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Neurosurgical relevance of the dissection of the diencephalic white matter tracts using the Klingler technique

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ABSTRACT

Objective: The Klingler fiber dissection technique is a relevant and reliable method for neurosurgery to identify with accuracy the fine structure of the brain anatomy highlighting white matter tracts. In order to demonstrate the significance of the application of this technique, we aimed to observe the course and relations of the mammillothalamic and habenulo-interpeduncular tracts as there are very few papers showing these important diencephalic tracts.

Material and methods: Twelve formalin-fixed brains were dissected using the Klingler technique in order to expose the medial diencephalic surface. Diencephalic white matter tracts, particularly the mammillothalamic and habenulo-interpeduncular tracts, were dissected using wooden spatulas and metallic dissectors with different sizes and tips. Several measurements were performed in both dissected hemispheres relative to the mammillothalamic and habenulo-interpeduncular tracts.

Results: The course and length of these two tracts were visualized and the relations with other fiber systems and with the neighboring gray matter structures quantified and registered. The mammillothalamic tract approximately marks the anteroposterior coordinate of the anterior pole of the subthalamic nucleus in the anterior commissure – posterior commissure plane.

Conclusion: The present study helps to understand the three-dimensional architecture of the white matter systems of tracts when the Klingler technique is used. The numerical data obtained may be helpful to neurosurgeons while approaching brain paraventricular and ventricular lesions and deep brain stimulation. Finally, the anatomical knowledge can lower surgical complications and improve patient care particularly in the field of neurosurgery.

1. Introduction

The white matter of the brain was described early in the history of the anatomy probably due to its fibrous structure and color [1]. In 1671, Niels Stensen proposed the scraping method for the dissection of the brain white matter [1]. This method was used by numerous anatomists in the two following centuries to describe numerous white matter fiber tracts, which allied to improved brain preservation with fixation chemicals, lead to more accurate drawings and photographs as it was reviewed in [1,2]. After the development of the staining techniques and of the sectioning with the aid of the microtome at the end of the 19th century, fiber dissection was partially abandoned [1,2]. However, using conventional histological techniques and brain sections, the white matter fiber tracts are difficult to recognize. As a result, medical students, neuroscientists, and neurosurgeons have difficulty to appreciate fiber tracts courses, relationships and connections. Although neuroanatomical textbooks and atlases describe fiber tracts, there is a lack of photographs allowing an appropriate understanding of the three-dimensional architecture of the white matter [3]. Computer-based imaging is now one of the main tools used to characterize the functional connections in the living human brain. Somehow, paradoxically, fiber dissections are emerging as the starting point to interpret the images provided by these new techniques [1,2].

Joseph Klingler developed a technique based in the freezing of formalin-fixed brains [4,5]. This methodology, known by his name (Klingler's technique), allows an easier separation of the white matter from the gray matter, hence facilitating the dissection of fiber tracts. Rather unexpectedly, this method was not widely applied [2] perhaps due to the difficulty in obtaining satisfactory speci-

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mens, the need of a good knowledge of the brain anatomy and the excessive time required to perform such dissections [1,2].

As a consequence, a large amount of the figures of the white matter fiber bundles of the brain in anatomical and neuroanatomical textbooks are generally photographs of brain sections or, more frequently, schematic diagrams [6–9]. In addition, most of the descriptions in these textbooks are based mostly in small laboratory animals or non-human primates and, although useful, minor details may not be applicable to humans [2]. Only very recently, the Klingler's technique was reborn due to the interest of neurosurgeons and some anatomists [1,2,10].

We think that the Klingler's method has the potential to reveal many interesting features of the white matter fiber tracts. It is also important to obtain three-dimensional dissected fiber tracts that can be used for educational purposes, both in pregraduate and postgraduate neuroanatomical studies [11–13]. We decided to dissect the mammillothalamic tract (MTT) and habenulo-interpeduncular tract (HIT), as their course and relations are relatively difficult to discern due to the difficult discrimination from the adjacent structures [14,15]. The MTT links the mammillary body to the anterior nucleus of the thalamus and it is involved in cognition [14,15]. The HIT, also known as fasciculus retroflexus, is an important link between the limbic forebrain and the brainstem [14,15]. Due to their position, the MTT and HIT seem to be pivotal in the regulation of cholinergic and/or serotonergic projections from the limbic system to the cerebral cortex [16]. The knowledge of the anatomy of these tracts is also important for deep brain stimulation procedures and for the planning, monitoring and surgery of tumors, and other resective interventions in the diencephalon [1,2]. Due to these reasons, we quantified the diameter of HIT and MTT and the location of MTT relative to the anterior commissure – posterior commissure plane.

2. Materials and methods

The study was approved by the local ethical commission. Human cadavers donated themselves for research purposes according to the Portuguese Act 274/99. Dissections were performed in the dissection room at the Unit of Anatomy, Department of Biomedicine, Faculty of Medicine of the University of Porto.

2.1. Human specimens

Twelve male cadavers donated to the Unit of Anatomy, Department of Biomedicine, Faculty of Medicine of the University of Porto (Porto, Portugal) that had a non-neurological cause of death were selected for brain extraction. Cadavers were injected with a 10% formalin solution within the femoral artery. Following several months of fixation, the brains were removed from each cadaver as previously described in detail [13,17].

2.2. Preservation technique

The Klingler method was used with minor adjustments [4,13,18]. After a total period of two months or longer in a 10% formalin solution, ten brains were washed for 24 h in fresh cold water. The arachnoid membrane, pia-mater and vascular structures were carefully removed from the brains. The specimens were placed in a plastic vessel and stored in a deep freezer at -15° C for a period of a month. Before the dissection, brains were thawed under running cold water for 24 h.

2.3. Dissection technique

The cerebral hemispheres were bisected in the midline through the corpus callosum. Before the dissection started, major anatomical landmarks on the medial surface of the cerebral hemisphere were identified. In the initial steps of dissection, hand-made wooden spatulas and metallic dissectors with different sizes and tips for superficial and deep dissection were used to remove the cortex of the medial surface of the cerebral hemispheres. This procedure was performed simply due to the different consistency between gray and white matter that allowed their differentiation. The ependymal coverings over the medial surface of the thalamus and the hypothalamus were removed cautiously and the dissection continued by removing the core of the gray matter of the thalamus. The major fiber tracts were exposed by peeling off parallel to the axonal direction from the one end of the tract to the other, i.e. along its anatomical course. Once the fiber tracts were identified, due to the removal of the gray matter and overlying white matter, the dissection continued in a stepwise manner in order to completely isolate the MTT and HIT and all the structures in their relation [2,13,19–21]. The MTT was identified running upward from the mamillary body and it was traced carefully until entering the anterior nucleus of the thalamus. The HIT was identified in the dorsal-most part of the diencephalon, beginning in the habenular region, passing medially and afterward inferiorly to the caudal pole of the medial part of the thalamus and terminating in the interpeduncular region of the midbrain. We must highlight the importance of previous training and attention necessary to avoid artifacts and damage during the procedure.

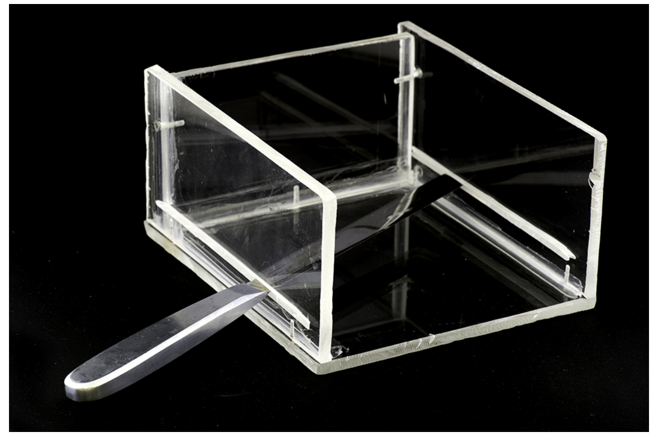

Fig. 1. Photograph of the acrylic device built for the accurate placement of a knife in order to obtain brain coronal and horizontal sections at regular and fixed distances.

2.4. Serial coronal and horizontal brain sections

The remaining two brains were serially cut to obtain coronal and horizontal sections using an acrylic device for the accurate placement of a knife (Fig. 1) in order to do sections at regular and fixed distances. The coronal and horizontal sections were used to identify the relative position of the tracts.

2.5. Measurements in relation to the mammillothalamic and habenulo-interpeduncular tracs

Several measurements were performed in both dissected hemispheres with a digital Vernier caliper with the main purpose of characterizing the MTT and its relations. The measurements consisted in: a) tract diameter; b) distance between the posterior border of the anterior commissure and the centre of the MTT and c) distance between the posterior border of the posterior commissure and the centre of the MTT according to the anterior commissure – posterior commissure (AC – PC) plane. Mean values and standard deviations of these values were calculated.