



# Existence and features of the myodural bridge in *Gallus domesticus*: indication of its important physiological function

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## Abstract

The myodural bridge (MDB) is a dense connective tissue that connects muscles with the cervical spinal dura mater via the posterior atlanto-occipital and atlanto-axial interspaces. To date, the physiological function of the MDB has not been fully elucidated. Recent studies have identified the presence of the MDB in mammals, but very little information is available on the existence of the MDB in avifauna. We selected *Gallus domesticus* to explore the existence and the fiber property of the MDB in avifauna. We found that in this species, fibers originating from the ventral aspect of the rectus capitis dorsal minor are fused with the dorsal atlanto-occipital membrane and that numerous trabeculae connect the dorsal atlanto-occipital membrane with the cervical spinal dura mater. Furthermore, the occipital venous sinus is located between the trabeculae. The MDB is mainly composed of collagen type I fibers. Our results show that the MDB is present in *G. domesticus* and lead us to infer that the MDB is a highly conservative evolutionary structure which may play essential physiological roles.

**Keywords** Myodural bridge · Cerebrospinal fluid circulation · Collagen fiber · *Gallus domesticus*

## Introduction

The concept of the myodural bridge (MDB) was first proposed by Hack et al. (1995) who described the MDB as a dense fibrous tissue that connected the rectus capitis posterior minor (RCPmi) muscle with the cervical spinal dura mater (SDM) via the posterior atlanto-occipital membrane (PAOM). In subsequent years, extensive studies were carried

out on this subject. Nash et al. (2005) confirmed the myodural bridge of the RCPmi. The ligamentum nuchae (LN), obliquus capitis inferior (OCI), and rectus capitis posterior major (RCPma) muscles were also reported to participate in forming the MDB, via the posterior atlanto-occipital and posterior atlanto-axial interspaces (Mitchell et al. 1998; Pontell et al. 2013; Scali et al. 2011). In more recent years, researchers have shown that the MDB may be a universal existing structure in mammals and reptiles (Liu et al. 2017; Zhang et al. 2016; Zheng et al. 2017). These findings suggest that the MDB may have a significant physiological function. The MDB has been proposed to play vital roles in preventing in-folding of the SDM and may be related to cervicogenic headache (Hack and Hallgren 2004; Pareja et al. 2007). Sui et al. (2013) and Zheng et al. (2014) proposed that the MDB may play important roles in modulating the circulation of the cerebrospinal fluid (CSF). To date, however, there is no published data on the existence—or not—of the MDB structures in avifauna. The study of different animal species will facilitate our understanding of the function and evolution of the MDB.

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## Materials and methods

### Ethical considerations

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

Fifty-six healthy *Gallus domesticus* were used in this study. They were anesthetized by intraperitoneal injection using 10% chloral hydrate at an injection dosage of 3.5 ml/100 g body weight and then perfused with 10% formalin. Gross dissection was carried on 47 specimens; five specimens were used for sagittal dissection; and histological analyses were carried out on six specimens.

### Anatomical dissection in the sub-occipital region

A midline longitudinal incision was made from the occiput to the 13th cervical vertebra. The superficial neck muscles were removed to access the deep muscles, and the longissimus dorsi which originated from the lower margin of the occipital bone and terminated at the spinous process of the 13th cervical vertebra was cut and retracted to expose the rectus capitis dorsal major (RCDma, same as the RCPma in humans). Subsequently, the RCDma was removed to expose the rectus capitis dorsal minor (RCDmi, same as the RCPmi in humans). Similarly, the RCDmi was cut off from its cranial attachment, and the connections between the RCDmi and the dorsal atlanto-occipital membrane (DAOM, same as the PAOM in humans) were observed. The connections between the DAOM and the SDM were also observed. Photographic documentations were made using a Canon 7D camera (Canon Inc., Tokyo, Japan).

### Thick sagittal section of the sub-occipital region

After fixation in 10% formalin solution, five specimens were frozen at  $-60^{\circ}\text{C}$  for 7 days, then embedded in polyurethane foam, and frozen again at  $-60^{\circ}\text{C}$  for 1 h. The embedded specimens were serially sectioned into 2 mm-thick sagittal slices with a high-speed band saw, and the slices were washed with cold water. Photographs were taken using the Canon 7D camera.

### Histological slices and staining

Tissue samples containing the sub-occipital musculature, occipital bone, atlas, axis, and SDM were decalcified with

ethylenediamine tetraacetic acid disodium salt (EDTA). The EDTA solution was changed every 2 days until the bones of the tissues could be easily pierced by a needle (16 days). The samples were then washed in running water overnight, dehydrated in a graded series of ethyl alcohol, and passed through xylene before being embedded in paraffin wax. A rotary microtome (Leica Micro HM450; Leica Microsystems GmbH, Wetzlar, Germany) was used to make 10- $\mu\text{m}$ -thick sections. For analysis under the light microscope, the sections were placed on glass microscope slides, rehydrated, and treated with hematoxylin and eosin (HE) stain, Van Gieson (VG; picric acid and acid fuchsin) staining, Masson trichrome staining (Masson), and Picrosirius red (PRS) stain. The slides stained with PRS were also viewed under a polarizing microscope (Olympus BH-2; Olympus Corp., Tokyo, Japan). Images were digitized using the Microsoft composite software of the microscope.

## Results

### Anatomical dissection of the sub-occipital region

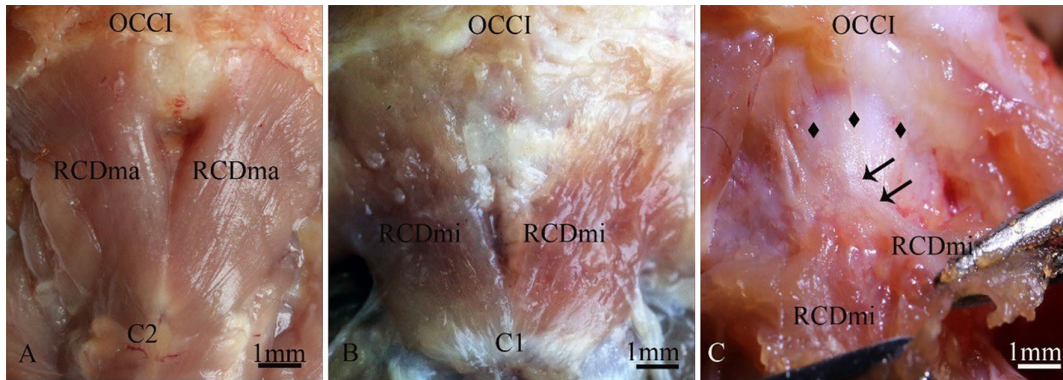
The RCDma and the RCDmi are located in the deep sub-occipital region (Fig. 1a, b). The RCDmi and the DAOM attach tightly to each other (Fig. 1c). The DAOM and SDM are connected to each other by many trabeculae (Fig. 2). Based on the shape of the trabeculae, the trabeculae could be classified as flake-like, cord-like, and mesh-like (Fig. 3). The cord-like trabeculae were present in all of the samples.

### Thick sagittal section of sub-occipital region

In the sagittal sections, the musculature of the sub-occipital region, cervical vertebrae, and spinal cord were clearly displayed. Fibers derived from the RCDmi fused with the DAOM, and trabeculae directly connected the DAOM with the SDM. These connections are similar with the MDB in humans (Fig. 4).

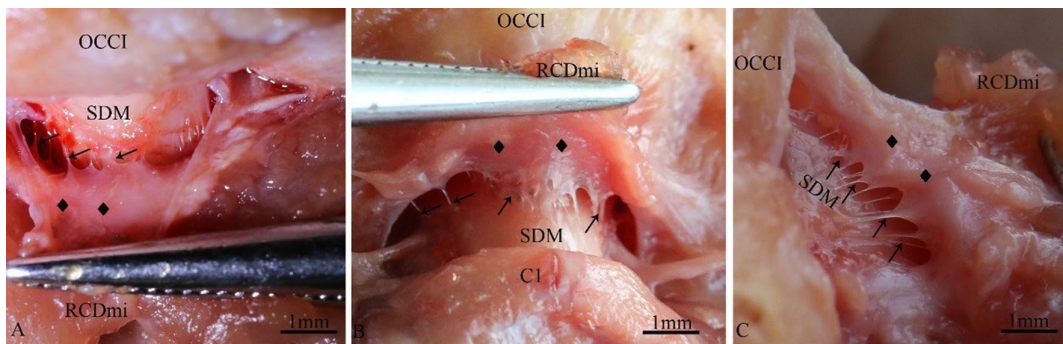
### Histology studies

Specimens stained with HE show that the dense fibers from the deep aspect of the RCDmi fuse with the DAOM. The trabecular meshwork emerges from the DAOM, and then the trabeculae extend out of the trabecular meshwork. The trabeculae pass through the venous sinus and then directly insert into the SDM (Fig. 5). In the VG-stained sections, the muscular fibers are stained yellow, and the MDB fibers (connection between RCDmi, DAOM, and SDM) are stained red (Fig. 6). In the Masson-stained sections, the muscular fibers are stained red, and the MDB fibers are stained blue (Fig. 7). The results



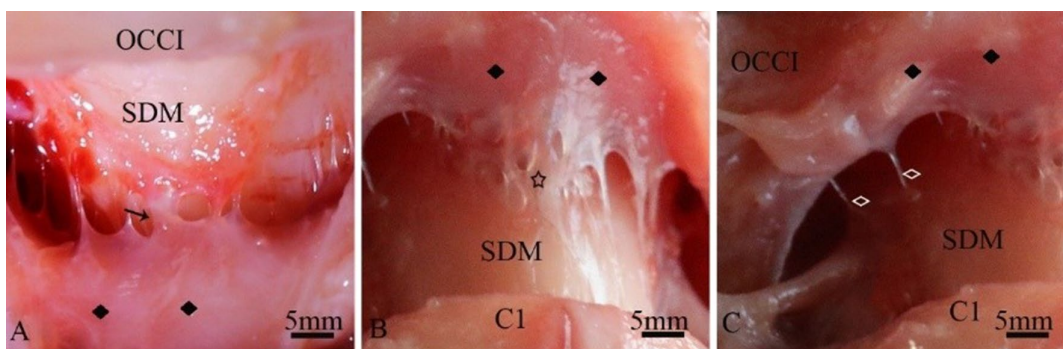
**Fig. 1** Anatomical dissection of the sub-occipital region of the domesticated chicken *Gallus domesticus* showing the connections between the rectus capitis dorsal minor (*RCDmi*) and the dorsal atlanto-occipital membrane (DAOM). **a** Dorsal aspect of the rectus capitis dorsal major (*RCDma*), **b** dorsal aspect of the *RCDmi*, **c**

the connection between the *RCDmi* and the DAOM. *OCCI* occipital bone, *C1* posterior arch of atlas, *C2* vertebral arch of axis. Filled diamond designates the location of the DAOM; black arrows indicate the connection between the *RCDmi* and the DAOM



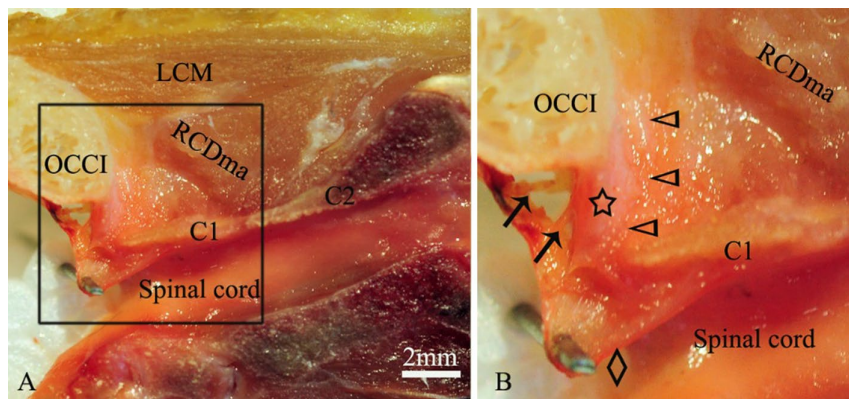
**Fig. 2** Anatomical dissection of the sub-occipital region of *G. domesticus* showing the connection between the spinal dura mater (*SDM*) and DAOM. **a** Cranial view, **b** caudal view, **c** lateral view. For abbreviations, see Fig. 1 caption. Filled diamond designates the location of the DAOM; black arrows in **a**, **b**, **c** indicate the many trabeculae connections between the DAOM and the SDM

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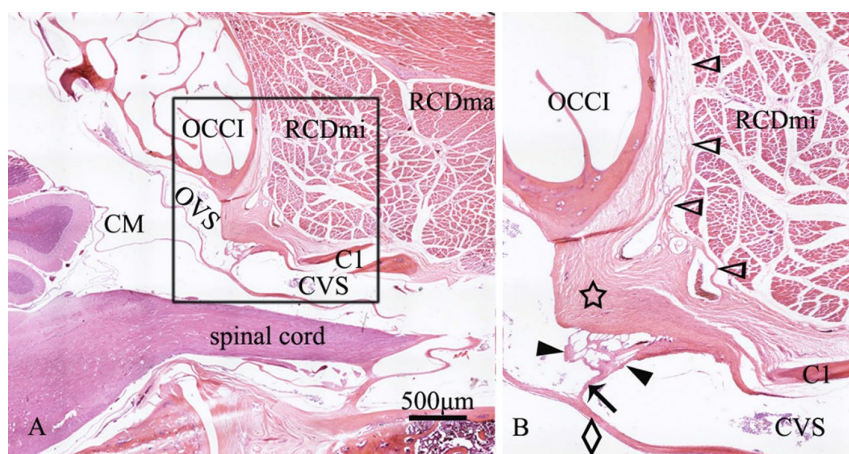
**Fig. 3** The morphological types of trabeculae connecting the DAOM and the SDM. **a** Cranial view, **b** caudal view, **c** lateral view. For abbreviations, see captions to Figs. 1 and 2. Filled diamond designates the DAOM. The different shapes of trabeculae are shown: **a** Arrows indicate flake-like trabeculae, **b** open star indicates mesh-like trabeculae, **c** open diamond indicates cord-like trabeculae

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**Fig. 4** Sagittal sections of the sub-occipital region of *G. domesticus* showing the myodural bridge (MDB). Area within square in **a** is shown enlarged in **b**. The RCDmi is fused with the DAOM, and the trabeculae structures originating from the DAOM directly insert into the SDM. *LCM* Longus collis muscle; for other abbreviations, see

captions to Figs. 1 and 2. Open diamond designates SDM; star designates the DAOM; arrows indicate the trabeculae; open arrowheads indicate the dense connection between the deep aspect of the RCDmi and the DAOM



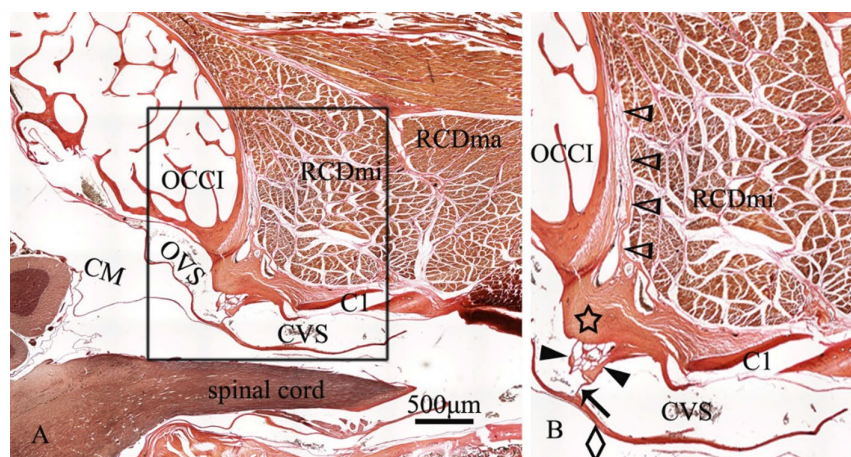
**Fig. 5** Prepared sagittal section of sub-occipital region of *G. domesticus* stained with hematoxylin/eosin. Area within square in **a** is shown enlarged in **b**. *CM* Cisterna magna, *OVS* occipital venous sinus; *CVS* cervical venous sinus; for other abbreviations, see captions to Figs. 1 and 2. Open star designates the DAOM; open diamond designates the SDM; filled arrowheads designate the trabecular meshwork; arrow

indicates the trabeculae. The fascial fibers (open arrowheads) originate from the RCDmi, extend directly into the atlanto-occipital interspace, then fuse with the DAOM (open star), and the DAOM extends into a trabecular meshwork (filled arrowheads). From the trabecular meshwork, the trabeculae (arrow) are sent through the venous sinus to connect with the SDM (open diamond)

of VG and Masson staining demonstrate that the MDB fibers in *G. domesticus* are collagenous fibers. Examination of the PRS-stained specimen under the polarized microscope determined that the fibers originating from the RCDmi are stained red and yellow, and the fibers connecting the DAOM and the SDM are stained red. This staining pattern indicates that the collagenous fibers of MDB are mainly composed of collagen I (Fig. 8).

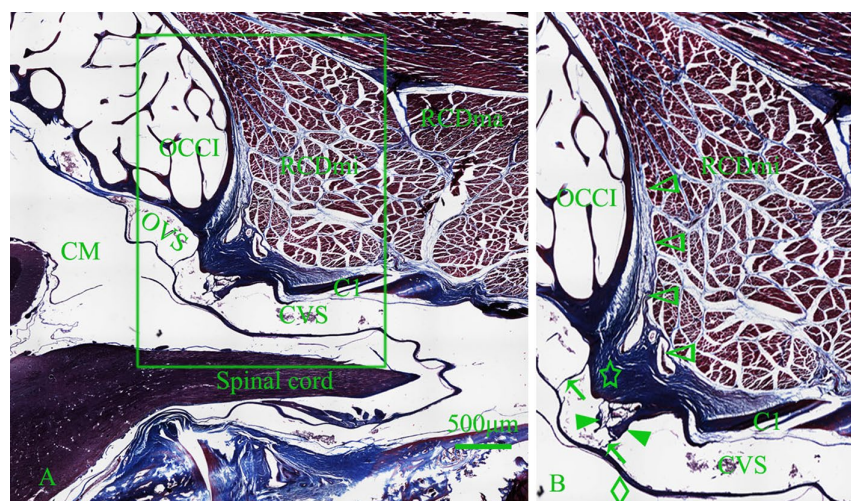
## Discussion

The concept of the MDB was initially proposed by Hack et al. (1995) as a dense connective tissue connection between muscles and the cervical SDM. The muscles involved are the RCPmi, RCPma, and OCI muscles through the posterior atlanto-occipital and atlanto-axial



**Fig. 6** Prepared sagittal section of the sub-occipital region in *G. domesticus* stained with Van Gieson's solution. Area within square in **a** is shown enlarged in **b**. For all abbreviations, see captions to Figs. 1, 2, and 5. Open star designates the DAOM; open diamond designates the SDM; filled arrowheads designate the trabecular meshwork; arrow indicates the trabeculae. The red fascial fib-

ers (open arrowheads) originate from the RCDmi, extend directly into the atlanto-occipital interspace, and then fuse with the DAOM (open star); the DAOM extends into a red trabecular meshwork (filled arrowheads). From the trabecular meshwork, the red-stained trabeculae (arrow) are sent directly through the venous sinus to connect with the SDM (open diamond)



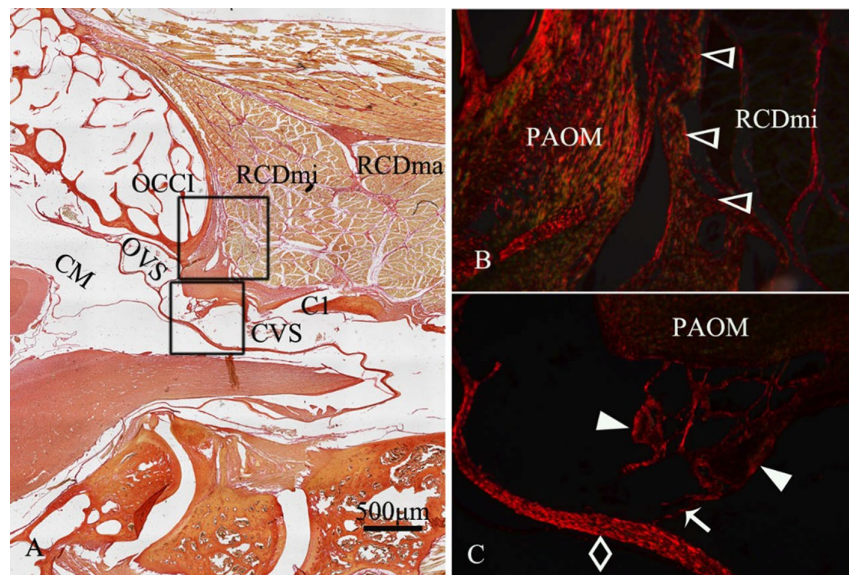
**Fig. 7** Prepared sagittal section of the sub-occipital region in *G. domesticus* stained with Masson preparation. Area within square in **a** is shown enlarged in **b**. For all abbreviations, see captions to Figs. 1, 2, and 5. Open star designates the DAOM; open diamond designates the SDM; filled arrowheads designate the trabecular meshwork; arrow indicates the trabeculae. The blue fascial fibers (open arrow-

heads) originate from the RCDmi, extend directly into the atlanto-occipital interspace, and then fuse with the DAOM (open star). The DAOM extends into a blue trabecular meshwork (filled arrowheads). From the trabecular meshwork, the blue-stained trabeculae (arrow) are sent directly through the venous sinus to connect with the SDM (open diamond)

interspaces (Scali et al. 2011; Scali et al. 2013a, b). Various researchers have reported that the LN also participates in the MDB (Mitchell et al. 1998; Zheng et al. 2014; Yuan et al. 2016). The connective tissue fiber bundles from the LN that participate in the MDB was designated the 'To Be Named ligament' by Zheng et al. (2014). The existence of the MDB in humans specifically has been confirmed in

a large number of experiments (Enix et al. 2014; Palomque-del-Cerro et al. 2017; Venne et al. 2017); the MDB is also present in mammals and reptiles (Zheng et al. 2017; Zhang et al. 2016).

Our results confirm, for the first time, that the MDB also exists in *G. domesticus*. It is therefore possible that the MDB is a universal structure in avifauna, mammals, and reptiles.



**Fig. 8** Prepared sagittal slice of the sub-occipital region in *G. domesticus* stained with Picrosirius red. Area within large square in **a** is shown enlarged in **b**; area within small square in **a** is shown in **c**. For all abbreviations, see captions to Figs. 1, 2, and 5. Open star designates the DAOM; open diamond designates the SDM; filled arrowheads designate the trabecular meshwork; arrow indicates the trabeculae. The fascial fibers (open arrowheads) originate from the RCDmi, extend directly into the atlanto-occipital interspace, and then fuse with the DAOM (open star). Under the light microscope, the DAOM

extends into a red trabecular meshwork (filled arrowheads), and then the trabecular meshwork extends into the red trabeculae. The trabeculae pass through the venous sinus and directly connect with the SDM (open diamond). Under the polarized microscope, the fascial fibers (open arrowheads) originating from the RCDmi are highlighted in red and yellow; moreover, the trabeculae are also red. Red and green in PRS stain indicates collagen type I while green indicates collagen type III. The results of the PRS staining indicate that the MDB fibers are mainly composed of collagen type I fibers

This highly conserved structure of the MDB implies that it may have important physiological functions. However, the MDB differs morphologically between mammals, reptiles, and avifauna. In mammals, the MDB consists of some dense connective fibers which originate from the suboccipital muscles and then pass through the DAOM to connect with the SDM. (Zheng et al. 2017). In crocodiles, a thick bundle of connective tissue trabeculae connect the pro-atlas, atlas, and the suboccipital muscles to the SDM (Zhang et al. 2016).

In this study on the MDB in *G. domesticus*, we observed that the RCDmi emits fibers that fuse with the DAOM; the DAOM, in turn, extends into a trabecular meshwork which emits the trabeculae that pass through the venous sinus to connect with the SDM. Based on the observed morphology of the trabeculae, they could be classified as flake-like, cord-like, and mesh-like. The cord-like type mainly exists near the nerve root. The MDB connection of the RCDmi and the DAOM in *G. domesticus* is similar to that of mammals, while the trabecular MDB between the DAOM and SDM is similar to that in reptiles.

Numerous speculations on the function of the MDB have been put forward over the years. Some researchers infer that the MDB may play vital physiology roles in keeping cervical balance, resisting SDM in-folding, monitoring dura

tension, and protecting the organism from cervical injury (Cailliet 1991; Uhlig et al. 1995; Rutten et al. 1997; Kulkarni et al. 2001; Palmgren et al. 2009; Cramer and Darby 2013; Luszyk et al. 2014). Others have proposed that the MDB may be involved in translating dural tension and maintaining the fluency of the subarachnoid space (Luszyk et al. 1982; Shinomiya et al. 1996; Hong et al. 2011; Pontell et al. 2013). Sui et al. (2013) hypothesized that the MDB may play important roles in modulating the circulation of CSF. When the RCPmi contracts, the dura mater is pulled by the MDB, leading to an alteration in the subarachnoid volume which in turn produces negative pressure similar to that produced by a pump. As a result, the MDB may have an effect on CSF circulation and is possibly a crucial power source for CSF circulation (Xu et al. 2016, Sui et al. 2013; Zheng et al. 2014).

In this study, we found that the dense connective fibers from the *G. domesticus* RCDmi is involved in the formation of the DAOM. The DAOM emits a cluster of trabecular meshwork; additionally, the trabecular meshwork emits trabeculae which then directly connect to the SDM. The occipital venous sinus was also found to be present among trabeculae. We also confirmed that the fiber properties of MDB are mainly composed of collagen type I. Based on

these findings, we propose that the MDB may perform two roles: first, when the head is extended or moved backward, the MDB will assist in maintaining the fluency of the occipital venous sinus by pulling on the venous sinus; secondly, with a pull on the epidural sac on the caudal side of the cerebellum, the MDB may provide force for the circulation of the CSF. Thereby promoting normal CSF circulation. The findings in this study provide comparative anatomical evidence for the CSF dynamic hypothesis proposed by Sui et al. (2013).

## Conclusion

This study confirms the existence of MDB in *Gallus domesticus*. Additionally, we demonstrated that fibers of the MDB in the specimens are primarily composed of collagen type I.

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**Author contributions** YRD conceived the study, performed the study, interpreted the data, prepared the figures, and wrote the manuscript. NZ supervised the research, performed the macroscopic analysis, and edited the manuscript. JG performed the scanning electron microscopy examination. TW performed the thick Sagittal section examination. OCS edited the manuscript. YZ, YZ, YXC, SYP, LCQ performed the anatomical dissections. SBY, HJS collected the research material, supervised the research, and edited the manuscript. All authors approved the last version of manuscript.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

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