A Stepwise Laboratory Manual for the Dissection and Illustration of Major Limbic Structures. Evidence from the Klingler’s Technique.

Spyridon Komaitis (skom.med@googlemail.com)
Department of Neurosurgery Evangelios Hospital, Athens, Greece https://orcid.org/0000-0002-7250-414X

Theodosis Kalamatianos
Hellenic Center for Neurosurgical Research, “Petros Kokkalis”, Athens

Evangelos Drosos
Department of Neurosurgery Evangelios Hospital, Athens, Greece

Aristotelis Kalyvas
Department of Neurosurgery Evangelismos Hospital, Athens, Greece

Maria Piagkou
National and Kapodistrian University of Athens

Theodore Troupis
National and Kapodistrian University of Athens

Nektarios Mazarakis
Department of Neurosurgery, Leeds General Infirmary

George Stranjalis
Department of Neurosurgery Evangelismos Hospital, Athens, Greece

Christos Koutsamakis
Department of Neurosurgery Evangelismos Hospital, Athens, Greece

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Abstract

OBJECTIVE: 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: Twelve (12) consecutive, discrete and easily reproducible laboratory anatomical steps are systematically described to reveal the intricate anatomy of the structures of the limbic system.

CONCLUSION: Surgical approaches for lesions or functional resections in and around limbic areas pose a challenging task for the neurosurgeon. By employing the fiber dissection technique, we were able to provide a stepwise and thorough laboratory guide for the gradual dissection and better comprehension of the morphology and spatial relationships of this specific system. Anatomical manuals like the present study raise interest and enrich anatomical knowledge on complex cerebral areas with the overarching goal to inform surgical practice.

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 fifth cerebral lobe incorporating various anatomical structures.[4, 6]

Broca’s legacy was followed by the pivotal experimental work and clinical observations of researchers like Christfried 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, 12, 16, 14] 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, 15, 20, 16]
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 in the pertinent literature with regard to its microsurgical anatomy, especially from an educational standpoint. Only a limited number of cadaveric studies have previously utilized the fiber dissection technique to elucidate the anatomy of the Jacob/Papez circuit.[17, 1, 8] 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 in 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 the 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 the Klingler's preparation (freeze-thaw process).[5, 10] All specimens were subsequently investigated through the fiber dissection technique with the aid of a microscope (OPMI Plus, Carl Zeiss) and by 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 6 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 mammilo-thalamic 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

Twelve (12) consecutive laboratory anatomical steps are systematically described to reveal the major structures of the limbic system. These steps derive from both medial to lateral and lateral to medial dissections and are summarized in Fig. 1 and Table 1.
Table 1
Stepwise dissection of major limbic structures. Steps and structures revealed.

| STEP | ORIENTATION OF DISSECTION | TECHNIQUE | STRUCTURES REVEALED |
|------|---------------------------|------------|---------------------|
| 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 HIPPOCAMPU S |
| 2    | MEDIAL TO LATERAL         | Remove the cortex of the cingulate gyrus. | SUPERIOR ARM OF THE CINGULUM |
|      |                           |            | INDUSIUM            |
|      |                           |            | GRISEUM/SUPRACOMMISSURAL HIPPOCAMPU S |
|      |                           |            | 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. | PRECOMISSURAL/POSTCOMMISSURAL FORNIX |
|      |                           |            | MAMMILOTHalam TRACT OF VICQ D’AZYR |
| 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 |
| STEP | ORIENTATION OF DISSECTION | TECHNIQUE | STRUCTURES REVEALED |
|------|---------------------------|------------|---------------------|
| 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 |
| 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 5mm ventral to inferior limiting sulcus to enter temporal horn of the lateral ventricle. | ALVEUS OF HIPPOCAMPUS |
| 9    | MEDIAL TO LATERAL | Dissect the fornix free from thalamus from anterior commissure to fimbria. | CHOROIDAL FISSURE STRIA TERMINALIS THALAMI |
| 10   | LATERAL TO MEDIAL | Remove ependyma of the roof and tip of the temporal horn. | AMYGDALA STRIA TERMINALIS (VENTRAL PART) TAIL OF CAUDATE NUCLEUS |
| 11   | 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/TAILOF HIPPOCAMPUS COLLATERAL EMINENCE SUBICULUM UNCAL GYRUS AMBIENT GYRUS DENTATE GYRUS |
| STEP | ORIENTATION OF DISSECTION | TECHNIQUE | STRUCTURES REVEALED |
|------|---------------------------|------------|---------------------|
| 12   | MEDIAL TO LATERAL         | Dissect the hippocampus from the parahippocampal gyrus along the hippocampal sulcus | FIMBRIODENTATE SULCUS  
HIPPOCAMPAL SULCUS  
MARGO DENTICULARIS  
FASCIOLA CINEREA  
GYRUS FASCIOLARIS |

**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 terminalis and represents the anterior continuation of the indusium griseum. (Fig. 1B, Fig. 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 indusium griseum – also known as the supra-commissural hippocampus - and the longitudinal striae are exposed over the superior surface of the Corpus Callosum (CC). (Fig. 1C, Fig. 2C)

**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 surface reveals the mammilothalamic tract of Vicq d’Azyr along with the precommissural fornix. (Fig. 1D, Fig. 2D)

**STEP 4**

The splenium of the corpus callosum is removed to reveal the atrium of the lateral ventricle. In this area and at the 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. 1E)

**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 posteromedial orbital lobule 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. (Fig. 1F, Fig. 3A,D)

**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. (Fig. 1G, Fig. 3B&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 in the topographical anatomy of the fimbria and dentate gyrus is allowed. (Fig. 1H, Fig. 3C)

**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 5mm inferior to the inferior limiting sulcus. In this way we enter the temporal horn of the lateral ventricle and identify the choroid plexus, the alveus of the hippocampus and the collateral eminence. (Fig. 1I, Fig. 3E&F)

**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. (Fig. 1K, Fig. 4A)

**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. The stria terminalis and the tail of the caudate nucleus can be seen terminating at the amygdalae region. (Fig. 1K, Fig. 4B&C)

**STEP 11**
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. (Fig. 1L&M, Fig. 5)

**STEP 12**

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. In 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. (Fig. 1N&O, Fig. 5D)

**Discussion**

The fiber dissection technique has emerged as an invaluable tool to unravel the fine spatial relationships and axonal connectivity of complex cerebral territories.[19, 7, 18] 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 with 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 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, 17, 8]

Here, we provide a systematic guide for the dissection and illustration of major limbic and paralimbic structures. By dividing the dissection process into 12 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.[11]

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

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 -areas that in essence make up what is known as the limbic system- 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.[9, 13]

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 in order to achieve surgical finesse and mastery. We are in an era where surgery is not regarded as a mere manual 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 on specific cerebral areas and can act as a roadmap for laboratory or intra-operative dissections.

Conclusion

Limbic and paralimbic structures exhibit a complicated anatomical architecture and therefore surgical approaches targeting lesions or functional resections in this area pose a distinct challenge. Here we provide for the first time in the pertinent literature a focused, stepwise laboratory manual for the gradual
dissection and better comprehension of the subcomponents of this specific system. Our aim is to provide the novice and experienced anatomist and neurosurgeon with a thorough but simplified roadmap for laboratory and intraoperative dissections.

Declarations

Disclosures

No funding was received for this study. The authors report no conflict of interest regarding the materials or methods used in this study or the findings specified in this paper.

Code Availability Not applicable

Author Contribution Conception and design: Komaitis, Koutsarnakis, Kalamatianos. Acquisition of data: Komaitis, Koutsarnakis, Kalamatianos. Analysis and interpretation of data: Komaitis, Kalamatianos, Koutsarnakis, Kalyvas, Drosos. Drafting the article: Komaitis, Koutsarnakis, Kalamatianos. Critically revising the article: Koutsarnakis, Kalamatianos, Stranjalis, Komaitis. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Koutsarnakis. Administrative/technical/material support: Stranjalis. Study supervision: Koutsarnakis, Stranjalis, Kalamatianos

Data Availability Not applicable

Ethics 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

Conflict of interest The authors declare that they have no conflict of interest

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Figure 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 mammillothalamic tract of Vicq D’Azyr as well as the pre-commissural and post-commissural part of the fornix are revealed. Inset: Close view of the mammillothalamic tract and the post-commissural fornix. E. 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. F. 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. G. 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. H. 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.. I. A No15 blade is used to make a longitudinal cut along the white matter of the temporal step, 5mm ventral to inferior limiting sulcus. The temporal horn of the lateral ventricle is entered and the alveus of the hippocampus is demonstrated. J. Medial view of the same specimen. The fornix is dissected free and the anatomy of the stria terminalis thalami and choroidal fissure is appreciated. K. Lateral view. The ependymal layer of the roof and tip of the temporal horn is removed to reveal the amygdala. L-M. 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. N-O. 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, Apx= Apex, CC= Corpus Callosum , Cg= Cingulate Gyrus, CgL= 
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, 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= Mammillary Body, MI= Massa Intermedia, Mtt= Mammillothalamic Tract, Pc= Posterior Comissure, 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
Figure 2
Steps 1-3 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. C. 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 mammillothalamic tract and the post-commissural fornix are also exposed. D. 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.

Figure 3
Steps 6-8 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) D-E-F. Lateral view: The cortex and u-fibers of the temporal operculum have been removed. The sagittal stratum is exhibited. A cut is made approximately 5mm 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. Ac= Anterior Commissure, CP= Choroid Plexus, PC= Posterior Commissure

**Figure 4**

Steps 9-10 of the dissection process A. The fornix is dissected free from the thalamus. The choroidal fissure and stria terminalis thalami are seen (INSET). B. 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. C. 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.
Figure 5

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) C-D. Medial views before (C) and after(D) 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, Cgl= 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

Figure 6

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.