

## Inclusion of human brain slides in resin stained with mainland technique

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### Abstract

Human brain slices were used which were stained and embedded in resin for retention of parts of the central nervous system (CNS). The application of the technique of embedding in resin prevents wear due to constant handling practical classes and research. Human brain, fixed in 20% formaldehyde, were cut into coronal sections and stained by the Mainland method. After staining, sections were dehydrated and accommodated in a pan of acetate and immersed in a liquid crystal resin solution. After catalysis the block was removed from the mold and its surface was planned and standardized using sandpaper in increasing weights. We observed brain sections stained by the Mainland allowing differentiation between gray and white matter, beyond the view of the basal ganglia and surrounding structures. Furthermore, the resin inclusion allowed the visualization through transparency, no wear of the cuts, increasing the life of the anatomical parts. This feature provides anatomy laboratories means for protection of parts against air, moisture and handling damage, further promoting the protection of those who use the parts, since the damages caused by the chemical formaldehyde used for fixation and preservation of anatomical specimens are known.

**Keywords:** brain mapping, central nervous system, mainland method, morphology.

### 1 Introduction

The central nervous system during its development provides an intensive and dynamic rolling to understand the process of development and maturation, it is essential to the correlation between structure and function, ie the development of specific function dependent on the ripening of its substrate neural anatomy corresponding (REED, 2005). This matured depends, in part genetic guidance, the degree of stimulation and adequate food (MORGANE, AUSTIN-LaFRANCE, BRONZINO et al., 1993). The brain maturation involves a series of temporally overlapping stages that follow a precise sequence, which differs from region to brain region and even within a particular region, varying from one species to another (MORGANE, MOKLER and GALLER, 2002).

With a mass of about 2 kg, corresponding to 3% of body weight, CNS of human beings although the smallest of the eleven organ systems is considered the most complex of all. The nervous system originates from the neural plate, a thickened area in the embryonic ectoderm (MOORE and DALLEY, 2007), this board will give rise to neural crest and neural tube, which form respectively the central and peripheral nervous system (MACHADO, 2006). The peripheral nervous system is composed of cranial and spinal nerves, ganglia and nerve endings. While CNS is composed of the brain and spinal cord. Although the surface of the cerebral hemispheres have a high variability, the main grooves formed in fetal life, have relatively constant locations, allowing precise references (DOOLING, CHI and GILLES, 1977). As the cerebral cortex is highly specific functional locations, identification of sulci and gyri is of great clinical and surgical

importance. This identification enables the understanding of the anatomy of the upper-lateral surface (convexity) of the brain, the location of lesions, surgical planning and ways to approach transsulcal (RHOTON JUNIOR, 2007). The anatomy of the cerebral convexity presents great practical importance, both clinically and surgery (GUSMÃO, RIBAS, SILVEIRA et al., 2001). Lesions of cerebral convexity in imaging allows correlate clinical and anatomical (correlation of signs and symptoms with the topography). This location also allows the surgical schedule as the main grooves can be represented in the skull surface using traditional methods of craniotopography (GUSMÃO, SILVEIRA and ARANTES, 2003).

The application of neuroimaging techniques has allowed for the presence of brain changes in vivo in samples of patients with neuropsychiatric disorders compared control groups of healthy volunteers matched for demographic variables, with quality increasing sensitivity and anatomic resolution (HSU, DU, SCHUFF et al., 2001). Techniques such as computed tomography (CT) and magnetic resonance images (MRI) provide data on the structural anatomy brain, allowing the detection of circumscribed or diffuse lesions, or quantitative evaluation of volume of different brain structures (TIEDE, BOMANS, HÖHNE et al., 1993). Since the methods of functional neuroimaging such as positron emission tomography (PET), the photon emission computed tomography (SPECT) and magnetic resonance imaging (fMRI), assess metabolic and biochemical processes in the brain (CAMARGO, 2001).

The Neuroanatomy is the branch of anatomy that studies the nervous system, basic content for the courses from different areas of health. For this study is necessary to use brains from unclaimed corpses. However, the brain is an anatomical structure most vulnerable to the whole body. Your purchase is also complicated by the fact that not always the corpse after death is well established thus preserving the brain. The most commonly used fixative is 10% formaldehyde is one where it can cause serious damage to health (MATTOS, SANTOS, ZULLO et al., 2008).

Show morphology of brain structures is accompanied by two major problems, brain tissue is inconvenient to handle and is easily destroyed when shown countless times. Besides the difficulty macroscopic demonstration of subcortical nuclei stained, and the subdivisions of the gray and white matter are difficult to delineate. These problems can be avoided by the methods described in this document that combines the advantages of staining brain tissue sections with the inclusion resin.

## 2 Materials and Methods

Stain techniques and inclusion in resin were developed in the Department of Anatomy, Academic Center in Victoria (CAV), Federal University of Pernambuco (UFPE) - Brazil. To perform this technique were used human brain from the collection of the Laboratory of Human Anatomy of the CAV - UFPE. The brains were fixed with formaldehyde solution 20% was cut with 1.5 cm thick, using a feedback timber and a 12-inch stainless steel knife. The brain sections were stained using Mainland method, which consists in successively submerging the brain ferric chloride solution for 20 seconds, potassium ferrocyanide for 15 seconds and nitric acid for 48 hours. After staining the sections run through an increasing ethyl alcohol, from 40% alcohol until the ethanol, leaving in each alcohol for a period of 24 hours. To complete the dehydration process, the pieces were immersed in glycerin for 48 more hours. Acetate were prepared forms, from radiographic films used, tailored to each section in particular. The forms were sealed with adhesive tape to initiate the process of embedment in resin. Acetate were prepared forms, from radiographic films used, tailored to each section in particular. The forms were sealed with adhesive tape to initiate the process of embedment in resin.

The acrylic resin was prepared in the resin liquid crystal (80%), styrene monomer (20%) and catalyst (1% of total volume). The brain slices were placed in the forms of acetate which contained as a base catalyzed resin (Figure 1). Only the pieces were then covered with a layer of fluid resin plus catalyst. The catalyst was an average time of 3 hours, depending on the temperature (Figure 2). After the total catalyst, the block of resin containing stained brain was cut sanded manually by a sequence of sandpaper 7, starting from the number 80, passing through the grit number 120, 360, 400, 600, 1200 and ending with the number of sandpaper in 1500 (Figure 3). After you have sanded the blocks have undergone a finishing polish with mass number 2.

## 3 Results

We observed brain sections stained Mainland method allowing differentiation between gray and white matter,

beyond the view of basal ganglia and surrounding structures. Furthermore, resin inclusion allowed the visualization through transparent wear-free cuts, increasing the life of the anatomical parts used in teaching and research. There

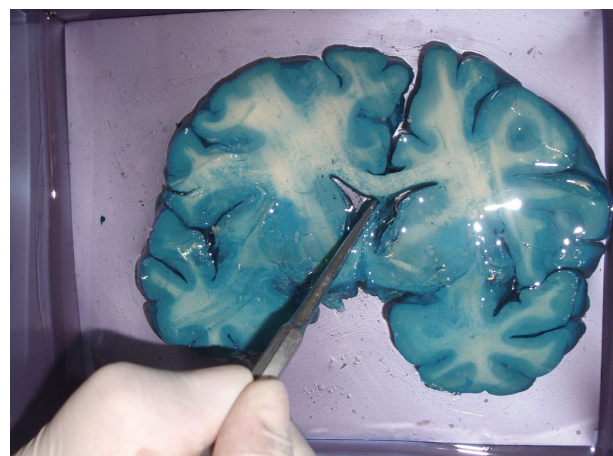


Figure 1. Inclusion the brain slide in resin.

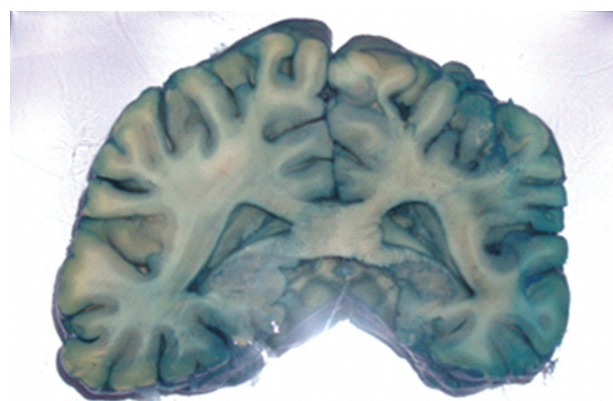


Figure 2. Brain slide immersed in resin after 3 hours of beginning the process.

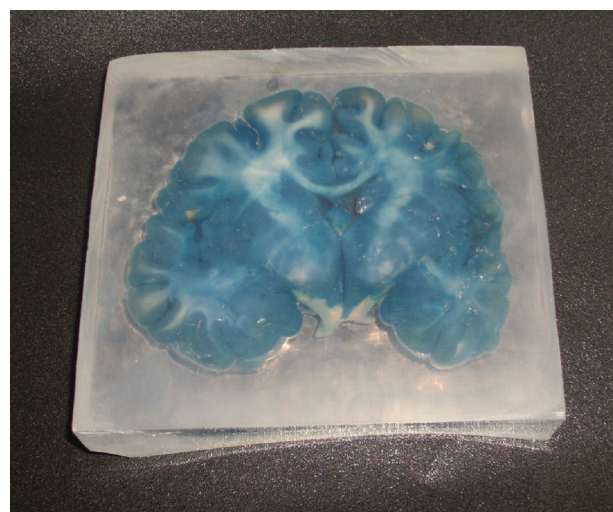


Figure 3. Start the process of sanding the resin block containing the brain slide.

are several anatomical techniques whose main objective is the preservation and display of parts, but some of these techniques require products that are difficult to acquire. The use of resin has been used in the laboratories of anatomy to repletion vessels and mold making bodies (RODRIGUES, 2005). A variety of techniques resin is used as the anatomical vinyl acetate (vinylite), polyester resin, methyl methacrylate, mercox, resin justin N° 17, Crystic resin 26C (RODRIGUES, 2005). The resin crystal is used in manufacture of swimming, surf boards, construction, among other applications. It is a translucent resin and the more affordable compared to other resins.

Developed in our Laboratory of Anatomy, the technique of embedding in resin parts of the CNS to facilitate the visualization of anatomical structures facilitating knowledge structures and aiding in the area of clinical medicine (Figure 4).

Anatomical characterization of geographically well-defined brain regions is particularly useful for the practice neuroanatomical for providing a better understanding of three-dimensionality their structures and injuries that affect (TIEDE, BOMANS, HÖHNE et al., 1993). Currently, new methods of neuroimaging by computed tomography and magnetic resonance have shown art anatomical details more precise (TIEDE, BOMANS, HÖHNE et al., 1993; BLACKSTAD, 1996).

However, study of human anatomy in medical schools continues use the body and its anatomical specimens dissected, as a basic and accurate knowledge of human body. Thus, use of resin blocks containing stained brain sections are practical instrument for the student's learning. In many anatomy courses, are occurring substitutions of traditional dissection of cadaveric material by material dissected from animals (REIDENBERG and LAITMAN, 2002). This is occurring due to the reduction of access to material bodies, and increases the number of students in Higher Education Institutes (INZUNZA, VARGAS and BRAVO, 2007). Considering the increasing difficulty in obtaining these bodies which allow dissection custom it is necessary to obtain anatomical material that enables the



**Figure 4.** Brain slide ready for use in the laboratory.

management and student learning and practical classes in anatomy.

The necessities of previous anatomical studies, performed in cadavers to perfect knowledge of anatomical structures during classes of human anatomy (LOSNER, 1994) are still the best way of learning. Using brain parts embedded in resin has the ability to view, handle and maintain the material with a long life. In addition to preventing accidents and preventing direct contact of the student/teacher with toxic substances such as formaldehyde.

The beginning of the century was characterized by a significant technological advance, with the emergence of sophisticated three-dimensional medical imaging techniques and the development of minimally invasive therapies in the area of surgery. Literally, were introduced in the medical world a new way to see the anatomy (PAALMAN, 2000). Ironically, at present, the anatomical knowledge is highly necessary; the morphological sciences in our country seem to be in an uncomfortable situation, due to the reduction of hours devoted to the morphological classes and reduction of materials from cadavers (INZUNZA, VARGAS and BRAVO, 2007).

#### 4 Conclusion

Finding new ways of presenting the material available in cadaveric institutions is essential for student learning, for the preservation of material already scarce, for protection of that individual who handles the play and perpetuation of knowledge. Thus, courses in anatomy, uses of spare brain stained and embedded in a resin provide adaptation of teachers, students and technical requirements of ethics committees and bodies to control unhealthy.

#### References

- BLACKSTAD, TW. Computer methods in neuroanatomy: determining mutual orientation of whole neuronal arbors. *Computers in Biology and Medicine*, 1993, vol. 23, p. 227-250. [http://dx.doi.org/10.1016/0010-4825\(93\)90023-T](http://dx.doi.org/10.1016/0010-4825(93)90023-T)
- CAMARGO, EE. Brain SPECT in neurology and psychiatry. *Journal of Nuclear Medicine*, 2001, vol. 42, n. 4, p. 611-23. PMID:11337551.
- DOOLING, EC., CHI, JG. and GILLES, FH. Ependymal changes in the human fetal brain. *Annals of Neurology*, 1977, vol. 1, n. 6, p. 535-41. PMID:560819. <http://dx.doi.org/10.1002/ana.410010605>
- GUSMÃO, S., RIBAS, GC., SILVEIRA, RL. and TAZINAFFO, U. Localização dos sulcos e giros da face súpero-lateral do cérebro na tomografia computadorizada e na ressonância magnética. *Arquivos de neuro-psiquiatria*, 2001, vol. 59, p. 570-76. PMID:11588637. <http://dx.doi.org/10.1590/S0004-282X2001000400016>
- GUSMÃO, S., SILVEIRA, RL. and ARANTES, A. Landmarks to the cranial approaches. *Arquivos de Neuro-Psiquiatria*, 2003, vol. 61, n. 2A, p. 305-8. PMID:12806519. <http://dx.doi.org/10.1590/S0004-282X2003000200030>
- HSU, YY., DU, AT., SCHUFF, N. and WEINER, MW. Magnetic resonance imaging and magnetic resonance spectroscopy in dementias. *Journal of Geriatric Psychiatry and Neurology*, 2001, vol. 14, p. 145-66. PMID:11563438 PMID:PMCID:PMCID1857299. <http://dx.doi.org/10.1177/089198870101400308>

- INZUNZA, O., VARGAS, A. and BRAVO, H. Anatomy and Neuroanatomy the Most Impair in the Curricular Reform. *International Journal of Morphology*, 2007, vol. 25, n. 4, p. 825-830.
- MOORE, KL. and DALLEY, A. F. *Anatomia orientada para a clínica*. 5. ed. Rio de Janeiro: Guanabara Koogan, 2007. p. 853-863
- LOSNER, A. Computer models of the brain - how far can they take us?. *Journal of Theoretical Biology*, 1994, vol. 17, p. 61-73.
- MACHADO, ABM. *Neuroanatomia Funcional*. 2. ed. São Paulo: Atheneu, 2006. p. 59-74.
- MATTOS, JP., SANTOS, MJ., ZULLO, JFD., JOAQUIM, AF., CHADDAD-NETO, F. and OLIVEIRA, E. Dissection technique for the study of the cerebral sulci, gyri and ventricles. *Arquivos de Neuro-psiquiatria*, 2008, vol. 66, n. 2A, p. 282-7. PMID:18545805. <http://dx.doi.org/10.1590/S0004-282X2008000200034>
- MORGANE, JP., AUSTIN-LaFRANCE, R., BRONZINO, J., TONKISS, J., DIAZ-CINTRA, L., KEMPER, T. and GALLER, JR. Prenatal malnutrition and development of the brain. *Neuroscience & Biobehavioral Reviews*, 1993, vol. 17, n. 1, p. 91-128. PMID:8455820.
- MORGANE, JP., MOKLER, DJ. and GALLER, JR. Effect of prenatal protein malnutrition on the hippocampal formation. *Neuroscience & Biobehavioral Reviews*, 2002, vol. 26, n. 4, p. 471-83. [http://dx.doi.org/10.1016/S0149-7634\(02\)00012-X](http://dx.doi.org/10.1016/S0149-7634(02)00012-X)
- PAALMAN, MH. Why teach anatomy?. Anatomists respond. *Anatomical Record*, 2000, p. 261:1-2.
- REED, UC. O desenvolvimento normal do sistema nervoso central. In NITRINI, R., BACHESCHI, LA. (Eds.). *A neurologia que todo médico deve saber*. 2. ed. São Paulo: Atheneu, 2005. p. 395-9.
- REIDENBERG, JS. and LAITMAN, JT. The new face of gross anatomy. *Anatomical Record*, 2002, vol. 15269, n. 2, p. 81-8.
- RHOTONJUNIOR, AL. The cerebrum. Anatomy. *Neurosurgery*, 2007, vol. 61, n. 1, p. 37-118.
- RODRIGUES, H. *Técnicas anatômicas*. 3. ed. Vitória, 2005. p. 193-200.
- TIEDE, U., BOMANS, M., HÖHNE, KH., POMMERT, A., RIEMER, M., SCHIEMANN, T., SCHUBERT, R. and LIERSE, W. A computerized three-dimensional atlas of the human skull and brain. *American Journal of Neuroradiology*, 1993, vol. 14, n. 3, p. 551-559.

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