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TEACHING ANATOMY

A stepwise laboratory manual for the dissection and illustration of limbic and paralimbic structures: lessons learned from the Klingler's technique

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Abstract

Background Three-dimensional relationships within the limbic and paralimbic areas are often hard to grasp. Relevant anatomical structures exhibit a complicated architecture and connectivity and therefore surgical approaches targeting lesions or functional resections in this area pose a distinct challenge.

Purpose To provide an educational, comprehensive, systematic and stepwise manual for the dissection and illustration of major limbic structures since there is a gap in the pertinent literature. Further, we aim to offer a thorough yet simplified roadmap for laboratory and intraoperative dissections.

Methods Twenty (20) normal adult, formalin-fixed cerebral hemispheres were studied through the fiber dissection technique and under the microscope. Stepwise and in tandem medial to lateral and lateral to medial dissections were performed in all specimens aiming to reveal the morphology and spatial relationships of major limbic and paralimbic areas.

Results Fourteen (14) consecutive, discrete and easily reproducible laboratory anatomical steps are systematically described to reveal the intricate anatomy of the limbic and paralimbic structures and their main connections.

Conclusion This study offers for the first time in the pertinent literature a focused, step-by-step laboratory manual for the dissection and illustration of the limbic and paralimbic structures. The overreaching goal is to supplement the novice and experienced anatomist and neurosurgeon with a thorough and systematic reference to facilitate laboratory or intraoperative dissections.

Keywords Limbic · Laboratory manual · Klingler's · White matter dissection · Papez · Paralimbic · Accumbens · Cingulum · Hippocampus · Dissection

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Introduction

The anatomical term "limbic lobe" (latin *limbus* meaning edge, border), was coined in 1878 by Paul Broca in his essay "*Anatomie compare des circonvolutions cerebrales: Le grand lobe limbique et la scissure limbique dans la serie de mammiferes*" [2]. The introduction of the "great limbic lobe", which marks a natural border between the cerebral hemisphere and diencephalon, would have an enormous impact on neuroanatomical literature for more than a century. From Broca's functional perspective, the limbic lobe represented an archaic structure primarily associated with olfaction in contrast to the high-order lobes linked to cognition, the latter prevailing over the atrophying limbic lobe during evolution. The term limbic lobe (*lobus limbicus*) is preserved to date (Terminologia Anatomica and Terminologia Neuoanatomica) to define a sixth cerebral lobe incorporating various anatomical structures [4, 6].

Broca's legacy was followed by the pivotal experimental work and clinical observations of researchers like Christrfied Jacob, James Papez and Ivan Yakovlev that would culminate in Paul Maclean's proposal for a discrete and integrated anatomo-functional system underlying our very emotional existence—the limbic system- the paleomammalian element of his triune brain theoretical conception [3, 13, 16, 18]. Since Maclean's proposal, the limbic system, one of the most pervasive models in modern neuroscience, has been extensively expanded and revised to incorporate distinct circuits, believed to subserve affective, mnemonic and behavioural functions [3, 17, 18, 22].

Despite the ongoing effort to elucidate the intricate anatomo-functional architecture and axonal connectivity of this particular network, indeed it seems that there is a paucity of the pertinent literature with regard to its microsurgical anatomy, especially from an educational standpoint. Only a limited number of cadaveric studies have previously utilised the fiber dissection technique to elucidate the anatomy of the Jacob/Papez circuit [1, 8, 9, 19]. In this context, our purpose is to offer for the first time a comprehensive, systematic and stepwise manual for the dissection and illustration of the limbic lobe and additional major limbic and para-limbic structures through the white matter dissection technique. This recently revitalised method has been incorporated into the neuro-anatomical and neurosurgical education as a valuable tool to acquire a three-dimensional perception of the topographical anatomy and axonal connectivity of the human cerebrum. Hence the present manual aims to provide a valuable resource for a better understanding of the spatial relationships of major limbic structures and assist the neurosurgeon in the mental grasping of the intricate regional anatomy encountered in the operating room.

Materials and methods

Twenty (20) adult, formalin-fixed cerebral hemispheres with no gross evidence of disease were treated with Klingler's preparation [5, 11]. According to the latter the brains were fixed in 10% formalin solution for a period of 4 weeks. Following the fixation period, the dura, arachnoid and vessels were carefully removed and the specimens were stored in a refrigerator in – 15 ℃ for a period of 14 days. The hemispheres were then thawed under running water for one day and stored in a fresh 10% formalin solution. All specimens were subsequently investigated through the fiber dissection technique with the aid of a microscope (OPMI Plus, Carl Zeiss) and using surgical micro-instruments including microscissors, micro-forceps and Penfield micro-dissectors.

The surface anatomy of the medial aspect of the hemisphere was initially observed and recorded to identify the main anatomical landmarks of the limbic lobe and major para-limbic structures according to the Terminologia Anatomica and Terminologia Neuroanatomica. Focused white matter dissections were then carried out in a reproducible medio-lateral and latero-medial stepwise manner with the goal to offer a concrete and thorough three-dimensional perception of the complex regional anatomical relationships. Each dissection was built upon the idea of gradually revealing and identifying six main structures/areas: (1) The limbic fissure (2) The Limbic Gyrus and underlying cingulum (3) The structures of the intralimbic gyrus (4) The insular region (5) The hippocampal-forniceal complex and the mammilothalamic tract and (6) The amygdala-caudate-stria terminalis complex. Multiple photos from different angles were obtained in each dissection step to document and vividly illustrate the regional anatomy and spatial relationships of the limbic structures.

Results

Fourteen (14) consecutive laboratory anatomical steps are systematically described to reveal the major limbic and paralimbic structures. These steps derive from medial to lateral, lateral to medial and basal dissections and are summarized alongside useful microsurgical comments in Table 1.

Step 1

In the first step, the cortex of the subrostral area (including the paraterminal and paraolfactory gyri) is carefully removed to reveal the prehippocampal rudiment. The prehippocampal rudiment, which corresponds to the precommissural hippocampus, is a thin lamina of gray matter located between the paraterminal gyrus and the lamina

Table 1 Stepwise dissection of major limbic structures. Steps/structures revealed and relevant microsurgical comments

Step	Orientation of dissection	Technique	Structures revealed	Microsurgical comment
0	Medial view	Appreciate superficial anatomy	Limbic sulcus Limbic gyrus Subcallosal area Cingulate gyrus Isthmus of cingulate gyrus Parahippocampal gyrus Uncus Rhinal sulcus Entorhinal cortex	
1	Medial to lateral	Remove the cortex of the subrostral area	Prehippocampal Rudiment/precommissural Hippocampus	
2	Medial to lateral	Remove the cortex of the cingulate gyrus	Superior arm of the cingulum Indusium griseum/supracommissural hippocampus Longitudinal striae (medial and lateral)	
3	Medial to lateral	Using a no10 blade remove the body of the corpus callosum from genu to splenium. Using a fine microdissector remove the medial aspect of thalamus and hypothalamus	Precomissural/postcompissural fornix Mammilothalam tract of vicq d' azyr, mesolimbic fibers	The substantia innominata is located in the triangle located between the anterior commissure the pre- and post-commissural fornix and the anterior perforated substance
4	Medial to lateral	Remove the splenium of the corpus callosum	Crus fornix Dentate gyrus/fasciola Cinerea/subsplenial gyrus (gyrus of andreas retzius) Parahippocampal gyrus with underlying inferior arm of the cingulum	
5	Medial to lateral then lateral to medial	Dissect the core of the hemisphere using a No10 blade along superior margin of caudate nucleus and isthmus Turn to lateral aspect of hemisphere and remove the cortex and U-fibers of the peri-insular region	Insular surface anatomy	The superior margin of the body/head of the caudate nucleus corresponds in the superficial level to the frontal operculum
6	Medial to lateral	Remove the medial cortex of the parahippocampal gyrus. Spare the superior cortex of the parahippocampal gyrus(Subiculum)	Inferior arm of the cingulum Subiculum	
7	Medial to lateral	Remove arachnoid of the basal cisterns to detach uncus from cerebral peduncle and posteromedial orbital lobule	Fimbria Anterior part of dentate gyrus	
8	Lateral to medial	Using a No15 blade cut along the white matter of the temporal stem 5 mm ventral to inferior limiting sulcus to enter temporal horn of the lateral ventricle	Alveus of hippocampus	The temporal horn can be entered by transgressing the temporal stem 3-5 mm ventrally to the inferior limiting sulcus of the insula
9	Medial to lateral	Dissect the fornix free from thalamus from anterior commissure to fimbria	Choroidal fissure Stria terminalis thalami	

Table 1 (continued)

Step	Orientation of dissection	Technique	Structures revealed	Microsurgical comment
10	Lateral to medial	Remove ependyma of the roof and tip of the temporal horn. Carefully remove periamygdaloid white matter	Amygdala Stria terminalis (ventral part) Tail of caudate nucleus	The amygdala is located at the level of the postero-medial part of the planum temporale, 3-6 mm ventral to the limen insulae
11	Basal	Meticulously dissect the area of the anterior perforated substance to reveal the ansa peduncularis. Carefully dissect the posterior orbitofrontal area to illustrate the nucleus accumbens	Ansa peduncularis, nucleus accumbens	The ansa peduncularis can be traced at the level of the posterior part of the anterior perforated substance
12	Basal	Remove the nucleus accumbens to reveal the mesocortical fibers running within the anterior limb of the internal capsule	Mesocortical fibers	The mesocortical fibers run within the anterior limb of the internal capsule at the level of the rostrum of the corpus callosum
13	Medial to lateral	Dissect the anterior part of the parahippocampal gyrus and hippocampus from free. Use a No10 blade to dissect the anterior part of the temporal stem below the amygdala	Head/body/tail of hippocampus Collateral eminence Subiculum Uncal gyrus Ambient gyrus Dentate gyrus	
14	Medial to lateral	Dissect the hippocampus from the parahippocampal gyrus along the hippocampal sulcus	Fimbriodentate sulcus Hippocampal sulcus Margo denticularis Fasciola cinerea Gyrus fasciolaris	The hippocampus can be detached from the hemisphere by dissecting it initially from the area of the collateral eminence (in order to detach it from the subiculum) and then from the choroidal fissure

terminalis and represents the anterior continuation of the indusium griseum (Figs. 1b, and 2b).

Step 2

The cortex of the cingulate gyrus is removed to reveal the superior arm of the cingulum, which typically extends from the subrostral area (pole of the cingulate gyrus) up to the level of the isthmus of the cingulate gyrus. In this step, the

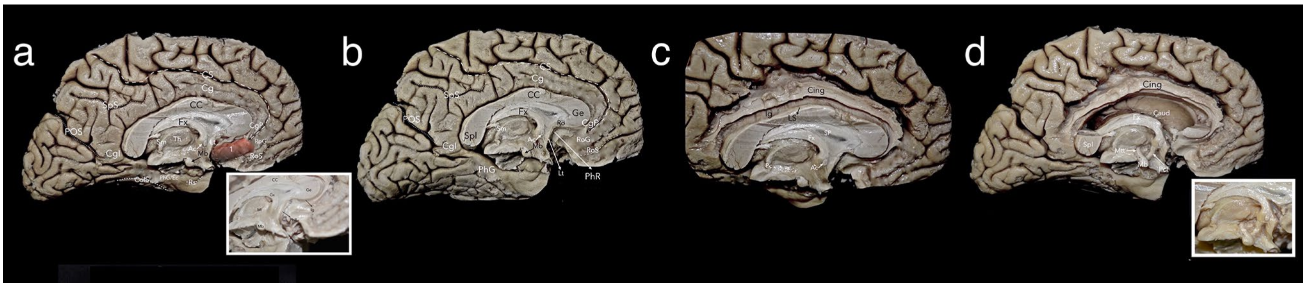

Fig. 1 Stepwise dissection of the limbic and paralimbic structures (left hemisphere). **a** The surface anatomy of the medial surface of the hemisphere is shown: The limbic sulcus is formed by the subcallosal sulcus, cingulate sulcus, sub-parietal sulcus, proximal calcarine fissure, anterior half of the collateral sulcus and rhinal sulcus. The limbic gyrus includes the paraterminal gyri, cingulate gyrus (cingulate pole and cingulate isthmus) and the anterior part of the parahippocampal gyrus is also seen. The subcallosal area (1- highlighted in red color) is seen ventral to the rostrum of the corpus callosum. The uncus is separated from the temporal pole by the shallow rhinal sulcus. **b** The subcallosal area is removed to reveal the prehippocampal rudiment, which is seen in the anterior surface of the rostrum and represents the continuation of the indusium griseum (lower left inset). **c** The cortex of the cingulate gyrus is removed and the superior arm of the cingulum is shown. The superior surface of the corpus callosum with the overlying indusium griseum (supra-commissural hippocampus) and the medial and lateral longitudinal striae can be appreciated. **d** The body of the corpus callosum is removed to access the intraventricular compartment. The gray matter of the medial surface of the thalamus and anterior hypothalamus is peeled away and the mammilothalamic tract of Vicq D'Azyr as well as the pre-commisural and post-commissural part of the fornix are revealed. Inset: close view of the mammilothalamic tract and the post-commissural fornix. *1* Subrostral area, *Ac* Anterior commissure, *Alv* Alveus, *Amg* Amygdala, *AP* Ansa peduncularis, *Apx* Apex, *CC* Corpus callosum, *Cg* Cingulate gyrus, *CgI* Isthmus of the cingulate gyrus, *CgP* Pole of the cingulate gyrus, *Cing* Cingulum superior arm, *CingI* Inferior arm of the cingulum, *ColS* Collateral sulcus, *CP* Cerebral peduncle, *Cpx* Choroid plexus, *CrS* Crural cistern, *CS* Cingulate sulcus, *Dg* Dentate gyrus, *Dg/SSG* Dentate gyrus/subsplenial gyrus, *Fc* Fasciola cinerea, *FdS* Fimbriodentate sulcus, *Fmb* Fimbria, *Fop* Frontal operculum, *Fx* Fornix, *Ge* Genu, *Gf* Gyrus fasciolaris, *GP* Globus pallidus, *Hip(h)* Hippocampus (head), *HpS* Hippocampal sulcus, *Ig* Indusium griseum, *Lg(a)* Insular long gyrus(anterior), *Lg(p)* Insular long gyrus(posterior), *Ln* Limen, *Lt* Lamina terminalis, *Mb* Mammilary body, *mcp* mesocortical pathway, *MI* Massa intermedia, *Mtt* Mammilothalamic tract, *NAcc* Nucleus accumbens, *Pc* Posterior commissure, *Phg/Ec* Parahippocampal gyrus/entorhinal cortex, *PhR* Prehippocampal rudiment, *POS* ParietoOccipital sulcus, *PrcF* Precommissural fornix, *PtcF* Postcommissural fornix, *Ro* Rostrum, *RoG* Rostral gyrus, *RoS* Rostral sulcus, *Rs* Rhinal sulcus, *Sg(p)* Short insular gyrus(posterior), *Sm* Stria medullaris, *SP* Septum pellucidum, *Spl* Splenium, *SpS* Subparietal sulcus, *SS* Sagittal stratum, *Ssg* Subsplenial gyrus, *STt* Stria terminalis thalami, *Sub* Subiculum, *Th* Thalamus, *TSt* Temporal stem, *Ug* Uncal gyrus

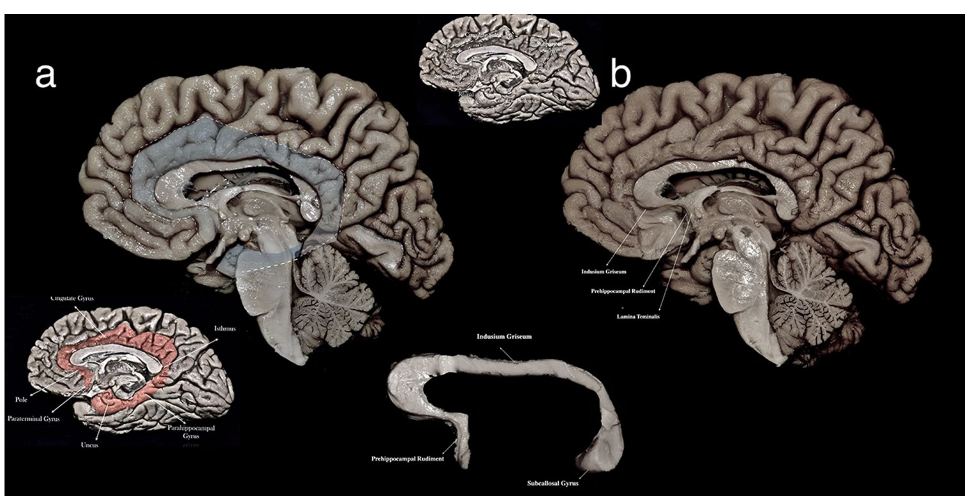

Fig. 2 Steps 1 of the dissection process. Medial views of a right hemisphere. **a** The anatomy of the limbic sulcus and limbic gyrus is illustrated (dotted line and blue color respectively). Inset (upper right): The limbic sulcus is delineated with its different segments. Inset (lower left): The limbic gyrus is highlighted in red colour and its different parts are displayed. **b** Step 1: The cortex of the subcallosal area is removed to reveal the prehippocampal rudiment, which lies in the anterior surface of the lamina terminalis

indusium griseum –also known as the supra-commissural hippocampus- and the longitudinal striae are exposed over the superior surface of the Corpus Callosum (CC) (Figs. 1c, and 3a).

Step 3

Dissecting the body of the CC from the level of the genu to this of the splenium along with the septum pellucidum reveals the anatomy of the lateral ventricle. The caudate nucleus, the superior aspect of the thalamus, the choroid plexus and the body of the fornix can be appreciated. Gradual dissection of the medial thalamic and hypothalamic surface reveals the mesolimbic fibers (connecting the ventral tegmental area to the ventral striatum) initially and the mammilothalamic tract of Vicq d'Azyr along with the precommissural fornix subsequently (Figs. 1d, 3b, and 4).

Step 4

The splenium of the corpus callosum is removed to reveal the atrium of the lateral ventricle. In this area and at the

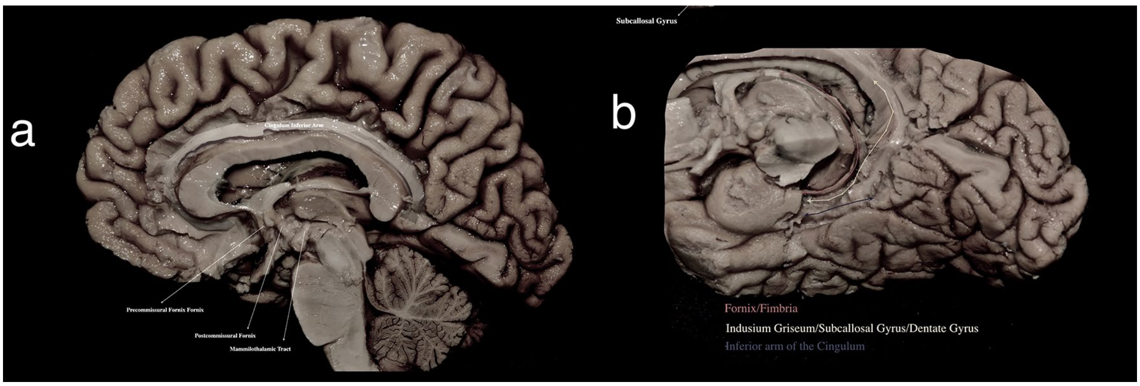

Fig. 3 Steps 2 and 3 of the dissection process. **a** Step 2: The cortex of the cingulate gyrus is peeled away and the superior arm of the cingulum, the superior aspect of the corpus callosum and overlying indusium griseum can be observed. The mammilothalamic tract and the post-commissural fornix are also exposed. **b** Step 3: After removing the splenium of the corpus callosum, three major limbic structures i.e. the crus fornix (yellow color), the dentate gyrus and the inferior arm of the cingulum (red color) can be appreciated. Inset (middle): The location of the prehippocampal rudiment, indusium griseum and subcallosal gyrus is illustrated

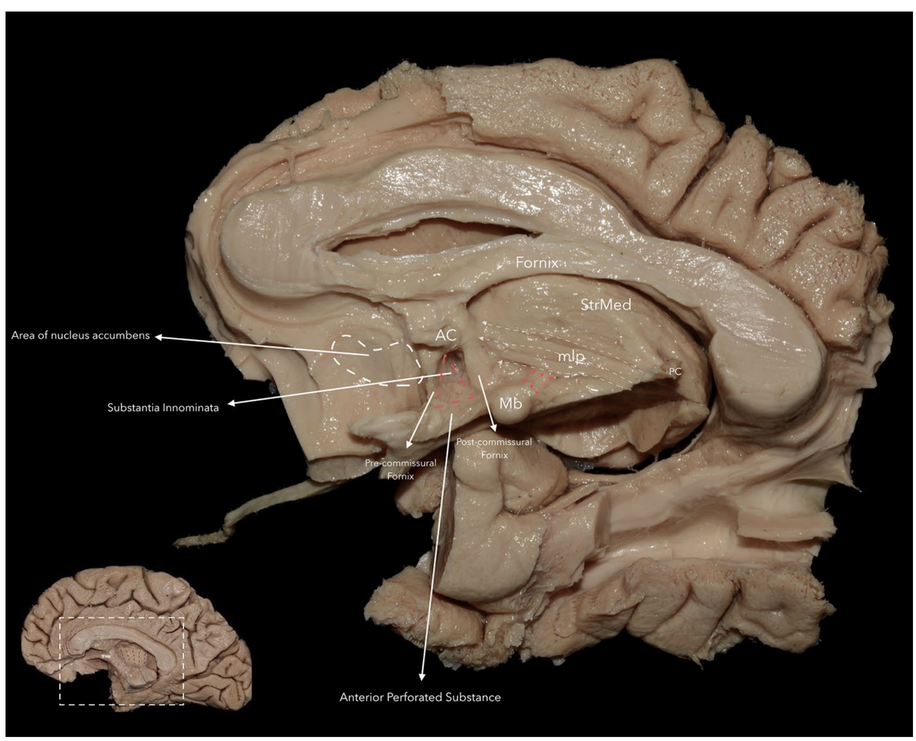

Fig. 4 Additional material. Medial view of the central core. The mesolimbic fibers connecting the area of the ventral tegmentum to the ventral striatum can be appreciated. This fibers travel lateral to the post-commissural fornix before terminating in the ventral(limbic) striatum. The substantia innominata is located in the triangle delineated by the anterior commissure superiorly, the precommissural fornix anteriorly. the post-commissural fornix posteriorly and the anterior perforated substance inferiorly(red intermittent lines). The mammilothalamic fibers are also evident running lateral to the mesolimbic fibers, emerging from the area of the mammillary body. (red arrows). *AC* Anterior commissure, *Mb* Mammillary body, *PC* Posterior commissure, *StrMed* Stria medullaris thalami

level of the isthmus of the cingulum three important limbic structures are seen to converge: the crus of the fornix, the dentate gyrus and the parahippocampal gyrus with the underlying inferior arm of the cingulum. The posterior part of the dentate gyrus including the fasciola cinerea along with the sub-splenial gyrus that are continuous posteriorly with the indusium griseum can be identified (Fig. 5a).

Step 5

The procedure continues with the core of the hemisphere being dissected free to illustrate more vividly the anatomy of the limbic system. A No10 blade is used to cut along at the level of the superior margin of the caudate nucleus superiorly, the isthmus posteriorly, the cerebral peduncle inferiorly and the fronto-orbito-polar area anteriorly. Then, moving in a lateral to medial direction, the cortex and superficial U-fibers of the peri-insular region are carefully removed. The final product of this step consists of the insular region enfolded within the temporal and frontoparietal opercula seen on the lateral aspect and the thalamus, fornix, parahippocampal gyrus/uncus identified on the medial aspect (Figs. 5b, 6a, and 7b).

Step 6

Upon removing the cortex of the medial aspect of the parahippocampal gyrus we expose the inferior arm of the cingulum seen to terminate in the entorhinal cortex. The cortex of the intraventricular aspect of the parahippocampal gyrus—known as the subiculum—is left intact (Figs. 5c, 6b and c).

Step 7

The arachnoid membrane of the basal cisterns is carefully removed with micro-forceps so as to dissect the uncus free from the cerebral peduncle and the posteromedial orbital lobule. In this way, a thorough look at the topographical anatomy of the fimbria and dentate gyrus is allowed (Figs. 5d, and 6c).

Step 8

Turning the specimen on the lateral side we proceed by removing the cortex and U-fibers of the superior and middle temporal gyri to expose the inferior limiting sulcus and the temporal stem. Further, we cut with a No15 blade the white matter of the temporal stem approximately 3–5 mm inferior to the inferior limiting sulcus. In this way, we enter the temporal horn of the lateral ventricle and identify the

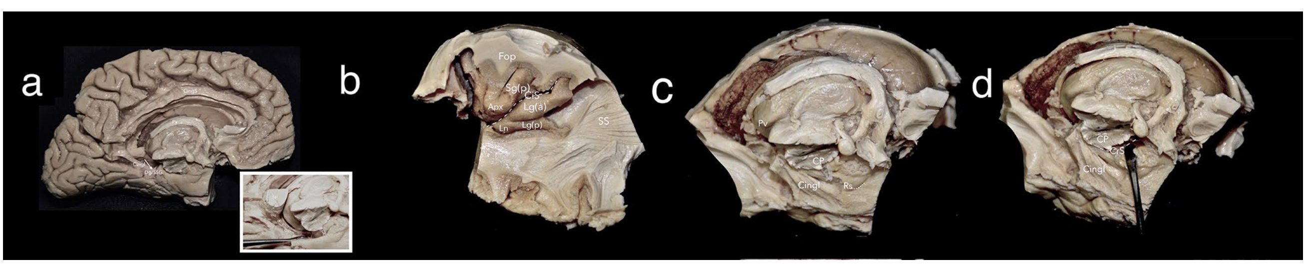

Fig. 5 **a** The splenium of the corpus callosum is removed and the crus fornix, the posterior part of the dentate gyrus with the fasciola cinerea and the subsplenial gyrus of Andreas Retzius are exhibited. Inset: Close view of the converging configuration of the crus fornix, the dentate gyrus and the subiculum/inferior arm of the cingulum. **b** The core of the hemisphere is dissected away and a perpendicular cut is made along the superior margin of the caudate nucleus and at the level of the isthmus of the cingulate gyrus. The hemisphere is turned in the lateral view and the cortex along with the superficial U-fibers of the peri-insular area are removed. The superficial anatomy of the insula and the fibers of the sagittal stratum are revealed. **c** Medial view of the same specimen. The medial parahippocampal cortex is removed while sparing the superior parahippocampal cortex. The inferior arm of the cingulum and the subiculum can be appreciated. **d** The arachnoid membrane of the crural and ambient cisterns is removed to detach the medial temporal lobe and the posteromedial orbital lobule from the cerebral peduncle. This maneuver exposes the fimbria and the anterior part of the dentate gyrus. *1* Subrostral area, *Ac* Anterior commissure, *Alv* Alveus, *Amg* Amygdala, *AP* Ansa peduncularis, *Apx* Apex, *CC* Corpus callosum, *Cg* Cingulate gyrus, *CgI* Isthmus of the cingulate gyrus, *CgP* Pole of the cingulate gyrus, *Cing* Cingulum superior arm, *CingI* Inferior arm of the cingulum, *ColS* Collateral sulcus, *CP* Cerebral peduncle, *Cpx* Choroid plexus, *CrS* Crural cistern, *CS* Cingulate sulcus, *Dg* Dentate gyrus, *Dg/SSG* Dentate gyrus/subsplenial gyrus, *Fc* Fasciola cinerea, *FdS* Fimbriodentate sulcus, *Fmb* Fimbria, *Fop* Frontal operculum, *Fx* Fornix, *Ge* Genu, *Gf* Gyrus fasciolaris, *GP* Globus pallidus, *Hip(h)* Hippocampus (head), *HpS* Hippocampal sulcus, *Ig* Indusium griseum, *Lg(a)* Insular long gyrus(anterior), *Lg(p)* Insular long gyrus(posterior), *Ln* Limen, *Lt* Lamina terminalis, *Mb* Mammilary body, *mcp* mesocortical pathway, *MI* Massa intermedia, *Mtt* Mammilothalamic tract, *NAcc* Nucleus accumbens, *Pc* Posterior commissure, *Phg/Ec* Parahippocampal gyrus/entorhinal cortex, *PhR* Prehippocampal rudiment, *POS* ParietoOccipital sulcus, *PrcF* Precommissural fornix, *PtcF* Postcommissural fornix, *Ro* Rostrum, *RoG* Rostral gyrus, *RoS* Rostral sulcus, *Rs* Rhinal sulcus, *Sg(p)* Short insular gyrus(posterior), *Sm* Stria medullaris, *SP* Septum pellucidum, *Spl* Splenium, *SpS* Subparietal sulcus, *SS* Sagittal stratum, *Ssg* Subsplenial gyrus, *STt* Stria terminalis thalami, *Sub* Subiculum, *Th* Thalamus, *TSt* Temporal stem, *Ug* Uncal gyrus

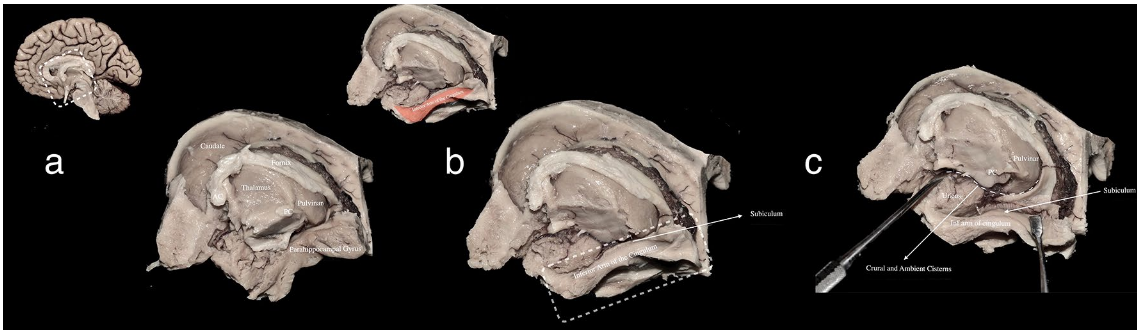

Fig. 6 Steps 6–7 of the dissection process. **a** Medial view: The central core has been dissected free from the rest of the hemisphere along the superior margin of the caudate nucleus and perpendicular to the level of the isthmus. **b** Medial view: The medial cortical layer of the parahippocampal gyrus has been removed to reveal the inferior arm of the cingulum. The superior cortical surface of the parahippocampal gyrus, which corresponds to the subiculum, is left intact. (Step 6). **c** Medial view: The arachnoid membrane of the basal cisterns has been dissected to reveal the fimbria and the anterior part of the dentate gyrus. (Step 7). *Ac* Anterior commissure, *CP* Choroid plexus, *PC* Posterior commissure

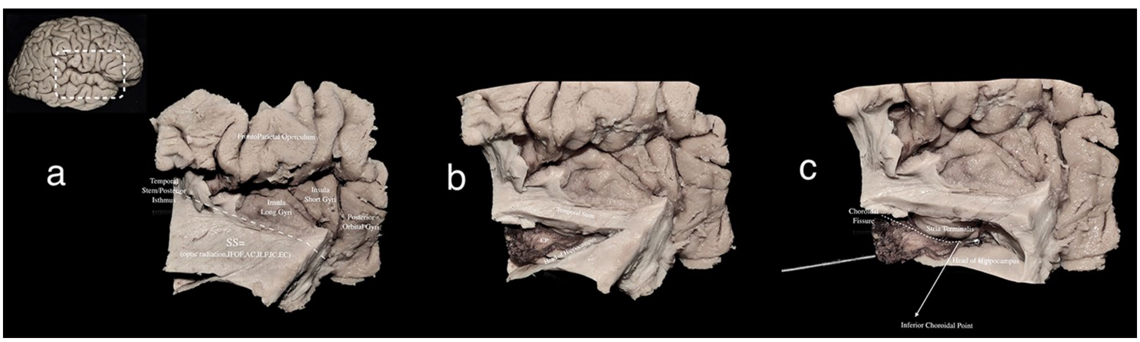

Fig. 7 Step 8 of the dissection process. **a**–**c** Lateral view: The cortex and u-fibers of the temporal operculum have been removed. The sagittal stratum is exhibited. A cut is made approximately 5 mm ventral to the inferior limiting sulcus (marked with the dotted line) and the temporal horn of the lateral ventricle is encountered. The alveus of the hippocampus, the choroid plexus and the stria terminalis can be identified. The inferior choroidal point is also evident

choroid plexus, the alveus of the hippocampus and the collateral eminence (Figs. 8a, 7b and c).

Step 9

The fornix is gradually dissected off the thalamus starting from the level of the anterior commissure and up to the level of the fimbria thus revealing the anatomy of the choroidal fissure. In addition, the stria terminalis connecting the amygdala to the septal nuclei and anterior hypothalamus is vividly illustrated (Figs. 8c, 9).

Step 10

Again, coming from a lateral-to-medial direction and upon removing the ependymal layer at the level of the tip of the temporal horn, the grey matter of the amygdala becomes evident lying over the anterior part of the temporal stem. Careful dissection of the white matter of the anterior temporal stem (underlying the planum temporale) can help to further illustrate the anatomy of the amygdaloid nucleus. The stria terminalis and the tail of the caudate nucleus can be seen terminating at the amygdalae region (Figs. 8c, 10, 11 and 12).

Step 11

At this stage and while facing the basal aspect of the frontal and temporal areas the amygdaloid nucleus is evident. Using meticulous dissection of the area of the anterior perforated substance reveals the fibers of the ansa peduncularis connecting the amygdaloid nucleus to the septal region. The ansa can be appreciated running parallel to the optic tract. By removing the cortex and superficial white matter of the posterior fronto-orbital area, the nucleus accumbens is revealed alongside the Accumbofrontal fibers that connect the latter to the fronto-orbital area. The area where the accumbofrontal fibers enter the nucleus Accumbens represents the ventral or limbic striatum (Fig. 13).

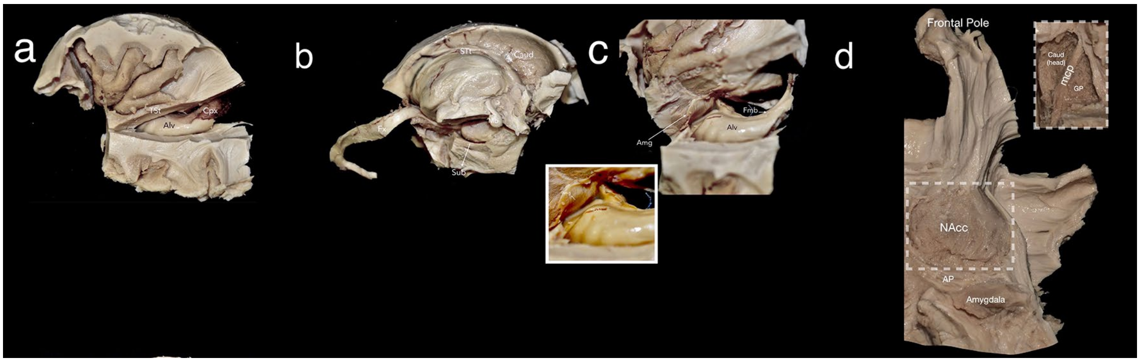

Fig. 8 **a** A No15 blade is used to make a longitudinal cut along the white matter of the temporal step, 5 mm ventral to inferior limiting sulcus. The temporal horn of the lateral ventricle is entered and the alveus of the hippocampus is demonstrated. **b** Medial view of the same specimen. The fornix is dissected free and the anatomy of the stria terminalis thalami and choroidal fissure is appreciated. **c** Lateral view. The ependymal layer of the roof and tip of the temporal horn is removed to reveal the amygdala. **d** Basal view of left hemisphere. After removing the superficial layer of the anterior perforated substance and the cortex and superficial white matter of the fronto-orbital area, the nucleus accumbens and the ansa peduncularis can be observed. By removing the nucleus accumbens, the mesocortical fibers running between the head of the caudate and the ventral globus pallidus become evident. *1* Subrostral area, *Ac* Anterior commissure, *Alv* Alveus, *Amg* Amygdala, *AP* Ansa peduncularis, *Apx* Apex, *CC* Corpus callosum, *Cg* Cingulate gyrus, *CgI* Isthmus of the cingulate gyrus, *CgP* Pole of the cingulate gyrus, *Cing* Cingulum superior arm, *CingI* Inferior arm of the cingulum, *ColS* Collateral sulcus, *CP* Cerebral peduncle, *Cpx* Choroid plexus, *CrS* Crural cistern, *CS* Cingulate sulcus, *Dg* Dentate gyrus, *Dg/SSG* Dentate gyrus/subsplenial gyrus, *Fc* Fasciola cinerea, *FdS* Fimbriodentate sulcus, *Fmb* Fimbria, *Fop* Frontal operculum, *Fx* Fornix, *Ge* Genu, *Gf* Gyrus fasciolaris, *GP* Globus pallidus, *Hip(h)* Hippocampus (head), *HpS* Hippocampal sulcus, *Ig* Indusium griseum, *Lg(a)* Insular long gyrus(anterior), *Lg(p)* Insular long gyrus(posterior), *Ln* Limen, *Lt* Lamina terminalis, *Mb* Mammilary body, *mcp* mesocortical pathway, *MI* Massa intermedia, *Mtt* Mammilothalamic tract, *NAcc* Nucleus accumbens, *Pc* Posterior commissure, *Phg/Ec* Parahippocampal gyrus/entorhinal cortex, *PhR* Prehippocampal rudiment, *POS* ParietoOccipital sulcus, *PrcF* Precommissural fornix, *PtcF* Postcommissural fornix, *Ro* Rostrum, *RoG* Rostral gyrus, *RoS* Rostral sulcus, *Rs* Rhinal sulcus, *Sg(p)* Short insular gyrus(posterior), *Sm* Stria medullaris, *SP* Septum pellucidum, *Spl* Splenium, *SpS* Subparietal sulcus, *SS* Sagittal stratum, *Ssg* Subsplenial gyrus, *STt* Stria terminalis thalami, *Sub* Subiculum, *Th* Thalamus, *TSt* Temporal stem, *Ug* Uncal gyrus

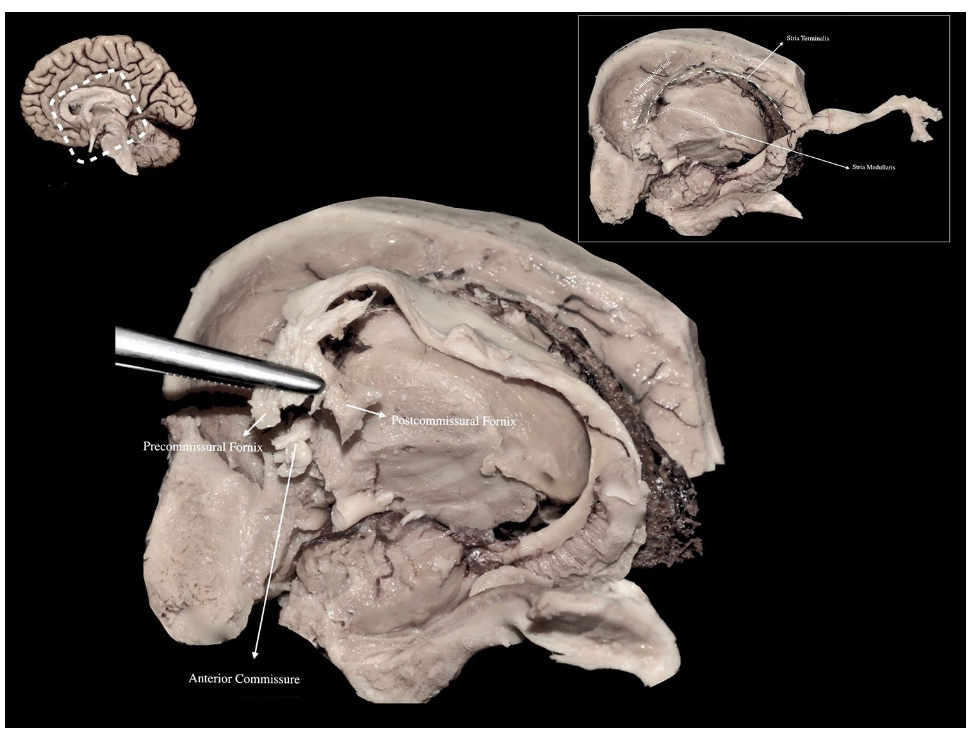

Fig. 9 Step 9 of the dissection process. **a** The fornix is dissected free from the thalamus. The choroidal fissure and stria terminalis thalami are seen (INSET)

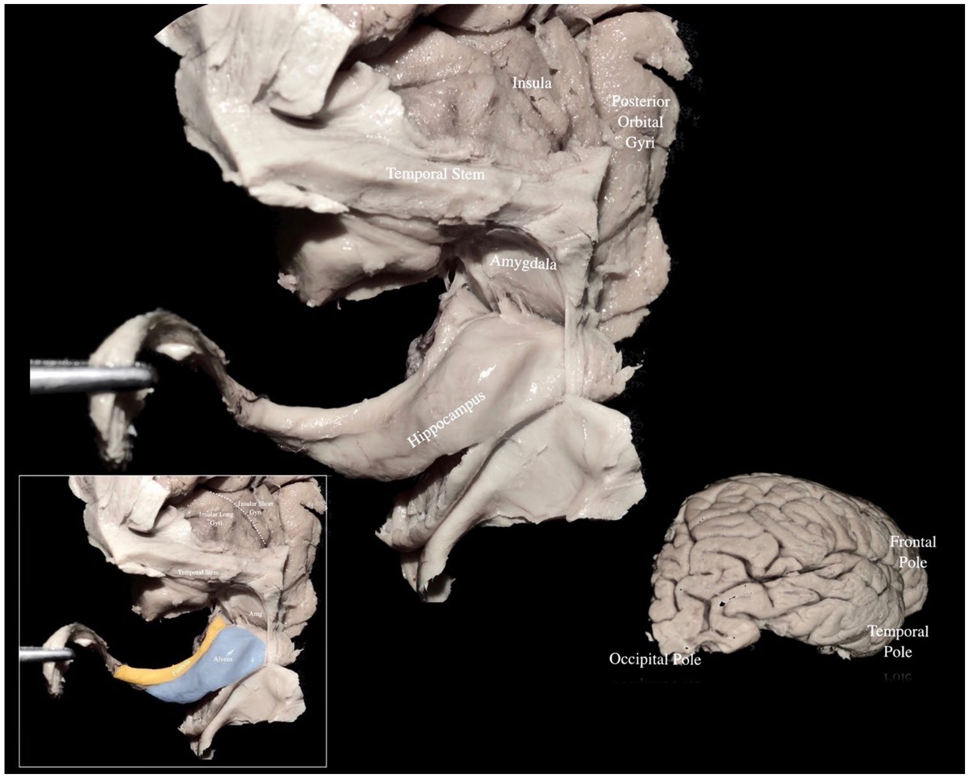

Fig. 10 Step 10 of the dissection process. The ependymal layer of the temporal horn is removed. The amygdala can be identified at the level of the tip of the temporal horn. Inset: The hippocampus and fimbria are illustrated in blue and yellow color respectively

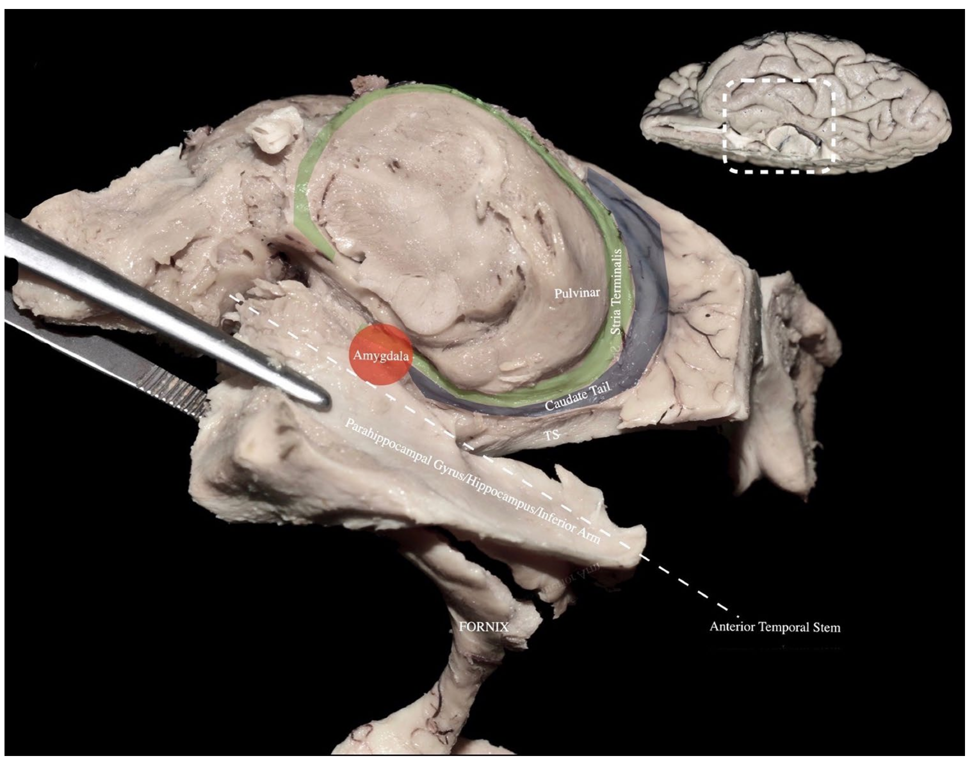

Fig. 11 Basal view of the specimen: The tail of the caudate nucleus and the stria terminalis thalami, can be seen to terminate in the area of the amygdala. The interrupted line, represents the level of the anterior part of the temporal stem

Step 12

After removing the nucleus accumbens, the ventral aspect of the head of the caudate nucleus and the ventral globus pallidus can be observed. Between them, the fibers of the mesocortical pathway (integrated within the anterior limb

Fig. 12 Additional material. Lateral view of the central core. Multilayered dissection simultaneously illustrating the anterior insula, the external capsule, the lentiform nucleus and thalamus. The intermittent line represents the posterior and part of the inferior isthmus. By removing the peri-amygdaloid white matter, the amygdala can be clearly observed lying at the tip of the temporal horn

of the internal capsule), connecting the ventral tegmentum to the ventral prefrontal cortex can be appreciated.

Step 13

The most anterior part of the parahippocampal gyrus along with the hippocampus are dissected free from the rest of the specimen. A No10 blade is used to cut the anterior part of the temporal stem at the level of the amygdala. At the end of this step, the parahippocampal gyrus/hippocampal complex can be studied from different angles. From a superolateral view, the head, body and tail of the hippocampus are seen. The alveus, corresponding to the intraventricular surface of the hippocampus as well as the fimbria representing the continuation of the fornix can be readily identified. Additionally, parts of the parahippocampal gyrus including the collateral eminence at the floor of the temporal horn as well as the subiculum representing the superior aspect of the parahippocampal gyrus in which the hippocampus lies can be observed. From a medial view the hippocampal sulcus, the uncus with its different parts (i.e. uncal and ambient gyri), the subiculum and the dentate gyrus are appreciated (Figs. 8d, 14a, 15, and 16).

Step 14

In the last step, the hippocampus is dissected free from the parahippocampal gyrus along the hippocampal sulcus. Upon completing this step, the structure of the hippocampus can be thoroughly studied. On the medial surface, the dentate gyrus demarcated superiorly by the fimbrio-dentate sulcus and the inferiorly by hippocampal sulcus inferiorly is illustrated. The different parts of the dentate gyrus including the margo denticularis anteriorly and the fasciola cinerea and gyrus fasciolaris posteriorly are evident (Figs. 14b, c, 16b, and 17).

Discussion

The fiber dissection technique has emerged as an invaluable tool to unravel the fine spatial relationships and axonal connectivity of complex cerebral territories [7, 20, 21]. This method has evolved during the last 70 years and has been recently employed as a gold-standard procedure for the exploration, illustration and better understanding of the human brain anatomy and connectivity alongside novel tractographic techniques. In addition to its theoretical significance, it also serves as a “navigation tool” for the neurosurgeon by revealing useful 3-dimensional information that can be extrapolated to real-time operative settings mainly in the field of neuro-oncology, brain mapping and epilepsy.

Numerous studies have previously focused on the gross as well as microscopic anatomy and functional role of the limbic system. Nonetheless, most of the existing literature relies on classical 2-dimensional and fragmentary

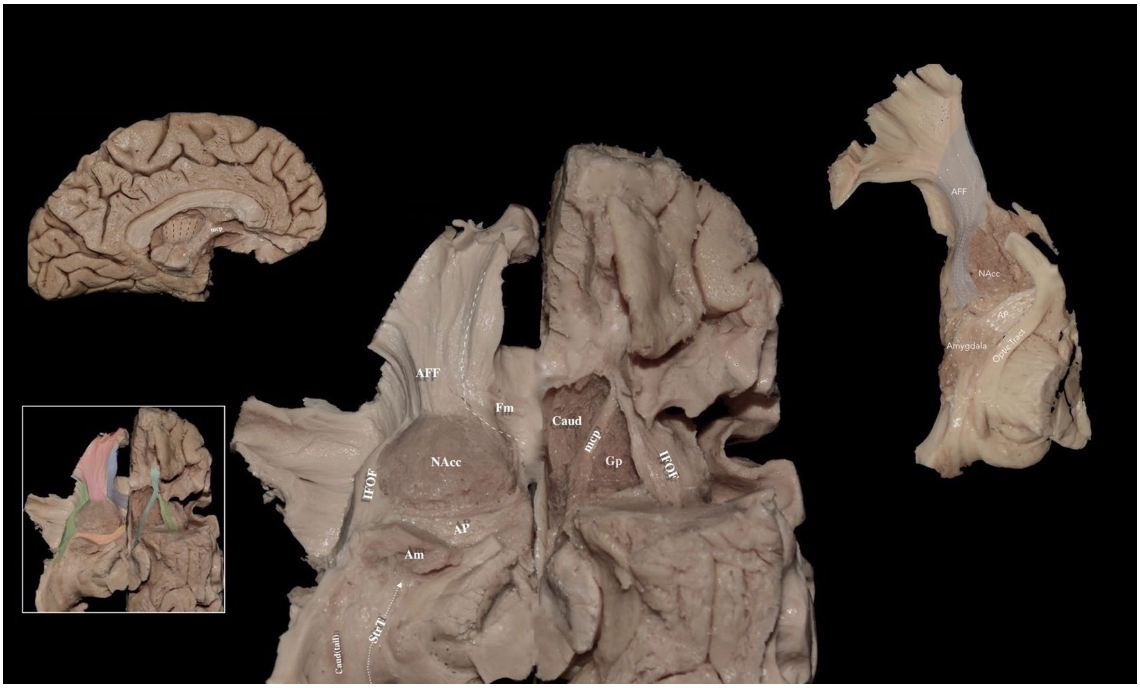

Fig. 13 Additional material. Basal view. Multilayered dissection. The following limbic/paralimbic structures and connections can be appreciated: (1) The amygdala and the ansa peduncularis connecting the latter to the septal region. (2) The nucleus accumbens. The accumbofrontal fasciculus connects the latter to the ventral prefrontal cortex and fronto-orbital prefrontal cortex. (3) The mesocortical pathway traveling within the anterior limb of the internal capsule and connecting the ventral tegmentum to the ventral prefrontal cortex. The inferior occipitofrontal fasciculus travels tangential to the inferolateral border of the nucleus accumbens. The fibers of the forceps minor can also be observed slightly medial to the accumbofrontal fasciculus. Insets: Left inferior: The ansa peduncularis is highlighted in orange color. The mesocortical pathway in cyan. The accumbofrontal fasciculus in red. The occipitofrontal fasciculus in green. The forceps minor in blue. Left Superior: Medial view of a left hemisphere. The Mesocortical fibers can be appreciated with respect to the fibers of the genu of the internal capsule (intermittent black lines). Right: Basal view of a right hemisphere. Advanced dissection stage. The accumbofrontal fasciculus and the ansa peduncularis can be clearly observed. The fibers of the ansa peduncularis run tangentially to the optic tract. *AFF* Accumbofrontal Fasciculus, *Am* Amygdaloid Nucleus, *AP* Ansa peduncularis, *Caud(tail)* Tail of the caudate nucleus, *Fm* Forceps Minor, *Gp* Globus Pallidus, *IFOF* Inferior Occipitofrontal Fasciculus, *mcp* Mesocortical pathway, *NAcc* Nucleus Accumbens, *StrT* Stria Terminalis

anatomical illustrations that can be difficult to decipher and translate into the 3-dimensional level. Very few authors indeed have used the white matter dissection technique to reveal the structures typically described under the umbrella term "limbic" [1, 8, 9, 15, 19, 23]. The studies implementing the white matter dissection technique to study the structures of the limbic lobe are summarized in Table 2.

Here, we provide a systematic guide for the dissection and illustration of major limbic and paralimbic structures and connections. By dividing the dissection process into 14 distinctive and consecutive steps we aim to offer a simplified yet comprehensive approach to understand the highly complex topographic anatomy of this area both in the context of an anatomy laboratory and during real operative scenarios. As anatomical experiments heavily depend on the operator's experience and usually lack reproducibility, stepwise anatomy manuals may compensate for these factors and significantly increase the credibility of findings in a laboratory context. This approach has been solidified through previous publications from our laboratory [12].

To our knowledge, this is the first attempt to offer a stepwise manual for the dissection of the limbic and paralimbic structures and relevant pathways that can be employed as an educational supplement for both novice and experienced anatomists as well as neurosurgeons.

Strengths and limitations of the study

The fiber micro-dissection technique following the preparation of the specimens according to Klingler's technique has been established as a gold-standard method to examine, validate and extend anatomical evidence deriving mainly from tractographic studies. As proven by Zemmoura and colleagues this technique helps simultaneously preserve the anatomical integrity of the nerve axons while facilitating the separation of the fiber tracts in a laboratory setting and under the surgical microscope. In the recent literature, the

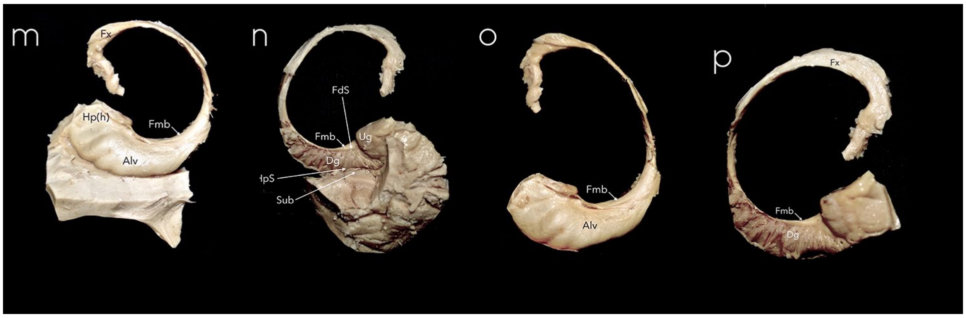

Fig. 14 **a**, **b** The anterior part of the parahippocampal gyrus with the hippocampus are dissected away from the hemisphere. For this purpose, a No10 blade is used to cut the anterior part of the temporal stem below the level of the amygdala. In this way, the hippocampus and subiculum and the adjacent structures including the collateral eminence, the uncal, ambient and dentate gyri can be observed in a medial to lateral (M) and lateral to medial (L) view. Lower left inset: close view of the spatial relationships of the head of the hippocampus with respect to the amygdala. **c**, **d** In the last dissection step, the hippocampus is dissected free from the parahippocampal gyrus along the hippocampal sulcus. The fimbria, fimbriodentate and hippocampal sulci as well as the different parts of the dentate gyrus (including the margo denticularis, the fasciola cinerea and the gyrus fasciolaris) are illustrated. *1* Subrostral area, *Ac* Anterior commissure, *Alv* Alveus, *Amg* Amygdala, *AP* Ansa peduncularis, *Apx* Apex, *CC* Corpus callosum, *Cg* Cingulate gyrus, *CgI* Isthmus of the cingulate gyrus, *CgP* Pole of the cingulate gyrus, *Cing* Cingulum superior arm, *CingI* Inferior arm of the cingulum, *ColS* Collateral sulcus, *CP* Cerebral peduncle, *Cpx* Choroid plexus, *CrS* Crural cistern, *CS* Cingulate sulcus, *Dg* Dentate gyrus, *Dg/SSG* Dentate gyrus/subsplenial gyrus, *Fc* Fasciola cinerea, *FdS* Fimbriodentate sulcus, *Fmb* Fimbria, *Fop* Frontal operculum, *Fx* Fornix, *Ge* Genu, *Gf* Gyrus fasciolaris, *GP* Globus pallidus, *Hip(h)* Hippocampus (head), *HpS* Hippocampal sulcus, *Ig* Indusium griseum, *Lg(a)* Insular long gyrus(anterior), *Lg(p)* Insular long gyrus(posterior), *Ln* Limen, *Lt* Lamina terminalis, *Mb* Mammilary body, *mcp* mesocortical pathway, *MI* Massa intermedia, *Mtt* Mammilothalamic tract, *NAcc* Nucleus accumbens, *Pc* Posterior commissure, *Phg/Ec* Parahippocampal gyrus/entorhinal cortex, *PhR* Prehippocampal rudiment, *POS* ParietoOccipital sulcus, *PrcF* Precommissural fornix, *PtcF* Postcommissural fornix, *Ro* Rostrum, *RoG* Rostral gyrus, *RoS* Rostral sulcus, *Rs* Rhinal sulcus, *Sg(p)* Short insular gyrus(posterior), *Sm* Stria medullaris, *SP* Septum pellucidum, *Spl* Splenium, *SpS* Subparietal sulcus, *SS* Sagittal stratum, *Ssg* Subsplenial gyrus, *STt* Stria terminalis thalami, *Sub* Subiculum, *Th* Thalamus, *TSt* Temporal stem, *Ug* Uncal gyrus

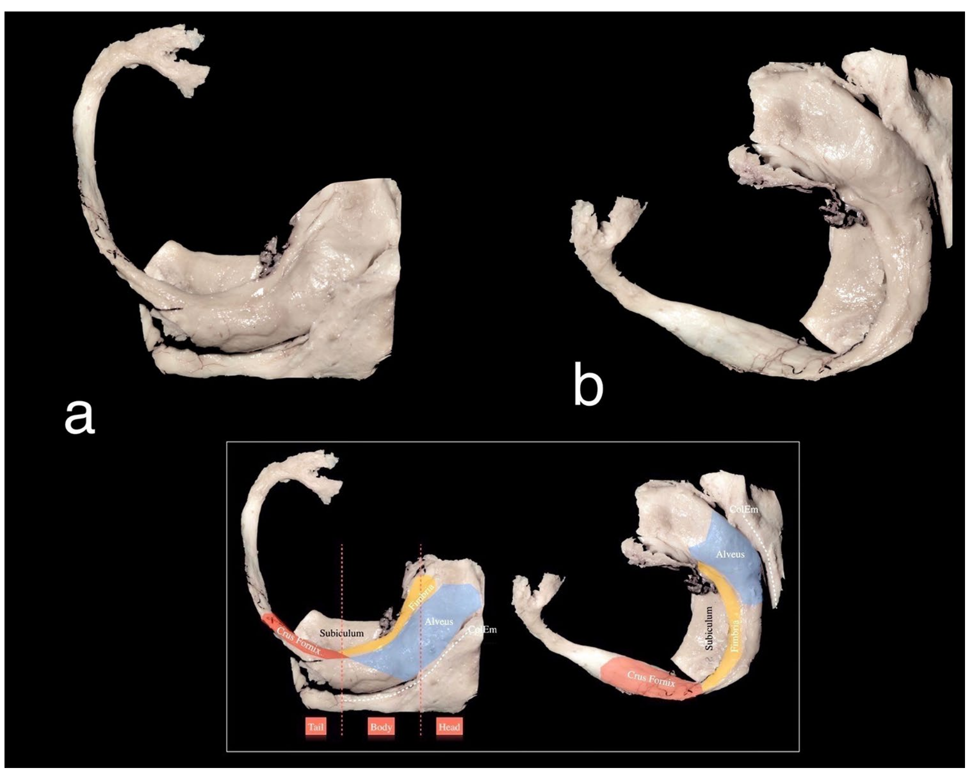

Fig. 15 Close-up view of the hippocampus and subiculum. **a-b** Lateral and superior view respectively. The anatomy of the head, body and tail of the hippocampus and the fimbria is displayed. Inset: The alveus, fimbria and crus fornix are highlighted in blue, yellow and red color respectively. Dotted lines are used to delineate the different parts of the hippocampus (head, body, tail)

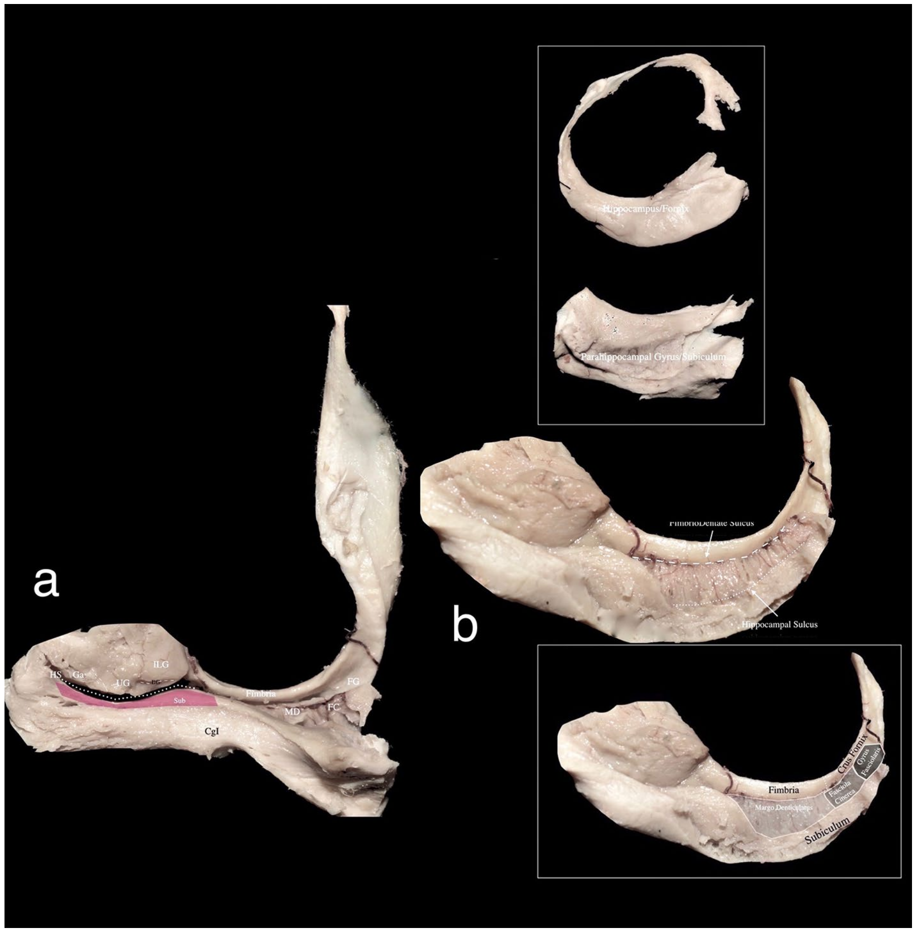

Fig. 16 a-b Medial views before (**a**) and after(**b**) removing the parahippocampal gyrus The subiculum, the different parts of the uncus, the fimbriodentate and hippocampal sulci as well as the different zones of the dentate gyrus (lower inset) are identified. *Bg* Band of giacomini, *CgI* Cingulum inferior arm, *ColEm* Collateral eminence, *FC* Fasciola cinerea, *FG* Gyrus fasciolaris, *Ga* Ambient gyrus, *HS* Hippocampal sulcus, *ILG* Intralimbic gyrus, *MD* Margo denticularis, *UG* Uncal gyrus

fiber dissection technique has been repeatedly accredited with high accuracy in illustrating fiber trajectory as well as termination pattern and a high spatial resolution even in complex anatomical areas such as the limbic lobe.

However, the fiber micro-dissection method is an expensive and extremely time-consuming technique requiring patience, excellent three-dimensional anatomical perception and dexterity. In this context, it is highly dependent on the experience and skillset of the operator. Additionally, poor quality of the specimens and inadequate or incorrect preparation can sometimes reduce the quality of the dissection and significantly affect the repeatability of the process. Finally, the spatial resolution of the data provided by the fiber dissection technique is lower in comparison to histology, optical coherence tomography and polarized light imaging.

Challenges in the surgery of limbic and paralimbic areas and the value of anatomy laboratory manuals in modern neurosurgery

Surgical treatment of lesions or functional resections for epilepsy in or around the limbic system pose a distinct challenge for the neurosurgeon. The deep location, the vicinity to critical neurovascular structures, the complex regional anatomy, the eloquence of the involved cortico-subcortical areas and the ill-defined anatomical borders make visibility, surgical maneuverability and effective intraoperative dissection arduous. Surgery of insular and peri-insular regions, amygdala and hippocampus, parahippocampal gyrus, cingulate isthmus and cingulate gyrus requires not only flawless surgical skills and optimal bimanual dexterity but a profound, thorough and detailed knowledge of the regional operative anatomy in each case [10, 14].

A distinction, however, has to be made between "static" anatomy, operative anatomy and intraoperative anatomy. The first entity is mainly conveyed through university anatomical lectures and texts. The second is mastered through dedicated and subspecialized laboratory work and the latter one, which is what the neurosurgeon actually has to face and decipher in the theatre and which is greatly influenced and usually distorted by the characteristics of the lesion, is patiently learned in real operative settings. Undoubtedly, there is and has to be a linear and progressive relationship between these three entities and the surgeon has to gradually develop from one to the other to achieve surgical finesse and mastery. We are in an era where surgery is not regarded as a mere manual

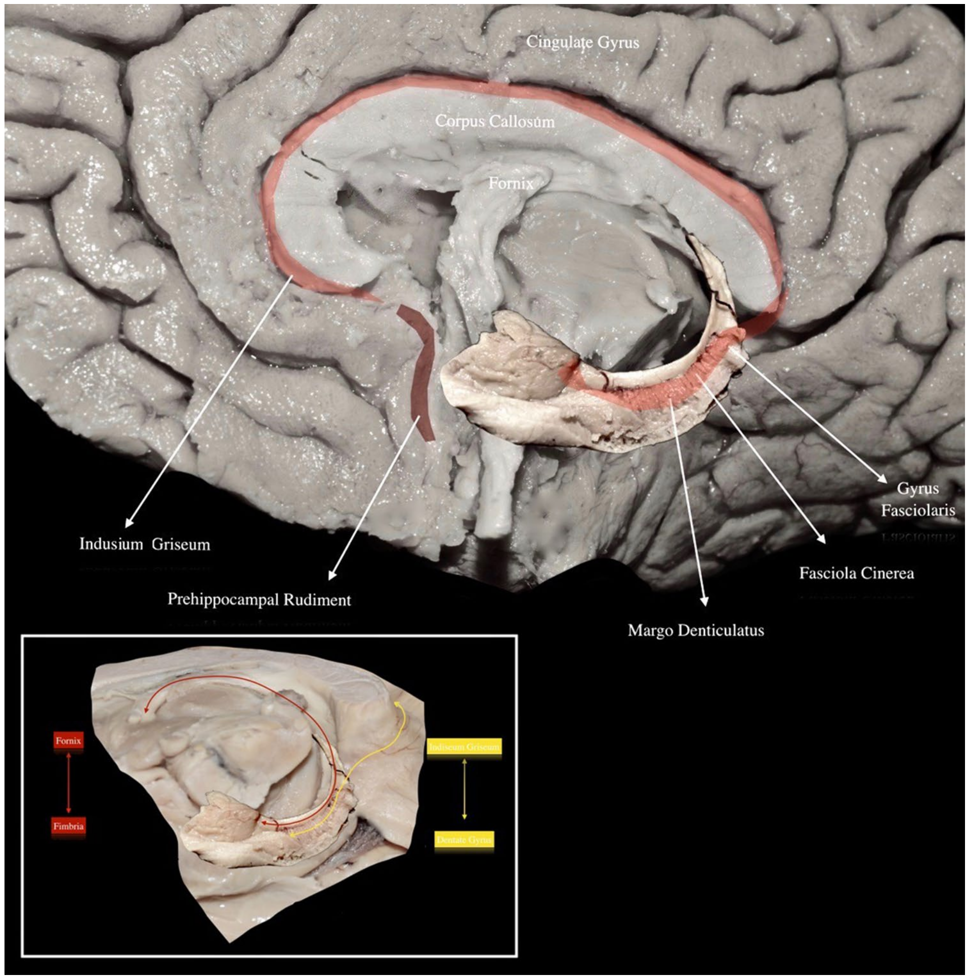

Fig. 17 Anatomy of the intra-limbic gyrus in a right hemisphere. The different parts of the intra-limbic gyrus are identified i.e. the dentate gyrus also known as the sub-commissural hippocampus, the indusium griseum or supra-commissural hippocampus and the prehippocampal rudiment or precommissural hippocampus. The dentate gyrus is formed by the margo denticularis, the fasciola cinerea and the gyrus fasciolaris. The latter gradually transits to the indusium griseum in the subsplenial area. The indusium griseum is continuous with the prehippocampal rudiment at the level of the subcallosal gyrus

Table 2 Main studies implementing the White matter dissection technique for the study the anatomy of the limbic structures [1, 8, 9, 15, 19]

Study/author	Year	Technique	Number of specimens	Main target of the study
Current study		White matter dissection		To offer a step-by-step laboratory guide for the systematic dissection of the limbic and paralimbic structures
Shah et al. [19]	2012	White matter dissection	10 hemispheres	To illustrate the anatomy and connectivity of the structures traditionally assigned to the Papez circuit using the fiber dissection technique
Alarcon et al. [1]	2014	MRI + Neuronavigation + White matter dissection	10 hemispheres	To study the anatomy and connectivity of the central core of the brain, including diencephalic and limbic structures
Pascalau et al. [15]	2018	White matter dissection + DTI	Fiber dissection:10 hemispheres DTI: 23 patients	Proposal of fiber tracking protocol for limbic tracts validated by fiber dissection
Haladaj et al. [9]	2019	White matter dissection	40 hemispheres	To study the anatomy and anatomical variations of the dentate gyrus in the human brain
Ferreira Jr et al. [8]	2020	MRI tractography + Structural MRI + White matter dissection	5 hemispheres	To describe the anatomy of the Papez circuit as a possible target for non-motor target for functional neurosurgery through dissections and ultra-high MRI and tractography

technique that is elegantly transmitted from the master to the students by submission and sermon but has evolved into a proper scientific specialty. The renowned doctrine "see one, do one, teach one" that conveys the notion of a "confidence based" neurosurgical practice belongs to the past. Novel operative techniques and approaches derive from robust scientific evidence and original laboratory investigations and propagate through a safe, effective and reproducible intra-operative implementation. This fact documents and supports the concept of what we call "evidence based" surgery. To this end, focused anatomy manuals like the current study provide a simplified yet thorough guide that enriches anatomical knowledge, raises interest and awareness of specific cerebral areas and can act as a roadmap for laboratory or intra-operative dissections.

Conclusion

Limbic and paralimbic structures exhibit a complicated and intricate anatomical architecture and connectivity. Their understanding poses significant challenges even for the experienced neuroscientist. Here, we provide for the first time in the pertinent literature a focused, stepwise laboratory manual for the gradual dissection and better understanding of the subcomponents of this specific system. Our aim is to provide novice and experienced anatomist and neurosurgeon with a thorough, systematic yet simplified roadmap for laboratory and intraoperative dissections.

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Data availability Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest The authors report no conflict of interest regarding the materials or methods used in this study or the findings specified in this paper. The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the Bioethical Committee of the Medical School of the National and Kapodistrian University of Athens.

Informed consent This is a cadaveric study not involving patients or patient-related information. All cadaveric specimens used in the current study were obtained by the providing company after strict self-consent or consent from the legally authorized representatives or next of kin of the donors.

Consent for publication Not applicable.

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