RESEARCH ARTICLE

Equine myodural bridges
An Anatomical, integrative and functional description of myodural bridges along the spine of horses: Special focus on the atlanto-occipital and atlanto-axial regions.

Authors
Vibeke Sødring Elbrønd [1], Rikke Mark Schultz [2]
Affiliations
[1] IVH, SUND, KU, [2] RMS Equine Practice, Karlebovej 22, DK- 2980 Kokkedal.
Corresponding author:
Dr. Vibeke Sødring Elbrønd,
Section of Pathobiology, Dept. of Animal and Veterinary Sciences, University of Copenhagen, Dyrlægevej 100, DK-1870 Frederiksberg C
E-mail: vse@sund.ku.dk

Abstract

Introduction: Socalled myodural bridges (MDB) linking the suboccipital muscles to the dura mater have been described in the human, canine, small ruminants, monkeys, rodents, porpoises, crocodiles, sperm whales, chickens and lately in equines. They are believed to have biomechanical functions and might also play a role in head/neck pathology and the pumping function of the cerebrospinal fluid. Up to now these bridges have only been described briefly in the horse in terms of their anatomy, and then only in relation to Ehlers-Danlos syndrome. The aim of this study was therefore to investigate and thoroughly describe the anatomy, biomechanics and integration of this complex throughout the equine spine, with special focus on the upper neck, the cervicothoracic- and the lumbosacral transitions. Pathology in these regions is well recognized in horses.

Material and methods: Horses were dissected, the heads and spine prepared in several anatomical planes, the heads MR-scanned and histology performed on the AO and AA MDBs.

Results: Gross anatomical observations showed that muscle-membrane-spinal dura mater connections (MDB) were evident in the full equine columna vertebralis, and were specifically developed in the upper cervical, the cervicothoracic and the lumbosacral transitions. In the upper cervical region, the m. rectus capitis minor and major and the m. obl. cap caudalis attached tightly to the dorsal intervertebral AO and AA membranes. On the ventral membrane surface there were trabecular connections to dura mater. The two membranes differed markedly in the amount of elastic fibers giving them different biomechanical function. The structures of the AO MDB were evident on the MRI scans.

Conclusions: Horses, like humans, other mammals, a reptile and a bird to date, have myodural bridges, which are tightly integrated with surrounding structures as well as the biomechanics of the upper neck. In addition, similar structures are present throughout the whole spine.

Keywords: Myodural bridge, Atlanto-occipital, Atlanto-axial, Dura mater connections,

1. Introduction

Based on dissections in humans and later also MR-imaging several research groups have described fibrous structures linking the suboccipital muscles or the nuchal ligament to the spinal dura mater directly, or through the dorsal atlanto-occipital (AO) and atlanto-axial (AA) membranes [1-7].

Certain issues regarding the specific nomenclature of these structures in the human dissections are presented in the review by Kahkeshani and Ward [7], to which end, it was concluded by this author that all the structures associated with this complex should be included in the general term "the myo-dural-bridge" (MDB), nomenclature that will also be adopted in this paper. The MDB connections have been described in the human as long ago as 1995 [8], where they were supposedly meant to anchor the spinal cord passively and actively in the vertebral- and dural canal and protect the dura mater from folding in on itself during cervical hyperextension. Focus up to now has been on the upper cervical regions, where the suboccipital muscles may modulate or influence dural tension through these myodural connections [3]. Indeed, it has also been stated that they may have a regulatory function on the pressure and circulation of cerebrospinal fluid [9][1]. Other reports focus on the role of the MDB in connection with cervicocephalic pain syndromes and headaches, and go on to imply a role in postural control, cervical proprioception, balance and sympathetic tone [3, 10].

More recent studies have observed and visualized the myodural connections in dissections, histological sections and plastinated preparations including the siamese crocodile [11], *gallus domesticus* [12], in five different mammalian orders (carnivores [*Felidae et canis familiaris*], small ruminants, rats, guinea pigs, rabbits, monkey and the finless porpoise)[13, 14], and the sperm whale [15]. Furthermore, the MDB structures of the upper cervical region are now accepted as being an evolutionarily conserved structure, suspected of being mainly involved in the pumping and circulating of cerebrospinal fluid.

Inter- and intra-species variations in the MDB have been verified by several research groups [13, 16], identifying differences in the attachment of the muscles, the dorsal intervertebral membrane and the spinal dura-membrane complex [13, 14, 16]. Moreover, dissections of human cadavers [16], have identified three types of bridging structures between the suboccipital muscles and the membrane. The most frequent type is the tendon like structure that runs between the muscle and the membrane. A second, and less frequent type is the muscle bridge between the muscle and the membrane, and the third and least frequent type, is the fascia type, consisting of fascia (connective tissue) running directly from the muscle belly to the membrane complex.

Very little information about the equine MDB´s in terms of histology and morphology is to be found in the literature [17] [18]. This is surprising since horses are prone to upper neck injuries, such as occipital base fractures, pathology of the tendon of m. semispinalis as well as osseous changes at ligament insertions (e.g. funiculus nuchalis) on the occipital crest [19] and other pathological conditions of the AO and AA region, making such research highly relevant. From a biomechanical point of view hypomobility of the occiput-atlas-axis joint complex is very common due to uni- or bi-lateral extended or flexed lesions [20, 21] in this region. Furthermore, head and neck carriage are important in many equestrian sports, making anatomical and biomechanical knowledge of the AO and AA regions essential, especially when considering horse welfare. For example, the so-called practice of "roll-kur" where the horse´s nose is pulled into its chest during hyperflexion, has considerable biomechanical impact on this area [22]. Likewise, pressure from tack and the pull of bridles may similarly compromise this area. Finally, the use of the AO space as an injection site for cervical myelography is yet another subject in which attention to these fragile structures should be a point for concern [23-25].

Lastly, since the dorsal intervertebral space is wide in several regions of the horse, anchoring of the spinal cord and dura mater may conceivably be expected to also occur further down the spine. Yet, to our knowledge there are no publications or cited observations of similar myodural connections in the remaining part of the spine from C3 to the sacrum in humans, equines or any other species.

The aim of this study was therefore; i) to investigate and describe the presence of a contact between the suboccipital muscles and the spinal dura mater in the equine AO - and AA regions at both the macro- and microscopical level, ii) to investigate and describe the presence of similar connections throughout the spine, macroscopically, iii) to discuss similarities and variations between the regions studied as well as, iv) evaluate the function and interaction of the MDB's with regard to their biomechanical and physiological properties in these regions.

2. Material and methods

2.1 *Animals*

For this study a total of twenty horses of different breeds (riding horses, Icelandic horses and ponies) as well as different sex and age were used. The horses were euthanized for other reasons than this study by stunning and bleeding. Fifteen of the twenty horses were used to perform dissections, two were frozen and cut into transverse sections; the head and upper neck of one horse was frozen and divided in the mid sagittal plane and an MRI-scan was performed on two horse´s upper necks immediately after euthanization. Tissue from the equine AO and AA myodural connections was sampled for histology from three horses. The macroscopic transverse and sagittal sections of the frozen specimens were performed using a bandsaw. The location and direction of the sections were palpated and marked before freezing. After cutting, the sections were cleaned, studied, imaged and videotaped. The focus was directed both to the morphology, topography and the functional integration and interaction of the structures of the myodural bridges and the related neighbouring structures at rest and in motion.

2.2 *Dissections*

2.2.1 Upper cervical region:

Dissections of the AO and AA myodural bridges in a total of fifteen horses were initiated by skinning the upper neck and head regions. The dorsal superficial muscles (m. splenius, m. semispinalis, mm. longissimus cervicis, atlantis and capitis) of the neck were removed and the suboccipital muscles in the AO and AA regions (m. obliquus capitis caudalis (OCCa) and cranialis (OCCr) and m. rectus capitis dorsalis major (RDMa)) were approached and exposed from a dorsal direction. RDMa was dissected from the insertion on the spinous process on C2 and reflected in a cranial direction to expose the connections to the AA membrane and the underlying RDInt (m. rectus dorsalis intermedius) and RDMi (m. rectus dorsalis minor) (fig.1a). From the caudal origo on the tuberculum dorsale atlantis the RDMi was lifted in a cranial direction and the connections to the AO membrane were studied before separating it (Fig.1b,c). The OCCa was loosened from the insertion on the ala atlantis and reflected in a caudal direction to expose the AA membrane. Both membranes were located between the bilateral capsula articularis of the AO and AA joints (fig.1c, fig.3a). The membranes were cut at the caudal (AO) (fig.1d) and cranial border (AA) respectively, to expose; i) the dorsal intervertebral foramen, ii) the canalis spinalis with the dura mater, and iii) the dural-membrane connections, as well as iv) the connection to the capsula articularis limiting the AO and AA joints from the epidural space. The connection between the muscles, the membranes and the dura mater were tested manually by moving and stretching the structures one by one in different directions whilst observing and video-recording the effects.

Figure 1

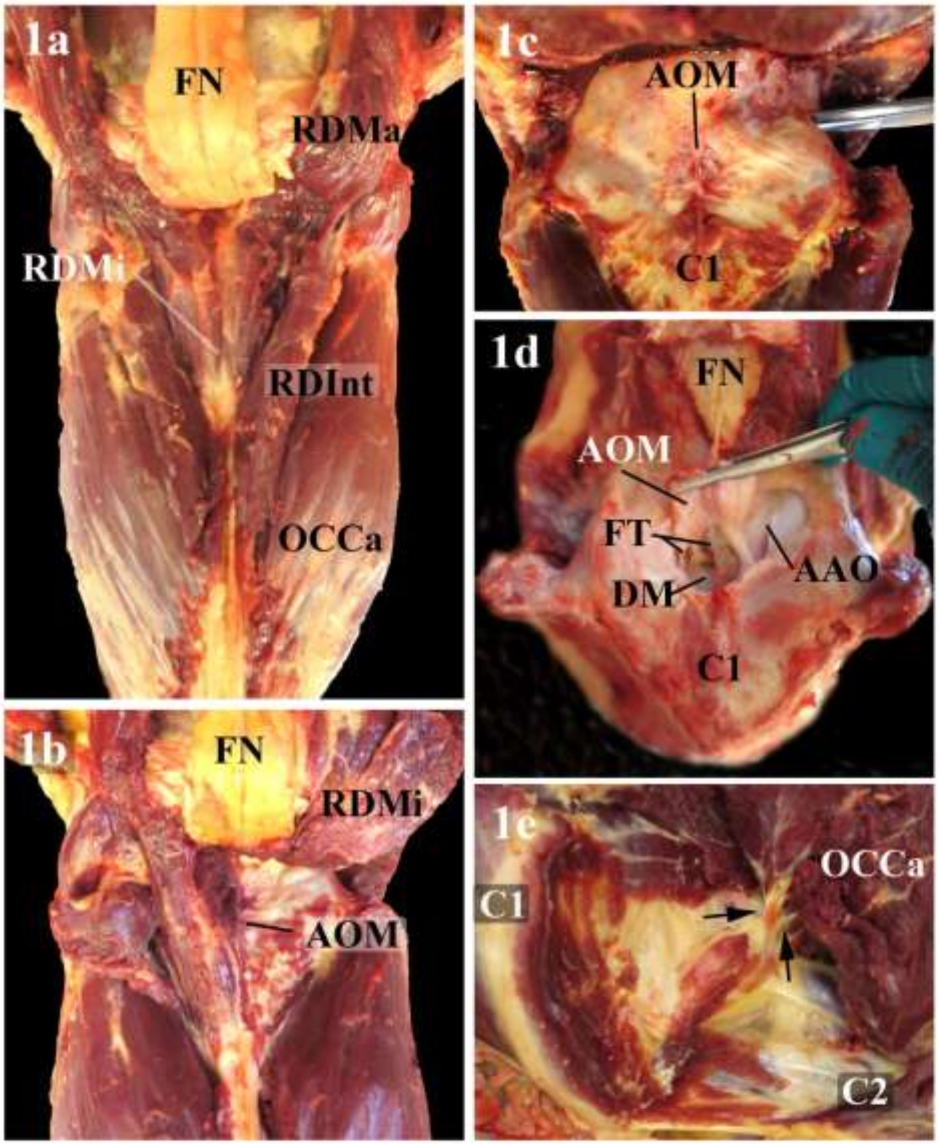

Fig.1. Dissection of the suboccipital region of the horse. Cranial is upwards. AAO (*articulatio atlanto occipitalis),* AOM (*atlanto-occipital membrane),* C1 - C2 (*vertebrae cervicalis primum et secundum),* DM (*dura mater*), FN (*funiculus nuchalis*), FT (*fibrous fibers and trabeculae*), RDInt (*m. rectus dorsalis intermedius*), RDMa (*m. rectus dorsalis major*), RDMi (*m. rectus capitis minor*), OCCa (*m. obliquus capitis caudalis*). Fig.1a, dorsal aspect of the FN, where the RDMa are reflected dorsally and the profound suboccipital muscles are presented. Fig 1.b, the right RDMi is reflected cranially and the AOM shown. The remnants of the muscle are visible on the membrane. Fig 1.c, presentation of the fibrous atlanto-occipital membrane. Notice the large collagen bundles arranged diagonally in the membrane. Fig.1d, the membrane is cut along the caudal bony border of the atlas and lifted. In the epidural space fibrous fibers, trabeculae and adipose cushions are visible. Notice that the epidural space is separated from the AAO with a membrane. Fig.1e, dissection of the atlanto-axial membrane. The OCCa muscle is reflected dorsally and the muscular and epimysial connections to the membrane are shown (black arrows).

2.2.2 Cervical, thoracic, lumbar and sacral regions:

Dissection of the cervical dorsal intervertebral membranes and the related structures caudal from C2, including the lumbo-sacral level, was performed with focus on the cervico-thoracic (C6,7-T1,2) and the lumbo-sacral transition (L6-S1). To study the dorsal intervertebral region, the dorsal muscular structures and ligaments were isolated and in the cervical and lumbar region these were, respectively, the mm. multifidi and the m. interspinales, both belonging to the spinocostotransverse muscle group. In the cervical region the dorsal elastic paraspinal ligaments, which were united with the lamina nuchalis were freed ventrally from their attachments to the medial rims of the two bilateral halves of the dorsal intervertebral membrane. The membrane was cut along the bony borders and the dorsal mesenteria-like sheet between the paraspinal ligaments was protected to further study the connections to the dura mater. In the lumbo-sacral region the membrane was approached between the processi spinosi from a dorsolateral angle. The interspinal muscle and fascia were cut open and a continuum between the fascia, the articular capsular and the membrane was observed and opened along the bony borders of the dorsal foramen to study the connections between the membrane and the dura mater.

Additionally, in three horses the demusculated bony spine was transected bilaterally in the horizontal plane with an oscillating saw taking care to protect the spinal cord, the dorsal dural connections, the dorsal intervertebral membranes and other supraspinal structures. Here the connection between the muscles, the membranes and the dura mater were tested manually by moving and stretching the structures one by one in different directions whilst observing and video-recording the effects.

2.3 MR-scanning (Esaote 0,19 Tesla):

The AO region of two of the horses, was MRI scanned (Esaote 0,19 Tesla) with a T1 weighting, immediately after euthanization. The neck was cut at the level of C5-C6 and the specimen put into a plastic bag. Before the scan, one head was positioned in a neutral posture and the other in flexion and extension postures, respectively.

2.4 Histology:

All layers of the myodural AO bridge (RCMi, atlanto-occipital membrane and the attached dura mater) were cut precisely along the osseous rim of the dorsal intervertebral foramen, kept aligned and lifted carefully to preserve the whole complex. Samples were pinned on a piece of spongostan and fixed in 4 % neutral buffered formalin (NBF). The samples were trimmed into transverse and sagittal sections, before being paraffin embedded using prolonged times for proper infiltration of the tight fascia structures. Each sample was cut into 4-7 μm thick sections and stained with Hematoxylin Eosin (so as to give an overview), van Gieson with and without Resorcin-fuchsin (so as to visualize muscle, collagen and elastic fibers) and alcian blue (in order to visualize the presence of hyaluronan - HA). The preparations were studied using a Leica DMR light microscope and all images were processed using Adobe Photoshop CX4 Extended version.

3. Results

3.1 Dorsal connection to the intervertebral membranes:

In this study we have focused on the anatomy and topography of the equine suboccipital region with particular emphasis on the connections between the suboccipital muscles, the dorsal intervertebral membrane, the spinal dura mater, the MDB's in the AO and AA regions. The myodural connections were verified at both the macroscopic and microscopic level. Dissections and transverse sections showed that the RDMi was attached to the AO membrane and the RDMa and OCCa muscles to the AA membrane (fig.1a,b,c,d,e, fig.3b,c,d). The musculo-membrane attachments were found to be tight trabecular and fibrous and of both peri- and epimysial origin (fig.1e, fig.3c,d). Dorsally in both regions contacts of pure fascial structures were observed. They were parts of the elastic nuchal (AO and AA) and paraspinal ligaments (AA) (fig.2a and fig.3a,b,d). In the mid-saggital plane a mesenteria-like sheet of areolar-like tissue was seen (fig.2a).

Figure 2

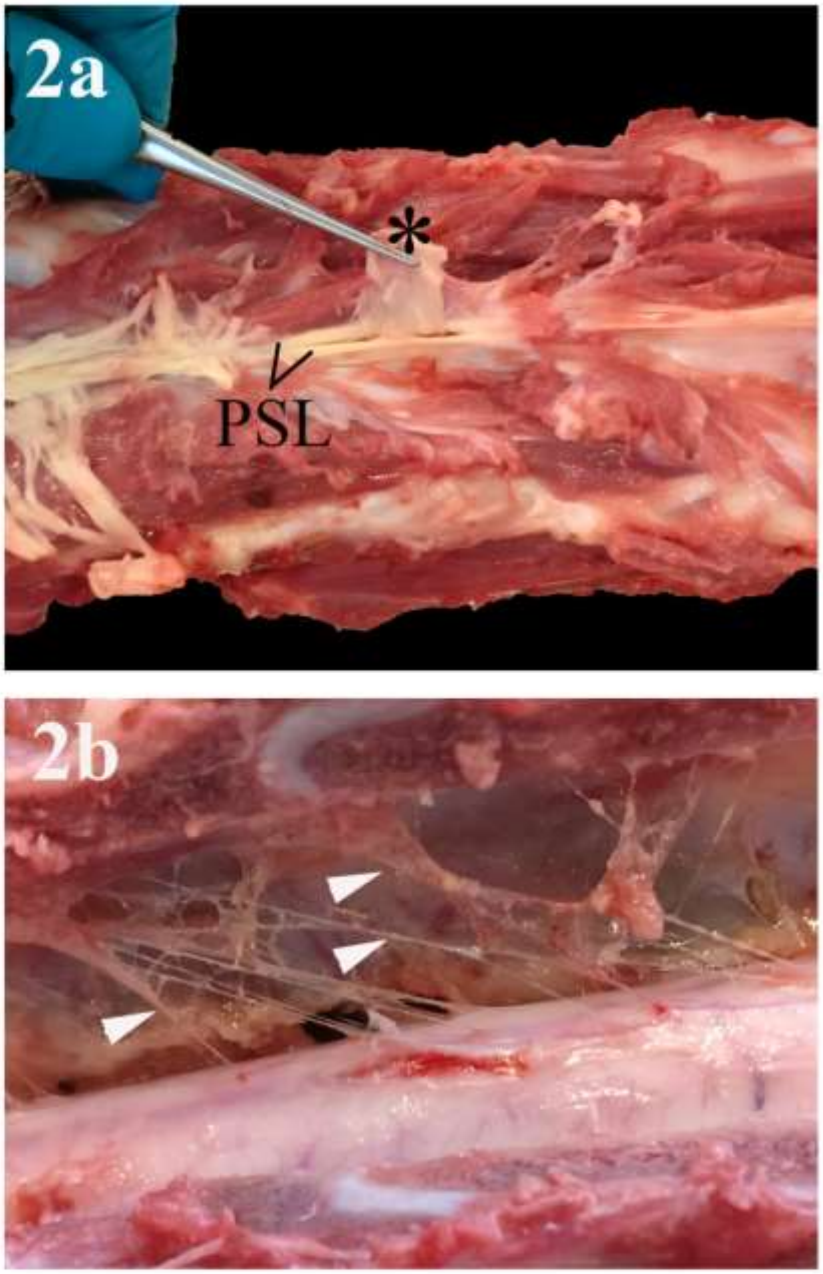

Fig.2. Dissection of the lower cervical vertebrae. Fig.2a, represents the bilateral PSL (*ligamentum paraspinalis*), running dorsally on the vertebrae. The ligament is in contact with the nuchal laminae (left). It is fused over the arcus vertebralis, whereas over the dorsal intervertebral membranes it splits in two. A mesenteria-like sheet of areolar tissue (*) is lifted from between the two halves of the ligament. Fig.2b represents the fibrous fibers (arrowheads) between the membrane and the dura mater in the lower cervical region.

The sheet was found to continue in a dorsal direction between the bilateral laminae and the funicular parts of the lig. nuchae. In the present study it was observed only in the cervical region. The dorsal muscular contact in the cervical region (C3-C7) was the m. multifidi and additionally the bilateral elastic paraspinal ligaments connected to the dorsal surface of the membrane (fig.2a). The ligaments fused latero-laterally in the midplane of the arcus vertebralis whereas they divided into two over the membrane and bordered the two halves of the membrane (see fig. 2a). The mesenteria-like sheet continued into the epidural space bringing vessels and nerves along with it. The dorsal continuation of the mesenteria-like sheet was as described in the AA region. In the thoracic region the m. multifidi, and in the lumbosacral region the m. interspinosus, presented the dorsal muscular membrane contact. In all the regions studied, the membrane was continuous with the joint capsule of the bilateral facet joints.

3.2 The dorsal intervertebral membranes and the dura mater:

In general, the size of the dorsal intervertebral membranes decreased in a cranial to a caudal direction, although in the lumbosacral transition the membrane size was found to increase again.

In this study several similarities, but also some differences, were found between the two most cranial membranes. The AO membrane, which closed the dorsal, semilunar shaped atlanto-occipital foramen, was found to be thick (1-2 mm), tight and fibrous with large collagen bundles interwoven in diagonal and oblique patterns crossing the midline at angles of 120/60 degrees (fig.1c, fig.3c). Remnants of the RCmi persisted between the collagen bundles after the dissection and reflected the very intimate contact (fig.1b,c). In a craniolateral direction the membrane continued into the joint capsules of the bilateral AO joints, and here it changed into a more smooth and elastic composition (fig. 1d). The AA membrane was, in contrast to the AO membrane, more elongated in shape, smooth on the surface, more elastic and the muscle attachments were tight but less invasive as compared to the AO membrane (fig.1e, fig.3a). In the abaxial direction the AA membrane covered the wide AA space and was also found to be in continuum with the laterally located AA joint capsules.

Between the ventral surface of the dorsal intervertebral membranes and the dura mater, multiple trabeculae of variable thickness and also collagen fibers, were found to span through the epidural space, fixing the dura mater in a dorsal position (figs.1d, 2b, 3c). The amount of epidural fibers varied between the body regions and was highest in the AO, AA, the cervicothoracic and the lumbosacral regions.

The contact between the AO and AA membranes and the dura mater was tested manually and found to be very tight. In vitro movements and positions of the head and neck were tested qualitatively on different dissected specimens (fig.3c,d), on transverse and sagittal sections and on the MR-scanned specimens (fig.3a,b). All tests clearly presented and visualized a close structural and functional connection and a tight interaction between the structures constituting the MDB, but also other surrounding structures such as the joint capsules (of the AA and AO joints) and facet joints, the local spinal nerves, the fascia sheets, the muscles and the ligaments. In the upper cervical region, lateral flexion of the head and neck tensed up the contralateral structures of both MDBs (including the dura). A head and neck flexion stretched the MDB (fig.4b) whereas a head and neck extension reduced the tension in the AO region and on the MDB (fig.4a). Simultaneously the AO membrane bulged ventrally into the epidural space and narrowed the subdural space markedly (MR scans). Axial rotation in the AA region tensed up the structures in a diagonal direction of the AA membrane, the paraspinal ligaments and the AA joint capsule (see fig.4c,d)

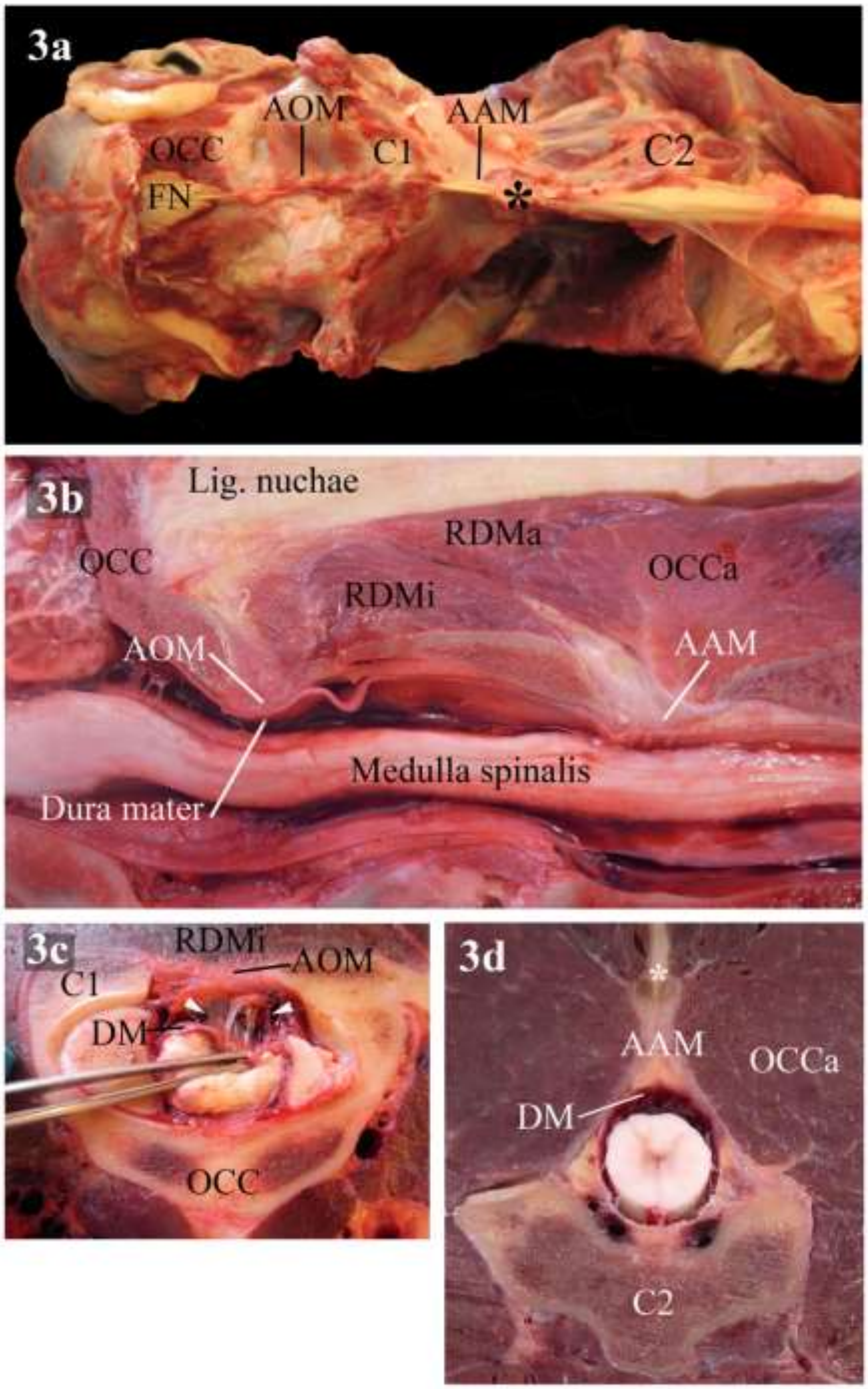

Fig.3. Macroscopical presentations of the AO and AA regions. AAM (*atlanto-axial membrane)*, AOM (*atlanto-occipital membrane)*, AAO (*articulatio atlanto occipitale)*, C1 - C2 (*vertebrae cervicalis primum et secundum)*, DM (*dura mater*), FT (*fibrous fibers and trabeculae*), OCC (*os occipitale)*, OCCa (*m. obliquus capitis caudalis*), RDMa (*m. rectus dorsalis major*), RDMi (*m. rectus capitis minor*). Fig.3a. A dissected and demusculated equine suboccipital region presenting the membranes, the ligaments and the fascia covering the region. Fig.3b. is a saggital section presenting the MDB in the AO and AA regions. The muscle contacts, the membranes, and the contact to the dura mater are presented. Dorsally the lig. nuchae is visible. Fig. 3 c and d are transverse sections of the MDB in the AO (3c) and AA (3d) regions. In Fig.3c, the dura mater is stretched downwards and a multitude of fibrous fibres are present in the epidural space.

3.3 Histology/ microscopy:

Histology of the equine AO and AA MDB´s showed a peri- and epi-mysial contact from the suboccipital muscles to the AO and AA membranes and multiple trabecular structures from these to the dura mater (fig.5a,b).

An intimate contact between the peri- and epi-mysium of the suboccipital muscles and the membrane fibers in both the AO and AA region was present (fig.5a,b). In addition, large clusters of elastic bundles were arranged dorsally and cranial on the AA membrane, representing the attachment of the paraspinal ligaments (fig.5b). The dorsal intervertebral AO and AA membranes themselves were found to be multi-layered with mainly large interwoven collagen bundles (fig.5a,b). The mid layer of the two membranes was found to be the thickest. In the AO membrane this was composed of large collagen bundles arranged at oblique angles. Blood vessels and nerves were located in the clusters of areolar tissue in between these collagen bundles. In the deepest, most ventral layer, smaller collagen bundles were enclosed by areolar tissue containing numerous blood vessels and some nerve sections (fig.5a,b).

Crimping and undulation of the collagen bundles was present in both membranes but only in the AA membrane a solid network of thin elastic fibers, surrounding the collagen bundles, was present. Between the collagen fiber bundles minor islets of areolar tissue were observed in both membranes (fig.5c,d).

The alcian blue stain, which indicates the presence of HA, was found to surround both the larger and smaller collagen bundles. Intense staining was present in the areolar (loose irregular connective) tissue and the structures within (fig.5a). Moreover, the larger bundles of elastic tissue exhibited a very intense and homogenous stain.

Multiple trabecles in the epidural space (fig.5a,b) were observed connecting the membrane to the dura mater. Some trabecles comprised both connective tissue and smaller blood vessels and nerves, others solely connective tissue. The epidural space was in some locations filled with adipose cushions arranged around the trabeculae (fig.5a). The fat cushions were covered by a monolayer of flat squamous epithelium with a peripheral thin alcian blue positive staining.

These histological findings of the suboccipital region not only present the connections between the suboccipital muscle, the membranes and the spinal dura mater (the MDB), they also reveal these connections to be tight and strong contacts, yet also possessing a high degree of plasticity, enabling them to move freely whilst also being capable of withstanding tension forces.

3.4 Integrative observations:

In the upper cervical region, the fascia profunda was found to be a continuous layer with integrations and connections to the external muscles of the spine (RCmi, RCma, OCCa), fascia structures (ligaments, tendons and articular capsules) as well as the structures and membranes inside the canalis vertebralis. The fascia composition and appearance varied from dense collagenous (peri- and epi-mysium, AO dorsal intervertebral membrane) to more elastic and flexible (lig. flava, lig. paraspinale dorsale, ligamentum nuchae and dura mater), and finally to smooth irregular connective tissue connecting and lubricating neighboring structures, thereby enabling these structures to slide and glide and to work independently.

3.5 Magnetic resonance imaging:

The MR scan clearly revealed the individual structures and connections of the AO MDB. The different MR planes presented the RCMi muscle as overlying the membrane and being clearly differentiated from the structures of the MDB (fig.4a,b). The two head postures in flexion (fig.4a) and extension (fig.4b) clearly showed a difference in the tension of the AO membrane, being most tight during flexion.

Figure 4

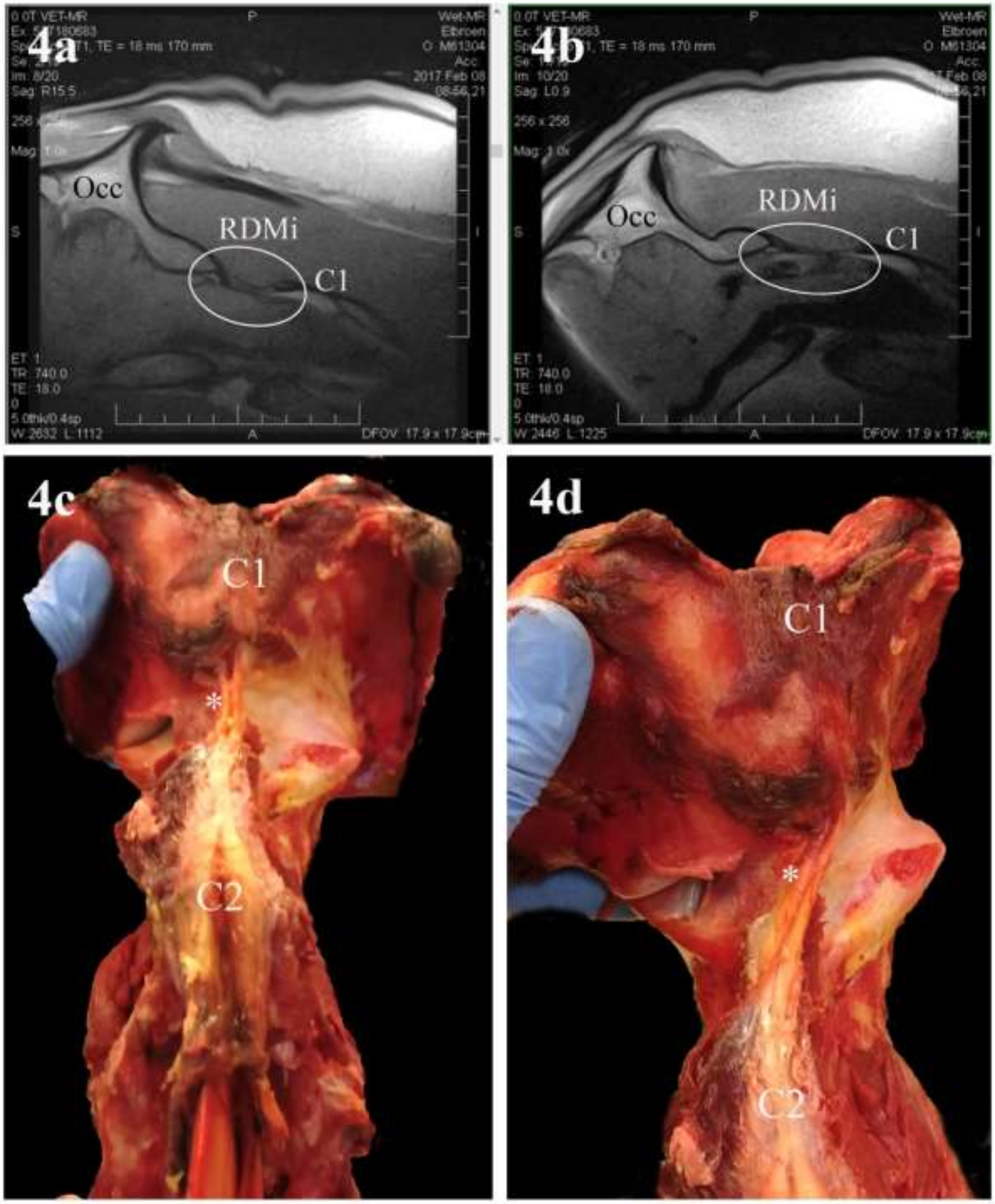

Fig.4. MRI scan of the MDB (white elipse) of the AO-region in an extended (fig. 4a) and a flexed (fig.4b) head posture. In the extension a bulging of the membrane towards the dura mater is visible. Fig.4c and d present the demusculated AA region. Atlas (C1) and axis (C2) and the elastic paraspinal ligaments (*) overlying the AA membrane and the capsula articularis of the left part of the AA joint is opened. In fig.4d the atlas is rotated. Stretching in a diagonal direction of the paraspinal ligaments, the AA membrane and the articular capsule is visible also presenting the tight integration between the structures.

Figure 5

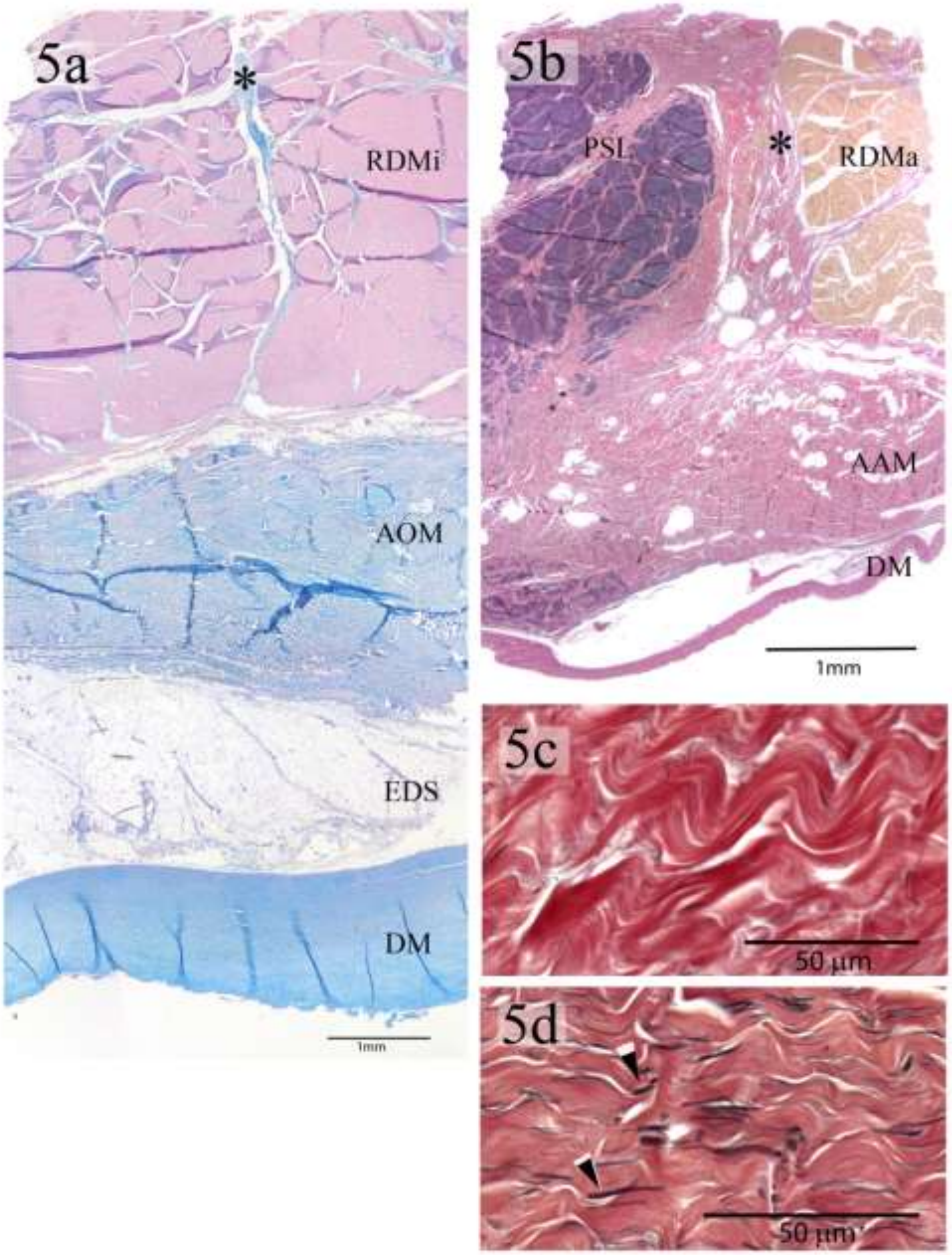

Fig.5. A microsopy section of the MDB in the AO (Fig.5a) and the AA (Fig.5b) regions, as well as close-ups of collagen bundles from the two membranes, AO (Fig.5c) and AA (Fig.5d). AAM (*atlantoaxial membrane),* AAO (*articulatio atlant-occipitalis),* AOM (*atlanto-occipital membrane)*, DM (*dura mater*), EDS (*epidural space),* OCCa (*m. obliquus capitis caudalis*)*,* RDMa (*m. rectus dorsalis major*)*,* RDInt (*m. rectus dorsalis intermedius*)*,* RDMi (*m. rectus capitis minor*)*.* Fig.5a presents the tight contact between the AO membrane and the muscle peri- and epi-mysium. The AO membrane is stained with Alcian blue, which indicates the presence of hyaluronan and thereby the possibility for mobility between these structures. The membrane is overlying the epidural space which is filled with fat cushions arranged around the trablecles. The AA section (Fig.5b) is stained using van gieson and resorcein. The muscle and the elastic paraspinal ligament (PSL) are located dorsally to the membrane and the connection to the dura mater is presented. The close-ups, fig.5c (AO) and d (AA), present the crimping effect of the collagen tissue in both membranes and the related elastic fibers. A markedly higher amount of elastic fibers (black arrowheads) are present in the AA membrane.

4. Discussion

4.1 Overview:

This study has shown that MDB connections are present throughout the whole columna vertebralis of the horse. Furthermore, we reveal for the first time connections to the lig. nuchae as well as a mesenteria-like structure throughout the midline. Histology has revealed morphological differences between the compositions of the AO and AA membranes, which might be related to their function. The MDB's were closely integrated with the surrounding tissues and structures, which underlines the importance of looking at the entire region when studying its biomechanics.

4.2 Definition of components of the myodural bridge:

The connections between the suboccipital muscles and the dura mater were dissected, isolated and described for the first time in the human for the C0-C1 region by Hack and colleagues in 1995 [8]. Thereafter, the membrane-dura connection was given the name of the posterior atlanto-occipital membrane-spinal dura complex (PAOM-SDC). Hack and colleagues described the connections as a tendon like structure running between the suboccipital muscles and the dura mater allowing the involved structures to move as a unit. Since then, several research groups have elaborated on this early work and have studied the connections between the layers in the AA region, as well as the presence and inclusion of e.g the nuchal ligament [1-7, 16, 26]. Indeed, the inter- and intraspecies variations have been verified by several research groups focusing on; i) the attachment of the muscles ii) the intervertebral dorsal membrane, and iii) the dura-membrane complex [1, 12-16].

In this study, detailed macro- and microscopic anatomy has been visualized and described confirming the existence of equine MDB's in both the AO and AA regions. Our findings are consistent with those described in the literature [7, 8, 12-16]. The three distinct major components involved in the connection between the suboccipital musculature and the dura mater include the suboccipital muscles (RDMi, RDMa, OCCa), a connection from the muscles to the dorsal intervertebral membrane (the AO or the AA), and the membrane-dura mater connection.

Additionally, macroscopical studies of the dorsal intervertebral spaces along the whole columna vertebralis were performed to evaluate whether similar MDB complexes were present elsewhere. This focus was especially directed towards the biomechanically challenged regions; the cervico-thoracic and the lumbosacral transitions. In both of these regions and in several other places' connections resembling MDB's were seen.

4.3 The muscle-membrane connection:

The muscles contacting the dorsal intervertebral membrane in the horse differed between the body regions. The muscular part of the AO-MDB in the AO, was found in the present study to be the RCMi. This is comparable to findings for both humans and other mammals [8, 13, 27]. Furthermore, in the AA region the RCaMa as well as the OCCa is part of the MDB in both humans [4-6] and horses. The muscles involved in the mid cervical, towards the lumbar region, changed gradually from the m. multifidi to the m. interspinales. To the best of the authors' knowledge, similar connections to these have not been described in other species.

Three types of bridging structures between the muscle and membrane have been described in humans [16]. The most frequent type was the tendon like structure, the less frequent was the muscle type, and the least frequent, the fascia type. In the present study of the AO and AA regions the combination of the macro- and microscopical observations revealed that in general there is one large and a multitude of smaller fibrous muscle-membrane connections, derived from the peri- and epi-mysium. Inspection of the microscope sections for this region showed how the muscle contacts merged invasively into the membranes and between the collagen fibre bundles of the dorsal part of the membrane. We therefore assume that this type of connection represents both the muscular and fascial type and that in terms of the myofascia, these might be considered as being very similar.

4.4 The membranes:

The dorsal intervertebral membranes are regarded by most Anatomical textbooks as being the ligamentum interarcuata, or in more contemporary terms the ligamentum flava, and of having an elastic appearance. The AO and AA membranes seem in the present study to differ from this viewpoint, as the AO membrane is predominantly fibrous, and the AA membrane comprises a blend of fibrous and elastic components. The oblique collagen bundles in the AO membrane represent the tension lines in the membrane and refer to the direction of the contraction from the OCaCr and OCaCa muscles thereby indicating a lateral flexion of the AO joints, which is supported from studies of in vitro studies of dynamic mobilization of the cervical vertebrae. The intervertebral motions have been studied and discussed in terms of the biomechanics of these joints. Indeed, previously whilst studies on clean bones indicated a major flexion and extension of the AO joint [28], more recent studies of the characteristics of AO joint mobility which have included the muscles, fascia etc. has led to a change in perception towards one of lateral flexion as well [29], a new conclusion that is also supported by the fiber direction of the membrane.

In none of the publications studied by these authors, is there mention of the fiber direction of the collagen fibers in the AO membrane. We assume that the reason for this could be that they are very thin in the other species and it is therefore difficult to verify the lines of tension. In the horse the fascia is generally tight, fibrous and strong and consequently the AO membrane is very thick, and lines of tension are relatively easy to observe. Alternatively, this observation may be the result of the approach adopted in this study, in so far as the researchers in our group have a biomechanical approach to the fascia and the information it can provide.

The size of the dorsal intervertebral membranes varies with the breed studied, but in general the most cranial are the largest and they then decrease in size in a caudal direction. In the horse we observed in the lumbosacral junction, the membrane increased markedly in size compared to the adjacent membranes. This finding also lends support to the specific biomechanics of this region, which is the most flexible region of the back and essential for the horse with a high ROM at fast speeds e.g. canter [30].

4.5 Membrane-dura mater connection:

The connection between the membrane and the dura mater in the present study was found to be one with multiple fibers and trabecles. Some of these comprised both connective tissue and smaller blood vessels and nerves but others were built solely of connective tissue. The epidural space was in some locations filled with flexible adipose cushions arranged around the traversing trabeculae. This latter observation was to our knowledge not described in previous studies. The epidural fibers and trabecular were focused on in previous studies with respect to the differing result of the presence of a direct ligamentous connection between the suboccipital muscles and the dura mater or contact to the AO and AA membrane in between [4, 5, 13].

4.6 Membrane-nuchal connection:

One of the discussion points concerning the human MDB, is whether the ligamentum nuchae anchors to the dorsal intervertebral membranes or not [1, 7]. The equine nuchal ligament is, in contrast to that in the human, highly specialized and developed. It is differentiated into two separate parts, the funicular and the laminar and all subunits are bilateral. In the present study a thin mesenteria-like sheath of connective tissue was found to attach in the midline from the occiput and caudally on the dorsal surface of the vertebrae and the intervertebral membranes. It was found to be in contact with the funicular part of the lig. nuchae. In this way, tension in the mesenterium could be transferred to the muscles, the membranes and to the dura mater of the MDB. Bilateral to the mesenterium, and on the dorsum of the atlas and the AA membrane, the bilateral elastic paraspinal ligaments were shown to be present and to make contact with the nuchal ligament. The ligaments fused with the laminar attachment to the processi spinosi and overlaid and attached to the AA membrane, such that tension in the nuchal ligament was transferred into the paraspinal ligament and further on to the AA membrane and to the dura.

The mesenteria-like sheet in the dorsal midplane, which was observed in the equine cervical region, might be comparable with the human "To be named ligament" [1], which seems to be very similar in its position, although in contrast to the horse, fibrous. This species difference could be explained by the requirements of the long equine neck for a high degree of ROM, bouncing, shock absorption and oscillation compared to the reduced ROM and vertical position of the human neck.

4.7 Midline structures:

In some studies, there has been disagreement between research groups as to whether humans (and other species) have an intervertebral membrane or not [2, 12, 13, 15]. Lirk and colleagues [31] found a high frequency of improper dorsal midline closures of the fascia structures (here ligamentum flavum) in the cervical and cranial thoracic region, giving rise to thoughts of a direct ligament from the epimysium to the dura mater. In our study we observed the midline rims of the membranes to be integrated with the paraspinal ligaments forming a slit-like opening in the membrane through which the mesenteria-like sheath emerged into the epidural space. This construction might be seen as being an incomplete midline fusion, and therefore be approached as being a malformation, which might even be present in the AO and AA regions [1].

4.8 Integration:

The AO and AA membranes were found to interact with the surrounding fascia structures, such as; i) muscle epi- and peri-mysium, ii) inter-muscular connective tissue, iii) capsular articularis of the AO and AA joints on both the lateral and medial sides, as well as iv) the vertebral periosteum. The closely connected membrane and capsular articularis of the intervertebral joints also showed a functional relation. When tensing the membranes or the attached muscles, both sheets of the joint capsule were affected and followed the induced movement.

The fascia structures continued on the inside as the lining of the canalis vertebralis, and on the outside as a connection with the fascia profunda, and thereby with the myofascial and ligamentous structures. An explanation for this structural integration might be found in the embryology of the paraxial mesoderm, and the development of each somite (the dermo-, myo- and sclerotome), between which growth and development of the vertebrae, muscles and fascial structures are induced. In the canalis vertebralis the meningeal layers are developed as the innermost endo-meningeal layer related to the medulla spinalis, and the outermost ecto-meningeal layer, in contact with the vertebral fascia structures on the "inside" of the vertebrae. The condensation of the sclerotomes around the spinal cord and local mesenchyme still permits the inside - outside connection through the dorsal and lateral intervertebral foramina and thereby enables a persistent myodural contact to develop.

4.9 Motion, biomechanics and clinical relevance:

The histological results showed in both equine cervical regions that the majority of fascia structures were stained positive with the alcian blue staining showing the presence of hyaluronan in the peri- and epimysium, the membranes and the trabecular structures in the epidural space. This feature indicated the possibility of motion, sliding and gliding of the major as well as the minor structures in the MDB's [32, 33]. This arrangement improves the capacity of fine tuning, adjusting and transmission of the tension from surrounding structures to the MDB.

The AO membrane had mainly obliquely arranged collagen bundles which crossed over the midline in angles around 120/60 and the AA membrane resembling the articular membrane, being more elastic and possessing larger islets of elastic tissue. The functions of the AO and AA MDB's are speculated by several research groups. One of the major suggestions is pumping of the cerebrospinal fluid. Based on the histological composition of the AO membrane as fibrous, showing crimping effect of the collagen fibres but no elastic fibres the AO membrane is stiff and rigid withstanding traction and tension and a pumping effect of the cerebrospinal fluid is possible.

In both the AO and AA membrane the morphology indicates stability in traction and tension but the presence of numerous tiny elastic fibers around the collagen bundles provides the AA membrane with more elastic features than the AO membrane. In fascia tissue this specific composition gives rise to shock absorption, which could be relevant in the horse due to the long neck, the high ROM as well as oscillation during walking and trotting.

Scali 2011 [4] described how manual traction in the RCPMa had an effect on the dura mater all the way to the first thoracic vertebrae. The RCPma exhibits a connection similar to that of the RCPMi and exerts an even greater mechanical traction on the dura because of its larger cross-sectional area. Thus, any clinical manifestation arising from the RCPMi and its connection to the dura may be replicated and amplified by a connection between the dura and the larger RCPma. I this study different manual tractions and movements were conducted on the specimens. The movements in the muscles interact with and tense up the dura mater and the movements are observed in both membranes and the dura mater underneath (AO and AA). The movement in the RCDMi transferred to the dura mater and was observed in the AO region and similar with the obliquus capitis caudalis attached to the AA membrane. Latero-lateral flexion of the head - neck induces a visible tension in the dura mater. In MRI studies the membrane was clearly observed tightening up in a flexion of the AO joint.

The suboccipital epaxial muscles are regarded as being the major muscles in moving the atlas mainly in latero-lateral flexion [29], whereas the rectus muscles possess proprioceptive characteristics, mechanical traction of the membranes and possible pumping capacity of the cerebrospinal fluid.

Several functions has been proposed such as preventing infolding of the spinal dura mater [8] to trigger cervical neck extensors that would resist hyper flexion or hyper translation [34], play a role in maintaining the integrity of the subarachnoid space [5] or work as a pump to propel the circulation of the cerebrospinal fluid [9].

One of the suggested functions of these structures in humans is to regulate the tension of dura mater and thereby, also the pressure in the cerebrospinal fluid. Another suggestion is that they protect the dura mater by pulling it caudal in cervical extension. The purpose/function of these structures in the horse is still to be investigated.

Numerous equine studies of the anatomy, topography, and various diagnostic techniques in the AO- and AA regions, as well as the lower cervicals have been performed, but no attention has been paid to the myodural connections, even if they seem relevant in relation to these studies. Topics of direct relevance with regard to the MDB that come to these authors minds include, ultrasound guided puncture for myelography [23]; effect of ex vivo flexion and extension on intervertebral foramina dimensions [35]; movement associated reduction of spatial capacity of the equine cervical vertebral canal [36] and endoscopic anatomy of the cervical vertebral canal in the horse [24, 37]. Moreover, with the high incidence (34%) of horses having adverse effects after cervical myolography [37] maybe this invasive procedure should also be considered in terms of possible interference with the MDB.

Additionally, numerous studies of the biomechanics of the equine head and neck have been performed, due mainly to the important role of their function locally, but also with regard to their integral role in locomotion, as stated previously in a review by Zsoldos and Licka [22]. Indeed, the equine neck has developed through evolutionary processes to a design that; 1) balances the head as it stretches and reaches for the ground whilst grazing, 2) enables the horse to remain on guard and ever watchful for potential threats in its immediate surroundings, and 3) facilitates a rhythmic “bouncing” movement of the head when walking and trotting. To this end, the horse, in contrast to e.g. the dog and human, is specifically designed and fine-tuned towards energy “recycling”, and for this reason can perhaps be considered as being more or less one big bundle of fascia. Tight interconnections and -actions between fascia layers and anatomical structures in the horse span from the microscopical [33, 38] to the macroscopical level. The latter as seen in the mechanical stay-apparatus and in different dampening and shock absorbing structures [39, 40].

The structures in the upper neck are targets for a variety of pathological conditions as presented in [19]. Soft tissue trauma can occur in horses as the result of riding accidents, a fall, or being trapped in their box, as well as direct effects of different riding techniques that alter the way in which horses carry their head and neck. Thus, traumas to the nuchal ligament, the m. semispinalis, the suboccipital muscles etc. will without a doubt have an effect on the AO and/or AA myodural bridge as the present study has found them to be highly integrated during the motion tests performed. Further elaborating studies now need to be undertaken in order to quantitatively evaluate the consequences of this finding.

Dyson [19] has described some of the clinical symptoms associated with head and neck trauma as being: pain in the head and neck region, resistance against the use of reins, problems with flexing the head and lowering the head and neck, head shaking and rearing of the head, all of which have an influence on the biomechanics of the thoracolumbar region, as also noticed and reviewed by Zsoldos and Licka [22]. Previous studies of the equine kinetic myofascial lines [41] explain how these myofascial structures span from top to toe in the horse´s body, both whilst standing and in motion. The lines explain and confirm how changes in the 3-D myofascial body network can influence regions far distant from the primary problem. The line, which directly relates to the myodural bridges in the head and neck, but also with the thoracolumbar and lumbosacral regions, is the profound, deep dorsal myofascial kinetic line (Elbrønd, unpublished data). This line comprises muscles and fascia from the spinocostotransversal system, including the suboccipital muscles and the multifidi muscles. Other lines, such as the three superficial myofascial lines, the superficial dorsal, ventral and the lateral line, relate to the MDB’s indirectly via connections in the head and neck region and via the balance point around the temporomandibular joint. In the cervicothoracic and the lumbosacral region several lines (e.g. superficial helical and profound lines) have an influence on the structures closely related to the MDB’s [41]. However, to what extent the myodural bridges and their structures are affected remains an area of speculation. Moreover, since carriage of the head, neck and body is important in different equestrian sports, the function of these structures should now be the subject of further investigation. From a human clinic perspective, there is considerable knowledge of the effects of head and neck trauma and their relation to invalidating clinical symptoms e.g. headache [10, 42]. Thus, perhaps the human clinic expertise in combination with the present findings, which demonstrate a tight connection between structures in the equine head and neck with the dura mater, can now be used to bring a renewed and enlightened focus in the equestrian world to horses with undiagnosed head and neck problems or low performance, as well as the effects of tight fitting tack on and around the suboccipital area, the consequences of training in a hyperflexed neck position, and atlanto-occipital injections.

Conclusion

In the present study, myodural bridges between the suboccipital muscles, the dorsal intervertebral membrane and the dura mater in the dorsal atlanto-occipital and atlanto-axial spaces have been identified in horses, using both micro- and macroscopic studies, as well as the use of MRI. The connections mirror those found in humans, five other mammals, a crocodile, a bird and the sperm whale, as described in the literature. The dissections revealed a clear integration with the other structures in the region, indicating that the upper neck biomechanic has an influence on the dura mater, and might be integral to the proposed pumping effect on the cerebrospinal fluid. Furthermore, the present study has shown the existence of similar MDB's throughout the spine, with the muscular connections being the multifidi and interspinal muscles. Finally, it is most exciting to note that those areas associated with the largest degree of mobility, such as the cervico-thoracic and lumbo-sacral junctions, possess the tightest fibrous connections between the membrane and the dura mater.

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Abbreviations

AA: Atlanto-axial
AAM: Atlanto-axial membrane
AO: Atlanto-occipital
AOM: Atlanto-occipital membrane
DM: Dura mater
EDS: Epidural space
MDB: Myodural bridge
OCCa: M. obliquus capitis caudalis
OCCr: M. obliquus capitis cranialis
PSL: Ligamentum paraspinalis
RDMa: M. rectus capitis dorsalis major
RDMi: M. rectus capitis dorsalis minor

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