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Original Research Article

A Cadaveric Study of Anatomical Variations in The Limbic System of Human Brain

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Abstract:

Background: The limbic system plays a crucial role in emotion, memory, and behavior, and exhibits considerable anatomical variability with important clinical implications.

Aim: To study the morphological and morphometric variations of selected limbic system structures in the human brain using cadaveric specimens.

Methodology: A descriptive cadaveric observational study was conducted on 20 formalin-fixed adult human brain hemispheres. The hippocampus, dentate gyrus, and amygdala were dissected and assessed for morphological definition (ill-defined, moderately defined, well-defined). Linear measurements of length, width, and thickness were recorded using digital vernier calipers, and descriptive statistics were applied.

Results: The hippocampus was well-defined in 50% of specimens, moderately defined in 35%, and ill-defined in 15%. The dentate gyrus showed greater variability, being moderately defined in 45% of cases. The amygdala was predominantly well-defined (60%). Mean dimensions (±SD) were 35.2 ± 3.4 mm for hippocampal length, 28.6 ± 2.9 mm for dentate gyrus length, and 16.8 ± 2.1 mm for amygdala length, with moderate inter-specimen variability.

Conclusion: The study highlights consistent identification with notable anatomical variation in limbic structures, providing baseline data valuable for neuroimaging interpretation and neurosurgical planning.

Keywords: Limbic system, Cadaveric study, Hippocampus, Dentate gyrus, Amygdala.

Introduction

The human brain is a complex and highly specialized organ that takes care of the integration of sensory input, motor output coordination, and even more complex cognitive and emotional processes [1]. One of the brain's most important parts concerning emotions, memory, motivation, and even behavior is the limbic system, which has been one of the many subdivisions, both structurally and functionally, in the brain. It all started with Papez, who in 1937 made the famous circuit between the hippocampus and the hypothalamus through the cingulate gyrus, later on, the limbic system has been described as a large system with lots of interconnected structures that consist mainly of the hippocampus, amygdala, septal nuclei, fornix, mammillary bodies, parahippocampal gyrus, and anterior thalamic nuclei [2]. These structures do not exist as independent anatomical entities; instead, they build up an integrated network that supports emotional experiences, memory processes, learning, and even the most basic, such as autonomic functions. The complex interconnections of the limbic system with cortical and subcortical regions underline its role in both the normal functioning of the nervous system and the development of a number of neuropsychiatric disorders.

The limbic system shows great anatomical diversity across people which is a landmark fact for clinical application and brain research [3]. Individual differences concerning the limbic system can be extensive and range from one person to another thus leading to different functional outcomes. To be more precise these differences can mean, for example, different sizes, shapes, placements and dissolutions of the parts that make up the limbic system. Every neurosurgeon, neurologist, and radiologist should be aware of these subtle variations which can play a big role in the success of treating the temporal lobe, performing deep brain stimulation, and tackling with epilepsy and mood disorders [4]. Furthermore, the anatomical variability in the limbic system may be a causative factor for diverse individuals' cognitive abilities; emotional regulation and even the susceptibility to certain mental disorders like depression, anxiety, PTSD, and schizophrenia. Despite the considerable importance of clinical and research applications, the number of morphometric studies focusing on the human limbic system is small due to the specified reasons, ie, the complexity of the region and difficulties in visualizing its deep structures.

Cadaveric studies are the perfect way to scrutinize anatomical variations very closely and deliver insights that are frequently not possible to get via in vivo imaging methods only [5]. The older imaging techniques like magnetic resonance imaging (MRI) and computed tomography (CT) do give useful structural information but are limited by the resolution issues, especially when trying to look at small and intricate structures like the amygdala or the mammillary bodies [6]. Dissection of cadavers allows limbic structures to be directly visualized, measured, and compared, which leads to the documenting of variations in size, morphology, and spatial relationships with neighboring neuroanatomical areas [7]. Besides, cadaveric investigations lead to the broadening one's understanding of development and evolution of the limbic system, thus making one more knowledgeable about the human brain anatomy. The same studies are also aiding the process of refining the neuroanatomical models as well as boosting the accuracy of the stereotactic and functional neurosurgical interventions.

Noteworthy anatomical variability in the major limbic structures has been pointed out by several studies. For example, the hippocampus undergoes changes in its length, volume, and shape, which may be associated with the memory processing differences and the susceptibility to degenerative brain diseases. Likewise, the amygdala has variations in the organization of its subnuclei, which can be a factor of the emotional responses and the patient's predisposition to the certain types of mental disorders. The cingulate gyrus, parahippocampal gyrus, and fornix present not only significant interindividual differences in terms of connectivity and size, but they are also very important for the comprehension of neural circuitry and the information flow within the limbic network [8]. The state-of-the-art imaging techniques and histological methods notwithstanding, post-mortem studies are still the gold standard methods for detailed morphometric analysis especially when it is the case of subtle variations that could be missed by imaging.

The primary reasons for the adoption of cadaveric studies for the investigation of anatomical variations in the limbic system are multiple and complex. The first and the most important one is that it gives a very basic and detailed insight into the structural diversity which is the basis of functional heterogeneity in the areas of emotion, cognition, and behavior. The knowledge mentioned above is indispensable for better diagnosis, neurosurgery, and making decisions regarding neuropsychiatric disorders treatment. In addition, cadaveric dissections for anatomical studies play an important role in anatomy education by making the students, clinicians, and researchers receive the very difficult neuroanatomical relationships hands-on training, thus improving their spatial comprehension and clinical reasoning. The mapping out of anatomical variations in the limbic system has its importance due to the fact that it is an area that has the most significant impact on human behavior and cognition which is, therefore, a very important step in the integration of anatomical knowledge with clinical and psychological outcomes.

The limbic system's anatomical complexity and variability underscore its importance in neuroscience, neurology, and psychiatry. Cadaveric studies serve as a vital tool to document these variations, offering detailed morphometric data that complement imaging studies and enhance clinical understanding. By systematically analyzing the anatomical differences in limbic structures, researchers can contribute to a more comprehensive neuroanatomical framework, facilitating improved diagnostic, surgical, and therapeutic strategies. Therefore, investigating the anatomical variations of the limbic system through cadaveric dissections holds both academic and clinical significance, reinforcing the intricate link between brain structure and function.

Methodology

Study Design: The present study was designed as a descriptive cadaveric observational study aimed at identifying and documenting anatomical variations in the limbic system of the human brain. Cadaveric dissection was chosen as it allows direct visualization and accurate morphological assessment of deep-seated neural structures, which is essential for understanding normal anatomy as well as anatomical variations of the limbic system components.

Study Area: The study was conducted in the Department of Anatomy, Darbhanga Medical College, Laheriasarai, Darbhanga, Bihar. The department is well equipped with adequate dissection facilities, preserved cadaveric specimens, and standard anatomical instruments required for neuroanatomical dissection and measurement.

Study Participants: The study participants comprised formalin-fixed human cadaveric brain specimens obtained during routine undergraduate dissection classes in the Department of Anatomy.

Inclusion Criteria

- Formalin-fixed adult human brain hemispheres

- Intact cerebral anatomy
- Adequately preserved limbic system structures (hippocampus, dentate gyrus, and amygdala)
- Specimens free from gross anatomical damage
- Both right and left cerebral hemispheres included
- Specimens included irrespective of sex

Exclusion Criteria

- Brain specimens showing evidence of trauma
- Specimens with history or signs of surgical intervention
- Presence of gross pathological lesions
- Congenital malformations of the brain
- Advanced decomposition of specimens
- Distorted anatomy due to improper fixation or handling

Sample Size: The sample size consisted of 20 formalin-fixed human brain hemispheres. This sample size was considered adequate for a descriptive cadaveric study aimed at assessing anatomical variations, given the limited availability of well-preserved specimens and the detailed nature of the dissection process.

Study Period: The study was carried out over a period of one academic year, coinciding with routine dissection schedules in the Department of Anatomy. This duration allowed systematic dissection, observation, documentation, and measurement of the limbic system structures.

Procedure: Each formalin-fixed brain specimen was carefully removed from the cranial cavity and cleaned of residual meninges and blood clots. The cerebral hemispheres were separated along the midline using standard anatomical techniques. Dissection was performed stepwise following established neuroanatomical guidelines to expose the limbic system. The medial surface of each hemisphere was examined to identify the cingulate gyrus, hippocampal formation, and associated structures.

Special attention was given to the hippocampus, dentate gyrus, and amygdala. These structures were exposed by carefully opening the temporal horn of the lateral ventricle and gently removing overlying tissues. Morphological characteristics were observed and recorded. Each structure was categorized based on its visual clarity into ill-defined, moderately defined, or well-defined. This categorical assessment helped in documenting variations in structural prominence and delineation.

In addition to qualitative assessment, quantitative measurements were taken for all identified limbic system structures. Measurements such as length, width, and thickness were obtained using digital vernier calipers with appropriate precision. All measurements were taken thrice by the same observer, and the mean value was recorded to minimize observer error. Photographic documentation was performed wherever necessary for academic reference and record maintenance.

Statistical Analysis: The collected data were entered into a Microsoft Excel spreadsheet and analyzed using appropriate statistical software. Descriptive statistics were applied to summarize the findings. Categorical variables were expressed as frequencies and percentages, while continuous variables were expressed as mean and standard deviation. The results were presented in tables and descriptive formats to highlight anatomical variations in the limbic system. Statistical analysis was primarily descriptive, as the objective of the study was morphological documentation rather than hypothesis testing.

Result

In the study, brain hemispheres were represented in an equal number that accounted for a total of 20 specimens. The specimens were divided equally into right and left hemispheres, with 10 for each side. This gives 50% of the total sample per side. The equal representation of both hemispheres guarantees that the sample is uniform and enables a non-prejudicial comparison of anatomical characteristics between the right and left sides of the brain.

Table 1: Distribution of Brain Hemispheres Studied

Hemisphere Side	Number (n = 20)	Percentage (%)
Right	10	50
Left	10	50
Total	**20**	**100**

Table 2, the examined specimens are described in terms of the morphological appearance of the hippocampus, which ranges from a good to a poor anatomical definition. A total of 10 specimens (50%, n = 10) demonstrated the best-defined hippocampus, thus, the structural features were very clear and distinct. The case with moderately defined hippocampus was in 7 specimens (35%, n = 7), so only partially morphology characteristics were clear. On the other hand, an ill-defined hippocampus was reported in a small number of cases (15%, n = 3), which means the anatomical definition was not very clear. Thus, the findings show that the majority of specimens had the morphology of the hippocampus that was either satisfactory or well-preserved.

Table 2: Morphological Appearance of the Hippocampus		
Degree of Definition	**Number (n)**	**Percentage (%)**
Ill-defined	3	15
Moderately defined	7	35
Well-defined	10	50
Total	**20**	**100**

Table 3 presents the morphological characteristics of the dentate gyrus in the examined specimens and reveals different levels of structural definition. The dentate gyrus with a medium definition was the most prevalent finding with 9 specimens (45%) being the case, which means almost half of the samples had unclear anatomical boundaries. In other words, this category was followed by a well-defined dentate gyrus case represented by 7 specimens (35%) and thus in more than one-third of the cases, the morphology was completely pronounced. On the contrary, the ill-defined appearance was marked by 4 specimens (20%), thus a minority was characterized by their very low discernible structure. The whole findings support the idea that although the dentate gyrus was recognizable in all specimens, its morphological definition was very different from one specimen to another.

Table 3: Morphological Appearance of the Dentate Gyrus		
Degree of Definition	**Number (n)**	**Percentage (%)**
Ill-defined	4	20
Moderately defined	9	45
Well-defined	7	35
Total	**20**	**100**

Table 4 illustrates how the morphology of the amygdala varies according to the degree of definition seen in the specimens that were examined. The results indicate that the largest part of the amygdalae were well-defined, which comprised 12 specimens (60%), thus suggesting that in most cases anatomical delineation was clear. A mediocre-defined look was seen in 6 specimens (30%), indicating that the boundaries of the structures were to some extent unclear. A mere 2 specimens (10%) had an ill-defined morphology, meaning that this was the least common case in the sample. To sum up, the findings suggest that the amygdala is mostly well-defined morphologically, while there are just a few cases of moderate or poor definition.

Table 4: Morphological Appearance of the Amygdala		
Degree of Definition	**Number (n)**	**Percentage (%)**
Ill-defined	2	10
Moderately defined	6	30
Well-defined	12	60
Total	**20**	**100**

Table 5 provides an overview of the quantitative data obtained from the main limbic system structures, giving their mean length, width, and thickness along with standard deviations. Among the different structures, the hippocampus showed the longest average length (35.2 ± 3.4 mm), which implies its elongated shape, while its width and thickness were somewhat less impressive. The dentate gyrus, on the other hand, had overall smaller dimensions and a length of 28.6 ± 2.9 mm, width, and thickness, which was a reflection of its compact anatomical structure in the hippocampal formation. The amygdala, however, had a shorter average length (16.8 ± 2.1 mm) but relatively wider (11.2 ± 1.5 mm) and thicker (9.4 ± 1.3 mm) measurements, which supports its more rounded and bulkier configuration. The deviations in standard measurement of all structures suggest that the inter-specimen variability is moderate, hence, the anatomical consistency with the normal biological variation among the studies brain specimens is implied.

Table 5: Quantitative Measurements of Limbic System Structures (Mean ± SD)			
Structure	**Length (mm) Mean ± SD**	**Width (mm) Mean ± SD**	**Thickness (mm) Mean ± SD**
Hippocampus	35.2 ± 3.4	9.8 ± 1.2	7.6 ± 1.0
Dentate Gyrus	28.6 ± 2.9	6.4 ± 0.8	4.2 ± 0.6
Amygdala	16.8 ± 2.1	11.2 ± 1.5	9.4 ± 1.3

Discussion

This current cadaveric investigation reveals substantial gross anatomical proof regarding the variations in morphology and measurements of the principal limbic system structures, which include the hippocampus, dentate gyrus, and amygdala, and associates them with previous anatomical and radiological studies. The study's even-handed representation of the right and left cerebral hemispheres reduces the impact of laterality bias and enhances the connection with previous cadaveric studies, many of which have not always specified hemispheric distribution. Such meticulousness in methodology is necessary because even minor asymmetries in limbic structures have been detected in both cadaveric and imaging studies (Kier et al., 1995) [9].

Morphological assessment of the hippocampus in the present study revealed a predominance of well-defined anatomy across specimens, indicating relative consistency in its gross appearance. This finding partially contrasts with Parmar et al. (2018) [10], who reported qualitative variation with 81% well-defined, 15% moderately defined, and 4% ill-defined hippocampi. The higher proportion of clearly delineated hippocampi in the present work may be attributable to differences in dissection approach. While Parmar et al. relied mainly on medial visualization, the superior approach used in this study—by opening the roof of the temporal horn—allowed more comprehensive exposure of hippocampal contours. Similar observations have been noted in anatomical texts, which emphasize that visualization angle significantly affects perceived hippocampal boundaries (Kier et al., 1995; Wright, 2020) [11,12]. Quantitatively, the relatively larger mean length of the hippocampus observed in this study aligns with its elongated configuration described in both classical neuroanatomy and MRI-based morphometric analyses (Carr et al., 2017) [13]. Such consistency reinforces the validity of cadaveric measurements as baseline anatomical references.

In contrast, the dentate gyrus demonstrated greater qualitative variability, with most specimens classified as moderately defined, followed by well-defined and ill-defined forms. These findings show both agreement and contrast with the study by Haładaj (2020) [14], who categorized the dentate gyrus as well-developed (67.5%), underdeveloped (22.5%), or hypoplastic (10%). Although both studies confirm notable variability, the predominance of moderately defined dentate gyri in the present work suggests a more nuanced continuum rather than a dominance of fully developed forms. Differences in classification terminology and hemispheric sampling may explain these discrepancies, as Haładaj did not specify laterality. Morphometrically, the smaller dimensions of the dentate gyrus observed here correspond with its compact, folded structure described in developmental and adult anatomy (Humphrey, 1967; Wright, 2020). These findings are also consistent with volumetric MRI studies demonstrating relatively small dentate gyrus volumes even in healthy populations (Nakahara et al., 2020) [15].

The amygdala showed the highest proportion of well-defined morphology among the three structures studied, supporting its reputation as a discrete and easily identifiable limbic nucleus. Parmar et al. (2018) reported 69% well-defined, 11.5% moderately defined, and 19.5% ill-defined amygdalae, whereas the present study demonstrated fewer ill-defined cases. This difference may again be attributed to methodological variations, particularly the coronal dissection approach used here, which provides clearer delineation of the amygdala from surrounding temporal lobe tissue. Quantitatively, the finding that the amygdala exhibited greater width and thickness despite shorter length is consistent with its rounded, bulky morphology described in embryological and adult anatomical studies (Kier et al., 1995). These morphometric features also correlate with neuroimaging studies linking amygdalar volume alterations to emotional and behavioral disorders (Burkert et al., 2019) [16].

Overall, the moderate standard deviations observed in the measurements of all three limbic structures suggest normal biological variability rather than pathological change. This observation supports earlier conclusions that structural variation within the limbic system exists along a physiological spectrum (Carr et al., 2017). Importantly, establishing such baseline morphometric data is clinically relevant, as hippocampal atrophy is a hallmark of mesial temporal sclerosis, a common cause of drug-resistant epilepsy (Liu et al., 1995) [17]. Clear differentiation between normal anatomical variation and pathological alteration is therefore crucial for accurate radiological interpretation and surgical planning.

From a neurosurgical perspective, detailed knowledge of limbic anatomy is particularly important during approaches to temporomesial lesions. Surgical classifications and approaches emphasize minimizing damage to adjacent limbic structures to prevent postoperative cognitive and emotional deficits (Faust et al., 2014) [18]. The present cadaveric findings, especially regarding consistent landmarks such as the hippocampus and amygdala, provide valuable reference points that complement preoperative imaging. Moreover, understanding variability in smaller structures like the dentate gyrus may help explain individual differences in vulnerability to neurological and psychiatric conditions.

Despite its contributions, the study is limited by its reliance on first-order linear measurements and lack of volumetric or histological correlation. However, similar limitations are present in much of the existing cadaveric literature, highlighting the ongoing relevance of gross anatomical studies (Iwanaga et al., 2021) [19]. Future research integrating cadaveric dissection with imaging and histology would further clarify structure–function relationships within the limbic system.

The present study corroborates and extends previous anatomical research by demonstrating both consistency and variability in limbic system morphology and dimensions. By providing comparative qualitative and quantitative data, it strengthens the anatomical foundation necessary for clinical, radiological, and neurosurgical applications.

Conclusion

The present cadaveric study provides valuable insights into the morphological and morphometric variations of key limbic system structures, namely the hippocampus, dentate gyrus, and amygdala. The findings demonstrate that while these structures are consistently identifiable, they exhibit notable inter-individual variability in their degree of definition and dimensions. The predominance of well-defined hippocampal and amygdalar morphology, along with moderate variability in the dentate gyrus, highlights the complex yet organized nature of the limbic system. Quantitative measurements further establish baseline anatomical data reflecting normal biological variation. Such detailed documentation is clinically significant, as it aids in accurate interpretation of neuroimaging, guides neurosurgical planning, and helps distinguish normal anatomical diversity from pathological alterations. Overall, this study reinforces the importance of cadaveric investigations in enhancing the anatomical and clinical understanding of the human limbic system.

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