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Teaching images in Neuroanatomy: Value of the Klinger method

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SUMMARY

This paper presents a series of dissections created using the Klinger method. This method allows the dissection of white matter tracts in the central nervous system showing fiber orientation, three-dimensional relationships between grey and white matter components, and connections between distant neural centers. In addition, the use of the Klinger method increases the didactic value of conventional dissection techniques and allows students to visualize structural and functional relationships in an easy way.

Key words: Fiber dissection – Klinger's method – Association fibers – Auditory radiation

INTRODUCTION

By the middle of the nineteenth century, Louis Gratiolet was the first author to describe the presence of a large group of axons extending between different regions of the brain: the lateral geniculate nucleus and the striate cortex (referenced in Pearce, 2006). Today, precise identification of white matter tracts is essential for understanding the three-dimensional anatomy of the central nervous system. This is not a mere academic issue. Many degenerative, traumatic and postoperative disorders as well as tumors can be explained in the light of white matter tracts interconnecting neural centers (Peuskens et al., 2004). This applies not only to association and commissural fibers, but also to projection fibers connecting different levels of the central nervous system. For instance, knowledge of the connections between the different lobes of the brain is necessary to understand the spread of electrical stimulation and to develop surgical approaches valid for the management of refractory epilepsy (Sincoff et al., 2004).

A laborious but reliable white matter tract dissection technique was developed many years ago by Klinger (Klinger, 1935; Ludwig and Klinger, 1956). This technique has been used by many authors (Türe et al., 2000; Peuskens et al., 2004; Sincoff et al., 2004) with excellent results. The combination of Klinger's method and cross-sectional magnetic resonance imaging (MRI) (Kier et al., 2004a, b) has expanded our knowledge of the relationship between different tracts and has improved the accuracy of anatomical descriptions. The development of *in vivo* white matter tractography allows the production of images

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of biological tissues based on the characteristics of water diffusion (for a recent review, see Filler, 2009, and references therein). In particular, diffusion tensor MRI provides very accurate information about the connections between different parts of the living brain (Conturo et al., 1999; Lazar et al., 2003).

The anatomical data provided by increasingly sophisticated MRI techniques are of invaluable help from the medical and surgical viewpoints (Johansen-Berg and Rushworth, 2009; Yamada et al., 2009; Lazar, 2010). However, dissections of white matter tracts are seldom presented to our neuroanatomy students. The use of this type of dissection is very important since it gives the students direct visual information not only of connections, but also of functional interrelationships. Here we report part of our experience with Klinger's technique and show that it can be applied to the study of fascicle connectivity, spatial relations between gray and white matter centers, and sensory pathways. Examples of this work have been reported elsewhere (Ojeda and Icardo, 2004).

Material and Methods

Three adult human brains obtained from fixed cadavers were used in this study. The anatomical pieces included the right cerebral hemisphere in one case, and the head and the upper part of the neck in the other two cases. Following Klinger's method (Klinger, 1935; Ludwig and Klinger, 1956), the pieces were washed in tap water to eliminate most of the fixative. Then, they were frozen at -15ºC for a period of at least four weeks. Frozen water disrupts structures that, like gray matter, have a high water content. In addition, ice crystals create spaces between myelinated axons. If freezing temperatures are achieved progressively, the size of the ice crystals increases. This improves the ability to separate fibers. Upon thawing, the formation of dissection planes allows both removal of undesired components and the progressive isolation of the structures under study. Three to four additional cycles of freezing and thawing were performed throughout the dissection procedures. Freezing within these cycles was maintained for at least two days. Otherwise, the specimens were maintained in 10% formalin at room temperature. Dissection was performed with spatulas of different widths, fine dissecting forceps, and histological needles. When appropriate, the procedures were carried out under a dissecting lens. Digital photographs were obtained with an Olympus digital 800 camera (Olympus Imaging Corp.,).

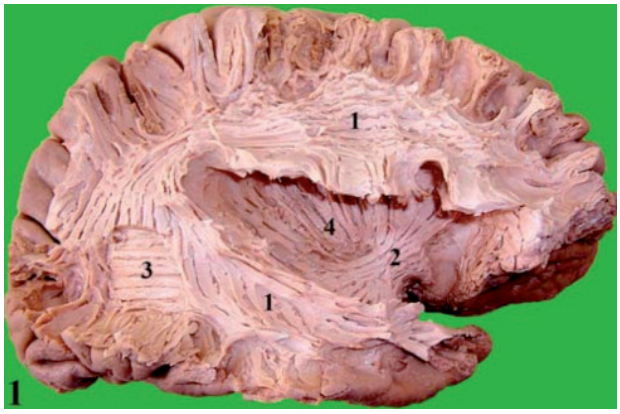
Results and Discussion

Specimen 1. Connection fibers

The right cerebral hemisphere was used (Fig. 1). Meningeal and vascular structures were stripped away. Initially, the gray matter from the frontoparietal and temporal opercula was removed to expose the insula. Just below the opercular cortex, the superior longitudinal fasciculus became apparent, bordering the lateral fissure (also, see Türe et al., 2000; Sincoff et al., 2004). Fiber spreading in the occipital and temporal lobes can clearly be observed. Further dissection of the superior longitudinal fasciculus in the occipitotemporal area revealed the presence of the inferior longitudinal fasciculus connecting the temporal and occipital lobes. This area is part of a structural core connecting the parietal cortex, the precuneus, and the cingulate cortex (Hagmann et al., 2008). The structural core was first demonstrated by diffusion spectrum imaging, constitutes a central area for brain connectivity, and appears to be involved in the integration of information across functionally different brain regions (Hagmann et al., 2008). The cortex of the insular gyrus was also removed. The uncinate fasciculus was first identified underneath the white matter of the limen insulae. The fibers could be followed bridging the orbital portion of the frontal lobe and the temporal pole.

Progressive dissection of the insular gyrus also

Fig. 1. Right cerebral hemisphere. Dissection of lateromedial connection fibers. 1, superior longitudinal fasciculus. 2, uncinate fasciculus. 3, inferior longitudinal fasciculus. 4, extreme capsule.



revealed the presence of the extreme capsule (Fig. 1).

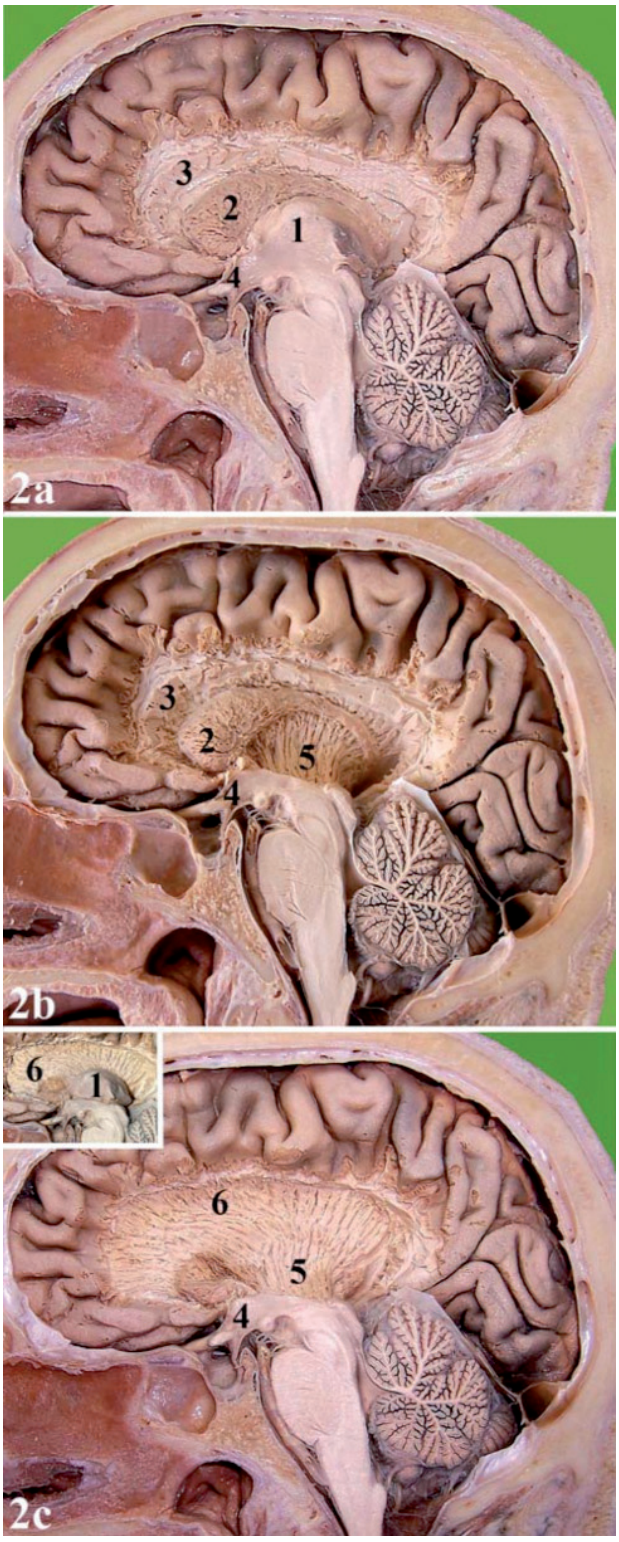
Dissection of the connecting fibers is laborious and time consuming. Care must be taken to avoid artifactual formation of tracts. In fact, errors are frequently made (Kier et al., 2004a, b) and we had to discard two other specimens because the fascicles were cut through by mistake. It should be stressed that the dissections presented here do not allow the visualization of individual fibers. However, they are very useful for showing large fiber groups and for illustrating their general arrangement. While very precise fascicle localization is important for surgical purposes (Türe et al., 2000; Kier et al., 2004a, b; Sincoff et al., 2004), rough fiber dissection is easier and quicker to perform, and has similar didactic value.

Specimen 2. Basal ganglia and internal capsule

A sagittal section of the head was used to perform a sequential dissection that, from the midline, allowed the spatial relationships between the thalamus, the caudate nucleus, and the internal capsule to be visualized (Fig. 2). First, the fornix and the septum pellucidum were removed and the corpus callosum was partially resected (Fig. 2a). Then, the thalamus was carefully scooped away and the fibers of the internal capsule were dissected (Fig. 2b). Next, the caudate nucleus and the remainder of the corpus callosum were removed (Fig. 2c). Fiber dissection of the corona radiata revealed the continuity of these fibers with those of the internal capsule.

This type of dissection allows the spatial relationships between the components under study to be taught in a very informative way. The didactic value can be increased if any of the removed components, such as the thalamus, can be put back into the dissection (Fig. 2c, inset). Progressive removal of structures with the subsequent three-dimensional information is also available in textbooks (Smith-Agreda, 2010), commercially available plastic models, and 3D rendering models (for instance, see anatomium.com/3dbrain; 3dscience.com). While the dissection of specimen 2 only shows a limited number of structures, it has the advantage of presenting real structures and can be used as a complement to the information provided by more sophisticated techniques.

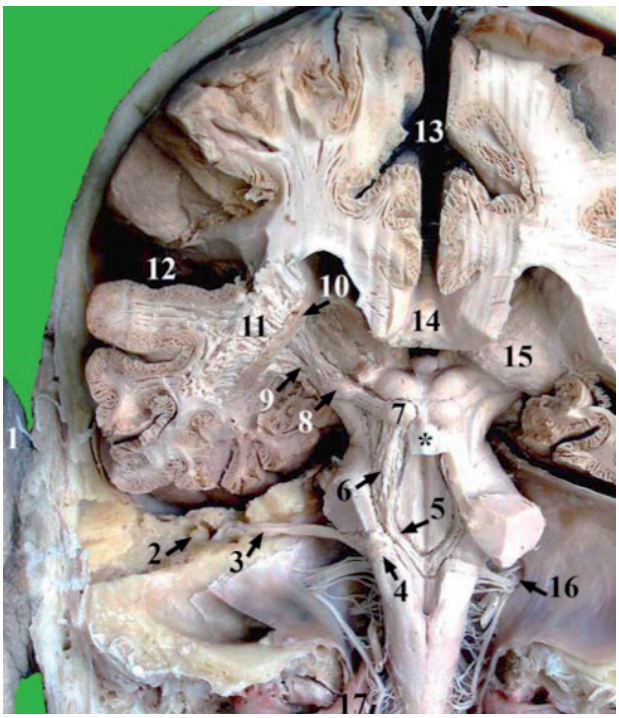
Fig. 2. Right half of head. The three panels show progressive lateral dissection of gray and white matter centers (see text). 1, thalamus. 2, caudate nucleus. 3, corpus callosum. 4, hypothalamus. 5, internal capsule. 6, corona radiata.



Specimen 3. The auditory pathway

The head and the upper neck were dissected from the dorsal side (Fig. 3). The squamous part of the occipital bone and the posterior half of the vertebral column were eliminated. The foramen magnum was cut open. The

Fig. 3. Ventral half of head. Dorsal projection. Several components of the ear and auditory pathway are exposed. 1, auricle; 2, head of malleus and superior ligament. 3, vestibulocochlear and facial nerves occupying the internal acoustic meatus. 4, cochlear nuclei. 5, dorsal acoustic striae. 6, lateral lemniscus. 7, inferior colliculus. 8, medial geniculate nucleus. 7 and 8 are bridged by the inferior brachium. 9, auditory radiation (sublenticular part of internal capsule). 10, body of caudate nucleus. 11, internal capsule. 12, lateral fissure. 13, great longitudinal fissure. 14, corpus callosum. 15, pulvinar. 16, jugular foramen. 17, vertebral artery. Asterisk, superior medullary velum.



cerebellum was excised by cutting through the cerebellar peduncles. Bone surrounding the internal acoustic meatus was eliminated to expose the vestibulocochlear and facial nerves. The roof of the tympanic cavity was also eliminated. On the left side, the pulvinar of the thalamus was excised and the auditory radiation (part of the sublenticular part of the internal capsule) was dissected. Fiber dissection was also performed along the lateral lemniscus, dorsal acoustic striae, and inferior brachium.

Dissection of specimen 3 combines conventional techniques and Klinger's method. This is applied here to the auditory pathway, but has also been used to show other sensory pathways such as that containing Meyer's loop and optic radiation (Kier et al., 2004b; Ojeda and Icardo, 2004; Sincoff et al., 2004). The combined method illustrates in a very informative way the connections between distant neural centers. It is a reliable method that captures the attention of students and facilitates the learning of structural and functional relationships in the brain.

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