3D anatomy of the quail lumbosacral spinal canal—implications for putative mechanosensory function

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Abstract: Birds are diverse and agile vertebrates capable of aerial, terrestrial, aquatic, and arboreal locomotion. Evidence suggests that birds possess a novel balance sensing organ in the lumbosacral spinal canal, a structure referred to as the 'lumbosacral organ' (LSO), which may contribute to their locomotor agility and evolutionary success. The mechanosensing mechanism of this organ remains unclear. Here we quantify the 3D anatomy of the lumbosacral region of the common quail, with a focus on establishing the geometric and biomechanical properties relevant to potential mechanosensing functions. We combine digital and classic dissection to create a 3D anatomical model of the quail LSO and use this to estimate the capacity for displacement and deformation of the soft tissues. We observe a hammock-like network of denticulate ligaments supporting the lumbosacral spinal cord, with a close association between the accessory lobes and ligamentous intersections. The relatively dense glycogen body has potential to apply loads sufficient to pre-stress denticulate ligaments, enabling external accelerations to excite tuned oscillations in the LSO soft tissue, leading to strain based mechanosensing in the accessory lobe neurons. Considering these anatomical features together, the structure of the LSO is reminiscent of a mass-spring based accelerometer.

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Abstract in German language: Vögel sind hochgradig angepasste und gewandte Wirbeltiere. Sie fliegen durch die Luft, rennen auf dem Land, bewegen sich im und unter Wasser, und sogar auf Bäumen. Es existieren Hinweise darauf das Vögel ein neuartiges Gleichgewichtsorgan im lumbosakralen Wirbelkanal besitzen. Diese Struktur wird "lumbosakrales Organ" (LSO) genannt und könnte zu ihrer Beweglichkeit und ihrem evolutionären Erfolg beitragen. Der genaue Mechanismus des mechanosensorischen Organs bleibt allerdings unklar. In dieser Arbeit erstellen wir eine 3D-Rekonstruktion der lumbosakralen Region der Wachtel. Wir untersuchen die geometrischen und biomechanischen Eigenschaften, welche für eine mögliche Mechanosensor-Funktion relevant sind. Wir kombinieren digitale und klassische Präparationen, erstellen ein anatomisches-genaues 3D-Modell, und verwenden das Modell um abzuschätzen, in wieweit die LSO Weichteile verschoben und verformt werden könnten. Wir beobachten ein hängemattenartiges Netzwerk von Ligamenten, welches das lumbosakrale Rückenmark stützt, direkt neben den vermuteten Mechanorezeptoren, den akzessorischen Loben. Der Glykogenkörper mit seiner relativ hohen Dichte hat das Potenzial Kräfte auf die Ligamente aufzubringen, und diese vorzuspannen. Damit könnten externe Beschleunigungen des Rennens oder Fliegens das LSO-Weichgewebe zum Schwingen anregen. Diese internen Schwingungen würden über die gedehnten Ligamente von den Neuronen der akzessorischen Loben erspürt, also direkt am Rückenmark und mit minimaler Zeitverzögerung. Wenn man die hier dokumentierte Anatomie als Einheit betrachtet, erinnert die LSO Struktur an einen Beschleunigungsmesser auf Basis eines Feder-Dämpfer-Masse Systems.
1. Introduction

Birds are diverse vertebrates with exceptional ecological range, occupying many habitats on Earth. Birds are capable of aerial, terrestrial, aquatic and arboreal (tree climbing) locomotion, and many species regularly use more than one locomotor mode. Avian species show specializations in limb proportions, foot morphology and body shape associated with their specific locomotor ecology and habitat (Barbosa and Moreno, 1999; Carrascal et al., 1990; Daley and Birn-Jeffery, 2018; Gatesy and Middleton, 1997; Grant, 1968; Heers and Dial, 2015; Norberg, 1979; Stoessel et al., 2013; Zeffer et al., 2003). However, despite diversity in body size and limb proportions (Gatesy and Middleton, 1997; Heers and Dial, 2015), birds share a consistent body plan, with similar basic arrangement of bones and joints. This suggests that it is not innovations in body form, that has been critical to the exceptional agility and locomotor diversity of birds.

Birds may possess a novel balance sensing organ in the lumbosacral spinal canal, a structure referred to as the 'lumbosacral organ' (LSO), which may contribute to their locomotor agility and evolutionary success (R. Necker, 1999, 2005; Reinhold Necker, 2006). While many tetrapods have enlargements in the cervical and lumbosacral spinal regions to support the sensorimotor demands of pectoral and pelvic limb function—the avian lumbosacral spinal canal is unique among vertebrates (Giffin, 1990, 1995). Birds exhibit particularly pronounced enlargement of the lumbosacral canal compared to other tetrapod species (Badawi et al., 1994; Giffin, 1990, 1995; Haziroğlu et al., 2001). The synsacrum of birds is a holistic structure formed by the fusion of lumbar and sacral vertebrae with each other and with the pelvic girdle (Figure 1) (Baumel, 1993). Within the avian synsacrum, the fused lumbosacral vertebrae form an enclosed and enlarged space, substantially larger than the spinal neural tissues contained within. Along with this unique bony morphology, the avian lumbosacral spinal region contains a suite of novel soft tissue morphological features (R. Necker, 2005; Sansone, 1977; Uehara and Ueshima, 1984). The combined features of the lumbosacral organ appear to be shared by all birds and is unique to birds among living vertebrates.

The LSO structure (Figure 2) includes segmental bilateral protrusions of neural tissue along the margins of the spinal cord, the accessory lobes of Lachi (Lachi (1889) as cited in Kölliker (1902)). These lobes occur near the intersections of denticulate ligaments, a network of ligaments that support the spinal cord (R. Necker, 2005; Reinhold Necker, 2006; D. Schroeder and Murray, 1987; Streeter, 1904). The spinal cord is dorsally bifurcated in this region, and supports a dorsally, centrally located glycogen body. The glycogen body is a gelatinous ellipsoid (Figure 2) composed of highly branched glucose polymers and cells of glial origin (C. Benzo and L. De Gennaro, 1983; L. De Gennaro, 1982; Imagawa et al., 2006; Koizumi, 1974; R. Necker, 2005). The fused lumbosacral vertebrae form a structure with distinctive, segmentally arranged, transverse semi-circular grooves (TG, or ‘semi-circular canals’ in R. Necker (1999)). The arrangement of the TG and accessory lobes is reminiscent of the mammalian vestibular system (R. Necker, 2005; Reinhold Necker, 2006). Each lobe may contain mechanoreceptors (Reinhold Necker, 2006; D. Schroeder and Murray, 1987; Yamanaka et al., 2008) with axons projecting to last-order premotor interneurons in the spinal pattern generating network (Eide, 1996;
Eide and Glover, 1996; R. Necker, 1999; Reinhold Necker, 1997; Rosenberg and Reinhold Necker, 2002). Collectively, these features suggest that the LSO functions as a balance sensing organ involved in the regulation of hindlimb sensorimotor control, independent of vestibular balance sensing in the head.

Although the anatomical features of the avian LSO have been known for decades, the putative mechanosensing function remains controversial and unresolved (Baumel, 1993; L. De Gennaro, 1982; Kölliker, 1902; Lachi, 1889; Rosenberg and Reinhold Necker, 2002; D. Schroeder and Murray, 1987; Streeter, 1904). Historically, several hypotheses have been proposed for lumbosacral organ function, including nutrition storage (Azcoitia et al., 1985; L. De Gennaro, 1961; Terni, 1924; Watterson, 1949) or a second brain (Streeter, 1904). More recently, mechanosensing is the prevailing hypothesis, but at least two theories exist on the nature of the mechanosensing process. Schroeder proposed a strain mechanosensing function, based on the location of the accessory lobes near the intersections of the denticulate ligaments, which could enable stimulation in response to ligament strain (D. Schroeder and Murray, 1987). Alternatively, Necker proposed a balance sensing function of the LSO based on the canal-like shapes within the bone and functional analogy to the mammalian inner ear (R. Necker, 1999, 2005). Necker suggests that cerebrospinal fluid (CSF) flow stimulates mechanosensors in the accessory lobes, enabling the sensation of body movement (R. Necker, 1999, 2005; Reinhold Necker, 2006).

Here we investigate the 3-dimensional anatomy of the quail LSO, with a focus on quantifying the geometric and biomechanical properties relevant to a potential mechanosensing function. Necker’s theory for balance sensing requires CSF flow to act upon the accessory lobes in response to body motion. Although it is feasible that CSF flow elicits a mechanosensory response in the accessory lobes, this proposed sensing mechanism does not explain several anatomical features of the LSO. We suggest an alternative mechanosensing hypothesis based on the anatomical features and Schroeder’s observations (D. Schroeder and Murray, 1987)—the inertial load of the glycogen body may act to tune and amplify localized oscillations of the spinal cord neural tissues, resulting in stress and strain in the denticulate ligaments which stimulates the accessory lobes to sense body motion (L. De Gennaro, 1982; L. De Gennaro and C. Benzo, 1987; Polák-Krašna et al., 2019). Here, we aim to quantify the morphology of the lumbosacral region of the common quail (Coturnix coturnix) to establish whether the topological and biomechanical features are more consistent with a fluid-flow or tendon-strain based mechanosensing function.

No tools are currently available to directly visualize the in vivo movements of neural soft-tissues in the lumbosacral region of birds. The region is inaccessible to direct instrumentation because it is fully enclosed in bone, and access would damage the tissues of interest and impair the mechanosensing function (R. Necker et al., 2000). The region is also difficult to image in vivo because of the broad range of contrasts between bones, neural tissue, pneumatized bone, and CSF, which each contribute to the hypothesized mechanosensing functions. For these reasons, we focus on establishing the geometric and tissue properties relevant to developing mechanical models of the mechanosensing function. We use a combined approach of digital dissection of a specimen using dif-
fusible iodine-based contrast-enhanced computed tomography (dice-CT) (Gignac and Kley, 2014; Metscher, 2009), complemented with a classic dissection via a stereo microscope to provide additional detail on the structure and potential deformability of the denticulate ligaments network. We overlay the measurements from classical and digital dissections to provide a complete 3D topology of the common quail lumbosacral organ.

2. Materials and Methods

![Image](https://example.com/image.png)

**Figure 1:** The anatomy of the synsacrum of the quail. (A) Schematic skeletal outline of a quail synsacrum, emphasized in green and turquoise. The schematic was modified from (commons, 2009). (B) A 3D view of the synsacrum, also showing a local coordinate system (x-red, y-green, z-blue, used in Section 3.1), and three planes of reference. The coordinate origin is located at the center between both femur head sockets, with the coronal plane in-parallel to the orientation of the denticulate ligament network (Figure 2). Abbreviations are explained in Table 4.

Three specimens were prepared for the classical dissection, and one specimen was prepared for the digital dissection (micro-computed tomography, and 3D segmentation).

For the digital dissection, one quail specimen was obtained from a commercial breeder (Fayre Game farm, UK) and prepared using fixation and staining procedures as recommended by Metscher (Metscher, 2009). The synsacrum was isolated and fixed for three days in 10% neutral-buffered formalin. The synsacrum was then immersed in 1% Lugol’s iodine solution (Sigma, Life science, L6146). The specimen was test-scanned to check contrast levels periodically and a final scan was taken after incubation in Lugol’s iodine for 49 days.

For the classical dissection, three adult, female common quail (*Coturnix coturnix*) (E. P. Reese and T. W. Reese, 1962) were obtained as frozen carcasses from a commercial breeder (Kitzingen, Germany). The birds’ average weight was (170 ± 5) g, the body size varied between 10-12 cm. All carcasses were dissected according to the practice described
in Gurtovoy et al. (1992). After slow thawing, the synsacrum were isolated and stored in a closed vessel at 4°C temperature, to minimize dehydration and soft-tissue autolysis.

2.1. Digital dissection, uCT scanning

The quail specimen was scanned with a micro-computer tomography machine (Skyscan 1172), operated at 80 kV and 124 μA. 1030 raw images (dark object against bright background) were taken within one 360° rotation, with an increment of 0.35°, and an integration time of 500 ms per image. Three images at each angle position were averaged. The raw images are 1000 by 668 pixels in size. Voxels of the original image stack are isotropic, with a 27 μm edge length. The images were back projected to generate 634 images (TIFF format, bright signal against black background). The resulting raw image size is 1000 by 1000 pixels.

2.2. Segmenting uCT data

The raw data was 3D segmented in Amira (version 6.5.0, Visage Imaging). The back-projected image stack was directly used in Amira, and required no further aligning. The contrast from dense bone tissue allowed labeling with a threshold function. The soft tissue inside the lumbar vertebral canal had not enough contrast for using the threshold function. Instead, soft tissues were segmented manually, with the ‘paintbrush’, the ‘magic wand’, and the ‘lasso’ tool. We switched working planes when necessary, depending on the shape and orientation of anatomical structures. The segmented structures were interpolated, from two to seven labeled slices.

2.3. 3D segmentation

Surfaces were individually rendered with the module ‘Surface Gen’. ‘Label Field’ files (AM format) were split, one for each segmented structure (Ruthensteiner and Heß, 2008). For rapid processing, 3D model surfaces were closed and the number of triangles reduced (Molnar et al., 2012). 3D segment surfaces were exported (STL format), and loaded in Autodesk Maya. Several of the resulting structures overlapped by a few triangles, and formed non-manifold edges. These overlaps were manually treated, by removing non-manifold edges, and reducing the number of polygons. Any remaining holes were manually closed, and smoothed with polygonal meshes with the ‘Sculpt Geometry’ tool (Buchanan et al., 2014). The final meshes were exported (STL format) and re-imported into Amira for the geometric morphometric analysis.

2.4. Digital dissection of denticulate ligaments

The denticulate ligaments are the smallest anatomical structures we were able to resolve with the digital dissection. The ligaments barely appeared in the pre-sliced raw data, with a thickness of a few voxels (27 μm voxel size). With a denticulate ligament thickness close to the imaging resolution, they required a dedicated segmentation. The first, main slicing direction was chosen approximately along the spinal canal direction, and only
a few slices contained recognizable denticulate ligaments. After this first segmentation of the spinal canal, the glycogen body, and the spinal cord, we re-sliced the raw data in-parallel to the expected orientation of the denticulate ligaments, and imported the new slices into Amira, which largely improved denticulate ligament visibility. This new set was used to segment the denticulate ligaments, with the ‘paintbrush’ tool. Both sets were merged by overlaying the commonly visible ventral processes (Figure 2b).

2.5. Classical Dissection

With the classical dissection, we exposed the fine structure of the denticulate ligaments. Synsacrum bone surfaces were cleaned of flesh. The movable thoracic vertebrae both from the anterior and posterior sides were cut off. The synsacrum’s surfaces were photographed (Nikon D5500, lens Nikon AF-S Nikkor 35 mm f/1.8G ED). Three perspectives are shown in Figure 3a-c. The remaining classical dissection was performed under a fluorescent stereo microscope (Leica M205 Fa, magnification 7.8-160x). Images from the microscope dissection were taken with the built-in camera (Leica DFC digital 7000-T, 2.8 megapixel sensor, pixel size 4.54 μm). Related pictures in Figure 3d, Figure 7, and Figure 8b have been cropped and annotated for clarity, and were not altered otherwise.

To simplify the classical dissection the bones of the pelvic girdle were cut off. The spinal canal wall was opened from dorsally, along the coronal plane aligned to the intervertebral foramina location. The spinal cord and the glycogen body were removed from the dorsal side of the spinal canal. The denticulate ligaments were photographed (Figure 3d), and ligaments were manipulated with a pair of forceps to indicate their stretchability and connectivity (Figure 7).

2.6. Measurements

Dimensions and distances between anatomical structures were measured applying landmarks to the 3D model in Amira (Figure 4). We measured in the transverse cut planes (Figure 4b), with a slight angle adjustment (Figure 4a). The cut planes were placed at the vertebral fusions (dashed lines, Figure 4a) and mid-vertebral regions (continuous lines). The glycogen body spans from the fourth lumbar to the second sacral vertebra (L4, S1, S2). We show a cut through the spinal canal in regions with and without glycogen body visible in Figure 4b and c, respectively.

2.7. Volume Measurements

Partial and full volumes of the spinal cord, the glycogen body, and the CSF were extracted in Autodesk 3ds Max. When volumes were separated by vertebrae, the transverse cut planes were used as separating planes, as shown in Figure 6a-c. At the position of the cut planes, the structures were split with the ‘Slice modifier’ function. Slicing volumes creates openings in the polygonal mesh. We closed these with the options ‘Cap holes’ function and ‘Smooth new faces’.
2.8. Ligament strain calculation

To explore the potential for soft tissue oscillations caused by external accelerations that could drive a mechanosensing process, we estimate the maximum possible displacement within the spinal canal, based on the available space. The resulting deformations would include all soft tissues, and the ligament network would produce countering forces, similar to a hammock stretched when loaded by a person (Figure 9). For simplicity, we assume that the soft tissue moves in parallel to the sagittal plane, driven by in-plane accelerations of the synsacrum.

We estimate the transversal ligament strain with a simplified model. The initial state assumes unloaded and non-stretched ligaments in planar orientation. We measured lengths \( L \) of left and right transverse ligaments of the three classical dissection specimen and one digital dissection specimen, and averaged values per vertebra (Table 3). The fluid space below and above, extracted from the digital dissection (Figure 5c), provided the vertical range, with additional heuristics—we averaged two neighbouring mid-vertebra values for the upward (dorsal) fluid distance estimate (Figure 5c, E-G and E-GH, i.e. the average of M-S3 and M-S2 for S3-S2). Otherwise, we would over-estimate the potential displacement based on the additional height of transverse semi-circular grooves between vertebrae. For downward (ventral) distance estimate, we directly used ventral distance in the CSF space below the spinal cord (Figure 5c, I-F). We then calculated the maximally stretched length \( l \) (at maximum vertical displacement) of a transverse ligament using the Pythagorean theorem, and its nominal strain \( e = \frac{l}{L} \).

2.9. Effective force from submerged, high-density glycogen body

All LS tissues are submerged in CSF, which itself has a specific gravity \( SG = \frac{\rho_{\text{CSF}}}{\rho_{\text{H2O}}} = 1.00 \), whereas the specific gravity of spinal cord tissue indicates a small tendency for sinking (Table 1). In comparison, glycogen is considerably denser, which suggests the glycogen body substantially loads the spinal cord and denticulate ligaments with a normal force \( F_N \) corresponding to the glycogen body’s effective, submerged weight. The estimated force value can assist future research characterizing the LS structure during external accelerations, in-silico or in physical, experimental setups, as it present the intrinsic force applied at the LS structure. For a (future) calculation of soft tissue deformation from force loading, more data will be required, including the externally applying forces caused by acceleration, the materials’ elastic, anisotropic properties, and the boundary conditions restraining the spinal cord on its anterior and posterior ends.

Here, we calculate the effective force applied by the glycogen body onto the spinal cord, and the spinal cord at the denticulate ligament, and assume a simplified system (1) at atmospheric pressure, (2) without effects by the height of a fluid column, and (3) with incompressible soft tissues.

The extracted soft tissue volumes are provided in Table 2. The effective, normal force
Table 1: Densities $\rho$ and specific gravity $\frac{\rho}{\rho_{CSF}}$ of soft tissue inside the LS spinal canal. Values marked in bold are calculated based on data provided in the referenced literature. We apply the glycogen density’s lower bound in our calculations.

| Tissue          | Density [g/cm$^3$] | Specific gravity | Reference                                      |
|-----------------|--------------------|------------------|------------------------------------------------|
| CSF             | 1.00               | 1.00             | Higuchi et al., 2004; Lui et al., 1998 (human) |
| Spinal cord     | 1.04               | 1.04             | Siegal et al., 1988 (rat spinal cord)          |
| Glycogen        | 1.40...1.48        | 1.40             | Scott and Still, 1970 (rat liver, human leukocytes) |

$F_{N,\text{eff}}$ of tissue submerged in CSF is calculated as:

$$F_{N,\text{eff}} = m_{\text{ti}} \cdot g - \rho_{\text{CSF}} \cdot V_{\text{ti}} \cdot g$$

$$= \rho_{\text{ti}} \cdot V_{\text{ti}} \cdot g - \rho_{\text{CSF}} \cdot V_{\text{ti}} \cdot g$$

$$= \Delta \rho \cdot V_{\text{ti}} \cdot g \quad (1)$$

where $m_{\text{ti}}$, $V_{\text{ti}}$, and $\rho_{\text{ti}}$ are the mass, volume, and density of the tissue of interest, respectively. $\rho_{\text{CSF}}$ is the density of CSF, and $g = 9.81 \text{ m/s}^2$ is the gravitational acceleration. The glycogen body’s specific gravity ($\text{SG}_{\text{GB}} = \frac{\rho_{\text{GB}}}{\rho_{\text{CSF}}}$) provides a similar understanding for its floating vs. sinking behavior when submerged in CSF.

2.10. Nomenclature

We assigned the intersection between lumbar and sacral vertebra numbering according to the maximum extension of the glycogen body, and the spinal canal (Can and Özdemir, 2011; Catala et al., 1995). We also matched the denticulate ligament networks from the classical dissection and the digital dissection by overlaying them. Similar nomenclature is used by R. Necker (2005), for pigeons.

3. Results

In this study, we characterize the geometry (length, surface area, volume), and the topology of the lumbosacral enlargement and its soft tissues. We report the morphometric data of the spinal cord hemispheres, the glycogen body, the denticulate ligaments, the CSF, and the spinal canal, for use in the development of physical models of the mechanosensing process. Based on the higher density of the glycogen body compared to the surrounding CSF, we calculate an estimate of its normal force applied to the spinal cord and the denticulate ligaments. We also estimate the maximum likely ligament strains from deforming the denticulate ligaments.

3.1. Morphometrics

The spinal canal data between vertebrae L2-S4 shows the following trends: (1) The overall height and width of the canal reduce from anterior to posterior, from 2.5 mm/3.2 mm (H/W at L2-L1), to 1.7 mm/2.2 mm in S5-S4 (Figure 5a). (2) The glycogen body is
co-located in the region of a substantial spinal canal extension in all directions, between the three vertebrae L4, S1, and S2. (3) Fused vertebrae are connected by transverse semi-circular grooves (TG), which substantially alter the canal shape. At these TGs, we observe maxima in vertical direction at dorsal (Figure 5a, diamond markers), with the largest vertical extension at S1-L4 (3.7 mm). Unlike the vertical extension, lateral extension maxima are found at the middle of vertebrae (Figure 5a). The S1 and the L4 vertebrae contain the largest lateral extension (MS1, ML4, 4.8 mm). Figure 5a shows that the spinal canal width is always greater than its height, in the lumbosacral region.

The glycogen body is 5.2 mm long (X-direction, Table 6), and has its maximum height of 2.5 mm at the dorsal fusion zone S1-L4, where the spinal canal height is maximal with 3.7 mm (Figure 5b, G-GH).

We provide two sets of vertical extension data of the spinal cord; a first from the intersection with the sagittal plane, and a second from the intersection with the parasagittal plane, at its highest point. We see a drop in vertical extension of the spinal cord, in the sagittal cut plane between L4 and S2, where it leaves an only 0.4 mm high bridge between both hemispheres (Figure 5b, GH-I). Here both hemispheres remain barely connected by spinal cord material. The vertical spinal cord extension in the parasagittal plane reaches its maximum between L3-L4, with a height of 1.9 mm (Figure 5b, GH-I). In sum, we see that the spinal cord’s width (Figure 5b, C-D) and height (Figure 5b, L-M) decrease from anterior to posterior, except in the region of the glycogen body.

The height of the CSF above the spinal cord fluctuates much, in the range of L1-S4 (Figure 5c, round markers). Transverse semi-circular grooves are located between dorsal and lateral surfaces inside the spinal canal. The CSF data shows TGs as spikes in the curve. The CSF has its highest vertical extension (1.5 mm) at TG L3-L4 (Figure 5c). The smallest height of CSF (0.3 mm) is found directly above the glycogen body, at S1 and TG S1-L4 (Figure 5c, E-G).

The vertical extension of CSF below the spinal cord reduces, in average, from anterior to posterior, from 0.4 mm to 0.1 mm, respectively (Figure 5c, I-F). Exceptionally, a large ‘dip’ is visible for about 2 vertebrae between S1-L4 and M-S1, where the fluid space below the spinal cord and the denticulate ligaments triples; it expands from 0.2 mm to 0.6 mm. The maximum below-expansion coincides with the maximum height of the glycogen body (Figure 5b, G-GH), and the maximum spinal canal height (Figure 5a, E-F).

The CSF fully surrounds the soft tissues in the spinal canal. The lateral expansion of CSF reduces from anterior (2x 0.4 mm) at L2-L1, towards posterior at S5-S4 (2x 0.15 mm). The data curve shows a zig-zag pattern, where lateral fluid expansions at the middle of vertebrae coincide with the location of spinal nerve foramina, also because of the height of the cut plane (i.e., MS1, MS2... Figure 5c, D-B). The remaining data at vertebrae fusions indicates the foothills of the TGs extending dorsally, into the spinal canal walls (i.e., S1-L4, S2-S1...). The most posterior segment S4 shows no peak lateral expansion. Its smaller foramina are shifted posterior, towards S4-S5.
Table 2: Digitally measured soft tissue volumes in [mm$^3$] and modelled effective normal forces ($F_N$ in [µN]) of the glycogen body, in order of vertebrae.

| Vertebra  | S4 | S3 | S2 | S1 | L4 | L3 | L2 | $\sum$ S4-L2 |
|-----------|----|----|----|----|----|----|----|-------------|
| Spinal cord Volume [mm$^3$] | 2.8 | 3.9 | 5.5 | 7.5 | 11.8 | 10.7 | 10.2 | 52.4 |
| Glycogen body Volume [mm$^3$] | - | - | 1.4 | 6.8 | 4.8 | - | - | 13.0 |
| CSF Volume [mm$^3$] | 3.3 | 5.6 | 8.3 | 11.5 | 10.8 | 14.3 | 10.8 | 64.6 |
| Spinal canal Volume [mm$^3$] | 6.1 | 9.5 | 15.2 | 25.8 | 27.4 | 25.0 | 21.0 | 130.0 |
| Glycogen body $F_{N,\text{eff}}$ [µN] | - | - | 5.5 | 26.7 | 18.8 | - | - | 51.0 |

3.2. Volume Measurements

The uCT scan ranges over seven vertebrae, from L2 to S4 (Figure 6a). An overview of the volume data is provided in Table 2 and Figure 6d.

From L2 to L4, the spinal canal increases from 21.0 mm$^3$ to 27.4 mm$^3$, respectively. The following posterior canal continuously decreases in volume, from S1 with 25.8 mm$^3$ to S4 with 6.1 mm$^3$ (Table 2 and Figure 6). The spinal cord volume decreases in average from anterior to posterior, from 10.2 mm$^3$ at L2 to 2.8 mm$^3$ at S4. The segmentation-based data shows a maximum at L4, with a volume of 11.8 mm$^3$. In average 50% of each spinal canal segment is filled with CSF (64.6 mm$^3$ of 130 mm$^3$). The spinal canal segments with the glycogen body contain relatively less liquid; 39% (10.8 mm$^3$) of L4 are fluid-filled, and 45% of the S1 spinal canal segment. In L4, the CSF occupies nearly the same volume as the spinal cord: 10.8 mm$^3$ and 11.8 mm$^3$, respectively.

Most of the glycogen body’s volume is located in S1, with a volume of 6.8 mm$^3$, or 52% of its total volume of 13 mm$^3$ (Table 2 and Figure 6). 11% (1.4 mm$^3$) of the remaining glycogen body are located in S2, and 37% (4.8 mm$^3$) is located in L4. Within the L4-S2 segments, the glycogen body occupies 19% spinal canal volume, and has half the volume of the sum of local spinal cord segments (13.0 mm$^3$ vs. 24.8 mm$^3$, 52%).

3.3. Normal forces by submerged glycogen body

Equation (1) indicates that forces applied by the dorsally positioned glycogen body depend linearly on the size and density of the glycogen body (Tables 1 and 2), leading to a net normal force of $F_{N,\text{eff}} = 13 \times 10^{-9} \text{m}^3 \cdot 0.40 \times 10^3 \text{kg/m}^3 \cdot 9.81 \text{m/s}^2 = 51 \times 10^{-6} \text{N} = 51 \text{µN}$, applied by the glycogen body, at the ventrally located spinal cord and the denticulate ligaments (Table 2). In comparison, the higher-than-CSF specific gravity (SG = 1.04) of spinal cord sections between L2, L1, and S4 ($\sum V_{L2,L1,S4} = 24.8 \times 10^{-9} \text{m}^3$) creates a normal force of $F_{N,\text{eff}} = 10 \text{µN}$ directed at the denticulate ligaments.

3.4. Denticulate ligament network

The denticulate ligaments in Figure 8a were segmented from uCT-data with a resolution between 1-5 voxels (27 µm voxel edge) per ligament diameter. Lateral longitudinal ligaments are thicker, around 3-6 voxels in diameter, and ventral longitudinal ligaments were between 1-2 voxels in diameter. To strengthen the digital dissection data, we studied the
Table 3: The calculated strain of transverse ligament from virtual, vertical soft tissue oscillations.

|                      | S3-S2 | S2-S1 | S1-L4 | L4-L3 |
|----------------------|-------|-------|-------|-------|
| Dorsal fluid space (up) [mm] | 0.48  | 0.37  | 0.41  | 0.56  |
| Ventral fluid space (down) [mm] | 0.19  | 0.33  | 0.52  | 0.19  |
| Transverse ligament length $L$, at rest [mm] | 1.21  | 1.51  | 1.61  | 1.67  |
| Transverse ligament length $L_{\text{up, virt}}$, stretched [mm] | 1.30  | 1.55  | 1.66  | 1.77  |
| Transverse ligament length $L_{\text{down, virt}}$, stretched [mm] | 1.23  | 1.54  | 1.69  | 1.68  |
| Nominal strain up $e_{\text{up}}$ [%] | 7.9   | 3.0   | 3.2   | 5.8   |
| Nominal strain down $e_{\text{down}}$ [%] | 1.3   | 2.4   | 5.2   | 0.7   |

denticulate ligaments with an additional classical dissection (Figure 8b). The network of denticulate ligaments consists of lateral and ventral longitudinal ligaments (D. Schroeder and Murray, 1987), all aligned in a paracoronal plane. Additional ligaments intersect in the transverse plane. Ligament nodes present the intersection points.

We measured the intersection angle in reference to the line of the ventral longitudinal ligament (Figure 8b, and Table 5). Transverse ligaments in the LSO region show changing pennate angles, with angles between 71° (S2-S1) and 90° (L3-L2). The glycogen body’s center of mass projects close to the transverse ligament-pair connecting to the ventral longitudinal ligament at 84° (S1-L4, Figure 8b).

3.5. Estimating strain in denticulate ligaments

Our calculations estimating denticulate ligament strains show that the lowest transverse ligament strain would occur during downward motion in L4-S3 (1 %), and the highest strain in S2-S3 during upward motion (8 %, Table 3, Figure 5d), with an average strain over all transverse ligaments of 5 % for upward motion, and 2 % for downward motion. During the classical dissection, we manipulated ligaments with a pair of forceps and observed that they are easily stretched to these extents without rupture (Figure 7).

4. Discussion

We generated a 3D anatomical model of the lumbosacral region of the common quail, including the soft tissue and bony spinal canal elements, with a focus on establishing the morphological topology and biomechanical properties relevant to potential mechanosensing functions. The goal of this effort was to establish quantitative and openly-accessible data on this inaccessible structure, for use in physical models and simulations. We observe a hammock-like network of denticulate ligaments supporting the spinal cord in the lumbosacral region, with a close association between the ligament intersections and the known locations of the accessory lobes. The glycogen body likely has higher specific gravity than the surrounding CSF and soft tissues, and a higher effective normal force compared to spinal cord in the same area (50 µN vs. 10 µN, respectively) suggesting potential to pre-stress the denticulate ligament network and allow external accelerations to excite oscillations in the LSO soft tissue. Oscillations in the glycogen body, the spinal cord, and the denticulate ligaments could allow mechanosensing in the accessory lobes’
neurons. Considering these anatomical features together, the structure of the LSO is reminiscent of an accelerometer.

Many studies have investigated potential functions of the lumbosacral organ of birds, and the constituent elements, including the glycogen body, the spinal canal-extension, the accessory lobes, the denticulate ligaments, and the CSF—yet, concrete evidence of its function has remained elusive. Early hypotheses assumed a nutritional or secretory function of the glycogen body (Azcoitia et al., 1985; C. Benzo and L. De Gennaro, 1981), or suggested function related to myelin synthesis for the spinal nerves of birds (C. Benzo and L. De Gennaro, 1981; L. D. De Gennaro and C. A. Benzo, 1976, 1978). Watterson suggested that the glycogen body plays an important role in the in-ovo development of spinal networks of birds, by wedging the spinal cord hemispheres apart during the development. However, none of these hypotheses have yielded conclusive evidence of LSO or glycogen body function.

Most recent work has supported the hypothesis of a mechanoreceptive function of the LSO. This hypothesis is based, in part, on the identification of mechanoreceptive neurons within the accessory lobes (Eide, 1996; Eide and Glover, 1996; Reinhold Necker, 2006; Rosenberg and Reinhold Necker, 2002; D. Schroeder and Murray, 1987; Yuko Yamana et al., 2012, 2013). Intraspinal mechanosensors are known to exist in many vertebrates (Dale et al., 1987; Henderson et al., 2019; Hubbard et al., 2016; Wyart et al., 2009), including the edge-cells in lampreys, which have been most directly characterized (Di Prisco et al., 1990; S. Grillner et al., 1981; Hsu et al., 2013; Rovainen, 1979). Marginal neurons with apparent developmental homology to lamprey edge cells have been observed in many vertebrates including reptiles, mammals and amphibians, and the accessory lobes of birds (S. Grillner et al., 1981; Hsu et al., 2013; D. Schroeder and Egar, 1990; D. Schroeder and Murray, 1987; D. Schroeder and Richardson, 1985; D. M. Schroeder, 1986a,b). The ultrastructure of the marginal nuclei, including the avian accessory lobes, suggests close association with denticulate ligaments and morphology similar to peripheral mechanosensors, and suggests a strain or pressure sensing function (D. Schroeder and Egar, 1990; D. Schroeder and Murray, 1987; D. Schroeder and Richardson, 1985; D. M. Schroeder, 1986a,b). These studies suggest widespread presence of intraspinal mechanosensors among vertebrates.

If the accessory lobes do act as intraspinal mechanosensors in birds, the sensory signals likely integrate directly with the lumbosacral spinal rhythm generation. The homologous edge cells of lamprey integrate sense of spinal bending directly with the rhythm generating circuits, entraining right/left alternation of activity (Di Prisco et al., 1990; S. Grillner et al., 1981; Hsu et al., 2013; Rovainen, 1979). Similarly, Eide found that chick accessory lobes project onto the contralateral ventral horn near lamina 8 (Eide, 1996; Eide and Glover, 1996), with potential synaptic targets including last-order premotor interneurons (Eide, 1996; Eide and Glover, 1996; Reinhold Necker, 2006). This suggests potential for the accessory lobes to play a direct role in coordinating hindlimb rhythm generation. Additionally, spinal cord lesion studies in chicks revealed that the circuits required for locomotor rhythm generation are completely localized to the ventral spinal cord (Ho and O’Donovan, 1993; Sholomenko and Steeves, 1987). These lines of evidence suggest direct integration of accessory lobes mechanosensing into the rhythm generating
interneuronal networks of the avian lumbosacral spinal cord.

Although the evidence for LSO mechanosensing in birds remains indirect, there is also separate experimental evidence that birds possess balance sensing in the body, independent from the vestibular system in the head. Birds retain the ability to reflexively compensate for body rotations even after labyrinthectomy and spinal cord transection to eliminate descending inputs influenced by vision and vestibular sense (Biederman-Thorson and Thorson, 1973). However, the definitive source of this extra-labyrinthine balance sense has remained unresolved.

We observe the following features of the avian LSO and suggest an updated hypothesis for its mechanoreceptive function. The spinal canal around the glycogen body shows a distinct modification of the fluid space and ligament geometry, centered around the glycogen body’s center of mass. The glycogen body has its largest height directly below the S1-L4 fusion zone (Figure 5b), and the largest ventral CSF space below this region, with 0.6 mm below, compared to 0.2 mm (posterior) and 0.4 mm (anterior) to the glycogen body. The network of denticulate ligaments shows a pennate angular arrangement with an angle close to perpendicular at the S1-L4 fusion (Figure 8a). The denticulate ligaments extend from the ventral processes to attach to the spinal canal walls at the at the fusion zones between vertebrae (Figure 8a,b, Figure 7, and D. Schroeder and Murray (1987)). The transverse ligaments directly extend from ventral processes and intersect with ventral longitudinal ligaments at varying pennate angles oriented ‘towards’ the S1-L4 fusion zone (Figure 8a,b). Ventral longitudinal ligaments and transverse ligaments were the thinnest ligaments observed, with a diameter between (27-54 µm), compared to the larger lateral longitudinal ligaments with diameters between 120-180 µm (Figure 7). The morphology suggests a varying and anisotropic stiffness of the denticulate ligament network, with the topology of a hammock centered directly underneath the glycogen body.

In most regions of the spinal column, spinal cord tissue is held relatively immobile by denticulate ligaments, membranes, the spinal canal itself, and the surrounding CSF (Loth et al., 2001; R. Necker, 2005; Polak et al., 2014; D. Schroeder and Murray, 1987). The CSF’s buoyancy supports the spinal cord, and protects it from injuries (Polak-Kraśna et al., 2019). However, within the avian LSO, the lumbosacral canal structure is modified in ways that are likely to enhance dorsoventral spinal cord motion, by the added mass of the glycogen body, the arrangement of the denticulate ligament network, and the increased dorsal and ventral fluid space within the bony canal.

The physical structure of the LSO presents a fluid-submerged spring-damper-mass system. As such, it would be susceptible to accelerating forces from locomotion, such as the vertical oscillations during flying and running. Under the influence of limb and body accelerations, the LSO soft tissues could physically oscillate, moving within the CSF. These oscillations could present multiple potential physical mechanisms for mechanosensation. First, Necker proposed the hypothesis that CSF flow through the transverse semi-circular grooves could elicit a mechanosensing response in the accessory lobes, based on analogy to the vestibular system in the mammalian inner ear (R. Necker, 1999, 2005; Reinhold Necker, 2006). However, the accessory lobes do not possess the stereocilia typical of hair cells of the inner ear, which are specialized for detection of fluid motion (D. Schroeder and...
Murray, 1987). Recently identified intraspinal mechanosensors in zebrafish do possess microvilli or cilia that extend into the CSF, consistent with potential for fluid sensing (Henderson et al., 2019; Hubbard et al., 2016; Wyart et al., 2009). Furthermore, fluid-based mechanosensing does not fully explain the novel anatomical features of the denticulate ligaments and glycogen body of the LSO. Alternatively, work by Schroeder suggests a strain mechanosensing hypothesis. Schroeder’s investigation of the ultrastructural features of the avian accessory lobes revealed a close association between the accessory lobes and denticulate ligaments and features consistent with strain or pressure mechanosensors similar to peripheral proprioceptors (D. Schroeder and Murray, 1987). Based on the morphology and biomechanical properties of the LSO, we suggest that the anatomy is more consistent with strain-based mechanosensing, producing accelerometer-like function. However, it is also possible that the LSO achieves both gyroscopic and acceleration sensing through a combination of multiple types of mechanosensing neurons in the accessory lobes with some responding to fluctuations in fluid flow or pressure, and some responding to strain in the denticulate ligaments. Specifically, the accessory lobes are found on the left and right side of the spinal cord (Reinhold Necker, 1997). Their pair-wise, segmental configuration opens the possibility for differential sensing, where rolling motions would be sensed as the difference in sensor activation between left and right, and/or pitching motion as the difference between proximal and distal lobes.

Future directions: If the LSO indeed acts like an inertial measurement sensor, this presents potential for morphological diversity in the LSO structure among birds, associated with different locomotor ecologies. Variations in the properties of the bony canal space, ligaments network and glycogen body could vary the directional sensitivity and frequency response characteristics of the LSO structures, acting to tune the LSO to specific locomotor demands. For example, a larger or higher density glycogen body would increase the sensitivity of the LSO-structure to external accelerations. Variation in the denticulate ligament network morphology could tune the directional sensitivity and frequency response characteristics. Variation in the canal space or transverse semicircular grooves’ prominence could enhance fluid flow, which could play a direct role in mechanosensing as suggested by Necker (Reinhold Necker, 2006), or, alternatively, tune the magnitudes of soft tissue oscillations by enabling fluid flow around the moving soft tissues.

Once a physical model of LSO mechanosensing has been developed, this would enable model-based predictions of morphological variations related to different locomotor ecologies. For example, swimmers and divers experience relatively low vertical accelerations, have few visual cues underwater, and might therefore require more sensitive angular sensing compared to translational sensing. Tree climbers must accurately balance in 3D—suggesting that they should have small glycogen body mass together with thin ligaments to enable rapid, high frequency responses to small angular fluctuations. Specialized runners experience high leg impact forces, which should require a robust network of ligaments to limit the excursions of the spinal cord, with an accentuated fluid space to act as a fluid damper. In additional to specialization of LSO structure between species, we might also expect changes through ontogeny to tune balance sensing to the changing locomotor capabilities and demands on juvenile body structures.
5. Conclusion

We have generated a three-dimensional reconstruction of the lumbosacral spinal canal of the adult *common quail*, including the soft tissue and the denticulate ligament network. Our morphological data is based on the combination of digital dissection of a contrast-enhanced uCT data set, and classical dissection of smaller soft tissue structures of the denticulate ligaments network below the spinal cord. Our morphometric analysis suggests an updated hypothesis for LSO mechanosensing, in which the combined structure of glycogen body, spinal cord, and denticulate ligaments could be tuned to oscillate in response to locomotor accelerations. The transverse semi-circular grooves may act as CSF reliefs to enable fluid flow around moving soft tissue. The combined LSO morphology is a fluid-submerged spring-damper-mass system that suggests an accelerometer-like balance sensing function.

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**Authors contribution:** MD and ABS conceived the original concept and provided supervisory support and feedback to VK. MD provided the quail CT scan. VK conducted digital and classical dissections, and prepared figures, video, and the 3d-model, with input from ABS and MD. VK and ABS analysed data, with feedback from MD. All authors contributed to interpretation, and writing and editing the manuscript.

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Figure 2: Anatomy of the lumbosacral enlargement. The 3D model is created from the digital dissection. (A) Right lateral view at the sagittal plane section of the lumbosacral spine. (B) Coronal section view, the canal is cut at half height, soft tissues are not cut.
Figure 3: Anatomy of the synsacrum, classic dissection. (A) dorsal, (B) right lateral, and (C) ventral view. A-C shows the intact synsacrum with a closed lumbosacral canal. Posterior left, and anterior right. (D) shows the spinal canal opened and from dorsal. Glycogen body and spinal cord are removed, to reveal the denticulate ligaments in the region of the glycogen body (S2 to L4). The denticulate ligaments are emphasized by dashed white lines, and the network’s nodes by circles. The tips of a pair of tweezer are inserted through the foramina of the S1 and L4 vertebrae.
Figure 4: The lumbosacral vertebral sections of a quail, schematic view. (A) Sagittal section through the spinal canal, right lateral view. Solid black curves present the cut planes at the vertebral fusion regions. Dashed black curves are the cut planes at the vertebral middle regions. (B) Transverse section through the vertebral column at the fusion region of the S1 and L4. The glycogen body spans between vertebrae S2 to L4. (C) Transverse section between L3 and L2.
Figure 5: (a) Height and width of the spinal canal. A-B (blue curve, square markers): spinal canal width. E-F (green curve, diamond markers): spinal canal height. (b) Morphometrics of the neural soft tissue. Abbreviations: C-D spinal cord width with square markers, G-GH glycogen body height with circle markers; GH-I middle of the spinal cord height shown with triangle markers, L-M spinal cord hemisphere height, diamond markers. (c) CSF space morphometry. E-G vertical distance between the glycogen body top and the spinal canal dorsal (circle markers), E-GH vertical distance between the spinal cord and the spinal canal dorsal in the range outside the glycogen body (circle markers), I-F vertical distance between the spinal cord ventral surface and the spinal canal (diamond markers), D-B horizontal, lateral distance between spinal cord and the spinal canal (square markers). (d) The strain calculation of the transverse ligaments at their maximum ventral and dorsal positions of the spinal cord, in reference to their resting position. Asterisk markers (black line) show the maximal transverse ligament strain at the dorsal position, and the blue line with hexagram markers the ventral position strain.
Figure 6: Volume measurements by spinal canal segments. A. Right lateral view at the 3D model of the spinal cord, the glycogen body, and the CSF surrounding both. Cuts are placed along the anatomical fusion zone, hence they are slightly tilted. The coordinate system’s origin is placed between the geometric center of both femur head sockets, with the x-axis aligned in-parallel with the denticulate ligament. The horizontal distance (x-axis) between the glycogen body’s center of mass and the femur head (coordinate system center) is 6.2 mm, the radial distance is 6.8 mm. B. Segment S1 of the spinal canal, and its contents. C. Right lateral view at the isolated segment S1 of the spinal canal. E. Data points show the volume measurements per spinal canal segment.
Figure 7: Top view at the open spinal canal, showing the network of denticulate ligaments. The glycogen body and the spinal cord were removed. The ligaments are attached to the spinal canal via ventral processes, at the fusion zones between vertebrae. The right lateral longitudinal ligament is raised by a pair of tweezers, and the deformation shows the attachment points with the spinal canal. Anterior is to the right.

Figure 8: Details denticulate ligaments. A) Digital dissection, top view at the opened spinal canal. B) Classical dissection, else identical to A. C) Transverse section through the spinal canal at the S1-L4 fusion region.
Figure 9: Potential for soft tissue movement and the resulting ligament deformation, schematic presentation. (A) Soft tissue in the (A) resting, (B) upper, and (C) lower position. Red indicated are the transverse ligaments. Black arrows show up- and down-movements of the glycogen body, the spinal cord tissue, and the ligament nodes. The nodes are defined as the intersection of the ventral transverse and ventral longitudinal ligaments. The dashed lines show the ventral transverse ligaments when deformed. The estimated angular deflection of the transverse ligaments are indicated by white range symbols.
A. Appendix
### Table 4: Scientific abbreviations and symbols.

| Abbreviation | Name                  | Abbreviation | Name                           |
|--------------|-----------------------|--------------|--------------------------------|
| A            | anterior              | a.isc.       | ala ischii                     |
| b.           | bone                  | a.p.i.       | ala preacetabularis ilii       |
| COM          | center of mass        | a.pr.i.      | ala preacetabularis ilii       |
| CSF          | cerebrospinal fluid   | an.          | antitrochanter                 |
| f.ac.        | foramen acetabuli     | bif.t.p.     | bifurcated transverse processes|
| f.h.         | femoral head          | c.i.d.       | crista iliaca dorsalis         |
| f.s.n.       | foramina spinal nerve  | c.s.s.       | crista spinosa synsacri        |
| g.b.         | glycogen body         | ex.c.sy.     | extremitas cranialis synsacri  |
| l.l.l.       | lateral longitudinal ligament | f.n.sp. | fused neural spines |
| P            | posterior             | i.           | ilium                          |
| sp.c.        | spinal cord           | is.          | ischium                        |
| TG           | transverse semi-circular grooves | p.    | pubis                          |
| v.c.         | spinal canal          | p.il.        | postacetabular ilium           |
| v.l.l.       | ventral longitudinal ligament | pr.tr.s. | processus transversus sacralis |
| v.p.         | ventral process       | pre.il.      | preacetabular ilium            |
| v.t.l.       | transverse ligament   | s.an.        | sulcus antitrochantericus      |
|              |                       | s.i.         | sync. ilioischadiaca           |
|              |                       | s.v.sy.      | sulcvent. synsacri             |
|              |                       | t.pr.        | tuberculum preacetabulare      |

### Table 5: Ligament intersection angles, numbers are mean(SD) angles over two or three classical dissection specimen.

|          | S3-S2  | S2-S1  | S1-L4  | L4-L3  | L3-L2  |
|----------|--------|--------|--------|--------|--------|
| 72(5)°   | 71(6)° | 84(4)° | 98(7)° | 92(10)°|

### Table 6: The length in [mm] of each vertebra and the glycogen body. Lengths were measured from the digital model, at the y-height of the denticulate ligament network.

|          | S4   | S3   | S2   | S1   | L4   | L3   | L2   | GB   |
|----------|------|------|------|------|------|------|------|------|
| Length x-direction [mm] | 2.2  | 2.3  | 1.9  | 2.2  | 2.4  | 2.2  | 3.1  | 5.2  |

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Figure 10: Snapshot of the 3D model of the lumbosacral region. The model contains eight objects, and is shared via SketchFab https://skfb.ly/6U6W/. 