

1 **Title page**

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6  
7 **Original article**

8  
9 **Structure and innervation of the equine supraspinous and interspinous ligaments**

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## 42 **Summary**

43 Pain related to the osseous thoracolumbar spine is common in the equine athlete, with  
44 minimal information available regarding soft tissue pathology. The aims of this study were to  
45 describe the anatomy of the equine SSL and ISL (supraspinous and interspinous ligaments) in  
46 detail and to assess the innervation of the ligaments and their myofascial attachments  
47 including the thoracolumbar fascia. Ten equine thoracolumbar spines (T15-L1) were  
48 dissected to define structure and anatomy of the SSL, ISL and adjacent myofascial  
49 attachments. Morphological evaluation included histology, electron microscopy and  
50 immunohistochemistry (S100 and Substance P) of the SSL, ISL, adjacent fascial attachments,  
51 connective tissue and musculature. The anatomical study demonstrated that the SSL and ISL  
52 tissues merge with the adjacent myofascia. The ISL has a crossing fibre arrangement  
53 consisting of four ligamentous layers with adipose tissue axially. A high proportion of single  
54 nerve fibres were detected in the SSL (mean = 2.08 fibres/mm<sup>2</sup>) and ISL (mean = 0.75  
55 fibres/mm<sup>2</sup>), with the larger nerves located between the ligamentous and muscular tissue. The  
56 oblique crossing arrangement of the fibres of the ISL likely functions to resist distractive and  
57 rotational forces, therefore, stabilizing the equine thoracolumbar spine. The dense sensory  
58 innervation within the SSL and ISL could explain the severe pain experienced by some  
59 horses with impinging dorsal spinous processes. Documentation of the nervous supply of the  
60 soft tissues associated with the dorsal spinous processes is a key step toward improving our  
61 understanding of equine back pain.

62

## 63 **Introduction**

64 Pain related to osseous and soft tissue pathology of the thoracolumbar spine is a common  
65 condition in the equine athlete (Denoix and Dyson, 2011). Over-riding or impinging DSPs  
66 (dorsal spinous processes) and osteoarthritis of the articular process joints are the frequently  
67 diagnosed bone related causes of back pain, usually localised between T15 and L1 (Walmsley  
68 et al., 2002; Girodroux et al., 2009; Cousty et al., 2010). Soft tissue lesions are mainly  
69 described in the M. longissimus dorsi, M. multifidus and SSL (Jeffcott, 1980; Gillis, 1999;  
70 Stubbs et al., 2010).

71 In man, the TLF (thoracolumbar fascia) is a commonly diagnosed source of non-specific  
72 lower back pain (Gibson et al., 2009; Willard et al., 2012; Schilder et al., 2014). Fascia  
73 performs an important role in transmitting mechanical forces between muscles. With acute  
74 inflammation, fascia tightens and becomes less flexible (Schleip, 2003). The detailed  
75 anatomy and sensory innervation of the TLF, SSL (supraspinous ligament) and ISL  
76 (interspinous ligament) have been investigated in man and in other animals; however, there is  
77 little information about these tissues in horses (Jiang et al., 1995a; Johnson and Zhang, 2002;  
78 Tsao et al., 2010; Tesarz et al., 2011; Hoheisel and Mense, 2015).

79 The general macroscopic appearances of the equine TLF, SSL and ISL have been described  
80 without concurrent description of the microscopic structure (Budras et al., 2003; Clayton et  
81 al., 2005; Liebich and König, 2007). The innervation of the epaxial musculature in horses has  
82 been detailed macroscopically to the level of the articular process joints; with little  
83 information regarding innervation of the tissues further dorsal (Vandeweerd et al., 2007).  
84 Therefore, the aims of this study were to describe the anatomy of the SSL, ISL and TLF of  
85 the equine thoracolumbar spine; and to assess the innervation of the ligaments and  
86 aponeurotic myofascial layers in microscopic detail. The authors hypothesise that the soft

87 tissues adjacent to the DSPs contain a dense sensory innervation similar to that described in  
88 humans.

## 89 **Material and Methods**

### 90 *Anatomical study*

91 The thoracolumbar spines of 10 skeletally mature horses (4-18 years) humanely destroyed for  
92 reasons unrelated to thoracolumbar pathology were included in the study. Informed owner  
93 consent for tissue retention was obtained and approval for the study was given by the local  
94 Committee on Research Ethics. The breeds represented were Thoroughbred (3), Warmbloods  
95 (1), Irish Sports Horse (3), Cob (1), Arabian (1) and Lusitano (1). There were six geldings  
96 and four mares.

97 Cadavers were placed in left lateral recumbency and the skin was removed, followed by  
98 reflection of the STF (superficial trunk fascia) and M. cutaneous trunci. The fascial  
99 attachments of the M. trapezius, M. latissimus dorsi and M. longissimus dorsi were  
100 determined before the muscle was separated from the fascia overlying the M. multifidus. The  
101 fibre arrangement of this fascia was documented before the ventral attachments were  
102 transected and the fascia reflected dorsally. The fascicles of the M. multifidus were detached  
103 from their ventral insertions on the mammillary processes to allow access to the ISLs.  
104 Meticulous anatomical dissection was performed to determine the structure and orientation of  
105 the ligamentous fibres of the SSL and ISL between T15 and L1. The relationship between  
106 different layers of fascia and their origins on the DSPs, the SSL and ISL was documented  
107 photographically.

### 108 *Histological study*

109 One tissue sample of the SSL and interconnecting ISL, including the inserting fascial planes  
110 and adjacent musculature, was harvested from each interspinous space between T15 and L1

111 in each specimen within two hours following euthanasia (Fig. 1). The sections were cut  
112 immediately adjacent to the bony margins of the DSPs from the level of the SSL to the most  
113 ventral part of the ISL at the intervertebral junction. Samples were fixed in 10% buffered  
114 formalin for 48-72 hours before being trimmed for paraffin embedding and tissue sectioning.  
115 Two slides per DSP interface were prepared for histological examination. One slide  
116 contained a transverse section of the SSL including the origin of related fascia and muscle  
117 layers. The second slide contained a dorsal and a ventral transverse section of the ISL and  
118 adjacent musculature (Fig. 1).

119 Hematoxylin and eosin (H&E) staining was performed for histomorphological evaluation of  
120 SSL, ISL, adjacent fascia and muscular tissue. Tissue was categorised as ligamentous,  
121 adipose or muscular. Digital photomicrographs were prepared (Nikon Eclipse i80 equipped  
122 with Nikon DS 5mc digital camera; 1600x1200 pixels) from which the cross-sectional area of  
123 the different tissue categories in each slide was determined (Image J, National Institute of  
124 Health; Schneider et al., 2012) and the percentage area of each tissue was calculated.  
125 Immunohistochemistry was performed following a previously described protocol; Ressel et  
126 al., 2015). For the quantitative evaluation of nervous tissue anti-S100 antibody (rabbit  
127 polyclonal anti-S100; Dako; dilution 1:100) was utilized. Nerves were ranked as small (single  
128 nerve fibre); medium (bundle of 2-5 nerve fibres) or large (bundle of  $\geq 6$  nerve fibres) (Fig.  
129 2C). The number of nerve fibres in collagenous, adipose or muscular tissue was documented  
130 and the neuron density within each tissue type was calculated (nerves/mm<sup>2</sup>) for each  
131 individual tissue section.

132 With four randomly selected tissue samples of the SSL and ISL, further staining was  
133 performed to detect contractile elements using anti-alpha smooth muscle actin [ $\alpha$ SMA]  
134 (mouse monoclonal clone 1A4; Dako; dilution: 1:300) and anti-desmin (mouse monoclonal  
135 clone D33; Dako; dilution: 1:100) antibodies. The presence of non-myelinated sensory nerve

136 endings was investigated with anti-human/equine Substance P (rabbit polyclonal; Biorbyt;  
137 dilution: 1:500). Tissue sections of normal spinal cord including nerve rootlets was used as  
138 control for S100 and Substance P, while smooth muscle of intestine served as control for  
139  $\alpha$ SMA and desmin.

140 Four additional sets of ISL and SSL tissue samples were collected from the interspinous  
141 spaces between T15 and L1 immediately post mortem, fixed in glutaraldehyde and processed  
142 rapidly for transmission electron microscopy looking for the presence of non-myelinated  
143 sensory nerves, as previously described (Finotello et al., 2016).

#### 144 *Statistical analysis*

145 Data were recorded in Excel (Microsoft Inc.)<sup>a</sup> and analysed in Minitab 17 Inc.<sup>b</sup> and STATA  
146 14 (StataCorp)<sup>c1</sup>. Descriptive analysis was performed for the nerve distribution in the SSL,  
147 ISL and adjacent tissues. Statistically significant differences in nerve fibre density between  
148 SSL and ISL sections, recorded tissue types (ligament, fat and muscle), different interspinous  
149 spaces (T15-L1) and between specimens were determined using the non-parametric Kruskal-  
150 Wallis test followed by the One-Sample Wilcoxon Signed Rank test. P values <0.05 were  
151 considered significant.

## 152 **Results**

### 153 *Anatomical study*

154 In the dorsal midline, the superficial trunk fascia (STF) merged with the SSL and the STF of  
155 the opposite side (Fig. 1 and 2A). Dorsally, the STF consists of three layers with the

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<sup>b</sup> Minitab Inc., State College, Pennsylvania, USA.

<sup>c</sup> StataCorp., College Station, Texas, USA.

156 superficial and the deep layers composed of areolar tissue with variable amounts of fat  
157 deposition (2-10mm thickness). The middle (main) layer of the STF is largely composed of  
158 firm collagenous tissue. Like the STF, the TLF (or deep trunk fascia) consists of three layers,  
159 two thin adipose outer layers and one substantial collagenous middle layer. The middle layer  
160 is composed of multiple tendinous fascicles, which angle axially to merge into and form a  
161 main component of the SSL (Fig. 1 and 2A).

162 The fascia of the *M. latissimus dorsi* merges into the STF in a cranio-abaxial to caudo-axial  
163 direction, becoming a major part of the STF. The TLF overlies most of the *M. longissimus*  
164 *dorsi* and may be referred to as the dorsal myofascia of the *M. longissimus dorsi*. In the  
165 caudal thoracic and lumbar spine (T17-L5), the *M. longissimus dorsi* is subdivided in a  
166 sagittal plane by an internal fascial layer originating from the TLF about 8-10 cm abaxial to  
167 the dorsal midline (Fig. 1A). The caudal aspect of the *M. longissimus dorsi* between T17-L5  
168 is covered by the *M. gluteus medius* with the two muscles separated by firm fascia arising  
169 from the TLF.

170 The ventral fascia of the *M. longissimus dorsi* forms fascicles that insert onto the caudal  
171 aspect of the articular and mammillary processes of each vertebra in the thoracolumbar spine.  
172 The fibre direction of these fascicles is cranioventral to caudodorsal, whereas the multiple  
173 fascicles of the *M. multifidus* are oriented from craniodorsal to caudoventral (Fig. 3). The  
174 fascicles of the ventral fascia of the *M. longissimus dorsi* span one to two DSPs before they  
175 blend into the SSL and the most dorsal aspect of the ISL (Fig. 2A and 3). The majority of the  
176 segmental fascicles of the *M. multifidus* arise from the caudolateral aspect of the DSPs with  
177 some scattered fibres originating from the abaxial layer of the ISL (Fig. 4). The largest of the  
178 segmental muscle bands of the *M. multifidus* cross three to four DSPs before attaching on the  
179 cranial aspect of a more caudal mammillary process.

180 The most dorsal aspect of the SSL consists of the fibres of the STF with the majority of the  
181 body of the ligament being composed of a crossing arrangement of the fibres of the merging  
182 TLF. The most ventral fibres of the SSL either continue into the ISL or are closely attached to  
183 the dorsal DSPs. The structures that merge to form the SSL between T15-L1 include the STF,  
184 TLF, ventral fascia of the M. longissimus dorsi, fascicles of the M. multifidus and the ISL.  
185 The TLF and the ISL fibres make up the majority of the structure of the SSL (Fig. 1 and 2A).  
186 The ISL consists of up to four ligamentous sagittal layers that blend into the SSL dorsally.  
187 The fibres of the two abaxial ISL layers course between the caudoventral aspect of the rostral  
188 DSP and the craniodorsal aspect of the caudal DSP. The fibres of the two axial ISL layers  
189 course in the opposite direction, from rostradorsal to caudoventral (Fig. 4). The abaxial layer  
190 is thinner and the fascicles smaller than those of the axial layer. A proportion of the fascicles  
191 of the M. multifidus and the DSP periosteum merge into the abaxial layers of the ISL.  
192 Especially in the thoracic spine, the abaxial layers of the ISL insert close to the articular  
193 process joint capsules. A core of loose adipose tissue lies between the axial ISL ligamentous  
194 layers (Fig. 2B).

#### 195 *Histological study*

196 The histological assessment of the SSL and ISL confirmed the previously described close  
197 association between the ligaments and adjacent fascial planes (Fig. 2A). A funicular core of  
198 the SSL was detected inconsistently in the most cranial section of the ligament (T15-T16) in  
199 some horses (n=6) but was not present in the majority of the sections examined (n=34). A  
200 clear distinction was not detectable between the fibres of the SSL and ISL in the transition  
201 area between the two ligaments. A core of adipose tissue was present in the midline between  
202 the axial ISL ligamentous layers (Fig. 2B).



203 The S100 immunostain revealed distinct single nerve fibres and fascicles in the SSL, ISL,  
204 adipose tissue and the musculature (Fig. 2C). The highest density of single nerve fibres was  
205 detected in the SSL (2.08 fibres/mm<sup>2</sup>) and in the M. multifidus (2.35 fibres/mm<sup>2</sup>). A high  
206 proportion of single nerve fibres was also detected in the ligamentous fibres (0.75  
207 fibres/mm<sup>2</sup>) and the adipose core (1.79 fibres/mm<sup>2</sup>) of the ISL. Medium and large sized  
208 nerves were evident mainly in the loose connective tissue between the SSL, ISL and the M.  
209 longissimus and multifidus (Table 1). Quantitative assessment of nerve fibres did not identify  
210 a significant difference in the density of nerve fibres between the tissues sampled from  
211 different interspinous spaces ( $p = 0.073$ ). A wide variation of neuron density within the SSL  
212 and ISL was found between the different horses ( $p = 0.001$ ). Kappa coefficient indicated  
213 good intra- and inter-observer agreement (0.93 and 0.74, respectively) for the quantitative  
214 assessment of nerves within the described tissues.

215 The staining with  $\alpha$ SMA and desmin antibodies confirmed that the SSL and ISL were largely  
216 free of contractile elements. Very few, scattered small  $\alpha$ SMA-positive fibres were detected  
217 throughout the sections examined (Fig. 5A). Substance P identified a high number of small  
218 nerve endings within the ligamentous tissue of the SSL and ISL, smaller than the single nerve  
219 fibres detected with the S100 immunostain (Fig. 2C and 5B). Transmission electron  
220 microscopy confirmed the presence of cytoplasmic cellular processes consistent with small,  
221 sensory nerve endings exhibiting a double membrane and mitochondria within the collagen  
222 bundles of the SSL and ISL sections (Fig. 6).

## 223 **Discussion**

224 The anatomical study demonstrated intimate connection between the SSL, ISL and the  
225 myofascia of the equine epaxial musculature. The oblique crossing arrangement of the M.  
226 longissimus dorsi and M. multifidus fibres and the crossed arrangement of the ISL fibres may  
227 indicate a shared biomechanical role of these structures, counteracting forces of distraction

228 along the equine thoracolumbar spine. As with human anatomy, a dense sensory innervation  
229 was found within the SSL and ISL, with the larger nerves located in the loose connective and  
230 muscular tissue.

231 The SSL is a well-defined structure with anatomical similarities across a diverse range of  
232 species including bipeds, pseudo-bipeds and small quadrupeds (Jiang et al., 1995b). In  
233 contrast, the anatomical structure of the ISL varies significantly between species. In the dog  
234 and cat the ISL is poorly developed, except dorsally where a thin double layer of distinct  
235 fibres merges with the TLF. A significant difference has been described in the fibre  
236 orientation between the ISL in baboons and humans with the well-developed, bilateral elastic  
237 fibres of the ISL in the baboon taking a direct craniocaudal course between the DSPs  
238 (Heylings, 1980). Like the equine ISL, the human ISL has been shown to be arranged in axial  
239 and abaxial layers with a fat filled slit-like midline cavity (Heylings, 1978). As in this study,  
240 the majority of the ISL fibres in humans and pigs are directed from craniodorsal to  
241 caudoventral with a thin, superficial layer of fibres directed in caudoventral to craniodorsal  
242 direction (Aspden et al., 1987; Scapinelli et al., 2006).

243 In man, the SSL and ISL maintain spinal stability by providing both mechanical constraint  
244 and neuromuscular feedback, while aiding force transmission from the TLF to the vertebrae  
245 (Aspden et al., 1987; Yahia et al., 1988). Both ligaments limit mainly the ventral flexion of  
246 the thoracolumbar spine in man and may play a similar role in the horse since the caudal  
247 thoracic and lumbar spine has been shown to be the least mobile region of the equine back  
248 (Prestar, 1982; Townsend et al., 1983; Hindle et al., 1990). In the thoracic spine, lateral  
249 bending is always accompanied by axial rotation which is limited by the rib cage (Townsend  
250 et al., 1983; Townsend et al., 1984; Faber et al., 2001a/b). The oblique crossing arrangement  
251 of fibrous bundles of the equine ISL is likely to counteract tensile and rotational forces of  
252 distraction between the DSPs in the caudal thoracic and cranial lumbar spine. Like in humans

253 and other quadrupeds, the M. multifidus provides intersegmental stability and stiffness in the  
254 equine thoracolumbar spine (Kaigle et al., 1995; Wilke et al., 1995; Stubbs et al. 2010). The  
255 close association between the ventral fascia of the M. longissimus dorsi, the multifidus fascia  
256 and the ISL suggest that all of these structures participate in the core stabilisation of the  
257 equine thoracolumbar spine.

258 Similar to the extensive neural network identified in the human SSL, ISL and TLF, the nerve  
259 endings identified in the horse likely provide a neurological feedback mechanism for the  
260 protection and stabilisation of the spine (Jiang et al., 1995a; Scapinelli et al., 2006; Tesarz et  
261 al. 2011). In the rat, induced inflammation in the TLF resulted in increased nociceptive input  
262 to the lumbar dorsal horn neurons with an increase in nociceptive fibres when compared to  
263 controls (Hoheisel et al., 2015; Hoheisel and Mense, 2015). Based on the histological study  
264 of the equine SSL, ISL and TLF, the free nerve endings identified adjacent to the collagen  
265 fibres are believed to be mainly responsible for the sensory innervation of these tissues. The  
266 high number of Substance P positive free nerve ending when compared to S100 positive nerve  
267 bundles indicate a higher prevalence of non-myelinated fibres within the structures examined,  
268 which was also confirmed by electron microscopy. In the horse, inflammation in the SSL and  
269 ISL associated with muscular pain or as a result of impingement of the DSPs could potentiate  
270 sensory stimulation in this region, explaining the high level of resentment to ridden exercise  
271 noted in many of these cases. Clinical studies have suggested that chronically active “kissing  
272 spines” may be associated with desmitis of the ISL or a compartment syndrome in the area of  
273 the interspinous space (Coomer et al., 2012). Further work is required to investigate the  
274 alteration in sensory innervation in diseased tissue to determine the role of myofascial pain in  
275 relation to back pain in horses. In addition, all surgical techniques to treat impinging DSPs in  
276 the horse result in trauma to the SSL, ISL and the fascial attachments (Walmsley et al., 2002;  
277 Perkins et al., 2005; Coomer et al. 2012). The influence of this disruption on the

278 biomechanics and the nociceptive feedback in the thoracolumbar spinal region has not been  
279 explored and could have long term implications for horses undergoing spinal surgery.

280 Interestingly, the highest density of small nerve endings was identified in the SSL and M.  
281 multifidus. The high density of small sensory nerve endings as well as larger, afferent nerves  
282 identified in the equine M. multifidus is consistent with the neuroanatomical pathway of  
283 nociception of the M. multifidus described in rats (Taguchi et al., 2007). The significance of  
284 the SSL in equine back pathology is controversial and lesions within the ligament are difficult  
285 to diagnose (Henson et al., 2007; Lamas, 2013). Nociceptive fibres within the SSL have been  
286 associated with lower back pain in human patients (El-Bohy et al., 1988). Based on the  
287 histological study of the equine SSL, pain associated with the SSL may be explained by the  
288 ligament's dense sensory innervation.

### 289 *Conclusion*

290 In summary, the anatomical features described in this study, particularly the oblique crossing  
291 fibre arrangement of the ISL layers and the myofascia of the M. multifidus and M.  
292 longissimus are likely to stabilise the equine thoracolumbar spine and function to resist  
293 tensile and rotational forces of distraction along the DSPs. The dense sensory innervation  
294 within the SSL and ISL could provide an anatomic basis for the perception of the severe pain  
295 experienced by some horses with DSP impingement. The documentation of the anatomical  
296 structure and nervous supply of the soft tissues associated with the DSPs is a key step toward  
297 improving our understanding of equine back pain.

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449

450 **Table 1**

451 Quantitative assessment of nerve distribution calculated as nerve fibre/mm<sup>2</sup> within ligament,  
 452 fat or muscle. Small = one nerve fibre; medium = 2-5 nerve fibres; large =  $\geq 6$  fibres. The  
 453 muscle adjacent to the SSL represents the M. longissimus dorsi and the muscular tissue  
 454 adjacent to the ISL consists of the fibres of the M. multifidus.

nerves/mm <sup>2</sup>	Supraspinous ligament (SSL)			Interspinous ligament (ISL)		
	ligament	fat	muscle	ligament	fat	muscle
small nerves						
mean/median	2.08 / 2.05	1.62 / 1.39	1.60 / 1.11	0.75 / 0.34	1.79 / 0.85	2.35 / 2.17
range	0.05 – 5.41	0.34 – 6.49	0.0 – 7.61	0.0 – 3.32	0.0 – 25.52	0.05 – 6.59
± SD	± 1.24	± 1.25	± 1.81	± 0.88	± 4.12	± 1.8
medium nerves						
mean/median	0.19 / 0.15	0.29 / 0.25	0.33 / 0.18	0.10 / 0.04	0.53 / 0.21	0.56 / 0.41
range	0.0 – 1.14	0.06 – 1.25	0.0 – 2.29	0.0 – 0.57	0.0 – 5.0	0.0 – 2.63
± SD	± 0.19	± 0.22	± 0.44	± 0.14	± 0.97	± 0.6
large nerves						
mean/median	0.05 / 0.04	0.15 / 0.11	0.10 / 0.06	0.05 / 0.01	0.36 / 0.07	0.22 / 0.19
range	0.01 – 0.15	0.0 – 0.64	0.0 – 0.96	0.0 – 1.04	0.0 – 4.64	0.0 – 0.6
± SD	± 0.03	± 0.13	± 0.16	± 0.17	± 0.82	± 0.18

455

456

457 **Figure legends**

458 Figure 1. Schematic illustration of the anatomical connection between the SSL, the ISL and  
459 the adjacent myofascial attachments at the level of T18. A. Transverse section of the caudal  
460 thoracic spine indicating the area of interest for histomorphological assessment (grey box) B.  
461 Detailed illustration of the anatomy and sites for tissue harvesting in the thoracolumbar spinal  
462 region. 1 = tissue section harvested from each interspinous spaces T15-L1, containing a  
463 transverse section of the SSL including the origin of fascia and muscle layers and the  
464 overlaying STF. 2 = dorsal transverse section of the ISL. 3 = ventral transverse section of the  
465 ISL. Each section includes about 50% ISL and 50% adjacent musculature.

466

467 Figure 2A. Hematoxylin and eosin transverse section (20x magnification) of the SSL with  
468 inserting fascial planes (I); ISL (II); STF (III); TLF (IV); M. longissimus dorsi (V); ventral  
469 fascia of the M. longissimus (VI).

470 Figure 2B. Hematoxylin and eosin (40x magnification) section of the ISL demonstrating the  
471 different components: muscle (arrow), ligament (white arrow), adipose tissue (arrowhead) of  
472 the ISL.

473 Figure 2C. Immunostain S100 (400x magnification) section of the ISL showing loose  
474 connective tissue with small (one single nerve fibre; black arrow); medium (bundle of 2-5  
475 nerve fibres; white arrow) and large (bundle of 6 or more; arrowhead) nerve fibres.

476

477 Figure 3. Photograph of the fascial attachments of the ventral fascia of the M. longissimus  
478 dorsi (white arrow) and the fascicles of the M. multifidus (black arrow) on the vertebral  
479 articular processes (a) (T17-L1). The fascicles of the M. multifidus arise from the caudal  
480 aspect of the DSPs and the abaxial layer of the ISL. The fascicles cross approximately three  
481 to four DSPs before they attach on the articular processes in the thoracolumbar spine. The

482 fascicles of the ventral fascia of the M. longissimus dorsi have their origin at the caudal  
483 aspect of the articular and mammillary processes of each vertebra, overlie the M. multifidus  
484 fascicles and span one to two DSPs before they blend into the SSL and the most dorsal aspect  
485 of the ISL further caudal.

486

487 Figure 4 A-C. Photographs of the ISL with attachments of the fascicles of the M. multifidus  
488 (m) to the abaxial layer of the ISL (a), overlying the axial layer of the ISL (b). The fibres of  
489 the two abaxial ligamentous layers course between the caudoventral aspect of the rostral DSP  
490 and the craniodorsal aspect of the DSP that lies caudal to the ISL. The fibres of the two axial  
491 layers course in the opposite direction, from dorsorostral to caudoventral.

492

493 Figure 5A. Alpha smooth muscle actin ( $\alpha$ SMA) stain (400x magnification) of a section of the  
494 SSL between T17-T18. The density of contractile elements is low within the collagen bundle  
495 shown. Black arrow = scattered contractile element. White arrow: negative fibroblast;  
496 arrowhead: internal positive control: smooth muscle cells of small blood vessel wall.

497 Figure 5B. Section of the ISL between T15-T16. Using Substance P stain (400x  
498 magnification) medium sized (arrow) and very small (arrowhead) single fibre nerve endings  
499 are present within the ligamentous tissue, smaller than the single nerve fibres that were found  
500 using the S100 immunostain. Small Substance-P positive nerve fibres are evident in  
501 transversal (arrowhead) or occasionally longitudinal (inset) section.

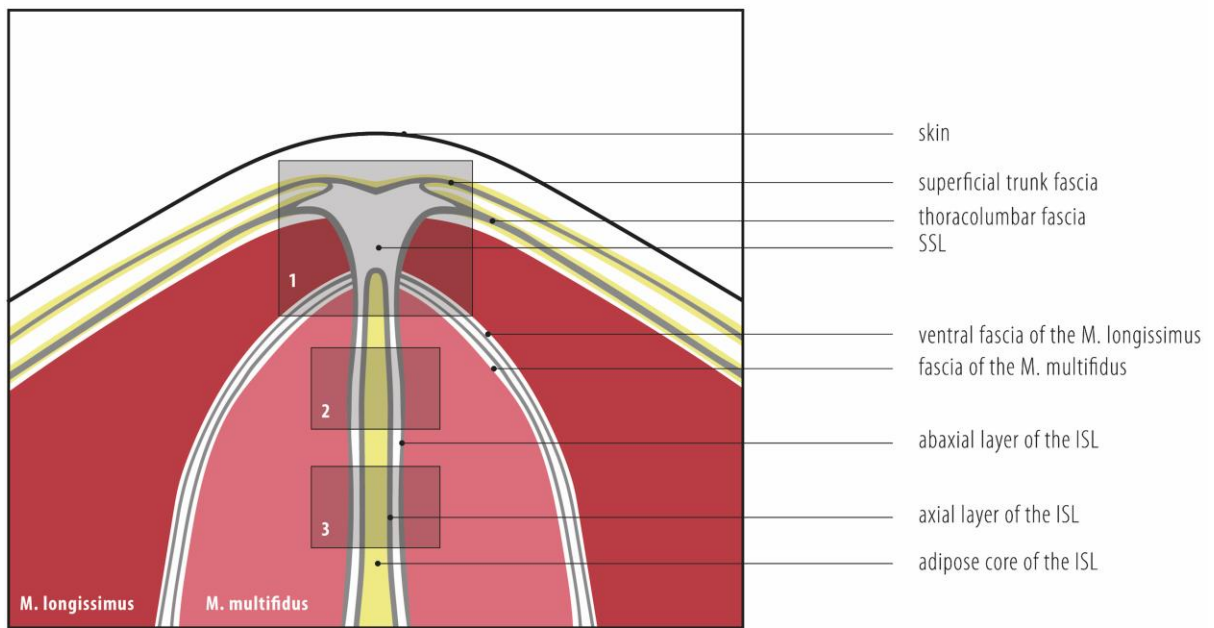
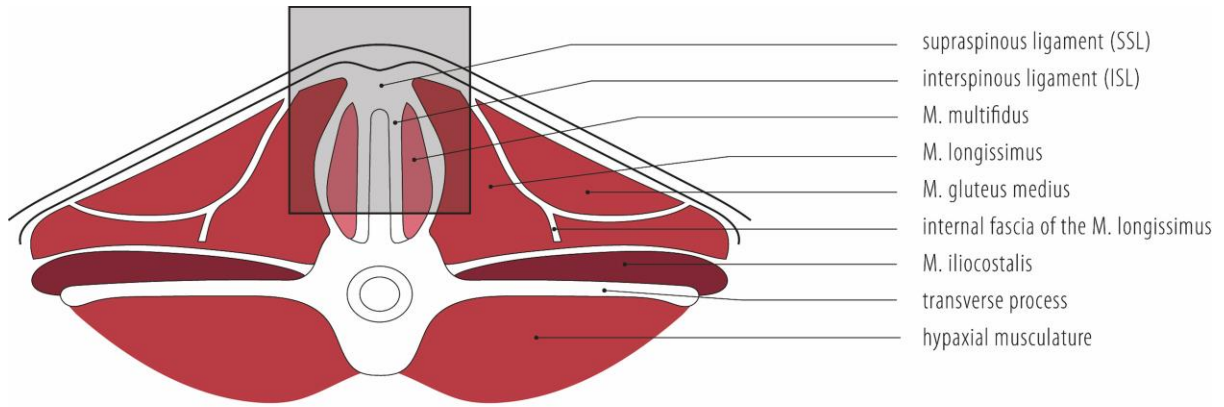
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503 Figure 6. Transmission electron microscopy image (scale bar = 1 micron) of the ISL at T18-  
504 L1. The arrow indicates cytoplasmic processes consistent with terminal unmyelinated fibres,  
505 scattered within collagen fibres (white circle). Inset: Higher magnification identify very small  
506 cytoplasmic processes characterised by double layered plasma membrane (arrowhead – scale

507 bar = 150 nanometers) consistent with small cytoplasmic processes identified by Substance P  
 508 stain (Fig. 5B).

509

510 Figure 1.

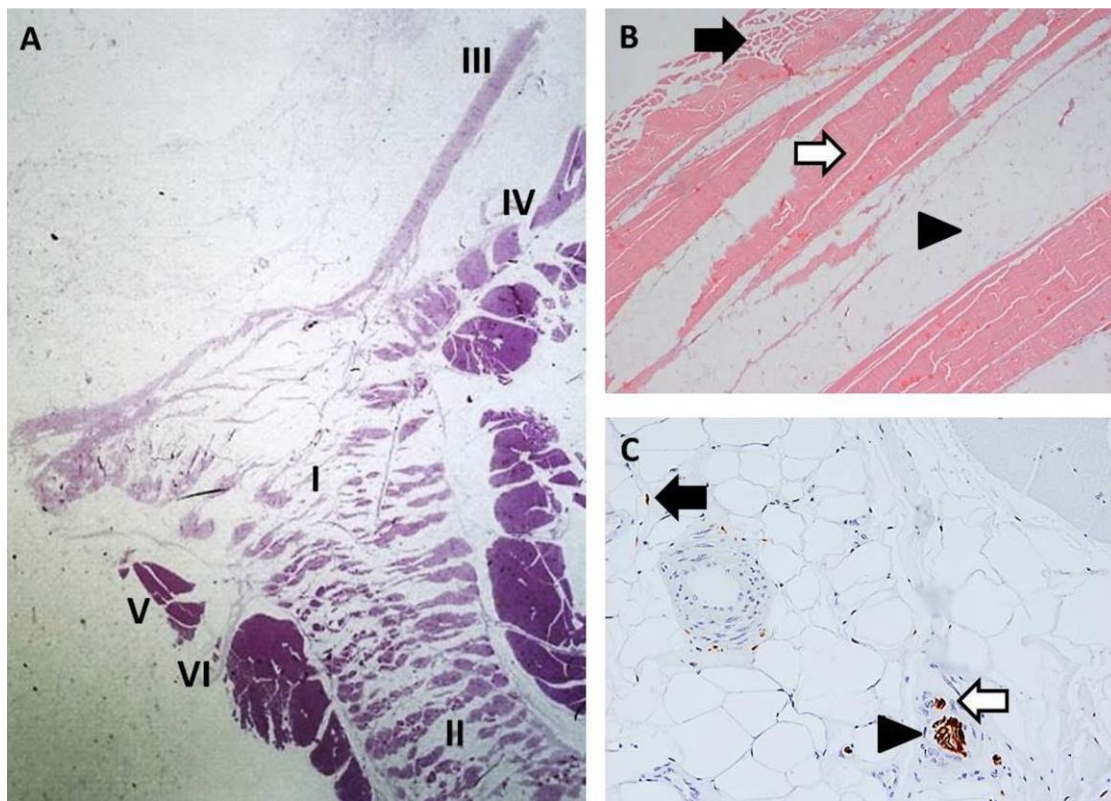


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514 Figure 2



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516



517 Figure 3



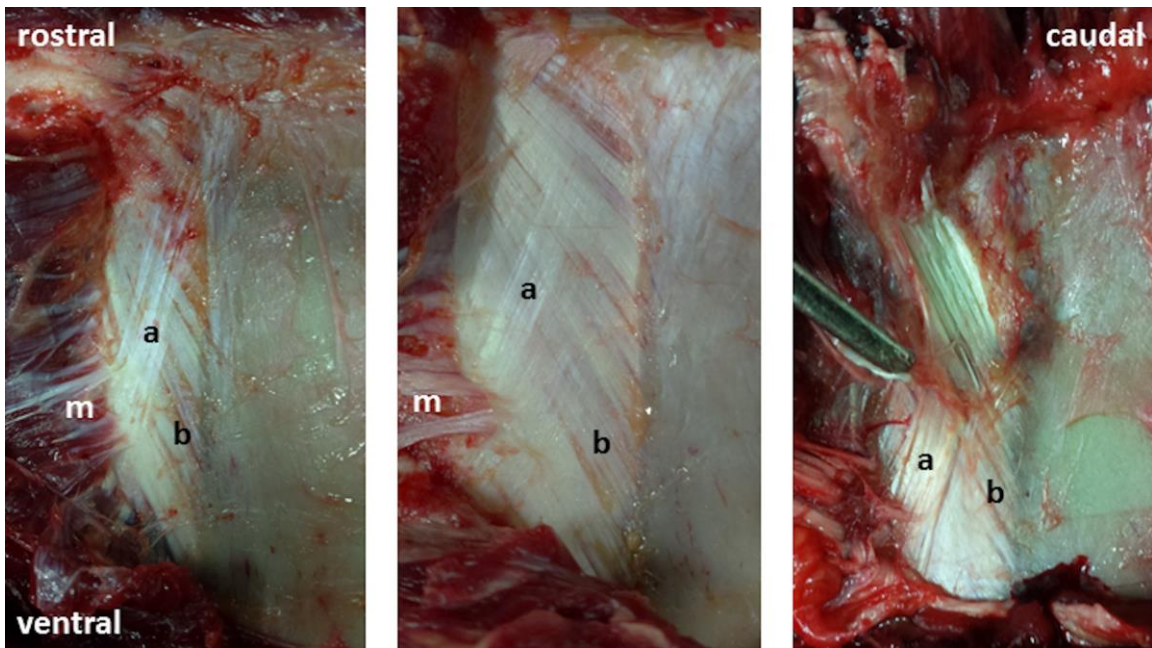
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522 Figure 4

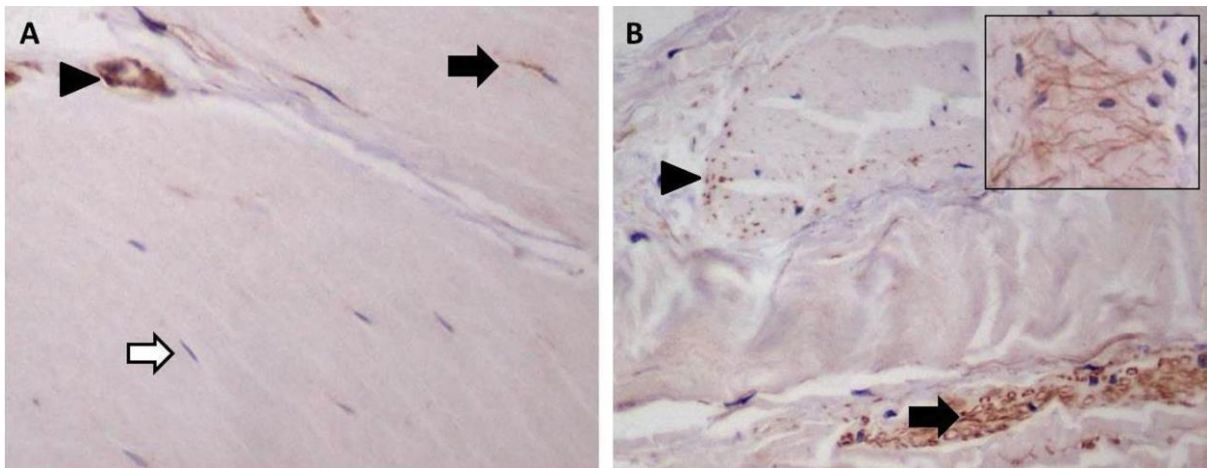


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525 Figure 5

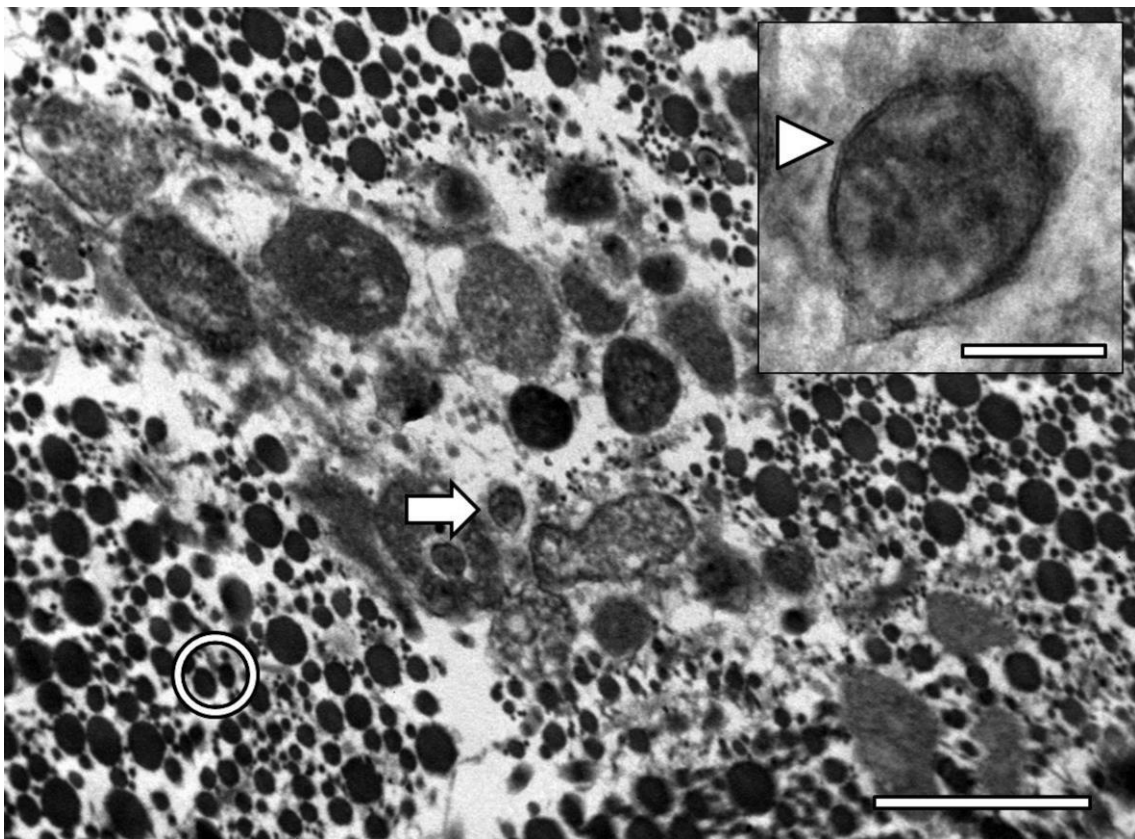


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527

528

529 Figure 6



530