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MICROSURRGICAL ANATOMY OF THE BASIS NUCLEI AND WHITE FIBERS ASSOCIATED WITH THREE-DIMENSIONAL IMAGES

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Universidade de São Paulo Experimental Pathophysiology Abstract: The microsurgical approach to the basal ganglia (NB) was performed from the lateral surface of the cerebral hemispheres (HC) to the medial surface in order to preserve the related structures. Our objective was to perform microdissection of NB in three HC fixed in formalin under a surgical microscope to analyze the topographic anatomy of this region in relation to the white fibers of the brain (FB). The white fiber (FB) microdissection technique used was Klingler. During and after the microdissections, photographs were taken and three-dimensional (3D) images were produced, to better understand the depth of the anatomical structures. The anatomical details of the topographic arrangement of the NB with the FB were described. From the microdissection of the NB, in relation to the FB, the images generated in 3D provided a better understanding of the relationship between the superficial and deep structures of this region, contributing to the depth relationship of the structures studied.

Keywords: basal ganglia; white fibers; threedimensional images; microsurgical anatomy

INTRODUCTION AND GOALS

The dissection of the basal ganglia and white fibers is essential for teaching neuroanatomy and neurosurgical practice, as it provides a better understanding of possible anatomical structures and injuries that can damage or provide better surgical access to cortical structures (Rhoton, 2002; De-Castro et al., 2005; Fernández-Miranda et al., 2008; Herbet et al., 2018). Their anatomical relationship is very well described in traditional teaching methods such as textbooks and atlases (Mooer et al., 2001; Dangelo and Fattini, 2003). However, it is known that microsurgical dissection of white fibers requires study techniques to better understand this area, as microdissection has been a technique used to understand and teach the anatomy of the

white fibers of the brain and its application for teaching anatomy. microsurgical (Di Carlo et al., 2019). A better understanding of the white matter of the brain can help science understand the post-surgical recovery of patients (Briggs et al., 2021).

The basal ganglia are anatomically situated in a central position in each hemisphere (Ribas and Oliveira, 2007) in relation to the insula lobe. Regarding its morphology, five basal nuclei are considered: the caudate nucleus, the putamen, the globus pallidus, the amygdaloid body and the claustrum (Prada, 2014).

The main function of the basal ganglia is motor control. However, the amygdaloid body and the claustrum are not directly related to motor function, the first would be related to emotional behavior and memory, while the second has no known function. The globus pallidus has two subdivisions: the medial globus pallidus and the lateral globus pallidus, which together with the putamen constitute the lentiform nucleus and this, together with the caudate nucleus, forms the so-called striatum (Machado and Haertel, 2014). The white fibers that surround the basal nuclei are: the extreme capsule, external capsule and internal capsule.

One of the most used techniques for understanding the brain structures of the nuclei with the connection of white fibers is the Kingler technique. The Klingler technique was discovered in 1935 by Joseph Kingler, he was an anatomist who developed a method of brain fixation which is named after him. It is a modified method of brain fixation and dissection, based on freezing and thawing of brain tissue, subsequent peeling of white matter fibers, and gradual exposure of white matter tracts. This fact makes it an invaluable tool for an in-depth understanding of the complex anatomical organization of the cerebral hemispheres. The brain freezing method allows the expansion of white fibers caused by ice crystals, allowing the separation of white matter and gray matter. This technique is still widely used today for macroscopic dissection of the brain's banking fibers, which allows and facilitates knowledge of brain connections (Kingler, 1935; Kingler and Gloor, 1960; De Castro et al., 2005; Fernandez-Miranda et al, 2008; Yagmurlu et al. 2016).

In this study, we performed microdissections on the lateral and medial surface of the brain using the macroscopic dissection technique of bank fibers developed by Klinger in 1935. In addition to macroscopic dissection, we also performed photographic documentation with stereoscopic three-dimensional anaglyph images (3D), as 3D images facilitate the understanding of dissected structures and their spatial relationship, in addition to generating a low-cost image archive. by incorporating stereoscopy Therefore, into our study, we sought to provide a more detailed and accessible view for teaching gross anatomy, especially neuroanatomy.

MATERIALS AND METHODS

For this study we used three cerebral hemispheres (HC) from cadavers fixed in 10% formaldehyde and dissected under a surgical microscope with an amplitude of 10 to 40x applying the Klingler technique to dissect the white fibers.

The dissection of the white fibers of two cerebral hemispheres took place from the lateral surface to the medial surface in order to verify all layers of anatomical structures from the insula lobe to the medullary white center of the brain in the region of the basal ganglia. Once the dissection process of the two cerebral hemispheres was completed, numerous photos were taken, with the aid of a Nikon camera with an 18-55mm lens for photodocumentation and development of 3D images. To create three-dimensional images, we use specific software (Anamaker 3D©) available free of charge to generate threedimensional anaglyph images. In the third cerebral hemisphere, a transverse section was made to expose the basal ganglia and their relationship with the white matter of the brain was described as shown in Figure 1.

The human brains used in this work belong to the Laboratory of Functional Anatomy Applied to Clinics and Surgery (LAFACC) of the Faculty of Medicine of ``Universidade Federal de Uberlândia``. The specimens were obtained in accordance with Law 8,501 of November 30, 1992, which regulates the use of unclaimed corpses for teaching and research purposes in medical schools. Our research does not require approval from the Ethics Committee, as it is a study carried out in the anatomy laboratory and material that was being prepared for practical anatomy classes. The study was conducted respecting the principles of the 2013 Declaration of Helsinki (WMA,2013). The pieces did not contain any apparent macroscopic pathology. In the present work we adopted the nomenclature of Anatomical Terminology, from the Brazilian Society of Anatomy (2001).

RESULTS AND DISCUSSION

In this study, the relationship between the basal ganglia and the white matter was demonstrated using the Klingler technique to highlight the white fibers of the brain relating them to the basal ganglia. The entire process was photo documented, from the images generated we used free software to generate 3D anaglyph images. Using the Klingler method, we obtained the topographical arrangement of the white fibers in relation to the basal nuclei Figure 2. 3, 4 and 5. The use of 3D anaglyph images allowed us to create three-dimensional images to facilitate the understanding of anatomical structures with greater clarity and depth of the arrangement of white fibers in relation to the base nuclei

that are frequently used in the study of brain connections and microsurgical training for future neurosurgeons Figure 6. In our research there were no measurements or comparisons that would lead to statistical analysis.

ARRANGEMENT OF THE BASAL GANGLIA IN CROSS-SECTION OF THE BRAIN

The nuclei were identified in three cerebral hemispheres, one of which was made in a transverse section (Figure 1) and in the others we dissected from the lateral to the medial face (Figures 2, 3, 4, 5 and 6).

The main nuclei identified were schematized in Figure 1. In the cross-section of one of the HC, it was possible to observe the arrangement of the basal ganglia from the insula lobe (Figure 2). In this work, we chose to identify only the caudate, lentiform nuclei (putamen and medial globus pallidus (internal segment) and lateral globus pallidus (external segment) and the claustrum. The substantia nigra and the subthalamic nucleus, although they are also considered basal ganglia, were not targets of our dissection, as we used cerebral hemispheres that did not contain the complete diencephalon or the brain stem.

The insula lobe delimits from lateral to medial the white center of the brain in which we can centrally locate the basal ganglia. Delimiting the insular cortex in Figure 2. The subcortical white matter is called the extreme capsule, composed of short association fibers that connect with the insula gyri and the opercular gyri that project to the Sylvian fissure. The extreme capsule has functions linked to speech. Located between the extreme capsule and the external capsule there is a thin layer of gray matter called the claustrum.

The claustrum can be divided into the dorsal claustrum and the ventral claustrum. The function of the claustrum is related to the cortical system responsible for the integration of visual, somatosensory and motor information, due to its topographic positioning being related to the supplementary motor area and the posterior parietal area. After the external capsule, medial to the claustrum, the putamen is observed, which is located between the external capsule and the internal capsule (Figures 2 and 3). The putamen together with the caudate nucleus forms the dorsal striatum. Medial to the putamen is the internal capsule, which is an important tract of projection fibers that originates in the cerebral cortex and projects between the lentiform nucleus formed by the putamen and globus pallidus nuclei and the head of the caudate nucleus (Figure 3). The fibers of the internal capsule project between the precentral cortex and the brain stem (Figure 4 and 5). The posterior branch of the internal capsule forms the corticospinal tract as shown in Figure 5 and is well described in the literature (Ebeling and Reulen, 1992; Winn, 2016).

MICRODISSECTION OF WHITE FIBERS IN ANATOMICAL RELATION TO THE NUCLEI

The dissection procedure began from the lateral surface of each cerebral hemisphere, with the removal of the superior and temporal gyri (superior, middle and inferior) to access the insula lobe via Silvio's fissure, the gray matter was removed with the help of of spatulas. The dissections were carried out under a microsurgical microscope with a magnification of 10x to 40x, which facilitated the observation of the dissociation of the white fibers in relation to the basal ganglia. During the microdissection process, we took step-by-step photographs of the dissection starting from the removal of the white fiber layers (FB) and exposure of the basal nuclei (NB). Figures 3, 4 and 5. The images were taken with a semi-professional Nikon D3200

camera and 18-55mm lens.

The microsurgery technique in this work was first developed based on the work of Yassargil at the end of the 19th century, this technique combined with Kingler's technique allowed a better macroscopic visualization of anatomical structures. Another author who has combined the Kingler and Yassargil technique and influenced several researchers to study the microdissection of white fibers was the neurosurgeon Rhoton, who brought great visibility to these structures for the knowledge of neurosurgeons, who considers this microdissection technique a milestone for the training of future neurosurgeons in microneurosurgery, being mandatory training for medical residency (Rhoton, 2002).



Figure 3 – phases of the left cerebral hemisphere dissection process demonstrating the beginning of the dissection process. In (A) the frontal (LF), temporal (LT), parietal (LP) and occipital (LO) lobes are observed. In B, after partial removal of the lobes, the insula lobe (2) and 1 (inferior frontal) are exposed. In C, the superior longitudinal fasciculus (1) and the corona radiata (2) and gyri of the insula are exposed, circled in red. In D, a view of the right cerebral hemisphere is observed, with the following structures: External capsule (CExt), Corona Radiata (CR), Inferior Longitudinal Fascicle (FLI) and Uncinate Fascicle (FU).

Superior longitudinal fasciculus (FLS) is composed of four segments, being an essential connecting pathway between the



Figure 1 - A) schematic drawing representing the coronal section of the telencephalon with the arrangement of the basal ganglia in relation to the insula lobe. Looking from lateral to medial we have: Insula lobe, Claustrum, Putamen, Globus Pallidus (external segment (lateral) and internal segment (medial).



Figure 2: Cross-section of a cerebral hemisphere. Note that in image 2A, the basal nuclei were demarcated, which are represented at a higher magnification in Figure 2B. Observe from lateral to medial the following structures: A) Lobe of the insula, B) Extreme capsule, C) Claustrum, D) External capsule, E) Putamen, F) Lateral globus pallidus, G) Medial globus pallidus and H) Internal capsule.

anterior and posterior regions of the brain. It establishes interconnections between the frontal, parietal and occipital lobes, including its arcuate portion, responsible for connecting these areas to the temporal lobe. In addition to its diverse functions, it plays a significant role in the integration of areas related to language, such as the inferior frontal lobe and the posterior portion of the temporal lobe (Figure 3 C).

Inferior longitudinal fasciculus (FLI) is a set of white fibers responsible for connecting the frontal region with the occipital region of the brain, the arrangement of the fibers passes horizontally parallel to the horn of the lateral ventricle in the temporal part, sending fiber projections from the inferior temporal gyrus to the occipital lobe. This fascicle is considered one of the main occiptotemporal association tracts. Authors such as Fernández-Miranda et al., 2008; From Benedictis et al. al., 2014; Latini, 2015; Palacios et al., 2016, in their work also applied the dissection of the ILF using the Klingler technique, with our dissections being in agreement with the authors above and also with Zemmoura et al., 2016, who described in detail the anatomy of these tracts of projection and association (Figure 3 D). In Figure 3D we can also see that the white fibers have close relationships with optical radiation (Figure 4 and 5). Both FU and FLI have cortical projections to anterior temporal structures. They provide an indirect anatomical connection between the posterior temporal and occipital areas and the frontal lobe.

Uncinate fasciculus (FU) is a white matter tract (Figure 3D, 4 and 5) connects the temporal lobe to the inferior frontal lobe, has an important semantic function, the removal of this fascicle can lead to damage to facial recognition, tumors in this region can affect to lasting deficits.. According to the literature, the FU connects part of the lateral, medial

and rectus orbital gyri, the subcallosal area with the anterior part of the superior, middle and inferior temporal gyri and the uncus, the arrangement of its fibers is similar to a hook around the gyrus of the insula at the bottom (Kier et al., 2004; Martino et al., 2011; Martino and DeLucas, 2014; Bhatia et al., 2017).

Corona radiata (CR) fibers are characterized as projection fibers that connect the cerebral cortex to the brain stem and thalamus with afferent and efferent fibers (Nieuwenhuys et al., 2008). Superiorly, they are continuous with the base nublei and inferiorly, they connect with the internal capsule (Figure 3 and 5). The fibers of the CR are arranged lateral to the lateral ventricles, the ventral callosal fibers and the caudate nucleus form a barrier between the corona radiata and the wall of the ventricle. Corona radiata may interfere with degenerative processes and cause cognitive decline, as described in the work of Hoza et al., 2015.

The fibers: Arcuate Fasciculus (AF), corresponds to a part of the FLS. The U-shaped Fibers (Fau) connect one turn to the other. Both fibers were highlighted in Figure 4 and have descriptions similar to those described in the scientific literature (Vavassori et al., 2023).



Figure 4- Dissection of the lateral view of the right brain with evidence of the white projection fibers of the brain and basal ganglia. Note the Superior Longitudinal Fasciculus (FLS); Arcuate fasciculus (FA), Inferior longitudinal fasciculus (FLI), Uncinate fasciculus (Fu), Corona Radiata (Cr) and putamen nucleus (Pt).



Figure 5 – Left lateral view in A and right lateral view in B. In A, the uncinate fasciculus (1) and the putamen nucleus (2) can be seen. In B, the arrows represent the following structures: green arrow (crown radiate), black arrow, bundles of fibers from the internal capsule, red arrows arcuate fascicle and blue arrows – optical radiation.

THREE-DIMENSIONAL ANAGLYPH (3D) IMAGES

The Klingler technique is excellent for understanding the white fibers of the brain in relation to the basal ganglia. It is a method of freezing the brain that results in an expansion of the white fibers during freezing, allowing greater ease during dissection to dissociate the fibers. white matter of the brain (Fernandez-Miranda et al., 2008). It is important to highlight that the process of dissecting the white fibers in relation to the basal nuclei results in the destruction of other fiber systems, so a more in-depth anatomical knowledge of these structures is necessary before starting the process of dissecting the white fibers, this detailed study before beginning dissection may result in less waste of anatomical structures.

For a better understanding of the neuroanatomy of this region, especially when applying microsurgical anatomy techniques, it is necessary that the student or resident has a great understanding of the spatial relationships, observing the structures that are deeper and those that are more superficial, which is challenging to achieve using traditional 2D textbooks and atlases.

Anaglyphic 3D stereoscopic imaging allows three-dimensional representations of an object

using two superimposed images taken of the same point of interest, from slightly different perspectives. To achieve the anaglyphic effect, we used two images at different angles and processed in Anamaker 3D software, the images were modified with a separate color filter before being superimposed. To view the images created in 3D, anaglyphic glasses are required with a red filter in one eye and a blue filter in the other, which, in combination with the slightly different viewing angle of each eye, allows the images to be integrated into the visual cortex. and, finally, the perception of three-dimensional content (Figure 6).

However, it is important to highlight that the application of the 3D anaglyph technique does not replace 2D images, as this technique only facilitates the perception of depth of anatomical structures, being a didactic material to aid in the teaching of neuronatomy (Kakizawa et al., 2007; Benet et al., 2016; Martins et. al. 2015).



Figure 6 – The image in letter A represents the dissection of the white fibers. In B we represent an example of a three-dimensional stereoscopic image in Anaglyph (3D) generated from the dissected piece in A. Anaglyph 3D glasses are required to view the image in B.

Using the Kingler technique, we were able to highlight the topographical arrangement of the white fibers in relation to the basal ganglia, thus understanding the anatomical connections of this region (Rhoton, 2002; Winn et al., 2016). We identified important association and projection fascicles, as illustrated in the images, using the Kinlger technique for dissecting white fibers and creating 3D images using Anamaker 3D software. The usefulness of white fiber dissection has been described in several studies (Kakizawa et al., 2007; Martino and DeLucas, 2014; Benet et al., 2016; Zemmoura et al., 2016).

Regarding the creation of 3D images, few articles used this technique to help students, residents or neurosurgeons understand the spatial relationships of these structures (Jacquesson et al., 2020).

Several studies on white fibers have developed images using tractography (Ebeling and Reulen, 1992; De Benedictis et al., 2014; Yagmurlu et al., 2015; Kier et al., 2004; Bhatia et al., 2017). In our study, we created a collection of 3D anaglyph images useful for assimilating the topography of white fiber tracts and basal ganglia in the human brain.

When using fiber dissection using the Kingler method and creating 3D images, we verified that this is a complex neural network, and very relevant in neuroanatomy, as it is an area that is related to motor control. Furthermore, we chose to focus our dissection on the basal ganglia and white fibers, as small structures are not among the easiest to dissect macroscopically. Thus, we verified that it is possible to be successful with the techniques presented.

Therefore, it is assumed that this combination of dissection and use of 3D images is suitable

for a better understanding of this anatomical region.

CONCLUSION

From what has been exposed, we believe we can conclude that the combination of dissection of the basal ganglia with 3D anaglyph images is effective for understanding and understanding the superficial and deep brain structures of the lateral side of the brain. This approach, together with traditional educational materials, offers a comprehensive methodology for teaching brain anatomy. Although our initial experience is positive, additional studies are needed to fully reveal its effects on learning human neuroanatomy.

Ethics Committee Approval: Our study does not require Ethics Committee approval, as it is an anatomical laboratory study on cadaveric brains. The study was conducted in accordance with the principles of the 2013 Declaration of Helsinki.

Informed Consent: Our study does not require consent from patients, as it is a study carried out in an anatomical laboratory with cadaveric pieces, in accordance with Law 8,501 of November 30, 1992, which regulates the use of unclaimed cadavers for teaching purposes and research in medical schools.

Conflict of interest: The authors declare no conflict of interest.

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