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Anatomy

# STAINED BRAIN SLICES: AN EFFECTIVE WAY TO UNDERSTAND NEUROANATOMY

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| **Dr. Swati Tiwari** | Associate Professor, Department of Anatomy, Maulana Azad Medical College, Bahadur Shah Zafar Marg, New Delhi-110002, India. |
| **Dr. Anita Mahajan*** | Director Professor, Department of Anatomy, Maulana Azad Medical College, Bahadur Shah Zafar Marg, New Delhi-110002, India. *Corresponding Author |
| **Dr. Sabita Mishra** | Director Professor, Department of Anatomy, Maulana Azad Medical College, Bahadur Shah Zafar Marg, New Delhi-110002, India. |

**ABSTRACT** Teaching neuroanatomy to the students by using classical teaching tools is a challenging task due to sheer complexity. Three-dimensional visualisation of the neuroanatomical architecture is quite difficult. As a teacher, one must keep innovating to facilitate understanding of the subject. The purpose of this study was to find an effective and practical method for teaching neuroanatomy using brain slices. Formalin fixed human brain slices were generated using a brain slicing knife The slices were stained with Alston's method, Prussian Blue, Mulligan's, Robert's and Astra blue dye method. The stains were compared on the basis of: quality of differentiation between the grey and white matter, reusability of solutions, potential hazard of chemicals and cost effectiveness. Alston's method proved to be the most suitable for staining brain slices. Voluntary feedback and tests were taken to get students' perspective on the efficacy of stained versus non stained slices as learning tool. The study revealed that stained slices facilitated better understanding of the neuroanatomy due to lasting visual impression and better spatial visualization leading to improvement in the average score of the students. Using stained brain slices for teaching sectional neuroanatomy was found to be very effective as well as cost efficient resource.

**KEYWORDS :** Brain, stained slices, Alston's method, neuroanatomy, teaching tool

## INTRODUCTION:

Anatomy of the brain is quite intricate with complicated interconnections. Understanding these connections is crucial for grasping brain function and addressing neurological disorders. Classical neuroanatomy teaching in medical colleges involves usage of formalin fixed human brain slices, where differentiation between grey and white matter structures is often difficult. Thus, students find it difficult to understand spatial relations and construct a three-dimensional picture. The term 'Neurophobia' and 'Achilles heel' have been coined by authors to describe the students' fear of neurosciences [1, 2].

Proficiency in neuroanatomy is relevant as it imparts expertise to interpret magnetic resonance and computed tomography images which further facilitate localization of vascular and space occupying lesions in the brain. The level of knowledge of various subdivisions of anatomy amongst the medical students is not at par with the basic standards for safe clinical practices. Refresher courses in anatomy have been suggested by authors to compensate for the reduced time devoted to basic sciences during MBBS curriculum [3, 4].

To improve the understanding of neuroanatomy, modern techniques like tractography, neuronal tracing techniques and virtual dissection softwares are available at advanced research institutes [5, 6, 7]. Lack of resources limits the access to such technology in the developing countries. Also, literature suggests that such techniques are better as an adjunct to routine cadaveric dissection [2, 8, 9].

Since formalin fixed brain slices at various levels are the mainstay of practical neuroanatomy teaching, the purpose of this study was to improve the differentiation between grey and white matter by staining the slices and further, to analyze the quality and effectiveness of these slices for teaching neuroanatomy.

## Methods:

This study was done using routine cadaveric teaching material available in the dissection hall of the Department of anatomy at our college after obtaining ethical approval from the institutional ethical committee.

Human cadaveric brains available in the department of anatomy were first fixed in 10% formalin for a period of 1 month. Transverse as well as coronal slices of brain (1 cm thick) were made. After measurements, the slices were stained with Alston's method, Prussian blue, Mulligan's, Robert's and Astra blue dye method as per the protocols described in literature [10]. The sections were mounted in Kaiserling's solution no. 3 [11]. Several parameters were used to compare the stain quality and efficacy (Table 1).

To test the efficacy of stained brain slices as a teaching learning tool, a total of 248 first year MBBS students volunteered to participate in the study. These MBBS students were divided into two groups. Group 'A' was shown components of basal ganglia on a set of unstained brain slices and group 'B' was taught same content on stained slices. Both the groups were subjected to the test for identification of structures. Now, groups were reversed and process was repeated on the topic "relations of lateral and third ventricles". The scores were analyzed and compared by using the paired student's t test with the help of SPSS 25 version. A Likert type validated questionnaire was given to see the students' feedback on the effectiveness of stained slices as a teaching learning tool.

## Observations:

### *Comparison Of Staining Methods:*

Based on indicators used to compare the staining protocols, the Alston's method was the most suitable for staining brain sections under the tropical climate settings of Northern India (Table 1; Figure 1). In Mulligan's, Prussian blue and Robert's method, the Mulligan's solution (containing phenol) has to be heated (60-65°C) which can be hazardous. In Braak's method constant shaking of the primary staining solution is required, staining time is longer and differentiation of gray and white matter was not as good. Astra blue dye was costly. Thus, we selected Alston's stain as stain of choice for preparing brain slices to teach neuroanatomy.

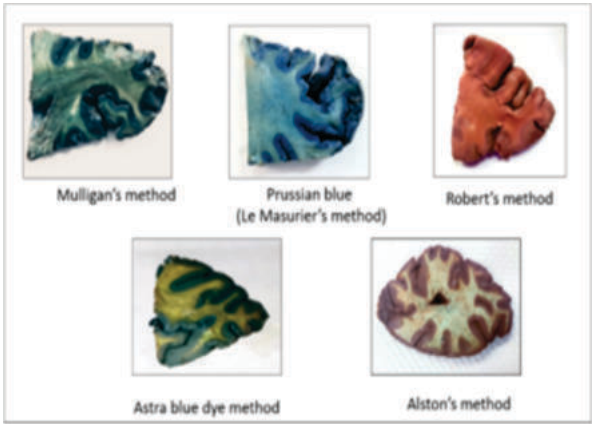


**Fig. 1a:** Colour differentiation between gray and white matter in the different staining methods.

**Fig. 1b:** Transverse section of brain before and after Alston's stain highlighting the grey matter in reddish brown colour.

***Quantitative Analysis Of Students' Performance:***

The mean test score of the students for stained slices was 9.41 out of 12 marks (S.D.=2.8) while for unstained sections it was 8.32 (S.D.= 2.7). It was found that teaching neuroanatomy on stained sections significantly increased the understanding and knowledge of the students. The gain is statistically significant at 'p' value <0.001.

***Qualitative Analysis Of Student Feedback:***

Usefulness and quality of stained brain slices was evaluated by feedback questionnaire given to the students. It revealed that 82.8% of students felt that they were able to better differentiate between grey and white matter in stained slices. 83.9% students had a better understanding and spatial visualisation of grey matter nuclei after being taught from stained slices. Majority opined that neuroanatomy should be taught using stained slices in future (Figure 2).

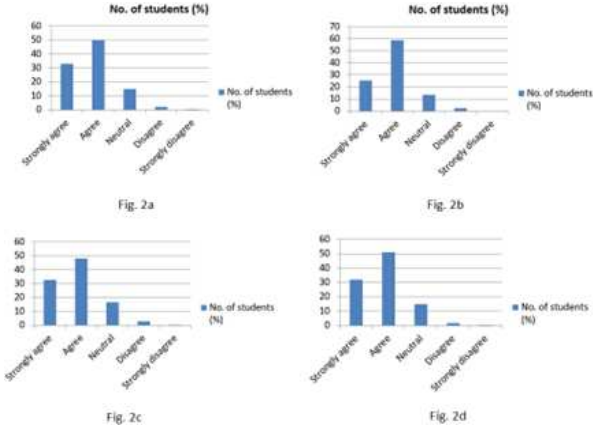


**Fig. 2:** Qualitative feedback from students on teaching neuroanatomy by stained sections.

**2a: Did the staining of the section improve the differentiation between grey and white matter of brain?**

**2b: Did staining of the section facilitate better understanding and spatial visualisation?**

**2c: Do you think that stained sections could be more effective self-learning tools compared to traditional unstained sections?**

**2d: Do you suggest staining of brain sections to demonstrate the various structures to MBBS students in future?**

## DISCUSSION:

A good teaching methodology ensures that students remain interested and become confident learners. The study was targeted to find out a simple yet effective teaching technique that will ease the understanding and visualization of neuroanatomical structures in the brain slices.

The differentiation between grey and white matter using the above-mentioned staining methods has a well-defined basis. Phenol present in Mulligan's solution dissolves the myelin of the white matter and forms a jelly like coat over it so the aqueous dye is unable to stain the white matter in Mulligan's method, Alston's method, Prussian blue and Robert's method. Depending upon the salt used in Mulligan's solution, the grey matter takes up specific colour [12]. In Alston's method, brick red colour is due to formation of cupric ferrocyanide. The Astra blue dye used in Astra Blue or Braaks's method, stains mucopolysaccharides rich areas of grey matter [13].

**Table 1: Comparison Of Different Staining Methods For Brain Sections**

| | Alston's method | Mulligan's method | Prussian Blue (Le Masurier) | Robert's Method | Braak's Astra blue dye method |
|---|---|---|---|---|---|
| Cost for chemicals | Cheap | Cheap | Cheap | Cheap | Costly |
| Heating required | No | Yes | Yes | Yes | No |
| Fumes | None | None | None | None | Offensive fumes of formic acid |
| Staining time | 25 min | 7 min | 13 min | 12min | 2 days |
| Colour of grey matter | Brick red colour: more life-like appearance | Grey black | Brilliant blue | Reddish brown | Bluish green |
| Colour of white matter | White | White | White | White | White |
| Details of gray matter nuclei | Best differentiation. Good contrast. | Not appreciable | Could be appreciate | Not appreciable | Could be appreciated |

Comparative analysis revealed that the Alston's stain gave the best results. The staining process is relatively simpler and faster with good differentiation. Suriyaprapadilok and Withyachumnarnkul, 1997 also found similar results [10]. Another study on dog brain compared three staining protocols (Alston's, Mulligan's and Prussian Blue method) and reported best results with Alston's stain [14]. One of the studies found that Roberts's method had the best differentiation, but, they did not use Alston's stain [15].

Brain slices mounted in Kaiserling's solution no. 3, retained the stain for longer time (at least five years). Thus, these stained brain slices could also be used to create museum specimens for display through which neuroanatomy can be taught over the years.

Brain slices stained with Alston's method, proved to be very effective in enhancing the contrast between grey and white matter. This led to better understanding of the topography and spatial relations. Teaching on stained slices definitely had a positive impact on the attitude of the learner towards neuroanatomy. Students felt motivated and actively engaged in the learning process. They developed a deeper appreciation for the spatial relationships by directly engaging with stained brain slices. Their improved knowledge was reflected in improved mean scores.

Digital modes of teaching such as virtual dissection tables, 3D software, 3D printers etc. are very effective teaching tools but are extremely costly and require specific infrastructure. In this study, human brain slices were stained with readily available stains. Our study demonstrated that even with limited economic resources, marked improvement can be achieved, thereby offering a comprehensive approach to mater the intricacies of the human brain.

## CONCLUSION:

Stained slices as non-digital 3D models are a valuable resource for improving the learning process among medical students for both large and small group teaching. In our study, Alston's stain is found to be the most suitable for formalin fixed brain slices. Stained slices enabled better spatial visualization of deeper nuclei in the brain. It is a great way to optimize the cadaveric resources which are limited in many institutes. It makes learning easy and interesting and thereby, strengthens the foundation of basic sciences among the medical students. A comprehensive understanding of complex neuroanatomical structures and their clinical relevance, potentially affects the quality of future healthcare professionals and their ability to adopt cutting-edge techniques in neurosurgery, neurology, and related fields.

### Limitations And Further Scope

The brain slices can also be plastinated to enable easier handling and

storage. The most suitable stain as regards plastination needs to be explored. Our findings about the perceptions of students may not be generalizable as the study was done only at one institute.

## Conflict Of Interest

The authors have no conflict of interest.

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