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Novel Technique for Extraction of the Hippocampus of Adult Male Wistar Rats

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ABSTRACT

The hippocampus is one of the most widely studied areas in the brain because of its remarkable neuronal cell plasticity, functional role in memory processing and learning, and its involvement in some neurodegenerative diseases. Despite being one of the most studied areas in the brain, it is not easily accessible in experimental animals' such as mice and rats. Techniques available for dissection are capital intensive and may not be possible in low income countries. This study described a novel technique for the extraction of the hippocampus. Three apparently healthy adult Wistar rats were used for this study. They were euthanized under anaesthetic condition with ketamine (75 mg/kg). Afterwards they were perfused with formosaline, the skull opened, and the brain harvested. A midline incision was made and the halves of the brain were separated. The medial surface of the halved cerebral hemisphere was faced upward then the cerebrum was dissected and the hippocampus neatly and manually harvested, weighed, grossly observed and histologically processed. Morphologically, the hippocampus is banana-shaped in the Wistar rat, while histologically it revealed normal cytoarchitectural arrangement. Using the manual extraction method, the hippocampus was isolated without distortion, indicating that hippocampus sample can be prepared. Hence, this convenient and accurate dissection technique can be used for histologic, histochemical, immunohistochemical and histopathological study of the hippocampus, especially in low income countries.

KEYWORDS: Wistar rats, hippocampus, dissection, extraction, histologic

INTRODUCTION

One important step in sample preparation for histological and histopathological evaluation is dissection of the brain tissue[1]. The usage of large number of experimental animals is gaining greater attention. Therefore there is a need to consider how to reduce the unnecessary use of animals and fully exploit each experimental animal[2] .The brain in mammals is anatomically organized into distinct regions that wields over specific physiological and behavioural functions. Various brain structures are of interest to neuroscientists' e.g. the olfactory bulb, prefrontal cortex, cerebellum, cerebrum, hippocampus, substantial nigra etc.

Despite the enthralled interest in several regions of the brain by the neuroscientist, some parts of the brain like the hippocampus, substantial nigra in experimental animals' e.g. mice, rats are small and not easily accessible[1], hence, a need to devise techniques to isolate these structures. The hippocampus is a paired structure, with mirror-image halves in both sides of the brain that plays important roles in long-term memory and spatial navigation. In primates and humans, the hippocampus is located deep within the medial temporal lobe, beneath the cortical surface[3]. It is made up of two interlocking grey matter folds, the cornu ammonis (or hippocampus proper) and the dentate gyrus separated by hippocampal sulcus and curve into each other.

It is not totally considered to be a sub-cortical structure even though it lies sub-cortically. In the axial and sagittal plane, it can be divided into three parts: the head or anterior expanded segment; the body or intermediate segment; and the tail or posterior thin curved segment. This general layout is the same across the full range of mammalian species, from hedgehog to human, although the details vary. In rats, the two hippocampi are similar to a pair of bananas, joined at the stems[4,5]. Its remarkable importance in memory processing and learning, neuronal cell plasticity, and it is involvement in some neurodegenerative diseases and psychiatric disorders[3,6] makes its study invaluable. Due to the difficulty in its accessibility, majority of studies today visualize the hippocampus by serial sectioning of the cerebrum.

Although Hagihara et al[7] and Sultan[8], have reported techniques for isolation of the hippocampus, this techniques may be difficult and not feasible in low income countries. Therefore this study describes a novel technique that can be used to isolate the hippocampus for both histological and histopathological studies.

MATERIALS AND METHODS

Ethical approval: Ethical approval was obtained from the Ahmadu Bello University Committee for Animal Use and Care with number of approval (ABUCAUC/2018/047).

Animals: Three Adult Wistar rats weighing between 120 to 130 g were purchased from the Animal House Centre, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. The rats acquired were housed in standard rat cages under suitable environmental conditions and fed with standard pelletized feed and water *ad libitum*. The rats were maintained in this condition for a period of one week (7 days) to acclimatize them prior to experimental study.

Materials: Razor blade (cleaned with Acetone), Curved forceps, Sharp forceps, Scalpel, Formol saline, Normal saline, Microtome, Microscope, Glass slides, Cover slips, Stains, Hand gloves, Spatula, Scissors, Hippocampal tool and Anaesthesia (Ketamine).

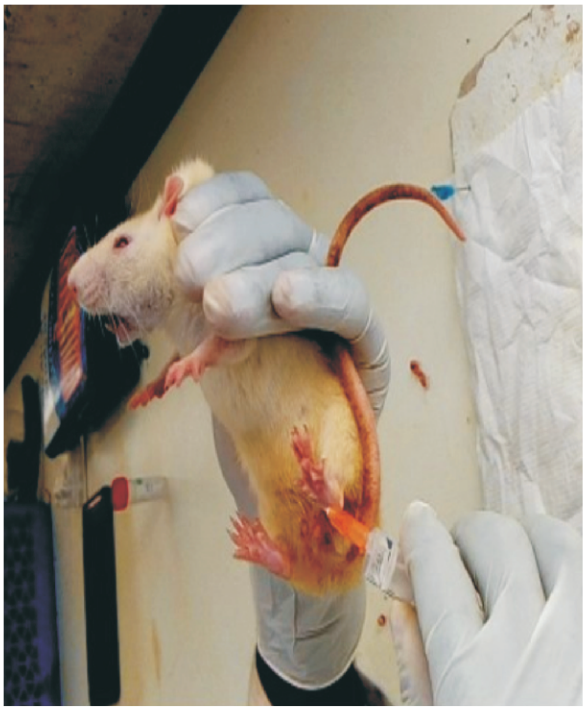

Figure 1: Intraperitoneal administration of anaesthetic agent (ketamine).

1. Anaesthetize using 75mg/kg of Ketamine or any anaesthetic agent of your choice (Figure 1).

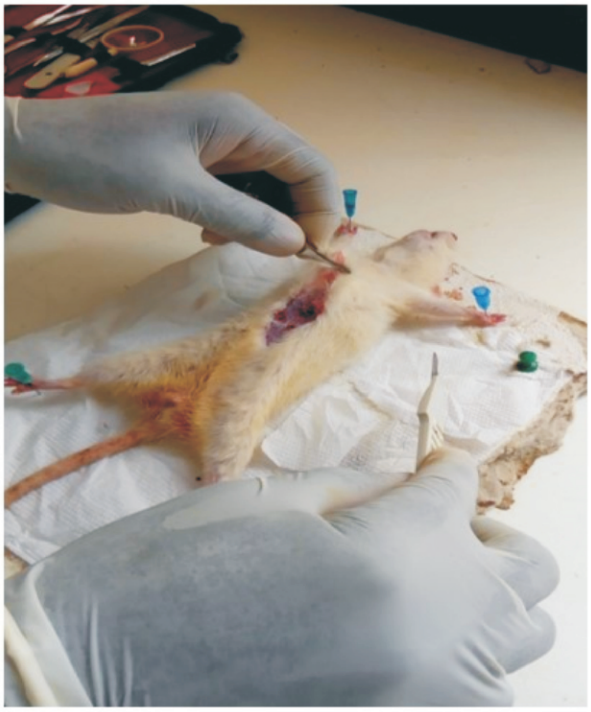

Figure 2: Transabdominal incision to expose the thoracoabdominal cavity. Take care not to lacerate abdominal organs.

1. Immediately after anaesthesia, make a cut through the abdomen inferior to the diaphragm to expose the thoracic cavity (Figure 2).

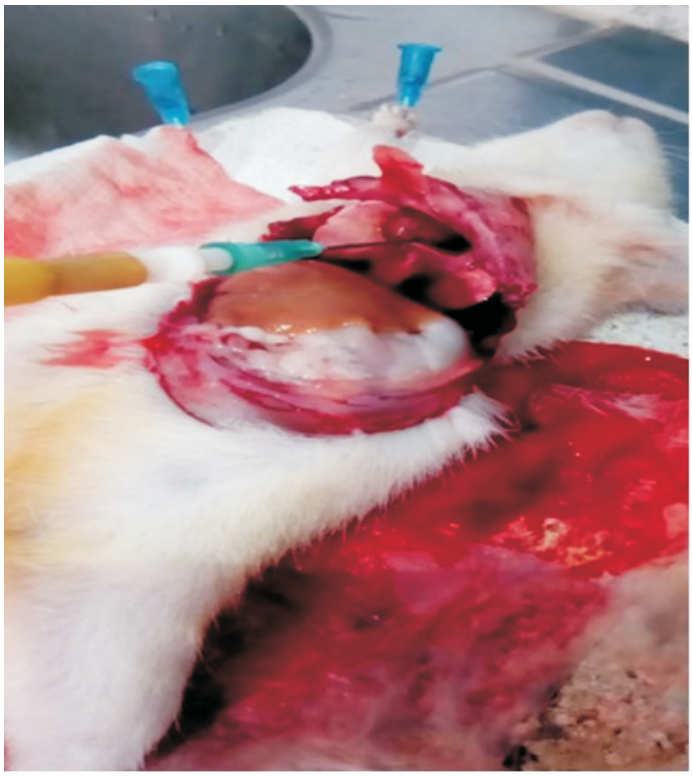

Figure3: Intracardiac perfusion of fixative (depending on your tissue processing and staining technique) through the left ventricle of the heart and drainage through the inferior vena cava.

1. Once the thoracic cavity of the rat can be accessed, perfuse the rat using the normal arterial system via the left ventricle of the heart with 10% formosaline (perfusion fixation) or any fixative of your choice depending on the processing and staining technique you intend to use after the blood has been flushed and drained through the inferior vena cava inferior to its entry into the right atrium of the heart using normal saline (Figure 3).

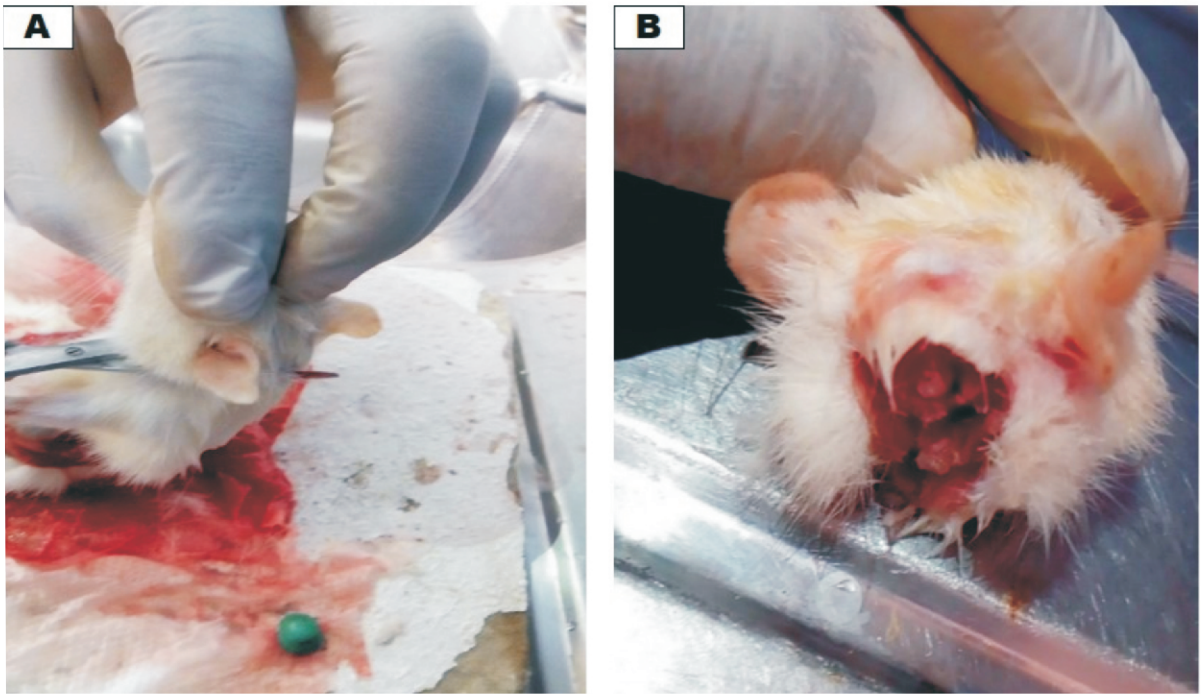

Figure 4: Separation of the head from the neck A) Large pair of scissors placed at the junction between the head and neck of the rats B) Decapitated head.

1. After 10 minutes or after proper fixation, decapitate the head using a large pair of scissors (Figure 4A & 4B).

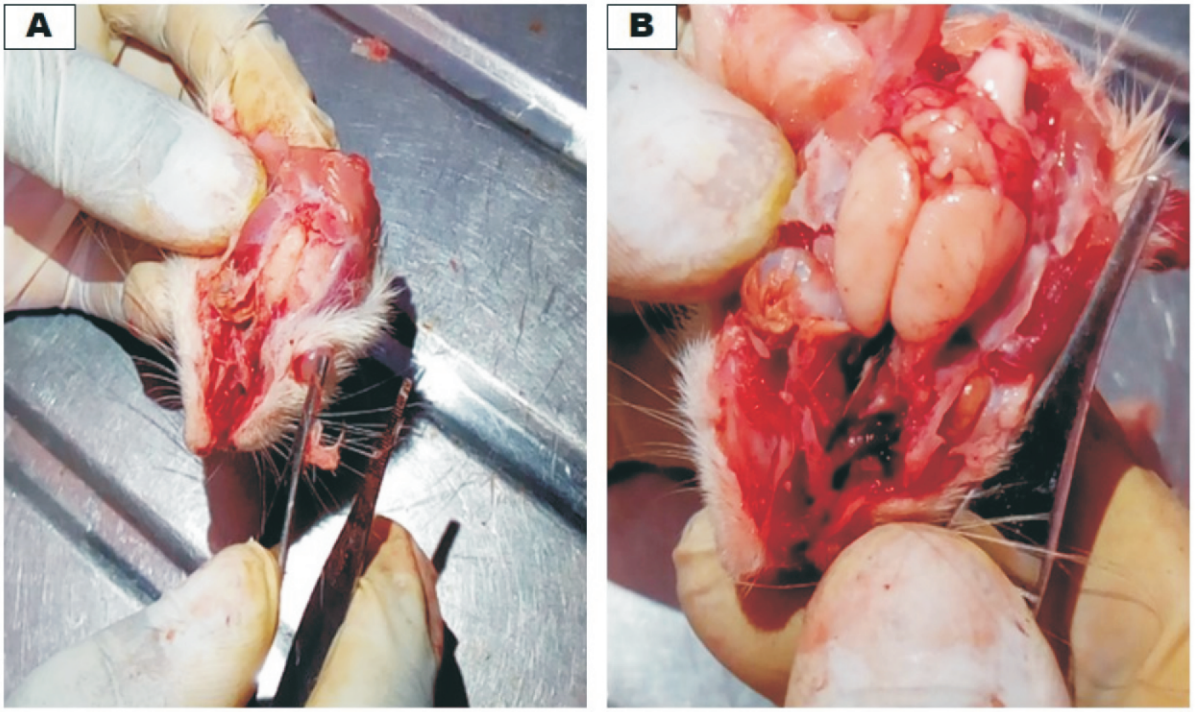

Figure 5: Removal of the brain from the skull. Avoid rupture of the brain by the bones of the skill while taking it out. A) Midline incison through the skull B) Brain within the skull

1. Once the head has been decapitated, make a midline incision through the skin of the head using a blade followed by a midline incision through the skull to isolate the brain (Figure 5A &5B; Figure 6).

Figure 6: Isolated brain with observable cerebrum and cerebellum and part of the medulla oblongata

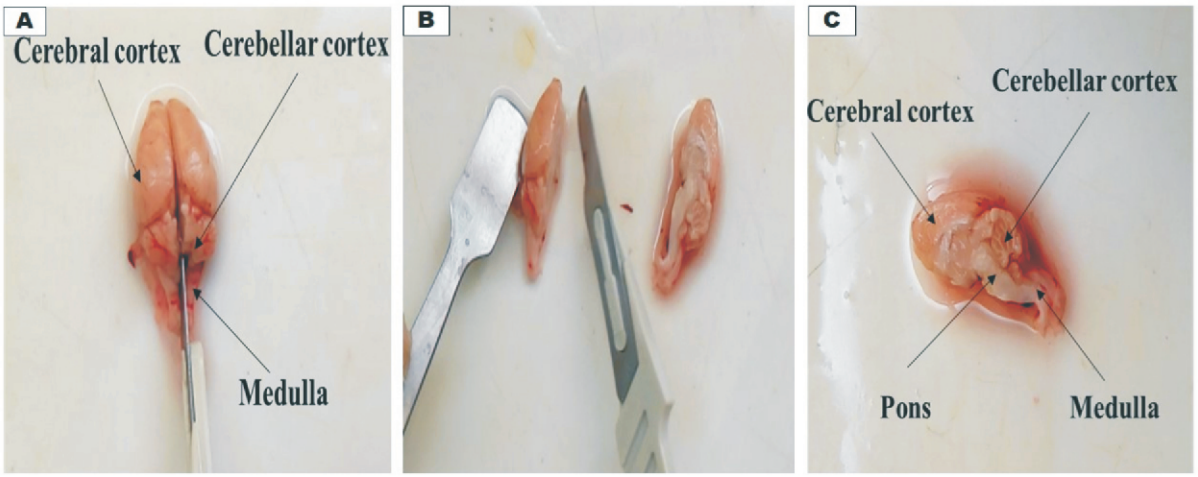

Figure 7: Brain placed on flat surface constantly bathed with phosphate buffer solution. A) Incision along the longitudinal fissure separating the cerebrum B) Halves of the separated brain C) One half of the brain turned with its medial surface facing upwards.

1. Once the brain has been carefully harvested from the skull, place it in a solution of 10% formosaline or the fixative of choice for some minutes depending on the fixative used to allow for proper fixation. Afterwards place it on a wet flat surface. On the flat surface, cut the brain along the longitudinal fissure of the cerebrum to separate them into halves using a surgical knife (Figure 7A & 7B). Turn the brain so that its medial surface is facing upwards to confirm that the cerebral cortex, cerebellar cortex, pons and medulla are visible ((Figure 7C).

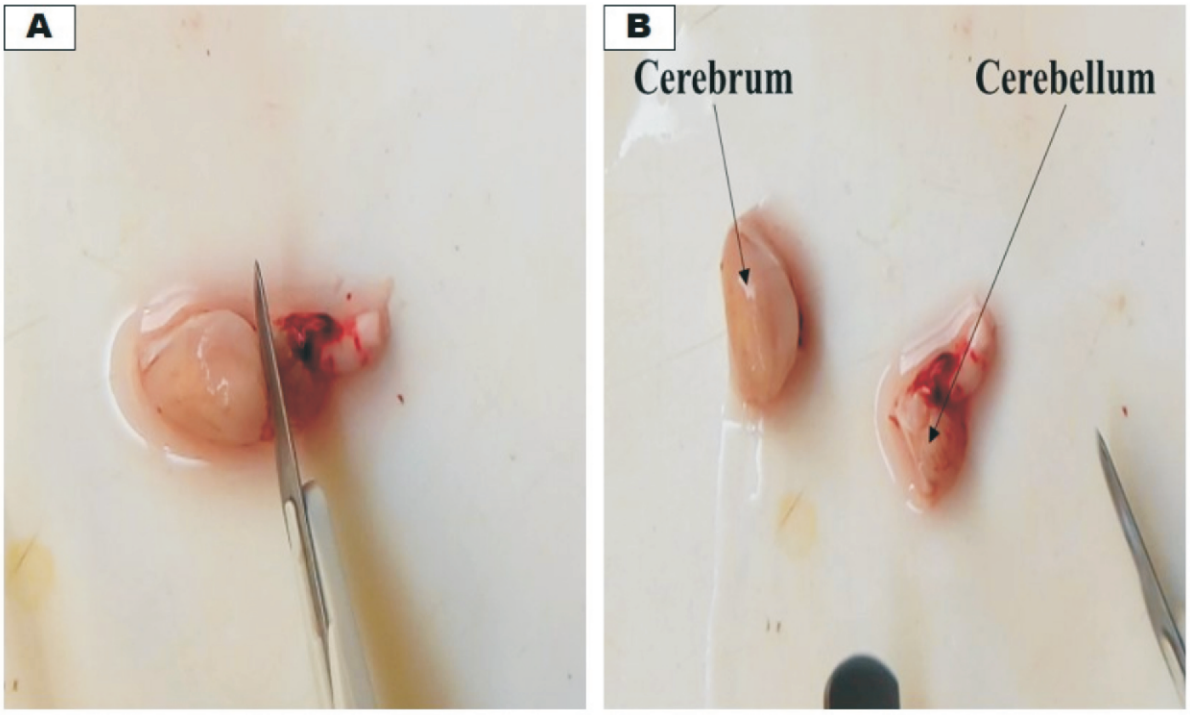

Figure 8: Separtion of the olfactory bulb and brain regions posterior to the lambda from the cerebrum A)Separation of midbrain and hindbrain from the cerebral cortex B) Cerebrum and cerebellum

1. As soon as the brain is separated into halves, dissect out regions posterior to lambda (midbrain, hindbrain, and cerebellum) as well as the olfactory bulb (Figure 8A & 8B). Pour phosphate buffer on the brain periodically to avoid desiccation.

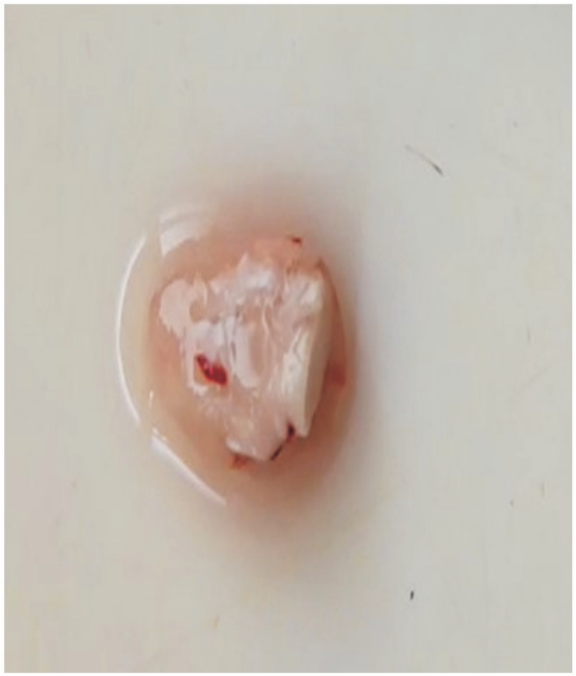

Figure 9: The cerebral cortex with its medial side turned upward

1. After dissection of regions posterior to the lambda and the olfactory lobe, turn the cerebral hemisphere with its medial side up(Figure 9).

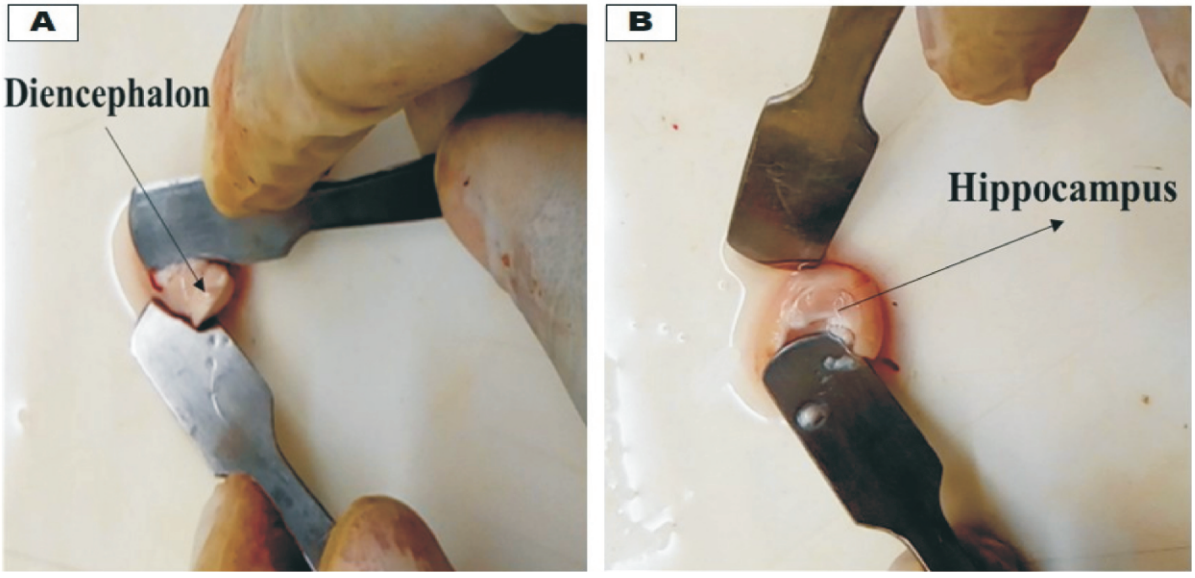

Figure 10: Removal of structures on the medial side of the cerebral cortex to expose the medial side of the hippocampus. A) Area of the diencephalon, parts of the basal ganglia and parts of the midbrain B) Medial side of the hippocampus.

1. Using forceps and spatula, carefully remove the area of the diencephalon (thalamus and hypothalamus) (Figure 10A). This exposes the medial side of the hippocampus (note the difference in colour between the cerebrum and hippocampus) (Figure 10B).

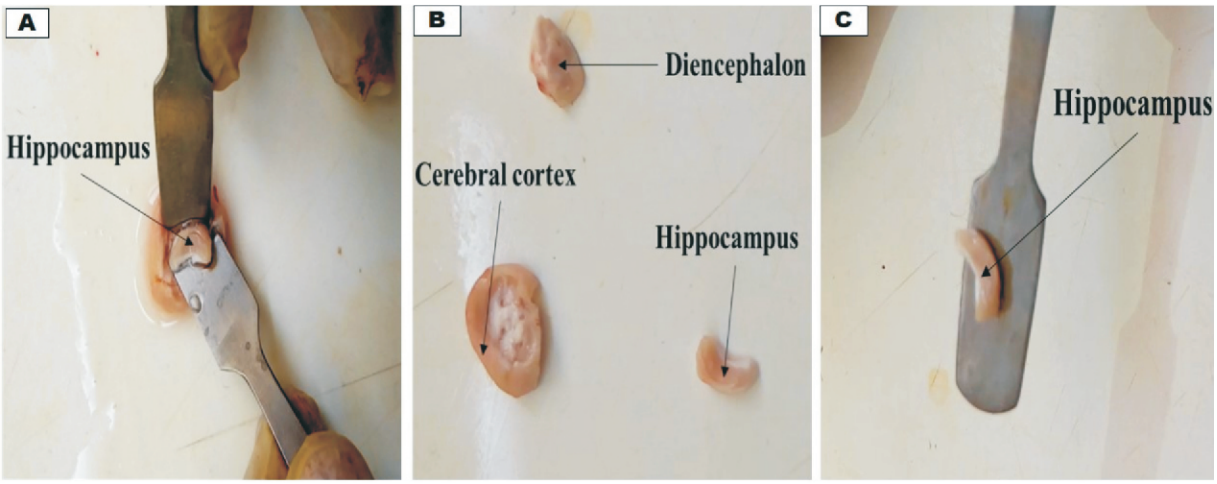

Figure 11: Isolation of the hippocampus (should be done carefully to avoid distortion) A) Use two blunt scalpels B) Cerebral cortex and subcortical structures C) Banana shape of the hippocampus.

1. Using two blunt scalpels, separate the hippocampus from the cerebrum (Figure 11A, 11B & 11C).
2. After isolation, the hippocampus can beweighed, grossly observed and histologically processed.

RESULTS

Physical observation: The result of the physical observation showed that the hippocampus can be weighed and is banana-shaped in the Wistar rat.

Histological Observation: From the histological observations, it was observed that the isolated hippocampus showed normal cytoarchitecture of the hippocampus with well-defined areas of the cornu ammonis (CA1-CA4), dentate gyrus and the subiculum as well as the layers of the hippocampus. The result for the Cresyl violet staining showed normal distribution of the Nissl substance.

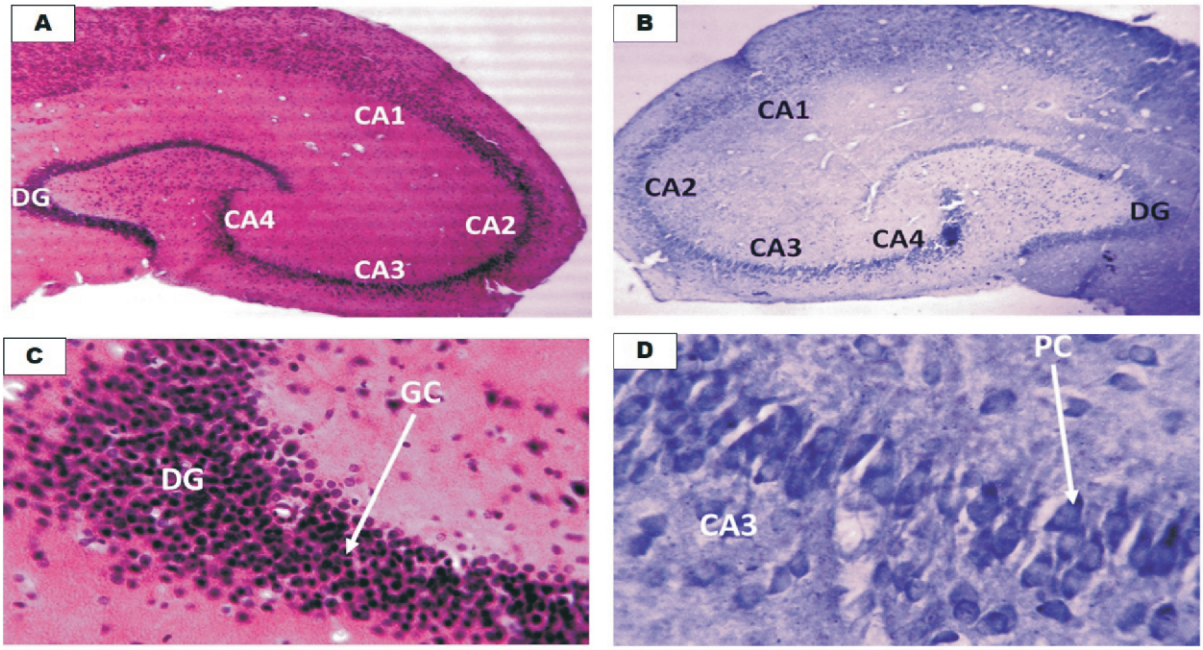

Figure 12. Photomicrograph of hippocampus section of adult Wistar rat (A) H and E (x40) (B) Cresyl violet stain (x40) (C) Dentate Gyrus (H and E x250) (D) CA3 (Cresyl violet stain x400). DG - Dentate Gyrus CA - Cornu Ammonis PC – Pyramidal cell GC- Granule cell.

DISCUSSION

The hippocampus is one of the most widely studied areas in the brain because of its important functional role in memory processing and learning. It is the earliest and most severely affected structure in several neuropsychiatric disorders such as Alzheimer's disease (AD), epilepsy etc[3]. Due to the difficulty in its accessibility, majority of studies today visualize the hippocampus by serial sectioning of the cerebrum. This method has proven to be hectic, time consuming and uncertain as the researcher cuts through the cerebrum continuously until he gets to the hippocampus. With the increasing consideration in regards to the usage of large number of rats, there is a need to consider how to reduce the unnecessary use of animals and fully exploit each experimental animal. Hence, techniques to isolate the hippocampus will be useful for investigating the events that occur specifically in this region as well as circumnavigating around this problems.

Here, we demonstrated a procedure to dissect the hippocampus efficiently from adult Wistar rat hippocampus and confirmed the precision of the technique for both histological, histopathological and histochemical evaluation. The histologic study revealed that the hippocampus was isolated without distortion, indicating that hippocampus sample can be prepared. Hence, this convenient and accurate dissection technique can be used for histologic, histochemical, immunohistochemical, histopathologic study of the hippocampus. Not only is it suitable for study of the hippocampus, it is less time consuming, aids the researcher in carrying out physical observation, morphometric and also allows for conservation of other important structures of the brain. This procedure permits increased utilisation of each rat ultimately leading to reduction of rats subjected to experimental study.

CONCLUSION

Using the manual extraction method, the hippocampus can be isolated without distortion, indicating that hippocampus sample can be prepared. This convenient and accurate dissection technique can be used for histologic, histochemical and histopathological study of the hippocampus, especially in developing countries.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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