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A Neglected Normal Anatomical Structure in Mammal: A Study in Bats and Tree Shrews

Una Estructura Anatómica Normal Desatendida en Mamíferos: Un Estudio en Murciélagos y Musarañas Arborícolas

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SUMMARY: The myodural bridge is a dense connective tissue connecting muscles and ligaments to the spinal dura mater in the atlanto-occipital interspace. Some researchers believe that the myodural bridge may play a vital physiological role. It is possible, for instance, that the prevention of spinal dura mater infoldings might be involved in regulated cerebrospinal fluid circulation. For instance, it is possible to prevent spinal dura mater infoldings, regulating cerebrospinal circulation. Bats are nocturnal and the only mammals that can perform a genuine and sustained flight, whereas tree shrews are arboreal mammals that often climb to a high altitude of about 10,000 feet. Both animals have lifestyles that are different from other previously studied mammals. The study of these two animals will shed further light on the existence of the myodural bridge in mammals. Gross anatomical dissection was used to observe the connections between the deep muscles of the neck and the dura mater at the level of the atlanto-occipital interspace. The existing structures were analyzed using conventional and special histological staining techniques. The suboccipital regions in bats and tree shrews contained the rectus capitis dorsal major (RCDma), rectus capitis dorsal minor (RCDmi), oblique capitis anterior (OCA), and oblique capitis posterior (OCP). Dense connective tissue connects the RCDmi to the posterior atlanto-occipital membrane (PAOM) and the latter to the spinal dura mater. The myodural bridge in these mammals shares a similar structure to the myodural bridge in humans. Histological analyses confirmed that the connective fibers of the myodural bridge were primarily type I collagen fibers. In this study, it is supplemented by the existence of the myodural bridge in mammals. This further demonstrates that myodural bridge widely exists in the normal anatomy of mammals. This provides morphological support for a comparative anatomical study of the physiological function of the myodural bridge.

KEY WORDS: Myodural bridge; Bat; Tree shrew; Morphology; Comparative anatomy.

INTRODUCTION

The myodural bridge (MDB) is a dense connective tissue that connects muscles and ligaments to the spinal dura mater. The muscles involved are the rectus capitis posterior minor (RCPmi), rectus capitis posterior major (RCPma), obliquus capitis inferior (OCI), and nuchal ligament. The existence of the MDB in humans has been established (Hack, 1995). Recently, studies have reported the existence of MDB in five other mammalian species. These are tree-dwelling primates (*Macaca mulatta*), land-based carnivores (*Canis familiaris* and *Felis catus*), cave-dwelling Lagomorpha (*Oryctolagus cuniculus*), rodentia (*Ratus norvegicus* and *Cavia porcellus*) and aquatic cetaceans (Indonesian finless porpoise). The authors hypothesized that the MDB might be a universal existing structure in mammals (Zheng *et al*., 2017; Liu *et al*., 2017). However, these studied mammalian species do not represent all mammals. There are about twenty-nine mammalian orders (Wilson & Reeder, 2005). In order to supplement and expand the study of the MDB in mammals, we studied two unique mammals, bats (Chiroptera) and tree shrews (*Tupaia belangeri*). Bats are nocturnal animals and have capable of sustained flight, whereas tree shrews are small arboreal mammals that can reach an altitude of 10,000 feet. Morphological differences in the MDB among mammals may exist according to their

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living environment, characteristics, and habitat. This study was therefore designed to further prove the universal existence of the MDB in mammals. Moreover, the properties of the MDB fiber were explored.

MATERIAL AND METHOD

All animals used in this study were collected with permission from the Chinese Authorities for Animal Protection, and the Ethics Committee approved the study of Dalian Medical University. All experiments were performed by the guidelines and regulations of Dalian Medical University.

Experimental animals. Eleven bats (Chiroptera) and eleven tree shrews (*Tupaia belangeri*) were used in this study. The animals all died naturally. We fixed and stored them in 10 % formalin. Six Bats and tree shrews each were used for gross dissection. Five Bats and tree shrews were used for histological studies.

Gross anatomical studies. A "T" shaped midline incision was made along the occipital bone to the back. The muscles in napex were dissected from the superficial layer to the deep layer, and the rectus capitis dorsal major (RCDma same as RCPma in humans) was exposed. Subsequently, the RCDma was reflected to reveal the rectus capitis dorsal minor (RCDmi same as RCPmi in humans). Similarly, the RCDmi was cut off from their cranial attachment, and the connections between the RCDmi and the dorsal atlanto-occipital membrane (DAOM, same as PAOM in humans) were observed. Furthermore, the connections between the DAOMand the spinal dural mater (SDM) were also observed. Photographic documentation was carried out using a Canon D-40 camera.

Histological studies. Tissue samples including the occiput and the cervical regions were fixed in 10 % formalin for 15 days. They were then transferred into ethylen ediaminetetraacetic acid (EDTA) solution for 12-15 days for decalcification. EDTA solution was changed at a three-day interval until the bones of the tissues were easily pierced by a needle. The decalcified tissue samples were washed overnight under running water, and the traditional paraffin sectioning method was carried out. The thickness of the sectioned slices was10mm and these slices were divided into three groups for Hematoxylin-eosin staining (HE), Masson trichrome staining, and Picrosirius red staining (PRS), respectively. The results were imaged using the NIKON Eclipse80i research light microscope. The Picrosirius red staining sections were also analyzed using the polarizing microscope.

RESULTS

Gross anatomical dissection. The suboccipital region of bats and tree shrews contains the RCDma, obliquus capitis anterior (OCA, same as obliquus capitis superior), and obliquus capitis posterior (OCP, same as obliquus capitis inferior) muscles. These muscles form the suboccipital triangle in bats and tree shrews (Bats, Fig.1: A, C; Tree shrews, Fig. 2: A, C). The cranial attachment of the RCDma was at the inferior nuchal line of the occipital bone and the bony area just below the inferior nuchal line. The caudal attachment is at the spinous process of the second cervical vertebra. The RCDma was reflected to expose the RCDmi, which lies deep in the RCDma. The cranial attachment of the RCDmi was below the inferior nuchal line of the occiput, and the caudal attachment was on the posterior tubercle of the first cervical vertebra (Bats, Fig.1: B, D; Tree shrews, Fig.2: B, D). Dense connective tissue connecting the RCDmi and the DAOM in bats and tree shrews can be seen following

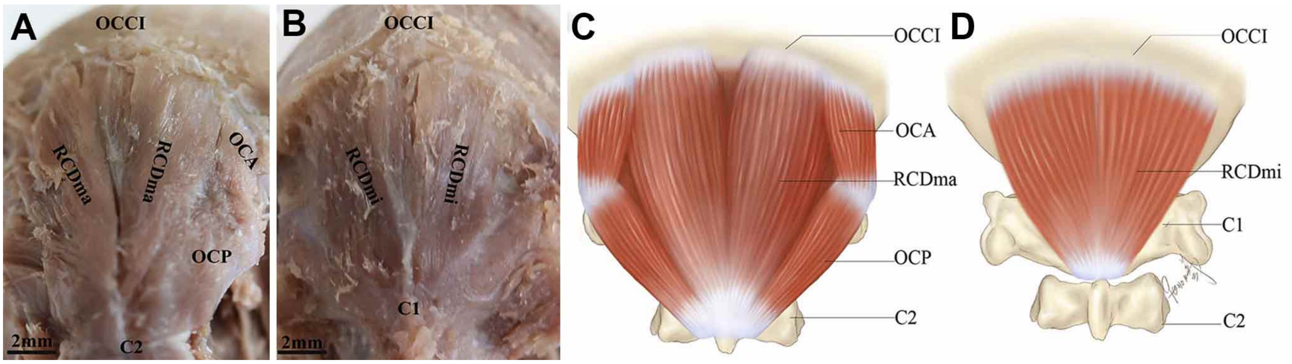

Fig. 1. The suboccipital region of the bats. (A: Suboccipital triangle; B: The rectus capitis dorsal minor; C: A diagrammatic drawing of suboccipital triangle; D: A diagrammatic drawing of the rectus capitis dorsal minor) OCCI: occipital bone; RCDma: rectus capitis dorsal major; RCDmi: rectus capitis dorsal minor; OCA: obliquus capitis anterior; OCP: obliquus capitis posterior; C2: spinous process of axis; C1: posterior arch of atlas.

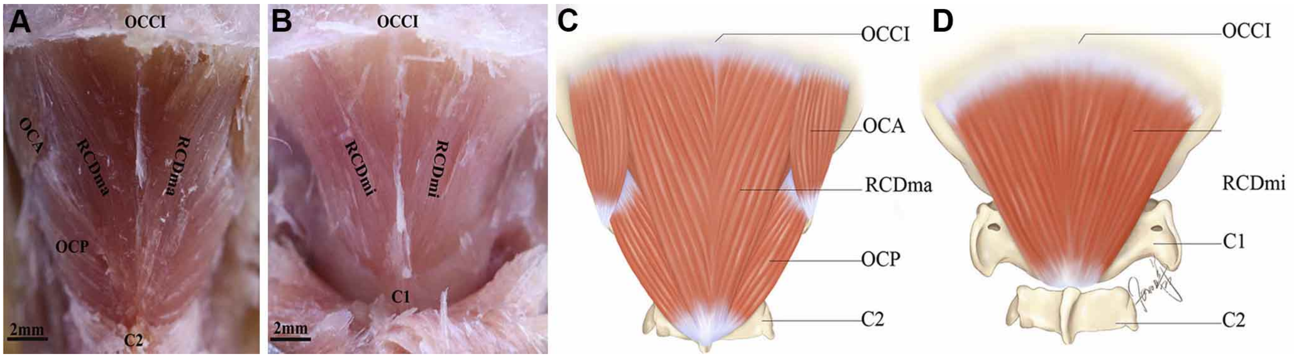

Fig. 2. The suboccipital region of tree shrew. (A: Suboccipitaltriangle; B: The rectus capitis dorsal minor; C: A diagrammatic drawing of suboccipital triangle; D: A diagrammatic drawing of the rectus capitis dorsal minor) OCCI: occipital bone; RCDma: rectus capitis dorsal major; RCDmi: rectus capitis dorsal minor; OCA: obliquus capitis anterior; OCP: obliquus capitis posterior; C2: spinous process of axis; C1: posterior arch of atlas.

the detachment of the RCDmi from its cranial attachment (Bat, Fig.3: A; Tree shrews, Fig.4: A). Subsequently, the DAOM was severed from its cranial attachment along the margin of the foramen magnum and gently reflected. The internal surface of the DAOM was merged with the spinal dural mater by dense connective tissues (Bat, Fig.3: A; Tree shrews, Fig.4: A). The dense connective myodural bridges found in this study are similar to the previously studied mammals.

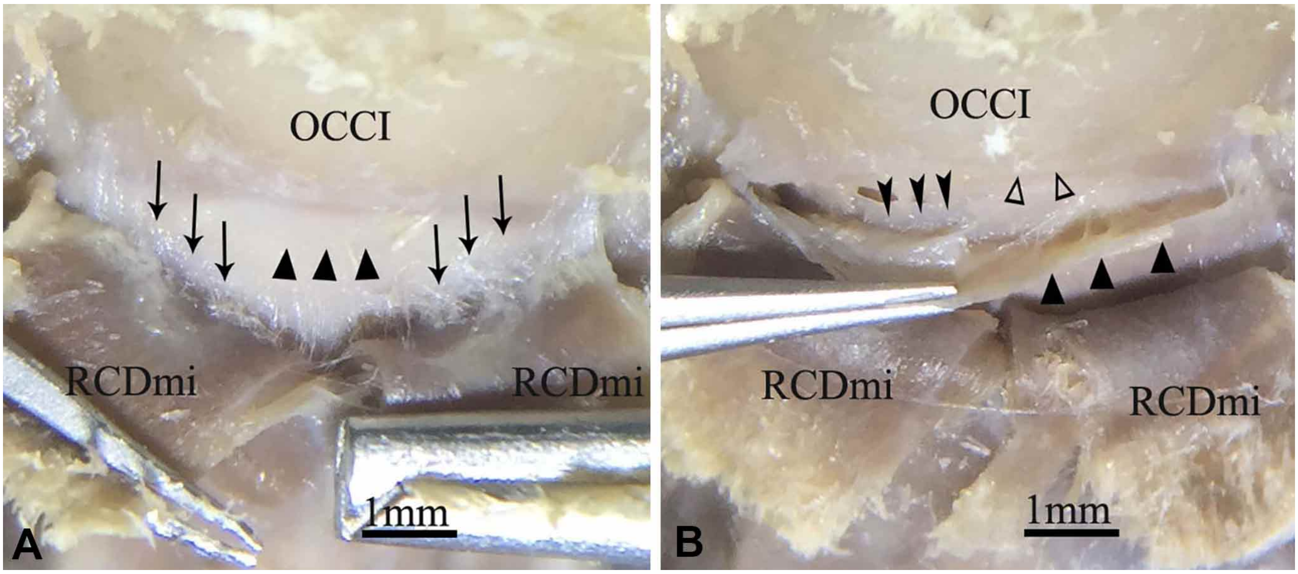

Fig. 3. Gross anatomy of the atlanto-occipital interspace of the bats. (A: Fibrous connection between the rectus capitis dorsal minor and the dorsal atlanto-occipital membrane; B: Fibrous connection between the dorsal atlanto-occipital membrane and the spinal dura mater) OCCI: occipital bone; RCDmi: rectus capitis dorsal minor; solid triangle: dorsal atlanto-occipital membrane (DAOM); arrow: Fibrous connections between the rectus capitis dorsal minor and the dorsal atlanto-occipital membrane; hollow triangle: spinal dural mater (SDM); filled arrowhead: Fibrous connection between the dorsal atlanto-occipital membrane and the spinal dura mater.

Histological studies. In bats, the HE-stained sections showed that the atlanto-occipital space extends from the inferior margin of the occipital bone to the atlas and was "C" shaped (Fig.5: A, a). In contrast, the tree shrew was "L" shaped (Fig.6: A, a). The posterior atlanto-occipital membrane does not completely span the atlanto-occipital space. It only exists in the upper and middle part of the atlanto-occipital space, which is closely connected to the spinal dura mater. Fibers emanating from the RCDmi fuse with the DAOM near the occipital bone, and at this point, the fibers appear to have two parts, one running to the posterior arch of the atlas (filled triangle) and the other directly connecting the spinal dura mater (hollow triangle) (Bat, Fig.5: A, a; Tree shrews, Fig.6: A, a). The fiber connection between the RCDmi and the

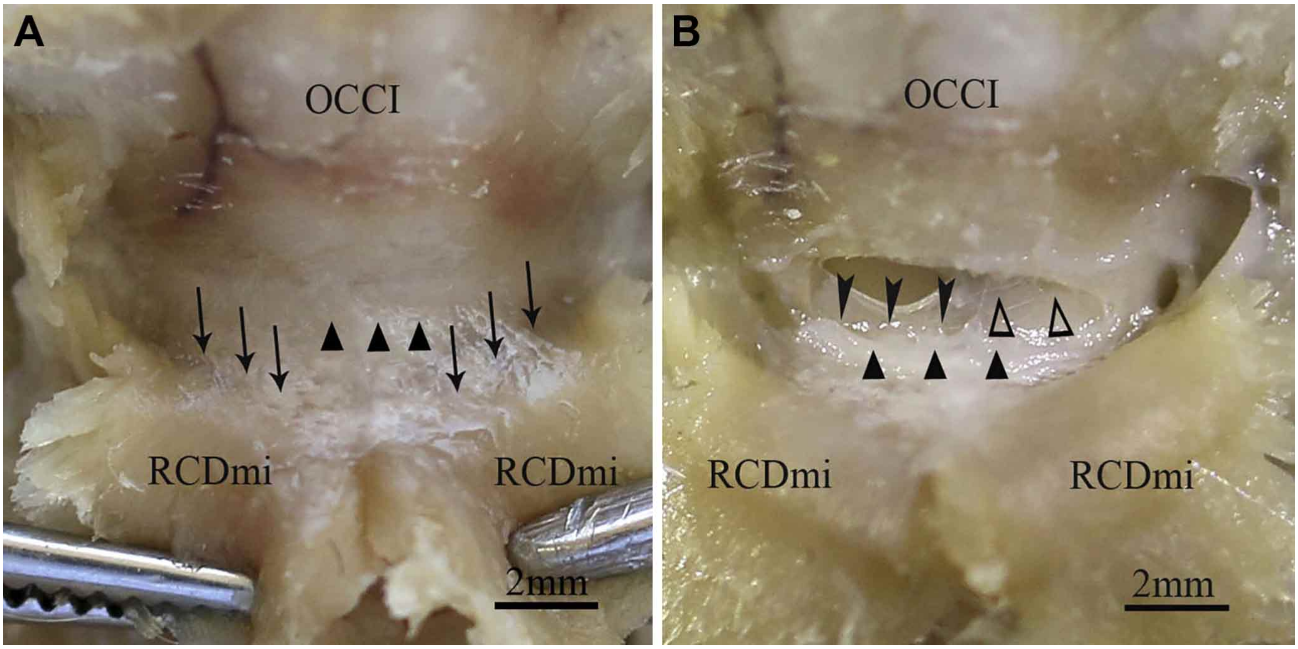

Fig. 4. Gross anatomy of the atlanto-occipital interspace of the Tree shrew. (A: Fibrous connection between the rectus capitis dorsal minor and the dorsal atlanto-occipital membrane; B: Fibrous connection between the dorsal atlanto-occipital membrane and the spinal dura mater) OCCI: occipital bone; RCDmi: rectus capitis dorsal minor; solid triangle: dorsal atlanto-occipital membrane (DAOM); arrow: Fibrous connections between the rectus capitis dorsal minor and the dorsal atlanto-occipital membrane; hollow triangle: spinal dural mater (SDM); filled arrowhead: Fibrous connection between the dorsal atlanto-occipital membrane and the spinal dura mater.

DAOM, as well as that between the latter and the spinal dura mater, constitutes the MDB. The results of the Masson trichrome staining showed that the fibers connecting the RCDmi to the dura mater are dense connective collagen fibers (Bat, Fig.5: B, b; Tree shrews, Fig.6: B, b). The results of the Picrosirius red staining were observed both under the light and polarized microscope. The fibers connecting the RCDmi to the spinal dura mater were revealed to primarily be type I collagen fibers (Bat, Fig.5: C, c, d; Tree shrews, Fig.6: C, c, d). This indicated that the MDB in bat and tree shrew is the strong type I collagen fibers.

DISCUSSION

The MDB refers to the dense connective tissue which connects the RCPmi, RCOma, OCI, and nuchae ligament to the spinal dura mater through the atlanto-occipital andatlanto-axial interspaces (Hack, 1995; Mitchell *et al*., 1998; Scali *et al*., 2011; Pontell *et al*., 2013). This structure is a normal and complex anatomical structure in humans (Zheng *et al*., 2017). Recently, researchers found this soft tissue connection in five other mammalian species. The authors hypothesized that the MDB might be widely present in mammals (Zheng *et al*., 2017; Liu *et al*., 2017).

In this study, we studied two unique mammals, bats, and tree shrews. The survival forms of these two kinds of mammals are quite different from those of other mammals because they can fly or reach an altitude of 10,000 feet. Bats are nocturnal animals and the only mammal capable of sustained flight (Wilson & Reeder, 2005). Tree shrews are small arboreal mammals found in tropical and subtropical regions. They can reach an altitude of 10,000 feet and have the appearance of a squirrel. Tree shrews are highly similar to primates and even humans in terms of their physiological anatomy and have been applied in many fields of medical research (Fan *et al*., 2013). The survival forms of these two kinds of mammals are very different from those of other mammals in that they are unique and representative.

In bats and tree shrews, gross anatomical dissection showed that suboccipital region of bats and tree shrews contains the RCDma, OCA and OCP. These muscles form the suboccipital triangle in bats and tree shrews. This is consistent with the majority of the mammalian suboccipital triangle muscle composition. Both gross anatomical dissection and histological staining showed dense fibrous connective tissues connected the RCDmi to the DAOM and the DAOM to the spinal dura mater. This soft tissue connection between them is similar to the human's or other mammalian MDB in the atlanto-occipital interspace. This study further confirmed

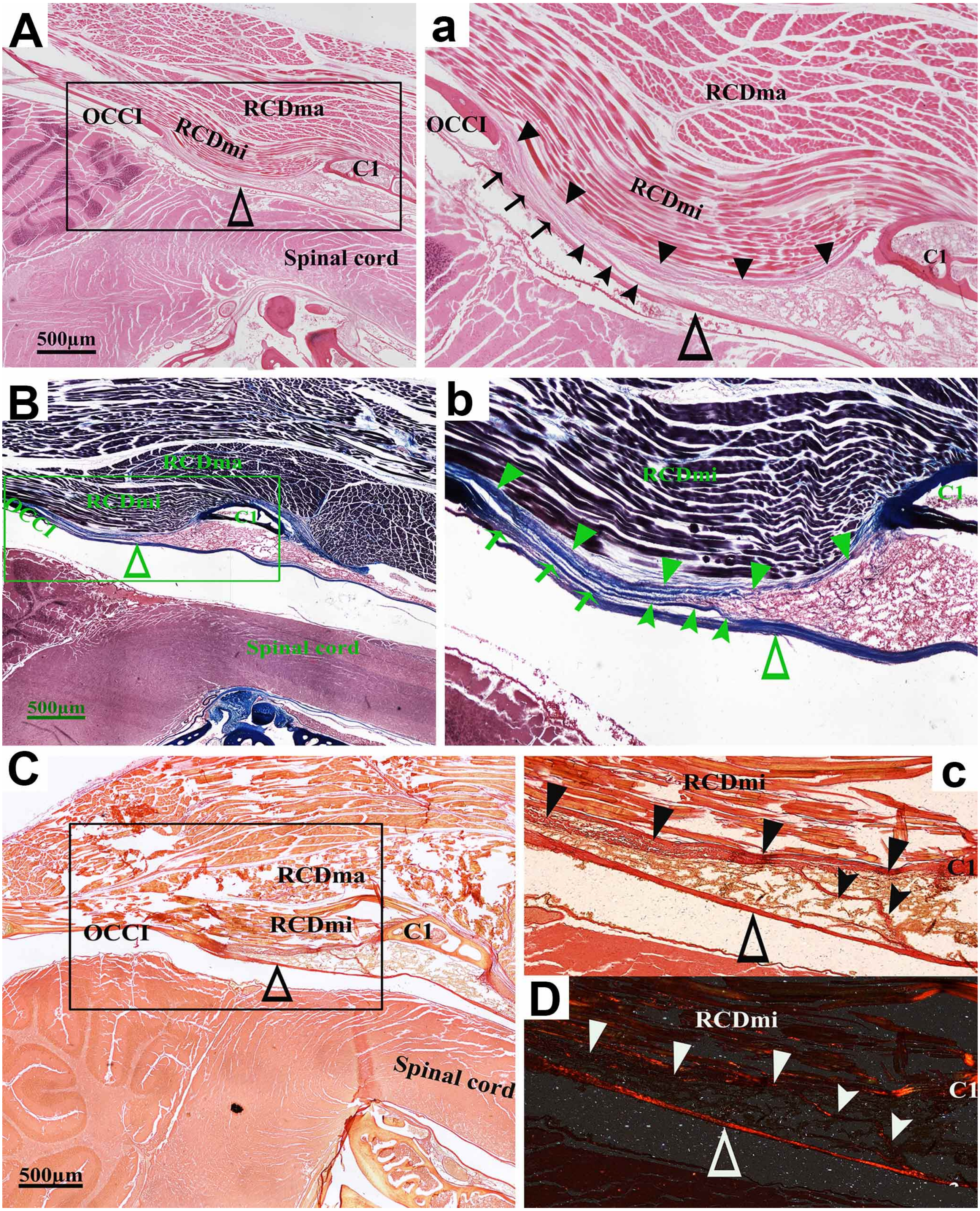

Fig. 5. Histological staining of the myodural bridge in Bats. (A, a: HE staining; B, b: Masson trichrome staining; C, c, d: Picrosirius red staining) OCCI: occipital bone; RCDma: rectus capitis dorsal major; RCDmi: rectus capitis dorsal minor; C1: posterior arch of atlas; hollow triangle: spinal dural mater (SDM); filled triangle: Dense fibers running from the RCDmi to the posterior arch of the atlas; arrow: Fibers originating from the dorsal atlanto-occipital membrane; solid arrowhead: Dense fibers running from the RCDmi to the spinal dura mater.

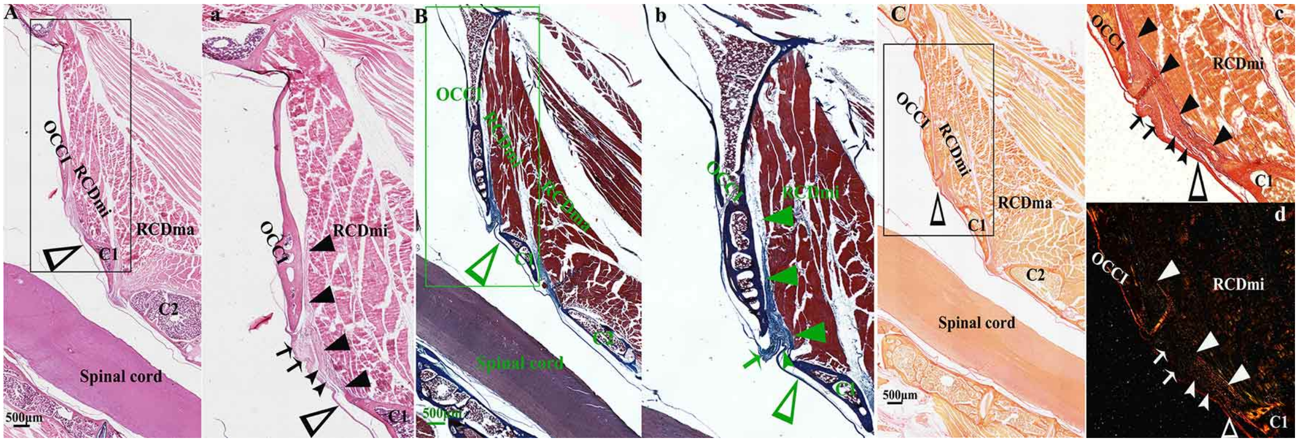

Fig. 6. Histological staining of the myodural bridge in Tree shrews. (A, a: HE staining; B, b: Masson trichrome staining; C, c, d: Picrosirius red staining) OCCI: occipital bone; RCDma: rectus capitis dorsal major; RCDmi: rectus capitis dorsal minor; C1: posterior arch of atlas; hollow triangle: spinal dural mater (SDM); filled triangle: Dense fibers running from the RCDmi to the posterior arch of the atlas; arrow: Fibers originating from the dorsal atlanto-occipital membrane; solid arrowhead: Dense fibers running from the RCDmi to the spinal dura mater.

the conclusion of Zheng *et al*. (2017) that the MDB is a normal universal anatomical structure in mammals.

Multiple functions have been ascribed about MDB. It is generally believed that the MDB is significant in cervicogenic headache, neck motor function, perception, and posture (Alix & Bates, 1999; Bogduk, 2001; Kulkarni *et al*., 2001; Hack *et al*., 2004; Fernández-de-Las-Peñas *et al*., 2007; Fernández-de-Las-Peñas, 2008; Grgic, 2007). Some researchers consider that the cervical muscles control the tension on the dura mater via the MDB (Shinomiya *et al*., 1996; Alix & Bates, 1999; Scali *et al*., 2011; Pontell *et al*., 2013; Venne *et al*., 2017), while some are of the view that the MDB prevents the in the folding of the dura mater during neck hyperflexion or hyper-translation (Burt *et al*., 1986; Hack,1995). Shinomiya *et al*. (1996) stated that the epidural junction of the neck could fix the spinal cord and prevent the occurrence of myelopathy. McPartland & Brodeur (1999) speculated that the rectus capitis posterior major muscle may play an essential role during head flexion by preventing poor cerebrospinal fluid circulation. Interestingly, using MR phase-contrast cine technique, the relationship between head turning and the flow rate and direction of the cerebrospinal fluid has been demonstrated (Xu *et al*., 2016).

Bats and tree shrews are similar to primates (Wilson & Reeder, 2005) and can be used as experimental models. Bats and tree shrews are unique mammals. For example, bats often hang upside down, and tree shrews often climb to a high altitude. According to the results of this experiment, MDB both exists in bat and tree shrew and is composed primarily of type I collagen fibers. Type I collagen fibers are mostly found in tendons and have high tensile resistance. The pull of the myodural bridge is likely to provide propulsion for cerebrospinal fluid. However, the dynamics may differ from that of humans and other mammals during the passive and active activity of the MDB. The role of the MDB clearly demands further studies in humans and other mammals.

CONCLUSIONS

Both bat and tree shrew possess the MDB, primarily made up of type I collagen fibers. This study provides strong evidence for the assumption that the MDB is a normal universal structure in mammals.

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RESUMEN: El puente miodural es un tejido conjuntivo denso que conecta los músculos y los ligamentos a la duramadre espinal en el espacio atlanto-occipital. Algunos investigadores creen que el puente miodural puede desempeñar un papel fisiológico vital. Es posible, por ejemplo, que la prevención de los pliegues de la duramadre espinal pueda estar involucrada en la circulación regulada del líquido cefalorraquídeo. En esta instancia, es posible prevenir los pliegues de la duramadre espinal, regulando la circulación cerebro espinal. Los murciélagos son animales nocturnos y los únicos mamíferos que pueden realizar un vuelo real y sostenido, mientras que las musarañas arborícolas son mamíferos arbóreos que a menudo ascienden a una gran altura de unos 10 000 pies. Ambos animales tienen estilos de vida diferentes a los de otros mamíferos previamente estudiados. El estudio de estos dos animales ofrecerá más información sobre la existencia del puente miodural en los mamíferos. Se realizó una disección anatómica macroscópica para observar las conexiones entre los músculos profundos del cuello y la duramadre a nivel del espacio atlanto-occipital. Las estructuras existentes se analizaron mediante técnicas de tinción histológica convencionales y especiales. Las regiones suboccipitales en murciélagos y musarañas arbóreas presentaban el músculo recto dorsal mayor de la cabeza (RCDma), el recto dorsal menor de la cabeza (RCDmi), el oblicuo anterior de la cabeza (OCA) y el oblicuo posterior de la cabeza (OCP). El tejido conjuntivo denso conecta el RCDmi con la membrana atlanto-occipital posterior (PAOM) y esta última con la duramadre espinal. El puente miodural en estos mamíferos comparte una estructura similar al puente miodural en humanos. Los análisis histológicos confirmaron que las fibras conectivas del puente miodural son principalmente fibras de colágeno tipo I. Esto demuestra además que el puente miodural existe ampliamente en la anatomía normal de los mamíferos. Esta investigación proporciona apoyo morfológico para un estudio anatómico comparativo de la función fisiológica del puente miodural.

PALABRAS CLAVE: Puente miodural; Murciélago; Musaraña de árbol; Morfología; Anatomía comparada.

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