eNeuro

Sensory and Motor Systems

Molecular Markers of Mechanosensation in Glycinergic Neurons in the Avian Lumbosacral Spinal Cord

Kathryn E. Stanchak,[1] Kimberly E. Miller,[1,2] Eric W. Lumsden,[1] Devany Shikiar,[2] Calvin Davis,[3] Bingni W. Brunton,[1] and David J. Perkel[1,4]

[1]Department of Biology, University of Washington, Seattle, WA, [2]Department of Psychology, University of Washington, Seattle, WA, [3]Department of Biology, University of Washington, Seattle, WA 98195, and [4]Department of Otolaryngology, University of Washington, Seattle, WA

Visual Abstract
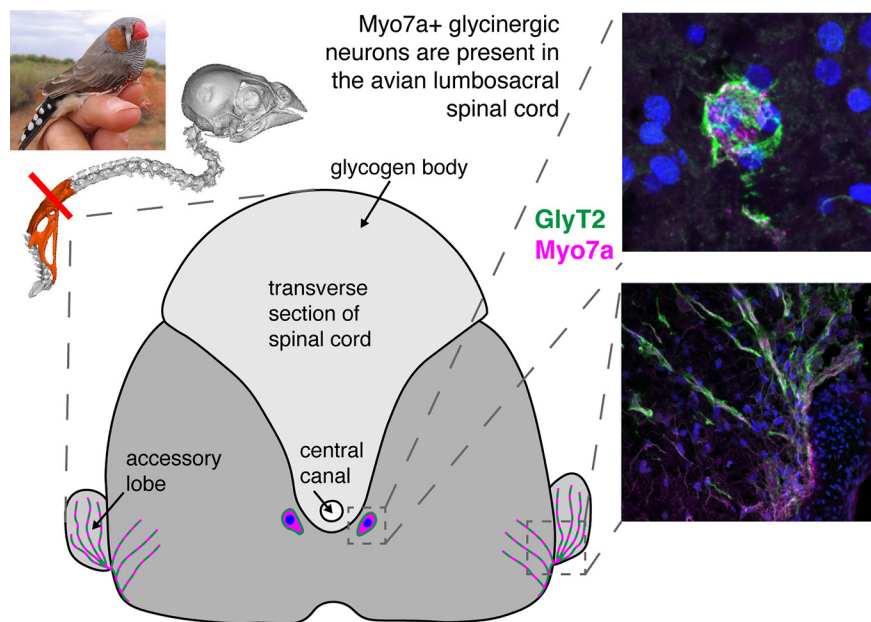
Significance Statement

The spinal cord can help control posture by sensing bending of the spinal column. In birds, the lower spinal column is fused and cannot bend. Notably, this region of the avian spinal cord has several specialized features compared with other vertebrates. We report neurons in the lower bird spinal cord that express proteins known to be involved in sensing mechanical signals. These neurons share characteristics of other spinal cord mechanosensory cells. This suggests that spinal cords across vertebrates are capable of mechanosensation and that spinal mechanosensory networks have evolved to allow unique functions, such as in the constrained lower spinal cord of birds. These data provide an avenue for molecular manipulations of these cells, permitting experimental tests of their role in controlling posture.

Birds are exceptionally adept at controlling their body position. For example, they can coordinate rapid movements of their body while stabilizing their head. Intriguingly, this ability may rely in part on a mechanosensory organ in the avian lower spinal cord called the lumbosacral organ (LSO). However, molecular mechanotransduction mechanisms have not been identified in the avian spinal cord. Here, we report the presence of glycinergic neurons in the LSO that exhibit immunoreactivity for myosin7a and espin, molecules essential for function and maintenance of hair cells in the inner ear. Specifically, we find glycinergic cell bodies near the central canal and processes that extend laterally to the accessory lobes and spinal ligaments. These LSO neurons are reminiscent of glycinergic neurons in a recently-described lateral spinal proprioceptive organ in zebrafish that detects spinal bending. The avian LSO, however, is located inside a series of fused vertebrae called the synsacrum, which constrains spinal bending. We suggest the LSO may be a modification and elaboration of a preexisting mechanosensory spinal network in vertebrates. A mechanistic understanding of its function may be an important clue to understanding the evolution and development of avian locomotion.

Introduction

Animals must be able to sense the position of their body to execute targeted movements, especially in response to environmental perturbations. Birds are often noted for their exceptional body-positioning abilities, including keeping their head still while their body twists and turns in response to wind or a swaying perch. As in other vertebrates, the vestibular organs of the inner ear and distributed proprioceptors affiliated with the musculoskeletal system provide sensory inputs to the avian body-positioning control system. Interestingly, birds are also hypothesized to have a mechanosensory organ within their highly-modified lumbosacral spinal cord, known as the avian lumbosacral organ (LSO), which may provide additional mechanosensory information (Necker, 2006). The LSO is found in all extant birds and may vary in morphology depending on a species' locomotor habits (Stanchak et al., 2020). However, the mechanotransduction mechanisms of the LSO remain unresolved.

It is becoming increasingly clear that vertebrates have spinal neurons that can provide sensory information on body positioning by detecting spinal bending. For instance, edge cells detect stretch at the lateral margin of the lamprey spinal cord (Grillner et al., 1984). Cerebrospinal fluid-contacting neurons (CSF-cNs) within the ependymal cells surrounding the central canal—found across vertebrates—sense alterations to CSF flow in the central canal because of spinal bending (Böhm et al., 2016; Orts-Del'Immagine et al., 2020). Recently, a lateral glycinergic proprioceptive organ has been described in zebrafish that detects stretching of neural tissue near intervertebral discs as the spine bends (Picton et al., 2021).

While these examples of spinal mechanosensory neurons all detect bending of the spine, the avian LSO lies within the synsacrum, a rigid series of fused vertebrae that prevent the spinal cord from bending (Fig. 1A–D). Indeed, the LSO has a striking set of specializations that make it anatomically distinct from the rest of the spinal cord. First, a glycogen body sits within a dorsal split of the spinal cord. The central canal passes through the base of the glycogen body such that the ependymal cells surrounding the central canal are fully encapsulated by glycogen body tissue (Fig. 1D,E). Second, the spinal cord has segmentally-repeating, laterally-projecting accessory lobes, which are protrusions of neural tissue that roughly align with transverse, canal-like recesses in the surrounding bone (Fig. 1D). Modified spinal membranes route cerebrospinal fluid through these recesses (Necker, 2006). Third, the entire neural structure is suspended within the synsacrum by a modified dentate ligament. At the ventrolateral corners of the spinal cord, the accessory lobes and the lateral dentate ligament adjoin the white matter (Figs. 1D, 2A–C). Based on these anatomic specializations, the LSO is hypothesized to work by transducing cerebrospinal fluid flow through mechanosensory neurons in the accessory lobes (Necker, 1999) or by acting as a pre-loaded vibratory structure (Kamska et al., 2020).

While some cellular-level neuroanatomy of the LSO has been described, little is known about the molecular features of putative mechanosensory cells. In 1905, Imhof (1905) illustrated cells and processes particular to the avian LSO, including around the central canal. Accessory lobe neurons project contralaterally, then both rostrally and caudally (Eide, 1996; Eide and Glover, 1996; Necker, 2006). Neurons within the accessory lobes produce a number of neurotransmitters, including glycine, glutamate, and GABA, as well as the enzyme choline acetyltransferase (Milinski and Necker, 2001). Processes from the neurons within the accessory lobes project into local lacunae, which are hypothesized to be part of a mechanosensory mechanism (Rosenberg and Necker, 2000, 2002;
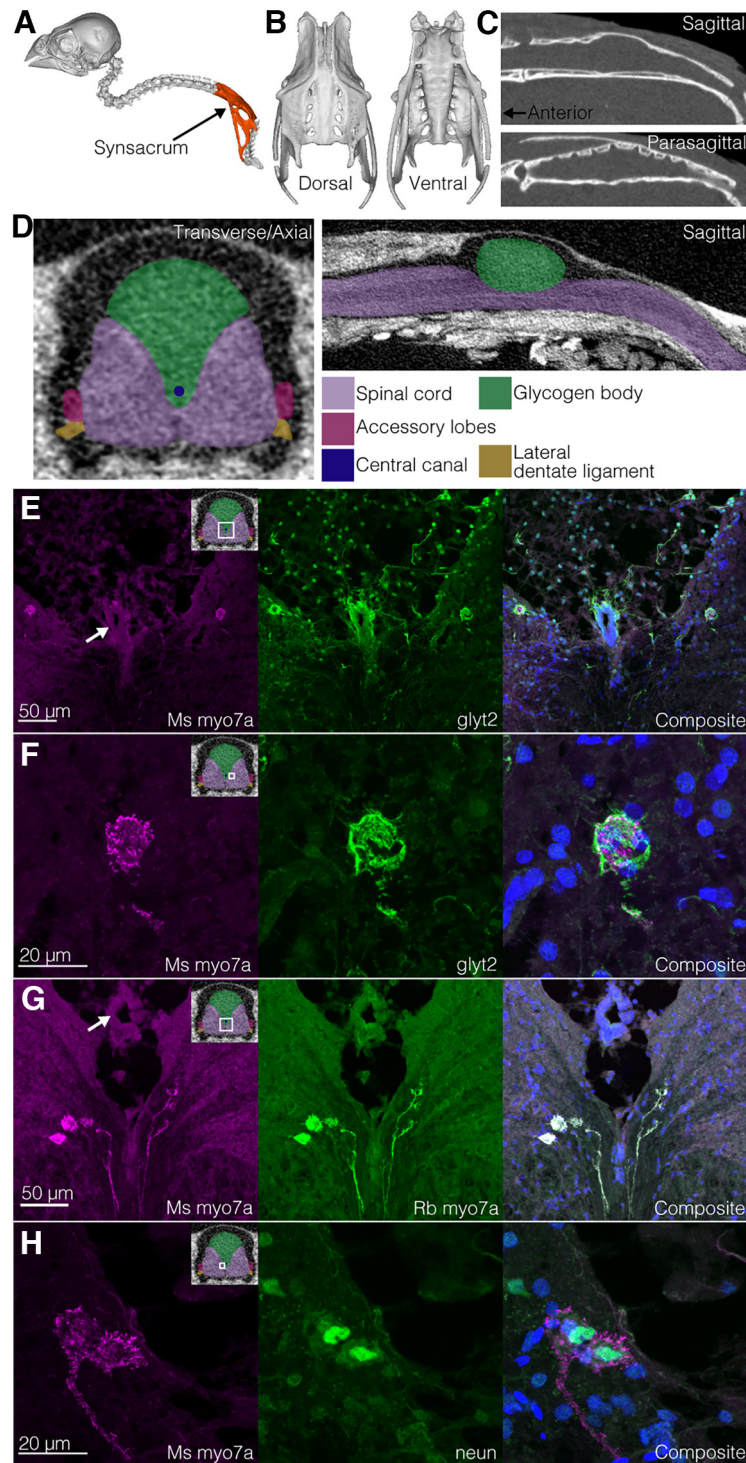
Figure 1. Glycinergic neuronal cell bodies near the central canal of the avian lumbosacral organ (LSO) display myosin7a immunoreactivity. *A*, The synsacrum (fused to the pelvic bones) is highlighted within a reconstructed CT scan of the zebra finch axial skeleton (Yale Peabody Museum specimen #125076). *B*, Dorsal and ventral views of the synsacrum, reconstructed from the CT scan. *C*, A sagittal section through the vertebral canal of the synsacrum CT scan shows the dorsal expansion of the vertebral canal that holds the glycogen body. A parasagittal section shows transverse canal-like structures on the internal dorsal side of the vertebral canal that roughly align with the accessory lobes (see Stanchak et al., 2020). *D*, A diagram of the neural tissue of the LSO based on dissection and histology observations and a contrast-enhanced CT scan (data from Stanchak et al., 2020). *E*, Cells near the central canal (middle) that are immunoreactive for both glyt2 and myo7a. *F*, Higher-resolution image of the right cell from *E*. *G*, Labeling with two different myo7a antibodies (mouse and rabbit). *H*, neun/fox3 labeling within the myo7a+ cells. White arrow in the first panel of *E* and *G* points to the ependymal layer surrounding the central canal. Upper right diagram in the first panel of *E–H* indicates approximately where in the transverse section the image was taken. In composite images of *E–H*, blue is DAPI.
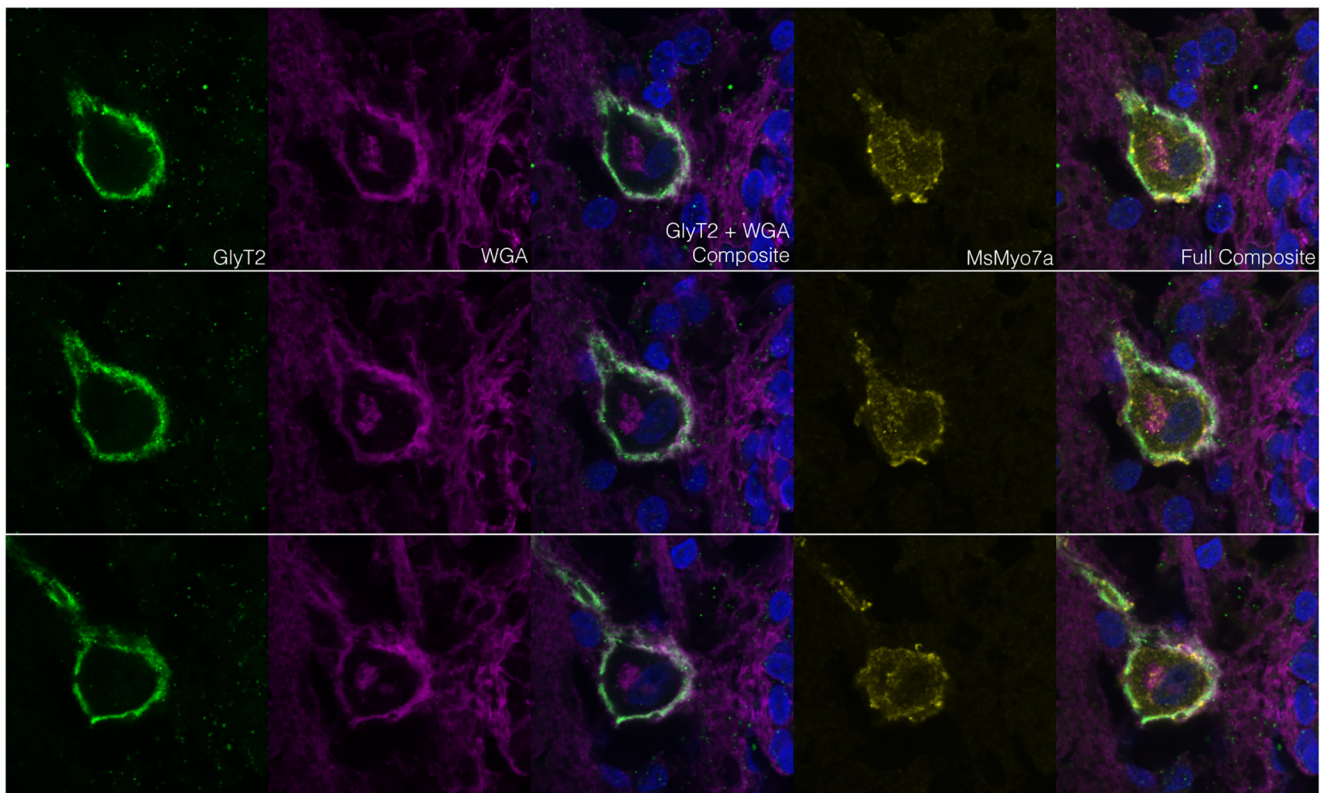
Figure 2. Glycine transporter 2 is located in the cell membrane of the neuronal cell bodies that display myosin7a immunoreactivity. Each horizontal row of images is a separate optical section along the z-axis of one cell. WGA is wheat germ agglutinin, a lectin that labels plasma membranes. In composite images, blue is DAPI. Nuclei of the ependymal cells of the central canal can be seen in the lower right of the composite images.

Necker, 2002). To date, no molecular mechanotransduction molecule or mechanism has been identified in the LSO.

Here, we describe neurons within the avian LSO that have molecular characteristics of known mechanosensory cells and are well positioned to transduce mechanical signals. These results provide critical evidence supporting the hypothesis that the LSO has a role in proprioception and balance in birds.

Materials and Methods

Tissue preparation

The spinal column was harvested from zebra finch (*Taeniopygia guttata*) individuals of either sex used for other experiments. Most were postfixed in 4% paraformaldehyde in phosphate buffer (PB) for 24 h. Results from seven individuals are reported here. Two spinal columns were alternatively harvested from transcardially-perfused individuals. In most samples, the LSO region of the spinal cord was dissected from the surrounding synsacrum bone and placed in 20% sucrose in PB for a minimum of overnight. The region of the LSO surrounding the glycogen body was then sectioned transversely at 20 μm on a cryostat in four alternating series with negative controls. For two samples (labeled with mouse-antimyo7a + rabbit-antiglyt2 and mouse-antimyo7a + rabbit-antimyo7a), the synsacrum

bone was first decalcified using 12% EDTA neutralized with NaOH before the entire spinal column was sectioned. We saw no difference in reported results between decalcified and undecalcified specimens, but we note that anti-myo7a labeling seemed sensitive to extended time prefreezing in other samples tested but not reported here.

Immunohistochemistry

Sections were briefly dried then washed 3× in 0.1% Triton X-100 in PBS (PBST) before they were blocked with 5% neutral goat serum (Abcam or Vector Labs). Sections were then incubated for double-labeling experiments with primary antibody (Ab) diluted in PBS, washed 3× in PBST, incubated with secondary Ab, then washed in PBST, PBS, and dH$_2$O and incubated for 10 min in 1:1000 aqueous DAPI (Biotium) before mounting with Fluoromount G (SouthernBiotech). Two timing protocols were used: a short protocol with a 1-h block, overnight incubation with primary Ab, and 2 h of incubation with secondary Ab; and a long protocol with overnight block, two-night incubations with primary Ab, and overnight incubation with secondary Ab. For some Abs, we altered dilutions across experiments (noted below), but we saw no difference in reported results.

Primary Abs used were rabbit-antiglyt2 (Alomone AGT-012; 1:400 n = 5 or 1:200 n = 1; 13/14 immunogen amino
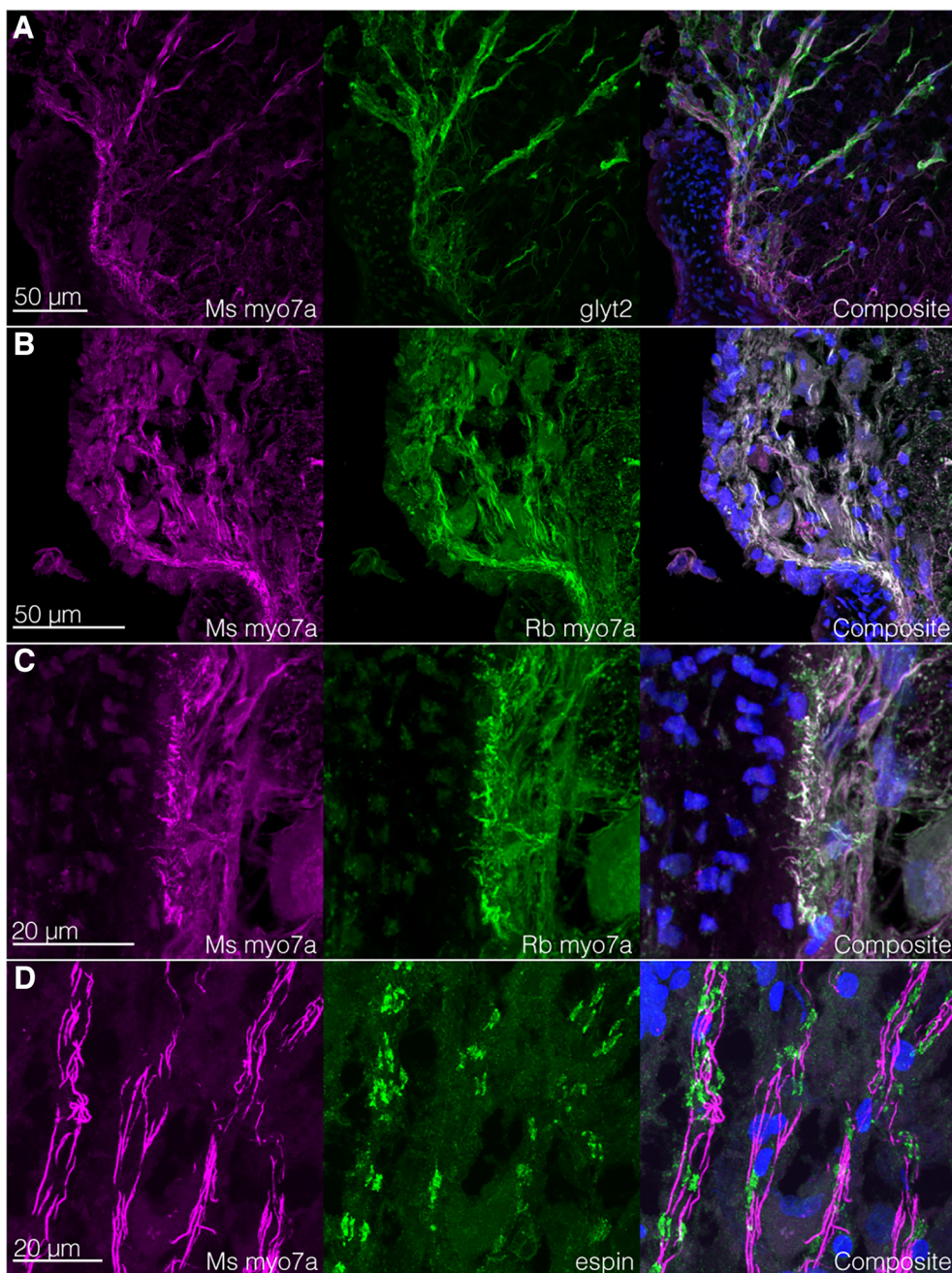
Figure 3. Lateral glycinergic processes are immunoreactive for proteins also present in inner ear hair cell stereocilia. **A**, Glyt2+ ventrolateral processes are immunoreactive for myo7a. Margin of spinal cord with dentate ligament is at lower left; upper left is accessory lobe. **B**, **C**, The accessory lobe processes (**B**) and terminations at the dentate ligament (**C**) double-label with two different antibodies for myo7a (mouse and rabbit). **D**, Myo7a-immunoreactive processes in the accessory lobe also react with anti-espin. **A–D**, In composite images, blue is DAPI. Upper right diagram in the first panel of each subfigure indicates approximately where in the transverse section the image was taken.

acids identical to *T. guttata* glyt2 according to BLAST-P search), mouse-antimyo7a (Developmental Studies Hybridoma Bank 138–1, 1:100 $n=5$ or 1:200 $n=4$; avian cross-reactivity listed by manufacturer), rabbit-antimyo7a (Proteus 25–6790, 1:400 $n=3$ 1:500 $n=2$; avian cross-reactivity listed by manufacturer), rabbit-antiespin (Invitrogen PA5-55941, 1:200 $n=5$; labeled interior side of ependymal cells in *T. guttata* spinal cord, where espin+ apical tips of CSF-

cNs are located; 54/64 immunogen amino acids identical to *T. guttata* espin according to BLAST-P search), rabbit-antineun/fox3 (Invitrogen 702022; 1:200 $n=3$ or 1:500 $n=2$; labeled cells in spinal cord as expected). The double-labeling experiments were rabbit-antiglyt2 and mouse-antimyo7a ($n=4$); rabbit-antimyo7a and mouse-antimyo7a ($n=5$); rabbit-antiespin and mouse-antimyo7a ($n=5$); and rabbit-antineun and mouse-antimyo7a ($n=5$).

Secondary Abs used were goat anti-mouse or goat anti-rabbit AlexaFluor 488, 568, and 647 (Invitrogen; 1:300). For two of the individuals double-labeled rabbit-antiglyt2 and mouse-antimyo7a, we applied a wheat germ agglutinin (WGA) membrane label conjugated to AlexaFluor 488 at 1:200 with the secondary Ab solution.

Slides were viewed and imaged on Nikon Eclipse epifluorescence, Olympus FV1000, and Nikon A1R confocal microscopes. All images presented in the text are confocal (Figs. 1, 3, Olympus FV1000; Fig. 2, Nikon A1R).

Results

Immunohistochemical assays of the zebra finch LSO demonstrated medial neuronal cell bodies and ventrolateral processes that expressed the sodium-dependent and chloride-dependent glycine transporter 2 (glyt2). The glycinergic medial cell bodies tended to be found near the central canal but not within the ependymal cell layer or the glycogen body that surrounds the central canal (Fig. 1D, E). Further immunohistochemical assays demonstrated that these medial glycinergic cells were also immunoreactive for myosin 7a (myo7a; Fig. 1E–G), an atypical myosin essential for proper inner ear hair cell function (Li et al., 2020) and neun/fox3, a neuronal cell body marker (Fig. 1H). Glyt2 expression in the myo7a+ cells of interest colabeled with the membrane-binding wheat-germ agglutinin, indicating that glyt2 is in the membrane of and not in presynaptic terminals adjacent to the myo7a+ cell (Fig. 2).

The glycinergic (glyt2+) ventrolateral processes traversed the gray matter to the white matter. Some processes extended into the accessory lobes, and others terminated at the margin between the dentate ligament and the spinal cord (Fig. 2A). These processes were also immunoreactive for myo7a (Fig. 2B,C). The processes within the accessory lobes were immunoreactive for both myo7a and espin antibodies, which in transverse sections colocalized in an alternating pattern (Fig. 2D). Espin is an actin-bundling protein, especially in sensory cells (Sekerková et al., 2011). Two different antibodies (from different manufacturers) for myo7a colabeled cellular structures (Figs. 1F, 3B,C). It was unclear from our sections whether these lateral processes extend from the medial cell bodies.

Discussion

The putative mechanosensory neurons in the avian LSO described here comprise a lateral group of neurons that express glyt2, as in the lateral spinal proprioceptive organ of zebrafish (Picton et al., 2021). We suggest these glyt2+ neurons in the avian LSO may be a modified version of the zebrafish lateral glycinergic proprioceptive organ. Notably, there is evidence that these lateral mechanosensory zebrafish cells initially develop from progenitors near the central canal (Picton et al., 2021), which may be a developmental connection between the medial avian cells described here and the zebrafish cells.

There is no clear connection between CSF-cNs and the glyt2+ cells reported here. CSF-cNs can be found ectopically (outside of the ependymal layer surrounding the central canal (Tonelli Gombalová et al., 2020; Jurčić et al., 2021). However, a recent transcriptome analysis of spinal dynorphin-lineage cells found a cluster with characteristics of CSF-cNs but comparatively low expression of glyt2 transcript (SLC6A5; Serafin et al., 2021, their Fig. 2). CSF-cNs are known to be GABAergic (Stoeckel et al., 2003; Wyart et al., 2009; Djenoune et al., 2014); a direct comparison of GABA and glycine in CSF-cNs and these avian glycinergic cells would be helpful for distinguishing whether or not these cells share a lineage. Pkd2l1, polycystic kidney disease 2-like 1 protein, is a known marker of CSF-cNs and is a required component of the mechanosensing mechanism in zebrafish (Sternberg et al., 2018). An assessment of pkd2l1 expression in the avian glycinergic neurons reported here would also be clarifying, although to our knowledge, as of this writing there are no commercially-available antibodies for pkd2l1 for avian tissue. The characteristics we have reported here could guide future explicit lineage tracing or single-cell characterization, facilitating a more thorough comparison with CSF-cNs.

Our observations that glycinergic neurons in the LSO have immunoreactivity for myo7a and espin are critical evidence of their mechanosensory potential. Both myo7a and espin are found in inner ear hair cell stereocilia, and myo7a is directly implicated in mechanotransduction in hair cells (Sekerková et al., 2011; Li et al., 2020). In addition, espin is also found in the cilia of CSF-cNs (as is the atypical myosin 3b; Desban et al., 2019). The CSF-cN cilium is essential for skilled locomotor activity (Gerstmann et al., 2022). The presence of myo7a and espin in these LSO glycineric neurons does not, however, explain how mechanical force may be transduced into electrical signals, i.e., the key force-transducing ion channel involved in mechanosensation is still unknown. In hair cells, mechanically-gated channels convert mechanical forces to electrical signals (for review, see Maoiléidigh and Ricci, 2019). The transmembrane channel-like proteins 1 and 2 (TMC 1/2) have been identified as the mechanosensitive components of the mechanoelectrical transduction apparatus in hair cells (Kurima et al., 2015; Jia et al., 2020). Piezo1 and piezo2 are mechanically-activated components of transmembrane channels (Coste et al., 2010, 2012) involved in homeostatic and somatosensory functions, respectively (for review, see Poole, 2021; Szczot et al., 2021). Pkd2l1, the marker of CSF-cNs mentioned above, is part of the polycystic family of transient receptor potential (TRP) channels, many of which are implicated in mechanotransduction (for review, see Christensen and Corey, 2007; Delmas et al., 2011). Pkd2l1 is required for CSF-cN function in zebrafish (Böhm et al., 2016; Sternberg et al., 2018), but interestingly does not appear to be required for CSF-cN function in mice (Gerstmann et al., 2022). Both protein and transcript assays for the above candidate channels (TMC 1/2, piezo2, pkd2l1) as well as putative mechanosensitive current properties of LSO glycinergic neurons could help identify the molecular structure of a mechanotransduction apparatus.

The fused vertebrae of the bird synsacrum prevent the lower spine from bending; therefore, the CSF-cNs and lateral proprioceptive spinal organ cannot function as has

been described in other vertebrates. Rather, the neuronal processes in the accessory lobes may sense cerebrospinal fluid flow (as hypothesized and summarized by Necker, 2006), while the processes at the junction between the spinal cord and the dentate ligament may detect differential vibration among tissue types (according to the vibration/suspension hypothesis by Kamska et al., 2020).

Our finding of the presence of myo7a-immunoreactivity and espin-immunoreactivity in LSO neurons is a starting point that critically enables testing of cellular, organ, and organismal-level mechanotransduction mechanistic hypotheses. Myo7a in particular may be a promising target for molecular manipulations. The ability of birds to impeccably control their body position may have helped them diversify into a clade with multifunctional locomotor specializations, including the capacity for terrestrial bipedalism as well as flight. Thus, continued research on the avian LSO may reveal foundational physiological and developmental mechanisms that enabled the evolution and diversification of avian locomotion.

References

Böhm UL, Prendergast A, Djenoune L, Figueiredo SN, Gomez J, Stokes C, Kaiser S, Suster M, Kawakami K, Charpentier M, Concordet JP, Rio JP, Del Bene F, Wyart C (2016) Csf-contacting neurons regulate locomotion by relaying mechanical stimuli to spinal circuits. Nat Commun 7:10866.

Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, Dubin AE, Patapoutian A (2010) Piezo1 and piezo2 are essential components of distinct mechanically activated cation channels. Science 330:55–60.

Coste B, Xiao B, Santos JS, Syeda R, Grandl J, Spencer KS, Kim SE, Schmidt M, Mathur J, Dubin AE, Montal M, Patapoutian A (2012) Piezo proteins are pore-forming subunits of mechanically activated channels. Nature 483:176–181.

Christensen AP, Corey, David P (2007) TRP channels in mechanosensation: direct or indirect activation? Nat Rev Neurosci 8:510–521.

Delmas P, Hao J, Rodat-Despoix L (2011) Molecular mechanisms of mechanotransduction in mammalian sensory neurons. Nat Rev Neurosci 12:139–153.

Desban L, Prendergast A, Roussel J, Rosello M, Geny D, Wyart C, Bardet PL (2019) Regulation of the apical extension morphogenesis tunes the mechanosensory response of microvilliated neurons. PLoS Biol 17:e3000235.

Djenoune L, Khabou H, Joubert F, Quan FB, Nunes Figueiredo S, Bodineau L, Del Bene F, Burcklé C, Tostivint H, Wyart C (2014) Investigation of spinal cerebrospinal fluid-contacting neurons expressing pkd2l1: evidence for a conserved system from fish to primates. Front Neuroanat 8:26.

Eide AL (1996) The axonal projections of the hofmann nuclei in the spinal cord of the late stage chicken embryo. Anat Embryol (Berl) 193:543–557.

Eide AL, Glover JC (1996) Development of an identified spinal commissural interneuron population in an amniote: neurons of the avian Hofmann nuclei. J Neurosci 16:5749–5761.

Gerstmann K, Jurčić N, Blasco E, Kunz S, de Almeida Sassi F, Wanaverbecq N, Zampieri N (2022) The role of intraspinal sensory neurons in the control of quadrupedal locomotion. Curr Biol 32:2442–2453.e4.

Grillner S, Williams T, Lagerbäck PA (1984) The edge cell, a possible intraspinal mechanoreceptor. Science 223:500–503.

Imhof G (1905) Anatomie und Entwicklungsgeschichte des Lumbalmarkes bei den Vögeln. Bonn: Friedrich Cohen.

Jia Y, Zhao Y, Kusakizako T, Wang Y, Pan C, Zhang Y, Nureki O, Hattori M, Yan Z (2020) Tmc1 and tmc2 proteins are pore-forming subunits of mechanosensitive ion channels. Neuron 105:310–321.

Jurčić N, Michelle C, Trouslard J, Wanaverbecq N, Kastner A (2021) Evidence for pkd2l1-positive neurons distant from the central canal in the ventromedial spinal cord and medulla of the adult mouse. Eur J Neurosci 54:4781–4803.

Kamska V, Daley M, Badri-Spröwitz A (2020) 3d anatomy of the quail lumbosacral spinal canal—implications for putative mechanosensory function. Integr Org Biol 2:obaa037.

Kurima K, Ebrahim S, Pan B, Sedlacek M, Sengupta P, Millis BA, Cui R, Nakanishi H, Fujikawa T, Kawashima Y, Choi BY, Monahan K, Holt JR, Griffith AJ, Kachar B (2015) Tmc1 and tmc2 localize at the site of mechanotransduction in mammalian inner ear hair cell stereocilia. Cell Rep 12:1606–1617.

Li S, Mecca A, Kim J, Caprara GA, Wagner EL, Du TT, Petrov L, Xu W, Cui R, Rebustini IT, Kachar B, Peng AW, Shin JB (2020) Myosin-viia is expressed in multiple isoforms and essential for tensioning the hair cell mechanotransduction complex. Nat Commun 11:2066.

Maoiléidigh DÓ, Ricci AJ (2019) A bundle of mechanisms: inner-ear hair-cell mechanotransduction. Trends Neurosci 42:221–236.

Milinski T, Necker R (2001) Histochemical and immunocytochemical investigations of the marginal nuclei in the spinal cord of pigeons (Columba livia). Brain Res Bull 56:15–21.

Necker R (1999) Specializations in the lumbosacral spinal cord of birds: morphological and behavioural evidence for a sense of equilibrium. Eur J Morphol 37:211–214.

Necker R (2002) Mechanosensitivity of spinal accessory lobe neurons in the pigeon. Neurosci Lett 320:53–56.

Necker R (2006) Specializations in the lumbosacral vertebral canal and spinal cord of birds: evidence of a function as a sense organ which is involved in the control of walking. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 192:439–448.

Orts-Del'Immagine A, Cantaut-Belarif Y, Thouvenin O, Roussel J, Baskaran A, Langui D, Koëth F, Bivas P, Lejeune FX, Bardet PL, Wyart C (2020) Sensory neurons contacting the cerebrospinal fluid require the Reissner fiber to detect spinal curvature in vivo. Curr Biol 30:827–839.

Picton LD, Bertuzzi M, Pallucchi I, Fontanel P, Dahlberg E, Björnfors ER, Iacoviello F, Shearing PR, El Manira A (2021) A spinal organ of proprioception for integrated motor action feedback. Neuron 109:1188–1201.

Poole K (2021) The diverse physiological functions of mechanically activated ion channels in mammals. Annu Rev Physiol 84:307–329.

Rosenberg J, Necker R (2000) Fine structural evidence of mechanoreception in spinal lumbosacral accessory lobes of pigeons. Neurosci Lett 285:13–16.

Rosenberg J, Necker R (2002) Ultrastructural characterization of the accessory lobes of lachi in the lumbosacral spinal cord of the pigeon with special reference to intrinsic mechanoreceptors. J Comp Neurol 447:274–285.

Sekerková G, Richter C-P, Bartles JR (2011) Roles of the espin actin-bundling proteins in the morphogenesis and stabilization of hair cell stereocilia revealed in cba/caj congenic jerker mice. PLoS Genet 7:e1002032.

Serafin EK, Paranjpe A, Brewer CL, Baccei ML (2021) Single-nucleus characterization of adult mouse spinal dynorphin-lineage cells and identification of persistent transcriptional effects of neonatal hindpaw incision. Pain 162:203–218.

Stanchak KE, French C, Perkel DJ, Brunton BW (2020) The balance hypothesis for the avian lumbosacral organ and an exploration of its morphological variation. Integr Org Biol 2:obaa024.

Sternberg JR, Prendergast AE, Brosse L, Cantaut-Belarif Y, Thouvenin O, Orts-Del'Immagine A, Castillo L, Djenoune L, Kurisu S, McDearmid JR, Bardet PL, Boccara C, Okamoto H, Delmas P, Wyart C (2018) Pkd2l1 is required for mechanoception in cerebrospinal fluid-contacting neurons and maintenance of spine curvature. Nat Commun 9:3804.

Stoeckel ME, Uhl-Bronner S, Hugel S, Veinante P, Klein M-J, Mutterer J, Freund-Mercier M-J, Schlichter R (2003) Cerebrospinal

fluid-contacting neurons in the rat spinal cord, a γ-aminobutyric acidergic system expressing the p2x2 subunit of purinergic receptors, psa-ncam, and gap-43 immunoreactivities: light and electron microscopic study. J Comp Neurol 457:159–174.

Szczot M, Nickolls AR, Lam RM, Chesler AT (2021) The form and function of piezo2. Annu Rev Biochem 90:507–534.

Tonelli Gombalová Z, Košuth J, Alexovič Matiašová A, Zrubáková J, Žezõula I, Giallongo T, Di Giulio AM, Carelli S, Tomašková L, Daxnerová Z, Ševc J (2020) Majority of cerebrospinal fluid-contacting neurons in the spinal cord of c57bl/6n mice is present in ectopic position unlike in other studied experimental mice strains and mammalian species. J Comp Neurol 528:2523–2550.

Wyart C, Bene FD, Warp E, Scott EK, Trauner D, Baier H, Isacoff EY (2009) Optogenetic dissection of a behavioural module in the vertebrate spinal cord. Nature 461:407–410.