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Innovative Brain Matrix Device-Hypomatrix for Histopathological Studies of Rat Hippocampus

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Authors' contributions

This work was carried out in collaboration among all authors. Authors VRN, PS and PR framed the concept, design of study or acquisition of data or analysis and interpretation of dat. Drafting the article or revising was done by Authors PR and PS. Final approval of the version to be published was done by authors PR and PS. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Rodents, such as rats, are frequently employed in neuroscience studies. Exact knowledge of the neuroanatomy and recognition of brain regions, as well as their interactions to nearby tissues, are required for precise technique and experiment success. In most studies, the hypothalamus of the rat brain is widely used to study many diseases. Brain sectioning is frequently the first stage in the dissection of certain brain structures. The aim of our study was to produce an innovative hypo matrix that would help compensate for the adversities that are caused by manual conventional grossing.

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Materials and Method: For the study 25 rat brain samples were obtained. 20 samples were immersed in formalin overnight and were grossed manually using conventional rat brain grossing method using blades and forceps. 5 rat brain samples were sliced using the hypomatrix which is a rectangular box with a lid containing blades that slice through the brain.
Results: The slides viewed under the microscope showed that the slices of hypothalamus obtained from the samples grossed using the hypomatrix presented a clear picture with least distortion and tissue damages.
Conclusion: The hypomatrix developed has shown better user handling, ease of use, and was better in terms of accuracy and efficacy. It will be helpful in reducing the time needed for the digestion and grossing of rat brain samples using conventional hand grossing hence showing improved time management.

Keywords: Conventional grossing; rat brain; histopathological application; rat hypothalamus; innovative hypomatrix.

1. INTRODUCTION

Rodents, such as rats, are frequently employed in neuroscience studies. Exact knowledge of the neuroanatomy and recognition of brain regions, as well as their interactions to nearby tissues, are required for precise technique and experiment success [1]. In tests involving behaviour, rats are frequently utilised. The animals are less fearful and more intelligent than mice, and their brains are bigger. Even though they don't "think" like humans, rats have some brain structures that are similar to the more basic parts of human brains, making them a useful model for some human behaviours [2]. In most studies, the hypothalamus of the rat brain is widely used to study many diseases. It produces neurohormones that either promote or inhibit pituitary hormone release.

The hypothalamus regulates circadian rhythms, appetite, thirst, and body temperature. Neurons from the rat hypothalamus can be used to examine neuroendocrine-related illnesses and disorders [3]. Numerous studies of hypothalamus have highlighted the importance of the connections that exist between the hypothalamic nuclei. Such connections mediate alterations in the function that occur in different physiological situations or post a localized lesion [4]. Brain sectioning is frequently the first stage in the dissection of certain brain structures. Specific equipment for sectioning fresh brains are needed for research that require undamaged neurons and membranes for biochemical, radiochemical, or electron microscopic examinations [5].

Sagittal slices from the whole brain are more challenging to prepare. Brain slices sliced by hand with the Stadle-Raggs slicer reveal tissue damage. Because the brain is a fragile tissue, consistent thickness and weight are difficult to achieve [6]. Many previous methods have a number of shortcomings that increase the manipulation time and, as a result, may have an impact on the quality of the tissue sample and separation accuracy [7]. Numerous studies of hypothalamus have highlighted the importance of the connections that exist between the hypothalamic nuclei. Such connections mediate alterations in the function that occur in different physiological situations or post a localized lesion [4]. Newer tools and techniques are being employed currently for precise and swift grossing and processing techniques [8]. The aim of our study is the development of a hypo matrix that would help compensate for the adversities that are caused by manual conventional grossing.

2. MATERIALS AND METHODS

2.1 Experimental Design

A total of 25 rat brain samples were collected and divided into two experimental groups. Group A consisted of 20 brain samples, which were processed following conventional grossing procedures to expose the hypothalamus. Group B included 5 brain samples, which were subjected to the novel Hypomatrix technique for tissue sectioning. After the tissue processing and staining, the resulting histological slides were analyzed by a qualified pathologist to assess the presence, precision and quality of the hypothalamus in rat brain specimens.

2.2 Rat Brain Sample Collection

Animal Ethics: All experimental procedures were carried out in compliance with ethical guidelines and protocols set forth by Institution's Animal Ethics Committee under the reference

number IHEC/SDC/UG-2038/22/GENPATH/205, ensuring the humane and responsible treatment of laboratory animals.

Rat Brain Specimens: A total of 25 Wistar rats were used for this study. Animals were euthanized using a humane method approved by the Animal Ethics Committee. Brain specimens were carefully excised from the skulls and immediately washed in a physiological saline solution to maintain tissue integrity and was transferred into formalin for overnight fixation for histopathological examination.

2.3 Experimental Groups

Group A (Conventional Grossing): In this group, 20 rat brain specimens were prepared for traditional grossing procedures. The brain specimens were meticulously dissected based on arbitrary measurements, revealing the hypothalamus region. And whilst using arbitrary measurements, all 20 brains while grossing was meticulously measured and recorded.

Group B (Hypomatrix Technique): For Group B, 5 rat brain samples were processed using the innovative Hypomatrix device. This novel technique allowed precise sectioning of the brain tissue, particularly focusing on the hypothalamus region.

2.4 Hypomatrix

The developed brain hypomatrix has a rectangular base as shown in Fig. 3. There were three chambers. The average of the dimensions of the rat brain were taken to determine the distance between the chambers and the length, breadth and height of the hypomatrix. From the side view the hypomatrix was half-moon shaped as shown in Fig. 4. The lid was equipped with sharp blades that would cut through the slits between the chambers, Fig. 5. Five rat brain samples were used to test the hypomatrix. The obtained slices of hypothalamus were stained and viewed under the microscope.

Fig. 1. Rat brain sample

Fig. 2. Rat brain grossing using routine conventional manual grossing

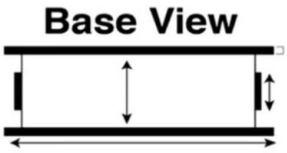

Fig. 3. Base view

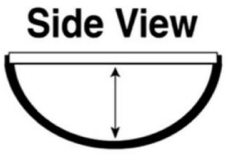

Fig. 4. Side view

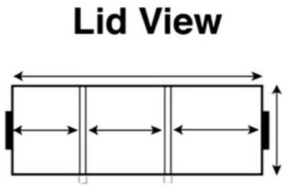

Fig. 5. Lid view

Table 1. Scoring criteria

Criteria	1	2	3
Tissue handling	Poor	Satisfactory	Good
Ease of cutting	Poor	Satisfactory	Good
Precession	Poor	Satisfactory	Good
User friendliness	Poor	Satisfactory	Good

2.5 Tissue Processing and Staining

Processing and Embedding: After grossing, the brain tissue specimens were processed following standard histological procedures, including dehydration, clearing, and paraffin embedding.

Sectioning and Staining: Paraffin-embedded tissue blocks were sectioned using a microtome. Sections were then mounted onto glass slides and subjected to routine histological staining techniques, such as Hematoxylin and Eosin (H&E) staining, to enhance tissue contrast.

2.6 Histopathological Analysis

Microscopic Examination: Prepared slides were examined under a light microscope by two pathologist who were blinded. The pathologist conducted a thorough evaluation of the hypothalamus region in both Group A and Group B specimens, documenting any observed differences in the quality and presence of the hypothalamus.

2.7 Data Analysis

The slides were scored based on tissue handling, ease of cutting, precision and user friendliness. (Table1). Two observers rated the slides for the precision of hypothalamus region. Both the observers were blinded about the groups. The interobserver agreement was analysed by performing kappa statistics and Independent t-test was used to analyse the statistical significance. A p value of less than 0.05 was considered statistically significant.

3. RESULTS

The results of the comparative analysis between the conventional grossing method and using the developed hypomatrix showed that there were favourable results obtained from the hypomatrix. From Fig. 6, the handling aspect of rat brain samples via conventional method showed a mean of 2.60±0.19 while hypomatrix showed a mean of 1.10±0.19, the mean of ease of handling of the samples in conventional grossing was 2.40±0.19, while it was 1.10±0.19 for hypomatrix. The user friendliness of the conventional method was comparatively low with a mean of 3.00±0.19 while that of the hypomatrix was 1.00±0.19. And in terms of accuracy and precision, the conventional grossing had a mean of 1.70±0.20 whereas for hypomatrix it was more accurate with a mean obtained of 1.20±0.20. The mean for the hypomatrix is closer to the value of 1 and hence denotes better results than the conventional method that has mean values higher than 1.

The slides viewed under the microscope showed that the slices of hypothalamus obtained from the samples grossed using the hypomatrix presented a clear picture with minimum distortion and tissue damages.

4. DISCUSSION

The developed design of this hypomatrix device for rat brain sectioning offers numerous advantages over the various other different techniques for sectioning fresh rat brain tissue slabs. We can avoid freezing the of the sample tissue that is employed in the method of microtome sectioning and for “punch” technique used for Eik-Nes and Brizzee as well as the immediacy of the slicing with the presented hypomatrix device eradicated the need for sample tissue freezing, except when very unstable compounds are to be studied [9,10].

Compared to the use of conventional methods for grossing and sectioning of brain samples, the hypomatrix showed more efficacy in user friendliness. With one push, the blades slice through the brain slices entirely leaving behind properly separated slabs. The hypothalamus is obtained undistorted and can be then used to carry out the required studies. The accuracy of the presented design was found to be better than the ones obtained with conventional grossing, the chances of wrong cuts and mistakes are greatly reduced. The device has also brought down the time required for the dissection.

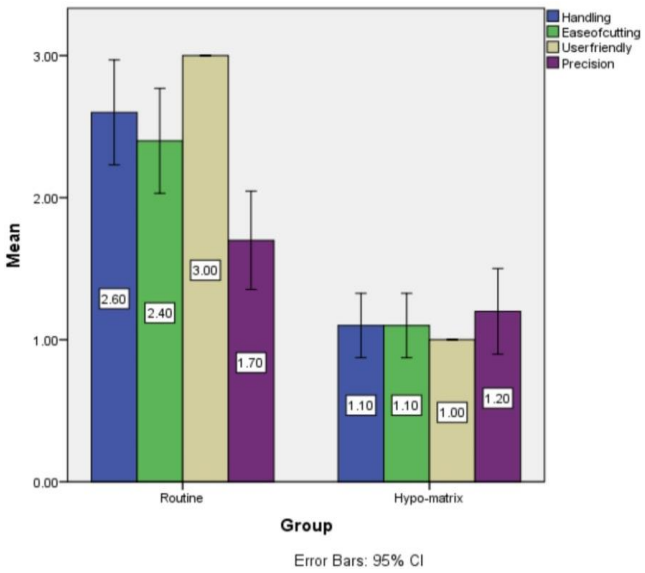

Fig. 6. Graph showing the differences in handling (blue), ease of cutting (green), userfriendly (yellow), and precision (purple) between the Routine (conventional grossing) and the hypomatrix

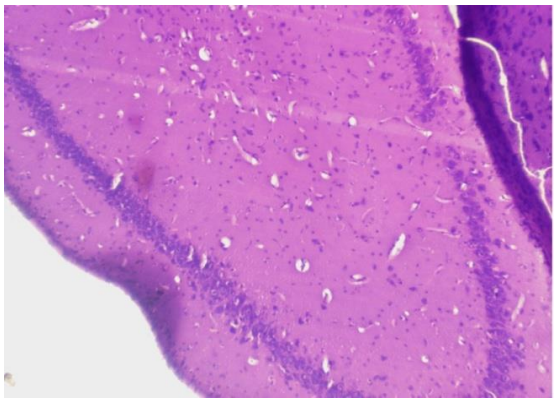

Fig. 7. Rat brain sample obtained viewed under the microscope

Addition to the rapidity, because of how quickly the slabs were prepared, we were able to bypass the highly laborious in-place cooling of the brain samples. The ability to quickly and easily access the cut slices is crucial once the slab has been cut through, and we discovered that this aspect was successfully achieved by creating the current design in two parts. This allowed the top lid to be removed, leaving the brain slices on the base resting all together in slices. Compared to Jacobowitz's fully single-piece design, this was a benefit [11]. Finally, the cost effectiveness of the presented hypomatrix is accounted to its simplicity and the minimal labour required for the device construction.

This study, aimed at evaluating the utility of the innovative Hypomatrix instrument for histopathological investigations, has yielded valuable insights. However, several limitations need to be considered when interpreting the results and implications of our research.

The Hypomatrix instrument could be improved further so as to apply it more widely. We currently have an optimised tool that can facilitate specific brain region-oriented studies, including but not limited to hypothalamus sections. The study of hypothalamus has been in great demand and thus motivated this design. As a result, the usefulness of the instrument is confined within the realm of this brain sub-division and could probably be less applicable for researchers studying on other parts of the brain. The study has another limitation in that it involved a relatively small number of people who might have hindered the extrapolation of the outcomes. The study reported positive results on how effective the Hypomatrix can be used to section and destroy the hypothalamus yet large samples need for different parts of brains to confirm and authenticate such findings. Therefore, we are currently involved in designing new cuts in the Hypomatrix for exact slicing off the parts of the brain.

5. CONCLUSION

The hypomatrix developed has shown better user handling, ease of use, and was better in terms of accuracy and efficacy. It will be helpful in reducing the time needed for the dissection and grossing of rat brain samples using conventional hand grossing hence showing improved time management. It has commonly and easily available materials making it a cost effective option. The slices can be readily and immediately obtained with accurate results and when viewed under a microscope shows a clear and distortion free view of the tissue. This hypomatrix can be an efficient and useful alternative to conventional hand grossing of rat brain samples.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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