ORIGINAL ARTICLE

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Vertical bundles of the white matter fibers in the pons revisited: preliminary study utilizing the Klingler technique

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Abstract

The inner structure of the pons contains several layers of transverse and vertical fibers and many nuclei. The vertical bundles are described as fibers of the corticospinal tract, corticonuclear tract, frontopontine tract and parieto-temporopontine tract organized in three layers. The aim of this study was to investigate the structure of the vertical bundles in the ventral pons using the modified Klingler method. Ten brain stem specimens were investigated. Specimens were fixed in 10% formalin, frozen for 24 h to separate nerve fibers by ice crystals, and then unfrozen again in 10% formalin solution. Afterwards, the specimens were dissected using a sharpened spatula. Results point to the existence of three main layers of vertical bundles and a small, constant, and superficial fourth fascicle that is yet to be described in the literature. We propose the name fasciculus longitudinalis superficialis (superficial longitudinal fascicle) for this group of vertical fibers of the pons.

Introduction

The inner structure of the pons is very complex, given that this compact structure contains many crossing neural tracts as well as many nuclei. Frontopontine (FPT), corticospinal (CST), parieto-temporopontine (PTT), corticonuclear (CNT), and cerebellopontine (CrPT) tracts are found in the ventral pons. These tracts are constructed from vertical (FPT, CST, CNT, and PTT) and transverse (CrPT) nerve fibers which cross each other in an inconstant manner (Clara 1959; De Simone et al. 2005; Fernández-Gil et al. 2010; Oh et al. 2003; Standring et al. 2008). The CST is located in the ventrolateral part of the pons. It is arranged in bundles of vertical fibers separated by transverse fibers and pontine nuclei. It is important to note that CST fascicles maintain somatotopic order along their course through the pons (Fernández-Gil et al. 2010; Hong et al. 2010; Hua et al. 2012; Jang 2011; Prats-Galino et al. 2012). Knowledge of

the precise anatomical localization of a lesion affecting the pons is crucial to the neurological diagnosis, but imaging a small and compact structure such as the pons or some other part of the brain stem remains problematic due to its complexity (Fernández-Gil et al. 2010; Jang 2011; Skadorwa et al. 2009). Pathologies that may require neurosurgical interventions, such as tumors or cavernous hemangiomas, should be localized in relation to the CST to avoid serious postoperative complications. However, even with modern tractography, this can be a difficult task (Kwon et al. 2011). It is therefore essential to localize the individual neural tracts in the pons.

Aim

The aim of this study was to investigate the spatial arrangement of the vertical nerve fibers in the pons.

Materials and methods

Ten human brain stem specimens of both sexes (five women, five men) aged 21–90 years were investigated using the modified Klingler method. The specimens, fixed in 10% formalin, were placed in a freezer at a temperature of − 20 °C

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for 24 h. The frozen specimens were then placed in 10% formalin solution again. After 24 h, the specimens were dissected in layers using a sharpened spatula and an anatomical needle under a surgical microscope with a magnification of up to 40×. After the removal of each nerve fiber layer, photographic documentation was carried out using a Fujifilm Finepix S1000FD camera.

Results

All of the specimens presented three main layers of vertical bundles in the ventral pons, which were separated by layers of transverse fibers (Fig. 1). In nine of the ten specimens (four women and five men), a small vertical superficial fascicle was observed. This fascicle was present on both sides of the pons in eight of those specimens. Altogether, this fascicle was present in 17 of the 20 sides investigated. This superficial vertical fascicle varied in course, size, and the number of smaller fascicles from which it was formed. It was homogeneous in six of the ten specimens dissected with a spatula, whereas it was composed of a number of smaller fascicles (varying from four to two) in three of the specimens (Fig. 2). The course of the superficial vertical fascicle was straight and ventral in four specimens, medioventrally diagonal in two cases, arcing to the lateral side of the pons in another two cases, and lateroventrally diagonal in the remaining case (Fig. 3). In all cases, the fascicle ran ventrally from the mesencephalon to the lower pons and merged with deeper vertical fascicles in its terminal segment.
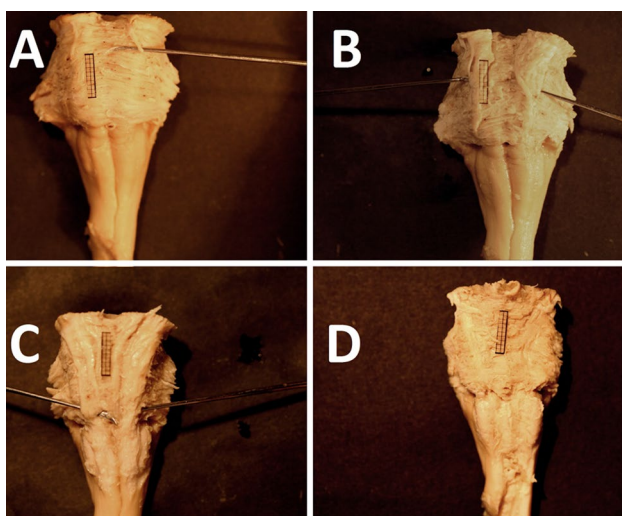

Fig. 1a–d Dissection of the layers of the pons to examine fascicles: **a** SLF; **b** FPT; **c** CST; **d** PTT
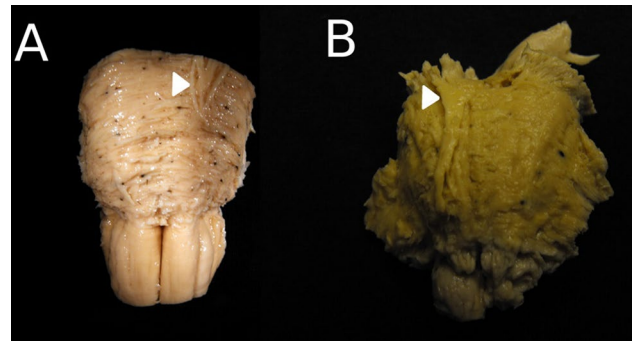

Fig. 2a–b Variations in superficial longitudinal fascicle (SLF; *arrowhead*) structure: **a** lesser fascicles that form the SLF; **b** homogeneous fascicle

Discussion

Studying the pons is a difficult task due to the large number of structures and crossing fibers located in such a small space. Although in vivo imaging techniques are commonly used to examine brain structures, such methods still unable to fully visualize the small, independent nerve fiber bundles that are present in the ventral part of the pons (Kwon et al. 2011; Virta et al. 1999). Recently, the importance of white matter dissection using the Klingler method was stressed, as it provides a means to validate the results of diagnostic imaging (Liakos and Koutsarnakis 2016). Indeed, although Zemmoura (2016) claimed that it is more important for a radiologist to carry out diffusion tractography with different tractography tools while appreciating their limitations than to perform dissection, dissection remains an important source of new anatomical information and discoveries. For example, an electron microscopy study of human white matter performed by the same author (Zemmoura 2016) showed that myelinated axons are preserved during Klingler's dissection, which provides strong support for the idea of validating tractography using this method. This may be supported by neuronavigation (Skadorwa et al. 2009). In this approach, dissection is validated by navigation or navigation is validated by dissection.

In the present study, during the layer-by-layer removal of the nerve fibers, the vertical nerve fibers in the ventral pons were observed to be arranged in three main layers, as also noted previously by other authors. The layers are arranged in the order FPT, CST, PTT (Fig. 4), with FPT being the most ventrally located tract according to various previous studies (Fitzek et al. 2014; Hong et al. 2010; Hua et al. 2012; Standring et al. 2008). Traditionally, the CTS has been functionally associated with voluntary motor control, while FPT and PTT have been linked to modulation of cerebellar function. During the dissection, it was
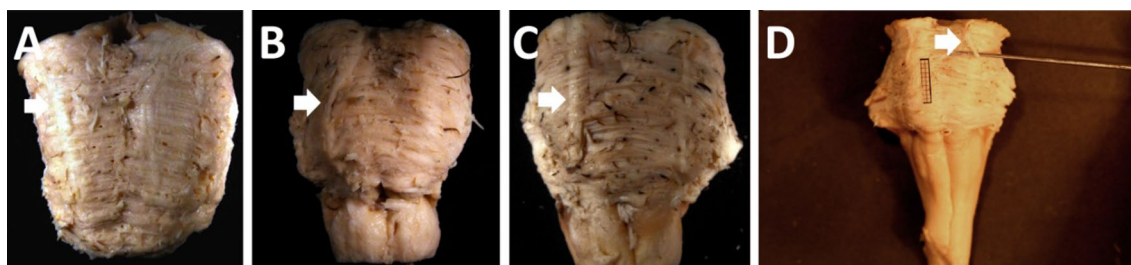
Fig. 3a–d Variations in the course of the superficial longitudinal fascicle (*arrow*): **a** medioventral, **b** arcing, **c** straight, **d** lateroventral
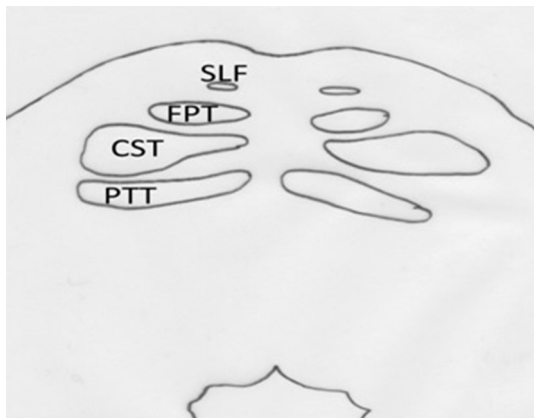

Fig. 4 Visualization of the transverse section of the pons at the level of the medial cerebellar peduncle. *SLF* superficial longitudinal fascicle, *FPT* frontopontine tract, *CST* corticospinal tract, *PTT* parieto-temporopontine tract

clear that the fascicles were connected by minor groups of fibers and so do not constitute separate entities. However, it was difficult to identify the spatial arrangement of these connections using the Klingler method. Serial transverse cross-sections with 3D reconstruction are necessary to visualize the internal structure of the vertical fascicles in the pons. An as-yet undescribed element in the superficial layer of the pons was discovered in the present work: a consistently observed fascicle (9/10 specimens, 17/20 sides) of vertical fibers located ventrally to the main group of vertical bundles, between superficial transverse fibers. For more than 50 years, this fascicle has been visualized in some neuroanatomical atlases and textbooks without any explanation or comment (Naidich et al. 2009; Peele 1961), whereas in other important academic publications, ranging from the studies of Max Clara to contemporary editions of Gray's anatomy, it has not been visualized at all (Clara 1959; Standring et al. 2008). We therefore propose a name for this fascicle—*fasciculus longitudinalis superficialis* (superficial longitudinal fascicle; SLF). The functional role of this fascicle remains unclear at this stage of our investigations. This structure should be measured and traced upward to the mesencephalon and the diencephalon, and then downward to the medulla. More detailed studies should show whether it is an independent fascicle of vertical fibers or a portion of frontopontine fibers that become separated from the main fascicle along its pontine course. Localization of SLF part in upper pons corresponds to the localization of fascicle fibers in medial 1/5 width of base of the pons (four specimens) or the middle part of the cerebral peduncle. This suggests that it originates in the motor and premotor cortex, a suggestion strengthened by the localization of the SLF in the medial and intermediate group of pontine fibers as described by Benagiano et al., thus making it a part of the cerebrocerebellar circuit (Benagiano et al. 2018). It may also resemble the aberrant pyramidal tract described by Kwon et al. (2011). Observation of its course, especially the ventral direction of its terminal segment, suggests the latter two options. However, the consistent presentation of this structure may indicate that it has a specific function. Our observations are rather incidental, as new studies of the structure of the brain stem using classical anatomical dissection are rare nowadays (Liakos and Koutsarnakis 2016; Skadorwa et al. 2009). While Klingler's technique is archaic, the arrangement of the vertical fascicles of the pons is invisible to standard MRI, meaning that the discovery of such minute structures as the superficial longitudinal fascicle using MRI is currently highly improbable. In conclusion, we can state that the vertical fibers of the ventral pons are arranged in four fascicles: the superficial, frontopontine, corticospinal, and parieto-temporopontine fibers.

Compliance with ethical standards

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