⁵ Subsequently, motion and geometrical distortion were corrected using the EDDY tool as implemented in FSL.

In vivo fiber dissection

We performed whole-brain deterministic tractography in the native diffusion-weighted imaging space using StarTrack software (<https://www.mr-startrack.com>). A damped Richardson-Lucy algorithm was applied for spherical deconvolutions.^{45,46} A fixed fiber response corresponding to a shape factor of $\alpha = 1.5 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ was adopted, coupled with the geometric damping parameter of 8. Two hundred algorithm iterations were run. The absolute threshold was defined as three times the spherical fiber orientation distribution of a grey matter isotropic voxel and the relative threshold as 8% of the maximum amplitude of the fiber orientation distribution.⁴⁷ A modified Euler algorithm⁴⁸ was used to perform the whole-brain streamline tractography, with an angle threshold of 35° , a step size of 0.5 mm and a minimum streamline length of 15 mm.

We co-registered the structural connectome data to the standard MNI 2 mm space using the following steps: first, whole-brain streamline tractography was converted into streamline density volumes where the intensities corresponded to the number of streamlines crossing each voxel. Second, a study-specific template of streamline density volumes was generated using the Greedy symmetric diffeomorphic normalisation (GreedySyN) pipeline distributed with advanced normalization tools (ANTs).^{48,49} This provided an average template of the streamline density volumes for all subjects. The template was then co-registered with a standard 2 mm MNI152 template using flirt as implemented in FSL. This step produced a streamline density template in the MNI152 space. Third, individual streamline density volumes were registered to the streamline density template in the MNI152 space template and the same transformation was applied to the individual whole-brain streamline tractography using the trackmath tool distributed with the software package Tract Querier⁵⁰ using ANTs GreedySyn. This step produced a whole-brain streamline tractography in the standard MNI152 space.

Percentage overlay maps

The fornix was dissected by using a one-region-of-interest approach by two examiners (KA, SJF) in every individual dataset using Trackvis (www.trackvis.org). The resulting reconstructions were binarized and converted to fiber density maps to generate percentage overlay maps using an in-house matlab script (Matlab R2021b). Results were visualized in FSL (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLeyes>).

Cadaver specimens

15 human brains were obtained from anonymized healthy body donations to the Anatomical Institute of the University of Zurich, Switzerland.

Multidirectional ex vivo fiber dissection

Nine brains were used for fiber dissection under the operating microscope. Brain preparation followed the technique originally described by Joseph Klingler⁵¹: in brief, the fresh specimens were fixed in a 5% formalin solution for at least 2 months. After fixation, the leptomeninges were removed under the operating microscope. This was followed by refrigeration for 7 days at a temperature of -10 to -15°C. After thawing, the brains were dissected following the technique of Klingler modified by Türe and colleagues using high magnification.^{51–54} The fiber dissection was performed from the superior and inferior aspect and from posterior in three specimens, respectively. Handmade soft wooden spatulas of various tip sizes, suction tips and microforceps were used to peel away the fibers. Specimen storage between different dissection steps was performed in 5% formalin solution at 5°C.

Morphometry of the psalterium

The length and the width of the psalterium were measured in the nine brain specimens used for fiber dissection under the operating microscope. The length (l) of the psalterium was measured from the unification of the crura fornicis to form the body of the fornix to the splenium of the corpus callosum. The width was defined as the distance between the right and left attachment of the crura fornicis to the splenium of the corpus callosum.

Multiplanar histological analysis

Six formalin-fixed specimens were used for histological analysis. The brains were cut through the splenium and caudal body of the corpus callosum into serial floating sections of 120 µm thickness with a mounting interval of 360 µm using a Vibratome (VT1000 S, Leica, Switzerland). The sections were oriented in the coronal (2x), sagittal (2x), and axial (2x) plane (Figure 4A). The floating sections were stained for myelin (1:900 dilution, FluoroMyelin Green, F34651, ThermoFisher) and counterstained with Hoechst 33342 (1:2000 dilution, H3570, Invitrogen, Carlsbad, CA) for 40 minutes at room temperature. After three washing steps for 20 minutes with phosphate buffered saline, the sections were mounted using ProLong Gold (Invitrogen, Carlsbad, CA). Whole-slide scans of the histological sections were produced by merging single images obtained with a Zeiss Observer. Z1 microscope coupled to a motorized stage (Carl Zeiss AG, Feldbach, Switzerland) at 10x magnification.

Results

In vivo fiber dissection (tractography)

We successfully identified and mapped the fornix in all participants. Individual dissections (<https://www.stephanieforkel.com/>) and the percentage overlay map (<https://neurovault.org/collections/12108/>) are provided online. In the percentage overlay map (Figure 1A) and tractography streamline reconstruction of the fornices (Figure 1B), we reliably identified the anterior and posterior columns of the fornix enclosing the anterior commissure, the body of the fornix underneath the corpus callosum, and the split of fornix into the crura fornices at the posterior end of the corpus callosum (splenium). The crura arched around the thalami and continued as fimbriae along the medial temporal lobe to terminate in the hippocampi. Commissural fibers between the crura fornices were, however, not evident.

Multidirectional ex vivo fiber dissection (Klingler technique)

The subsplenial region between the forniceal crura, where the forniceal commissure would be expected, was approached in a stepwise fashion. Fiber dissection was performed from ventral to dorsal, dorsal to ventral, and from caudal to rostral.

Dissection from ventral to dorsal (Figure 2): The entorhinal cortex as well as the cortex from the parahippocampal and fusiform gyri was peeled away, and the arcuate fibers were removed to expose the fibers of the parahippocampus and the inferior longitudinal fasciculus (Figure 2A). The mesencephalon, the thalamus, part of the hypothalamus and the caudate nucleus were removed to expose the columnae, body and crura of the fornices, and the roof of the lateral ventricles (Figure 2B). To expose the fimbria, the fibers of the parahippocampal gyrus and the inferior longitudinal fasciculus were removed as well as part of the cornu ammonis and the ependyma of the floor of the atrium and temporal horn (Figure 2C). The fornix was then dissected from the dorsal end of the fimbria along the crus towards the body (Figure 2D). By the end of the dissection the body of the corpus callosum was exposed (Figure 2E). We were able to identify the psalterium as a thin soft tissue membrane spanning between the right and left crus fornices in all specimens. It was limited rostrally by the merging bodies of the fornix, dorso-caudally it attached to the splenium of the corpus callosum and ventrally it abutted the velum interpositum. We were, however, unable to identify any crossing fibers between the crura fornices.

Dissection from dorsal to ventral (Figure 3A-C): Both hemispheres were cut down to the level of the corpus callosum, exposing the supracommissural hippocampus (Figure 3A). The callosal fibers were removed layer by layer until the ventricles and the crus fornices were

shining through. On one side the ventricle was opened to identify the caudate nucleus, the plexus, and the crus and the body of the fornix (Figure 3B). The removal of the remaining callosal fibers was performed and the underlying psalterium dissected without identification of crossing fibers between the fornices (Figure 3C).

Dissection from caudal to rostral (Figure 3D-E): The hemispheres were reduced up to the splenium of the corpus callosum. The splenium was fenestrated to visualize the lateral ventricle, the crus fornicis and the psalterium (Figure 3D). In the midline, the callosal fibers were separated and elevated from the psalterium (Figure 3E). There were no fibers identified crossing from one crus fornicis to the other.

Based upon the measurements in nine human specimens (Table 1), the psalterium had a mean length of 1.4 cm (SD 0.40 cm) and a mean width of 2.4 cm (SD 0.51 cm) (Figure 3F).

In summary, while the psalterium was visualized in all human brain specimens, it was not possible to identify commissural fibers between the two crura fornicis by further dissection of this membrane.

Multiplanar histological analysis

Representative examples of the myelin-stained histological appearance of coronal, sagittal and axial sections through the human splenium and caudal body of the corpus callosum are shown in Figure 4. In all three section planes, we identified a delicate bundle of commissural fibers extending between the crura fornicis. While the splenial fibers showed a caudo-convex trajectory and were running in parallel bundles, the interforniceal fibers had a net-like configuration. In comparison to a previous histological description of the human forniceal commissure,² this commissural system was found to be very discrete and of small extent in the rostrocaudal axis. The caudal bulbous expansion of the splenium wrapped around the caudal contour of the forniceal commissure. Rostrally, the forniceal commissure formed an increasingly thinner plate fading out where the fornix detached itself from the undersurface of the corpus callosum and came together with the contralateral fornix. The commissural fibers were enclosed by connective tissue, which also formed the boundary to the splenium of the corpus callosum in the midline and the fibers of the forceps major laterally.

Discussion

In this multimodal anatomical study, we characterized the morphology of the human forniceal commissure using advanced in vivo tractography, multidirectional ex vivo fiber dissection, and multiplanar histological analysis.

Virtual fiber dissection based on 7 Tesla diffusion-weighted imaging dataset of 180 participants did not identify interhemispheric connections between the crura fornices. Previous tractography literature has been inconclusive about the visualization of a forniceal commissure: while some studies of the fornices did not describe commissural fibers,^{30,32–34} others did describe a forniceal commissure.^{31,35,37,55} The absence of evidence in our study, however, might not be the evidence of absence. Previous reports indicated that fibers crossing the hemispheric midline have a different diameter.⁵⁶ In this case, the algorithm might not be able to trace commissural connections accurately.

Our stepwise dissections of nine previously frozen, formalin-fixed human brains under the operating microscope showed a fine triangular soft tissue membrane between the forniceal crura, but no clear commissural fibers. The soft tissue membrane, which we identified in all specimens, was consistent with previous descriptions of the psalterium.²⁹ There is only a small number of fiber dissection studies specific to this anatomical region: no fibers suspicious of a forniceal commissure were identified by Shah et al of 2012,²⁷ Destrieux et al 2013,²⁸ and Güngör et al 2017.³⁰ However, Tubbs et al. visualized the psalterium in twenty specimens, and in some cases dissected individual fine fiber-like bundles within the psalterium, which were designated as forniceal commissure.²⁹ We were not able to identify such fibers during the dissection under the operating microscope. While the fiber dissection technique by Joseph Klingler appears to be suitable for identifying larger and highly myelinated fiber tracts,^{54,57–59} it might be less applicable to fine, less myelinated, and cruciform fibers as is the case for the forniceal commissure. This may explain why we were unable to identify distinct commissural fiber tracts in the psalterium through ex vivo fiber dissection.

The microscopic anatomy of the forniceal commissure has been subject to extensive studies in NHP: the work of Amaral et al. on the commissural connections of the hippocampal commissure in macaques through the use of anterograde and retrograde labeling techniques revealed a number of differences in their organization compared to previous studies in rodents and lagomorphs.²¹ First and foremost, there was an overall reduction in the size of the commissural projections. Second, it was surprising that the hippocampus proper received no commissural input. The most prominent portions of the commissural fibers originated in the presubiculum and terminated in the medial entorhinal area on the opposite side. The entorhinal cortex, in turn, was the origin of homotopic commissural projections with additional

minor portions to the contralateral subiculum. Demeter et al. characterized the distinct hippocampal and parahippocampal origins of the fibers of fimbria, fornix, and the forniceal commissure in NHP.^{22,23} From their presubicular, entorhinal, and parahippocampal origins, the fibers of the forniceal commissure pass through the alveus – not the fimbria – into the medial (alvear) fornix (contrasted by the lateral (fimbrial) fornix), continue to the undersurface of the crus fornicis, and thence cross along the undersurface of the splenium to the crus fornicis of the opposite side, where they follow the same path in a retrograde sequence. The morphological characteristics of the fibers comprising the forniceal commissure were studied in detail by Lamantia and Rakic in myelin-stained histological sections of primate brains.³ In this work, the forniceal commissure was described to be composed of approximately 237,000 predominantly small and medium-sized myelinated axons, representing 0.4% of the telencephalic commissural axons. The fibers of the forniceal commissure could be differentiated morphologically from the fibers of the splenium by their smaller caliber and paler staining properties.

While early anatomical studies in humans contradicted the existence of a distinct forniceal commissure,¹⁷ the work of Gloor et al. described a well-defined voluminous tract ventral to the splenium with fibers of smaller caliber and paler staining than those of the splenium.² Remarkable in the description by Gloor et al. was in particular the thickness of the tract, corresponding in its greatest extension to about one fourth of the splenium.² This surpasses the representation in NHP severalfold and contradicts the expected phylogenetic trend of a decrease in the volume of the forniceal commissures relative to the splenium.^{2,3,21–23} In our multiplanar histological analysis, we reliably detected a subtle connective tissue membrane between the crura fornicis as the microscopic correlate of the psalterium. This membrane encapsulated fine cruciform fiber bundles, likely corresponding to the phylogenetic remnant of the forniceal commissure. In all six specimens, the forniceal commissure appeared much more discreet than described by Gloor et al.,² and as usually depicted in anatomical textbooks. The morphology of the structure identified as dorsal hippocampal commissure (i.e., forniceal commissure) in the work of Gloor et al.² resembles the appearance of the rostrally turning ventral part of the splenium in our examinations.

The function of the forniceal commissure both in health and disease remains largely elusive. Memory deficits have been observed in patients after splenial callosotomies, yet it is unclear to what degree the forniceal commissure and the splenium, respectively, contributed to these deficits.⁶⁰ A recent study postulated a role of the forniceal commissure in familiarity-based recognition memory.³⁷ This is consistent with observations in NHP which displayed impaired discrimination learning after lesion of the forniceal commissure.⁶¹ While certain authors attribute no or minor relevance to the forniceal commissure in mesial temporal lobe

epilepsy,^{25,26,62–66} others consider it as one of the most important pathways of contralateral seizure propagation and false lateralization of the ictal onset in extracranial EEG recordings.^{2,67–69} Ictal involvement of the forniceal commissure has been associated with the phenomena of pure amnesic seizures and transient epileptic amnesia.^{2,69} The forniceal commissure received additional attention as a component of forebrain commissurotomies for the therapy of intractable generalized seizures, yet failed to establish itself as an independent disconnection procedure.^{70–72} Our study provides a multimodal morphological characterization of the human forniceal commissure and might serve as a basis for future functional investigations.

Conclusions

Multimodal anatomical approaches indicate that the human forniceal commissure is much more delicate than previously described and presented in anatomical textbooks. This finding is consistent with the observed phylogenetic trend of a reduction of the forniceal commissure in NHP compared to non-primate eutherian mammals.

Acknowledgements

This study was supported by the Prof. Dr. med. Karl und Rena Theiler-Haag foundation (grant Nr.: F-86101-26-01) and the Horizon2020 Marie Skłodowska-Curie programme (SJF; Grant agreement No. 101028551). Many thanks to Ahmad Beyh for discussions.

Data Availability

The tractography data (Package X) is freely available as a preprocessed dataset from <http://www.bcblab.com/BCB/Opendata.html>. Individual dissections and the percentage overlay map of the fornices are available from <https://www.stephanieforkel.com/> and <https://neurovault.org/collections/12108/>, respectively. A 3D model of the fornix is available for digital exploration and 3D printing at <https://www.thingiverse.com/stephforkel/designs>.

Disclosure

We confirm that we have read the Journal's position on issues related to ethical publication and affirm that this report is consistent with those guidelines. None of the authors has any conflict of interest to disclose.

References

- 1 Ariens-Kappers CU, Huber GC, Crosby EC. The Comparative Anatomy of the Nervous System, Including Man. *Hafner, New York* 1936.
- 2 Gloor P, Salanova V, Olivier A, Quesney LF. The human dorsal hippocampal commissure. An anatomically identifiable and functional pathway. *Brain* 1993; **116 (Pt 5)**: 1249–73.
- 3 Lamantia AS, Rakic P. Cytological and quantitative characteristics of four cerebral commissures in the rhesus monkey. *J Comp Neurol* 1990; **291**: 520–37.
- 4 Thiebaut de Schotten M, Dell'Acqua F, Valabregue R, Catani M. Monkey to human comparative anatomy of the frontal lobe association tracts. *Cortex* 2012; **48**: 82–96.
- 5 Barrett RLC, Dawson M, Dyrby TB, *et al.* Differences in Frontal Network Anatomy Across Primate Species. *J Neurosci* 2020; **40**: 2094–107.
- 6 Ferrier D. The Croonian Lectures on Cerebral Localisation. *BMJ* 1890; **1**: 1349–55.
- 7 Smith GE. A Preliminary Communication upon the Cerebral Commissures of the Mammalia, with Special Reference to the Monotremata and Marsupialia. *J Anat* 1937; **71**: 528–43.
- 8 Villiger E, Piersol GA. 'The Brain And Spinal Cord.' A Manual For The Study Of The Morphology And Fibre Tracts Of The Central Nervous System. *The American Journal of the Medical Sciences* 1913; **146**: 908.
- 9 Baehr M, Frotscher M, Duus P. Duus' topical diagnosis in neurology: anatomy, physiology, signs, symptoms. *Thieme* 2005.
- 10 Scarabino T, Salvolini U. Atlas of Morphology and Functional Anatomy of the Brain. *Springer* 2006.
- 11 Cajal SR y., Ramón y Cajal S. Histologie du système nerveux de l'homme & des vertébrés. *Maloine* 1909.
- 12 Raisman G, Cowan WM, Powell TPS. The extrinsic afferent, commissural and association fibres of the hippocampus. *Brain* 1965.
- 13 Shipley MT. The topographical and laminar organization of the presubiculum's projection to the ipsi- and contralateral entorhinal cortex in the guinea pig. *The Journal of Comparative Neurology* 1975; **160**: 127–45.
- 14 Swanson LW, Cowan WM. The connections of the septal region in the rat. *J Comp Neurol* 1979; **186**: 621–55.
- 15 Blackstad TW. Commissural connections of the hippocampal region in the rat, with special reference to their mode of termination. *J Comp Neurol* 1956; **105**: 417–537.
- 16 Lorente de Nó R. Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. *Journal für Psychologie und Neurologie* 1934.
- 17 y Cajal SR, Kraft LM. Studies on the Cerebral Cortex-Limbic Structures. *Lloyd-Luke* 1955.
- 18 Van Hoesen GW, Pandya DN. Some connections of the entorhinal (area 28) and perirhinal (area 35) cortices of the rhesus monkey. I. Temporal lobe afferents. *Brain*

Research 1975; **95**: 1–24.

- 19 Van Hoesen GW, Pandya DN, Butters N. Some connections of the entorhinal (area 28) and perirhinal (area 35) cortices of the rhesus monkey. II. Frontal lobe afferents. *Brain Research* 1975; **95**: 25–38.
- 20 Van Hoesen GW, Pandya DN. Some connections of the entorhinal (area 28) and perirhinal (area 35) cortices of the rhesus monkey. III. Efferent connections. *Brain Research* 1975; **95**: 39–59.
- 21 Amaral DG, Insausti R, Cowan WM. The commissural connections of the monkey hippocampal formation. *Journal of Comparative Neurology* 1984; **224**: 307–36.
- 22 Demeter S, Rosene DL, Van Hoesen GW. Interhemispheric pathways of the hippocampal formation, presubiculum, and entorhinal and posterior parahippocampal cortices in the rhesus monkey: the structure and organization of the hippocampal commissures. *J Comp Neurol* 1985; **233**: 30–47.
- 23 Demeter S, Rosene DL, Van Hoesen GW. Fields of origin and pathways of the interhemispheric commissures in the temporal lobe of macaques. *J Comp Neurol* 1990; **302**: 29–53.
- 24 Wang ML, Wilson CL, Babb TL, Crandall PH. Functional connections in the human limbic system. *Springer* 1981: 82.
- 25 Wilson, CL. A comparative view of local and interhemispheric limbic pathways in humans: an evoked potential analysis. *Fundamental mechanisms of human brain function* 1987; 27–36.
- 26 Wilson CL, Isokawa M, Babb TL, Crandall PH, Levesque MF, Engel J Jr. Functional connections in the human temporal lobe. II. Evidence for a loss of functional linkage between contralateral limbic structures. *Exp Brain Res* 1991; **85**: 174–87.
- 27 Shah A, Jhawar SS, Goel A. Analysis of the anatomy of the Papez circuit and adjoining limbic system by fiber dissection techniques. *J Clin Neurosci* 2012; **19**: 289–98.
- 28 Destrieux C, Bourry D, Velut S. Surgical anatomy of the hippocampus. *Neurochirurgie* 2013; **59**: 149–58.
- 29 Tubbs RS, Bosmia AN, Gupta T, *et al.* The enigmatic psalterium: a review and anatomic study with relevance to callosotomy procedures. *Neurosurgery* 2015; **11 Suppl 2**: 322–8; discussion 328.
- 30 Güngör A, Baydin S, Middlebrooks EH, Tanriover N, Isler C, Rhoton AL Jr. The white matter tracts of the cerebrum in ventricular surgery and hydrocephalus. *J Neurosurg* 2017; **126**: 945–71.
- 31 Colnat-Coulbois S, Mok K, Klein D, Pénicaud S, Tanriverdi T, Olivier A. Tractography of the amygdala and hippocampus: anatomical study and application to selective amygdalohippocampectomy. *J Neurosurg* 2010; **113**: 1135–43.
- 32 Yeo SS, Seo JP, Kwon YH, Jang SH. Precommissural fornix in the human brain: a diffusion tensor tractography study. *Yonsei Med J* 2013; **54**: 315–20.
- 33 Postans M, Hodgetts CJ, Mundy ME, Jones DK, Lawrence AD, Graham KS. Interindividual variation in fornix microstructure and macrostructure is related to visual discrimination accuracy for scenes but not faces. *J Neurosci* 2014; **34**: 12121–6.

- 34 Sboto-Frankensteen UN, Lazar T, Bolster RB, *et al.* Symmetry of the fornix using diffusion tensor imaging. *J Magn Reson Imaging* 2014; **40**: 929–36.
- 35 Wei P-H, Mao Z-Q, Cong F, *et al.* In vivo visualization of connections among revised Papez circuit hubs using full q-space diffusion spectrum imaging tractography. *Neuroscience* 2017; **357**: 400–10.
- 36 Yağmurlu K, Zaidi HA, Kalani MYS, Rhoton AL, Preul MC, Spetzler RF. Anterior interhemispheric transsplenic approach to pineal region tumors: anatomical study and illustrative case. *J Neurosurg* 2018; **128**: 182–92.
- 37 Postans M, Parker GD, Lundell H, *et al.* Uncovering a Role for the Dorsal Hippocampal Commissure in Recognition Memory. *Cereb Cortex* 2020; **30**: 1001–15.
- 38 Vu AT, Auerbach E, Lenglet C, *et al.* High resolution whole brain diffusion imaging at 7T for the Human Connectome Project. *Neuroimage* 2015; **122**: 318–31.
- 39 Nozais V, Forkel SJ, Foulon C, Petit L, Thiebaut de Schotten M. Functionnectome as a framework to analyse the contribution of brain circuits to fMRI. *Commun Biol* 2021; **4**: 1035.
- 40 Moeller S, Yacoub E, Olman CA, *et al.* Multiband multislice GE-EPI at 7 tesla, with 16-fold acceleration using partial parallel imaging with application to high spatial and temporal whole-brain fMRI. *Magn Reson Med* 2010; **63**: 1144–53.
- 41 Caruyer E, Lenglet C, Sapiro G, Deriche R. Design of multishell sampling schemes with uniform coverage in diffusion MRI. *Magn Reson Med* 2013; **69**: 1534–40.
- 42 Glasser MF, Sotiropoulos SN, Wilson JA, *et al.* The minimal preprocessing pipelines for the Human Connectome Project. *Neuroimage* 2013; **80**: 105–24.
- 43 Sotiropoulos SN, Jbabdi S, Xu J, *et al.* Advances in diffusion MRI acquisition and processing in the Human Connectome Project. *Neuroimage* 2013; **80**: 125–43.
- 44 Andersson JLR, Skare S, Ashburner J. How to correct susceptibility distortions in spin-echo echo-planar images: application to diffusion tensor imaging. *Neuroimage* 2003; **20**: 870–88.
- 45 Smith SM, Jenkinson M, Woolrich MW, *et al.* Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 2004; **23 Suppl 1**: S208–19.
- 46 Dell'acqua F, Scifo P, Rizzo G, *et al.* A modified damped Richardson-Lucy algorithm to reduce isotropic background effects in spherical deconvolution. *Neuroimage* 2010; **49**: 1446–58.
- 47 Thiebaut de Schotten M, Dell'Acqua F, Forkel SJ, *et al.* A lateralized brain network for visuospatial attention. *Nat Neurosci* 2011; **14**: 1245–6.
- 48 Dell'Acqua F, Simmons A, Williams SCR, Catani M. Can spherical deconvolution provide more information than fiber orientations? Hindrance modulated orientational anisotropy, a true-tract specific index to characterize white matter diffusion. *Hum Brain Mapp* 2013; **34**: 2464–83.
- 49 Avants BB, Tustison NJ, Song G, Cook PA, Klein A, Gee JC. A reproducible evaluation of ANTs similarity metric performance in brain image registration. *Neuroimage* 2011; **54**: 2033–44.

- 50 Wassermann D, Makris N, Rathi Y, *et al.* The white matter query language: a novel approach for describing human white matter anatomy. *Brain Structure and Function*. 2016; **221**: 4705–21.
- 51 Klingler J. Erleichterung der makroskopischen Präparation des Gehirns durch den Gefrierprozess. *Schweiz Arch Neurol Psychiatr* 1935; 247–56.
- 52 Klingler J, Gloor P. The connections of the amygdala and of the anterior temporal cortex in the human brain. *J Comp Neurol* 1960; **115**: 333–69.
- 53 Ludwig E, Klingler J. *Atlas humani cerebri*. 1956.
- 54 Türe U, Gazi Yaşargil M, Friedman AH, Al-Mefty O. Fiber Dissection Technique: Lateral Aspect of the Brain. *Neurosurgery*. 2000; **47**: 417–27.
- 55 Catani M, Thiebaut de Schotten M. A diffusion tensor imaging tractography atlas for virtual in vivo dissections. *Cortex* 2008; **44**: 1105–32.
- 56 Tomasch J. The human posterior commissure. *J Comp Neurol* 1965; **124**: 43–50.
- 57 Serra C, Akeret K, Maldaner N, *et al.* A White Matter Fiber Microdissection Study of the Anterior Perforated Substance and the Basal Forebrain: A Gateway to the Basal Ganglia? *Oper Neurosurg (Hagerstown)* 2019; **17**: 311–20.
- 58 De Schotten MT, Dell’Acqua F, Forkel S, *et al.* A lateralized brain network for visuo-spatial attention. *Nature Precedings* 2011; 1–1.
- 59 Catani M, Robertsson N, Beyh A, *et al.* Short parietal lobe connections of the human and monkey brain. *Cortex* 2017; **97**: 339–57.
- 60 Phelps EA, Hirst W, Gazzaniga MS. Deficits in recall following partial and complete commissurotomy. *Cereb Cortex* 1991; **1**: 492–8.
- 61 Moss M, Mahut H, Zola-Morgan S. Concurrent discrimination learning of monkeys after hippocampal, entorhinal, or fornix lesions. *J Neurosci* 1981; **1**: 227–40.
- 62 Lieb JP, Babb TL. Interhemispheric Propagation Time of Human Hippocampal Seizures: II. Relationship to Pathology and Cell Density. *Epilepsia*. 1986; **27**: 294–300.
- 63 Lieb JP, Hoque K, Skomer CE, Song XW. Inter-hemispheric propagation of human mesial temporal lobe seizures: a coherence/phase analysis. *Electroencephalogr Clin Neurophysiol* 1987; **67**: 101–19.
- 64 VanLandingham KE, Lothman EW. Self-sustaining limbic status epilepticus.: II. Role of hippocampal commissures in metabolic responses. *Neurology* 1991; **41**: 1950–1950.
- 65 Wilson CL, Isokawa-Akesson M, Wang ML, Babb TL, Jnr JE. Lack of functional evidence for a human hippocampal commissure. *Electroencephalogr Clin Neurophysiol* 1985; **61**: S54–5.
- 66 Lacruz ME, García Seoane JJ, Valentin A, Selway R, Alarcón G. Frontal and temporal functional connections of the living human brain. *European Journal of Neuroscience* 2007; **26**: 1357–70.
- 67 Spencer SS, Marks D, Katz A, Kim J, Spencer DD. Anatomic correlates of interhippocampal seizure propagation time. *Epilepsia* 1992; **33**: 862–73.

- 68 Spencer SS, Williamson PD, Spencer DD, Mattson RH. Human hippocampal seizure spread studied by depth and subdural recording: the hippocampal commissure. *Epilepsia* 1987; **28**: 479–89.
- 69 Palmini AL, Gloor P, Jones-Gotman M. Pure amnestic seizures in temporal lobe epilepsy. Definition, clinical symptomatology and functional anatomical considerations. *Brain* 1992; **115 (Pt 3)**: 749–69.
- 70 Wilson DH, Culver C, Waddington M, Gazzaniga M. Disconnection of the cerebral hemispheres. An alternative to hemispherectomy for the control of intractable seizures. *Neurology* 1975; **25**: 1149–53.
- 71 Wilson DH, Reeves AG, Gazzaniga MS. 'Central' commissurotomy for intractable generalized epilepsy: series two. *Neurology* 1982; **32**: 687–97.
- 72 Harbaugh RE, Wilson DH, Reeves AG, Gazzaniga MS. Forebrain commissurotomy for epilepsy. *Acta Neurochirurgica* 1983; **68**: 263–75.

Tables

Specimen	Length (cm)	Width (cm)
1	2.2	2.9
2	1.4	2.4
3	1.1	2.4
4	0.9	1.9
5	1.7	2.2
6	1.6	3.4
7	1.3	2.2
8	1.1	1.7
9	1.2	2.2

Table 1. Morphometry of the psalterium:

Length and width (compare Figure 3F) of the psalterium in nine human specimens.

Figures

Figure 1. In vivo dissection of the fornix:

A. Selected slices of the percentage overlay map based on the fornix reconstructions in 180 participants from the HCP (bcbclab/packageX). Individual dissections (<https://www.stephanieforkel.com/>) and the full percentage overlay map (<https://neurovault.org/collections/12108/>) of the fornices are available online. **B.** Tractography streamline reconstruction of the fornix. A 3D model is available online (<https://www.thingiverse.com/stephforkel/designs>).

Figure 2. Fiber dissection of the psalterium from the ventral aspect:

Stepwise fiber dissection of the subsplenial region from the ventral towards the dorsal surface of the cerebrum using the Klingler method. **A.** Exposure of the inferior longitudinal fasciculus (ILF) and the fibers of the parahippocampal gyrus (fPHG) after removal of the entorhinal cortex (EC), cortex of the parahippocampal (cPHG) and fusiform (lateral to the PHG, not shown) gyri and the underlying arcuate fibers (AF). CA, cerebral aqueduct; CC, crus cerebri; Cing, cingulum; Isth, isthmus; LPS, lateral perforated substance; MB, mammillary body; MGB, medial geniculate body; OT, optic tract; PG, pineal gland; PPS, posterior perforated substance; Pu, pulvinar; SCC, splenium of the corpus callosum; SN, substantia nigra; TC, tuber cinereum; Tec, tectum mesencephali; Teg, tegmentum mesencephali. **B.** View on the roof of the lateral ventricles (RLV), crus (crF), body (bF) and column (coF) of the fornix, and the psalterium (Ps) after removal of the mesencephalon, thalamus, and parts of the hypothalamus and the caudate nucleus. **C.** Exposure of the dentate gyrus (DG), fimbria (Fi), gyrus fasciolaris (GF), the subcallosal trigone (SCT), inferior surface of the uncus apex (iUA), band of Giacomini (BG), and the external digitations of the hippocampal head (ED) after removal of the fibers of the parahippocampal gyrus, inferior longitudinal fasciculus, part of the cornu ammonis (CA) and the ependyma of the floor of the atrium (A) and the temporal horn (TH). **D-E.** Dissection of the fornix from the dorsal end of the fimbria (Fi) along the crus (crF) towards the body of the fornix (bF). No commissural fibers between the fornices were identified. Removal of the ependymal layer of the roof of the lateral ventricle (RLV) exposed the fibers of the body of the corpus callosum (BCC).

Figure 3. Fiber dissection from the dorsal and caudal aspects, and morphometry of the psalterium:

A. Exposure of the corpus callosum (CC) and the supracommissural hippocampus with its medial longitudinal stria (MLS), lateral longitudinal stria (LLS) and the indusium griseum (IG). **B.** Removal of the fibers of the body of the corpus callosum (BCC) until the lateral ventricle (LV) and the crus fornix (CF) were shining through (right). Further dissection of the remaining callosal fibers and removal of the choroid plexus and lamina affixa revealed the taenia fornix (TF), thalamus (Th), lamina terminalis (LT) and the caudate nucleus (CN) (left). **C.** Elevation of the psalterium (Ps) without identification of commissural fibers between the crura fornix (CF). The choroid plexus (CP) is fixed to the crus fornix via the taenia fornix (TF) and to the lamina affixa (LA) through the taenia choroidea (TC). The thalamus (Th) is shining through the choroid plexus and the lamina affixa. **D.** Fenestration of the splenium of the corpus callosum (SCC) and partial removal of the thalamus (Th) to visualize the lateral ventricle (LV), crus fornix (crF) and psalterium (Ps). **E.** Separation and elevation of the medial part of the splenium of the corpus callosum (SCC) from the psalterium (Ps). The unification of the crura fornix (crF) into the body of the fornix (bF) form the anterior border of the psalterium. **F.** The length (l) of the psalterium was measured from the unification of the crura fornix (crF) to form the body of the fornix (bF) to the splenium of the corpus callosum (SCC). The width (w) of the psalterium was defined as the distance between right and left attachment of the crura fornix (crF) to the splenium of the corpus callosum.

Figure 4. Multiplanar histology of the psalterium:

A. Schematic illustration of the histological section planes: coronal (B), sagittal (C) and axial (D). **B.-D.** Floating sections stained for myelin (1:900 dilution, FluoroMyelin Green, F34651, ThermoFisher) and counterstained with Hoechst 33342 (1:2000 dilution, H3570, Invitrogen, Carlsbad, CA): **B.** Coronal sections from caudal to rostral. BCC, body of the corpus callosum; bF, body of the fornix; CF, commissura fornicis; CG, cingulate gyrus; crF, crus fornicis; M, connective tissue membrane; SCC, splenium of the corpus callosum; SP, septum pellucidum. **C.** Sagittal sections from left to right. **D.** Axial sections from basal to dorsal.