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1 **Functional organization of vestibulospinal inputs on thoracic motoneurons responsible for**  
2 **trunk postural control in *Xenopus***

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37

38

39 **ABSTRACT**

40 In vertebrates, trunk postural stabilization is known to rely mainly on direct  
41 vestibulospinal inputs on spinal axial motoneurons. However, a substantial role of central  
42 spinal commands ascending from lumbar segments is not excluded during active  
43 locomotion. In the adult *Xenopus* a lumbar drive dramatically overwhelms the descending  
44 inputs onto thoracic postural motoneurons during swimming. Given that vestibulospinal  
45 fibers also project onto the lumbar segments that shelter the locomotor generators, we  
46 investigated whether such a lumbo-thoracic pathway may relay vestibular information and  
47 consequently, also be involved in the control of posture at rest. We show that thoracic  
48 postural motoneurons exhibit particular dendritic spatial organization allowing them to  
49 gather information from both sides of the cord. In response to passive head motion, these  
50 motoneurons display both early and delayed discharges, the latter occurring in phase with  
51 ipsilateral hindlimb extensor bursts. We demonstrate that both vestibulospinal and lumbar  
52 ascending fibers converge onto postural motoneurons, and that thoracic motoneurons  
53 monosynaptically respond to the electrical stimulation of either pathway. Finally, we show  
54 that vestibulospinal fibers project to and activate lumbar interneurons with thoracic  
55 projections. Altogether, our results complete the scheme of the vestibulospinal control of  
56 posture by illustrating the existence of a novel, indirect pathway, which implicates lumbar  
57 interneurons relaying vestibular inputs to thoracic motoneurons, and participate in global  
58 body postural stabilization in the absence of active locomotion.

59

60 **KEY POINTS**

- 61 • Vestibulospinal reflexes participate in postural control. How this is achieved has not  
62 been investigated fully.
- 63 • We combined electrophysiological, neuroanatomical and imaging techniques to  
64 decipher the vestibulospinal network controlling the activation of back and limb  
65 muscles responsible for postural adjustments.
- 66 • We describe two distinct pathways activating either thoracic postural motoneurons  
67 alone or thoracic and lumbar motoneurons together, the latter coordinating  
68 specifically hindlimb extensors and postural back muscles.

69

## 70 INTRODUCTION

71 Postural adjustments are essential to compensate body perturbations and perform  
72 trunk and limb movements that need to be spatially and temporally well coordinated  
73 (Massion et al., 2004). This stabilization of posture relies on the integration of multi-modal  
74 sensory inputs deriving from proprioceptive, tactile and visuo-vestibular signals (Amblard et  
75 al., 1990) together with voluntary and locomotor-induced intrinsic feed-forward commands.  
76 In particular the vestibular system, which encodes head motion, is known to interact with  
77 posturo-locomotor information to maintain body balance in space (Medrea and Cullen,  
78 2013; Cullen, 2012; Bagnall and Schoppik, 2018).

79 Posture stabilization is achieved principally by trunk and limb skeletal muscles  
80 innervated by thoracic and lumbar motoneurons (Deliagina et al., 2008; Beliez et al., 2015).  
81 In mammals such motoneurons (MNs) receive uncrossed monosynaptic and crossed  
82 polysynaptic vestibulospinal inputs, both originating from the lateral vestibulo-spinal tract  
83 (LVST; Shinoda et al., 2006; Kasumacic et al., 2010, 2015). Previous studies suggested that  
84 segmental and intersegmental spinal neurons receiving also vestibular inputs could be the  
85 neural substrate for motor coordination between thoracic and lumbar, and right and left  
86 skeletal muscles during vestibular-driven postural control of trunk and limbs (Krutki et al.,  
87 2003; Kasumacic et al., 2015). Beyond this basic description, very little is known about how  
88 vestibular inputs act on spinal motor circuits in order to produce appropriate counteractive  
89 body movements in response to unexpected head motion. This lack of information is  
90 probably due in most animal models to the combined complexity of distributed  
91 multifunctional muscles and segmental spinal networks that achieve postural adjustments.  
92 In the juvenile *Xenopus laevis*, axial muscles *dorsalis trunci* are located dorsally along each  
93 side of the vertebral column and are supposedly implicated particularly in body orientation  
94 (Vallois, 1922). On the one hand, during swimming *dorsalis* MN activity is rhythmically driven  
95 by the hindlimb movements-organizing spinal network, through the activation of an  
96 ascending lumbo-thoracic propriospinal circuitry. This results in the bilateral contraction of  
97 *dorsalis* muscles in synchrony with the bilateral contraction of hindlimb extensor muscles,  
98 leading to the vertebral axis stiffening responsible for posture stabilization during forward  
99 propulsion (Beyeler et al., 2008). On the other hand, vestibular signals directly control the  
100 left/right balance activation of *dorsalis* muscles as shown by the occurrence of both postural  
101 alteration and rolling swimming behavior after the unilateral destruction of the vestibular

102 sensory organs in the post-metamorphosis *Xenopus* (Beyeler et al., 2013). Therefore,  
103 vestibulospinal and propriospinal influences might interact to ensure the trunk stabilization  
104 during both active and passive motion. So far the functional organization of vestibulospinal  
105 inputs on thoracic MNs and its coordination with propriospinal circuits remains barely  
106 unknown.

107 We thus took advantage of the *Xenopus* model to investigate whether vestibular  
108 descending and lumbar ascending pathways somehow act conjointly to shape the postural  
109 command produced by thoracic MNs. We report anatomical and physiological evidence that  
110 *dorsalis* MNs receive both direct and lumbar-relayed vestibular inputs from vestibulospinal  
111 nuclei, and that lumbo-thoracic propriospinal neurons receive direct vestibular inputs too.  
112 Our findings further suggest that direct and lumbar-relayed vestibulo-thoracic pathways  
113 respectively ensure trunk only or trunk/hindlimb coordinated reflex responses according to  
114 passive head motion physical components.

115

## 116 **MATERIALS AND METHODS**

### 117 *Ethical approval*

118 Experiments were conducted on the South African clawed toad *Xenopus laevis*  
119 obtained from the *Xenopus* Biology Resources Centre in France (University of Rennes 1;  
120 <http://xenopus.univ-rennes1.fr/>). Animals were maintained at 20–22°C in filtered water  
121 aquaria with a 12:12h light/dark cycle. Experiments were performed on juveniles from stage  
122 64-66, according to external body criteria (Nieuwkoop and Faber, 1956). All procedures were  
123 carried out in accordance with, and approved by, the local ethics committee (protocols  
124 #2016011518042273 APAFIS #3612), and conform to the principles and regulations as  
125 described in the Editorial by Grundy (2015).

126

### 127 *Neuronal retrograde and anterograde tracing*

128 Application of neuronal anterograde or retrograde tracer crystals was performed  
129 following injection procedures already used in previous publications (Straka et al., 2001;  
130 Beyeler et al., 2008; Lambert et al., 2013). Wavelength-dependant dextran amine  
131 fluorescent dyes [10 kD Alexa dextran amine (AD) 488, 568 and 647nm; 3 kD tetramethyl-  
132 rhodamine dextran amine (RDA); Life Technologies] used in each type of tracing experiments  
133 are specified in corresponding figure legends.

134 Thoracic MNs were backfilled from identified dorsal trunk muscles (*dorsalis trunci*).  
135 Briefly, consecutive to anesthesia in a 0.05% MS-222 water solution, the skin covering the  
136 dorsal trunk muscles was dried and a tiny incision was performed allowing the intramuscular  
137 application of tracer crystals. Excess dye was washed out with cold Ringer's saline (93.5 mM  
138 NaCl, 3 mM KCl, 30 mM NaHCO<sub>3</sub>, 0.5 mM NaH<sub>2</sub>PO<sub>4</sub>, 2.6 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, and 11 mM  
139 glucose, pH 7.4). After recovering from anesthesia, juvenile *Xenopus* were kept in a water tank  
140 for at least 48h to allow tracer migration into MN cell bodies and dendrites (e.g., Fig. 1D-E).

141 Vestibulospinal or lumbo-thoracic propriospinal pathways that project onto *dorsalis* MNs  
142 were anatomically deciphered using isolated whole CNS preparations obtained after dissection  
143 according to surgery procedures described in Beyeler et al., 2008. Bilateral vestibulospinal  
144 descending neuronal groups were first identified with retrograde labeling (Fig. 3). After a tiny  
145 unilateral incision, fluorescent dye was applied in thoracic segment 2 (T2) on one side, in a  
146 ventro-medial position that corresponds to *dorsalis* MNs location in the thoracic cord (Fig. 1D),  
147 and tracer migration was allowed overnight in circulating Ringer's saline at 15-16°C at dark  
148 (Lambert et al., 2013) to label brainstem vestibulospinal cell bodies. To distinguish thoracic-  
149 projecting vestibular neurons from cervical-projecting vestibular neurons (Fig. 3), a second  
150 fluorescent dye, with a different emission wavelength, was applied in the ipsilateral first  
151 cervical hemi-segment (C1) the morning after T2 injection, followed by a 4-5h migration. Similar  
152 double dye injection was performed to label lumbar neurons projecting to T2 but the dye  
153 injection in C1 was performed prior to that in T2 (Fig. 5A). Concomitant fluorescent labeling of  
154 either the VIII<sup>th</sup> cranial nerve or the first lumbar ventral root (L1) allowed the identification of  
155 rostro-caudal anatomical markers (vestibular afferents and L1 MNs, respectively) on cross-  
156 sections during confocal imaging.

157 Vestibulospinal terminals onto backfilled thoracic MNs (Fig. 4C-E) were revealed with  
158 overnight anterograde labeling. Following tiny unilateral incision in brainstem dorsal surface on  
159 one side, dye crystals were applied either in rhombomere 4 at the VIII<sup>th</sup> nerve level in order to  
160 label LVST projections, or in rhombomere 5-6 at the X<sup>th</sup> nerve level to label tangential nucleus  
161 (TAN) projections, respectively. Lumbar propriospinal terminals onto thoracic MNs (Fig. 5D)  
162 were labeled with a similar procedure, where crystals were applied in a small incision  
163 performed at the ventral surface of a L1 spinal hemi-segment and close to the midline.

164

165 *Immunofluorescence labeling*

166 After fluorescent tracer migration, isolated CNS preparations were fixed in 4%  
167 paraformaldehyde at 4°C overnight, then incubated in a 20% [in phosphate-buffered saline  
168 (PBS) 0.1%] sucrose solution for 24h before being embed in Tissue-Tek (VWR-Chemicals) and  
169 frozen at -45°C in isopentane (for detail procedure see Lambert et al., 2018). Fluorescence  
170 immunohistochemistry on thoracic or lumbar spinal 20-µm cross-sections obtained with a  
171 cryostat (CM 3050, Leica) was performed with the same protocol as extensively described in  
172 Bougerol et al., 2015, and Lambert et al., 2018. Samples were incubated with the primary  
173 antibody for 48 h at 4°C. After rinsing, samples were incubated for 90 min at room  
174 temperature with a fluorescent dye-coupled secondary antibody. After washing, cross-  
175 sections were mounted in a homemade medium containing 74.9% glycerol, 25% Coon's  
176 solution (0.1 M NaCl and 0.01 M diethyl-barbiturate sodium in PBS), and 0.1%  
177 paraphenylenediamide. The mouse primary antibodies anti-synapsin (1:200; Synaptic  
178 Systems) were used to label presynaptic terminals and revealed with donkey secondary anti-  
179 mouse IgG coupled to Alexa Fluor 488 (1:200; Thermo Fischer).

180

#### 181 *Image acquisition and processing*

182 Cross-sections labeled with fluorescent materials were imaged using an Olympus  
183 FV1000 confocal microscope equipped with 488, 543 and 633 nm laser lines. Multi-image  
184 confocal stacks with 0.6 µm z-step intervals were generated using a 20x/0.75 oil objective  
185 and with 0.3 µm z-step intervals using a 60x/1.4 oil objective. Cell population images were  
186 obtained by orthogonal projection from multi-image stacks while synaptic apposition images  
187 were drawn from single confocal planes. Final images were processed with artificial  
188 fluorescent colors using Fiji (<https://fiji.sc/>) and Photoshop (Adobe Systems Inc.) softwares.

189

#### 190 *Brainstem and lumbar cord 3D reconstruction*

191 Following neuronal tracing, consecutive brainstem or lumbar spinal cord 40-µm slices  
192 were imaged with the confocal microscope using a 10x air objective to obtain multi-channel  
193 image z-stacks. In all preparations used in 3D reconstruction, every consecutive z-stack was  
194 subsequently used to generate a multi-channel orthogonal projection view. These latter images  
195 were then separated channel-by-channel to be reassembled in a rostrocaudal sequence of  
196 images for each wavelength channel. As a result, two rostrocaudal image sequences were  
197 produced for single brainstem or lumbar cords, displaying the segmental distribution of two

198 retrogradely labeled populations of neurons (*i.e.*, T2 vs. C1-projecting vestibulospinal neurons,  
199 or T2 vs. C1-projecting lumbar propriospinal neurons, respectively). As explained above, labeled  
200 vestibular afferents or L1 MNs were used as a rostrocaudal anatomical marker in 3D  
201 reconstructions. Every single-channel image sequences were computed in the Fiji *TrackEM2*  
202 plug-in to realign all single images and generate new sequences of aligned images. Thereafter,  
203 all brainstem and lumbar cord cross-sections were manually outlined from aligned image  
204 sequences, and the borders of vestibular afferents or L1 MNs acquired and stored similarly.  
205 Every single labeled neuron was then manually pointed, image after image, its coordinates  
206 being recorded automatically in a ROI tally sheet (Fiji *ROI Manager* plug-in). Subsequently, a  
207 final rostrocaudal image stack including consecutive cross-section borders with all neuron  
208 positions was generated with a homemade Fiji macro (Imagys; [http://www.incia.u-](http://www.incia.u-bordeaux1.fr/spip.php?article629)  
209 [bordeaux1.fr/spip.php?article629](http://www.incia.u-bordeaux1.fr/spip.php?article629)). This final stack was applied a segmentation process to  
210 restore tissue volume from single plane images and implemented thereafter into the ParaView  
211 5.5.0-RCA software to generate and export 3D visualizations (Fig. 3D, Fig. 5B). Brightness and  
212 contrasts were adjusted for illustration using Photoshop.

213

#### 214 *In vivo EMG recordings*

215 Procedures used for *in vivo* EMG recordings of dorsal trunk and hindlimb muscles were  
216 as described previously (Beyeler et al., 2008). Briefly, the EMG activity of bilateral trunk  
217 (*dorsalis trunci*) and ankle extensor (*plantaris longus*) muscles was recorded using pairs of  
218 50  $\mu$ m insulated wire electrodes connected through a grounded cable to a differential AC  
219 amplifier (Model 1700; AM-Systems) and digitized at 10 kHz with a CED 1401 interface  
220 (Cambridge Electronic Device). Data were displayed and stored using Spike2 software  
221 (Cambridge Electronic Device).

222

#### 223 *In vitro semi-intact and isolated preparations*

224 Animal dissection for *in vitro* preparations and nerve recording procedures were  
225 conducted as described extensively in Beyeler et al., 2008. Under anesthesia and after  
226 viscera and forebrain removal, the brainstem-spinal cord together with the thoracic ventral  
227 roots (Vr) and the motor nerves innervating ankle flexor (Flex; *tibialis anterior*) and extensor  
228 (Ext; *plantaris longus*) on both sides were dissected out from stage 64-66 juveniles. *In vitro*  
229 experiments were performed in carbogen-bubbled saline, either normal Ringer's (see above)



230 or a solution enriched in divalent cations (74.8 mM NaCl, 3 mM KCl, 30 mM NaHCO<sub>3</sub>, 0.5 mM  
231 NaH<sub>2</sub>PO<sub>4</sub>, 10.6 mM CaCl<sub>2</sub>, 4 mM MgCl<sub>2</sub>, and 11 mM glucose, pH 7.4) in order to raise  
232 neuronal spiking threshold and preserve only monosynaptic connections (Berry and  
233 Pentreath, 1976). In semi-intact preparations, the two otic capsules were kept intact and  
234 attached to the brainstem with the VIII<sup>th</sup> nerve. T2 Vr extracellular activity was recorded with  
235 borosilicate glass suction electrodes (tip diameter, 100 nm; Clark GC 120F; Harvard  
236 Apparatus) filled with Ringer's solution. Extracellular activity from Ext/Flex motor nerves was  
237 recorded using stainless *en passant* wire electrodes. Combined T2 Vr and Ext motor nerve  
238 activities were recorded in response to either vestibular nuclei or lumbar cord electrical  
239 stimulation in isolated preparations and in response to natural stimulation of vestibular  
240 endorgans in semi-intact preparations. Electrical stimulation of vestibular nuclei or lumbar  
241 regions consisted of single pulses delivered with a Grass stimulator S88 through a 2 MΩ  
242 monopolar stainless electrode (Micro Probe, Inc.). Stimuli (10-15 μs; 5-10 V) were always set  
243 with the minimal voltage amplitude capable of eliciting stable motor responses in normal  
244 saline. In some vestibular nuclei stimulus experiments, a ventral hemi-section was  
245 performed at C1 level to prevent composite vestibulospinal commands to activate  
246 downstream motoneurons; no differences in motor response were ever observed in this  
247 condition compared to intact isolated spinal cords, and data were pooled in the results.  
248 Natural stimulation of vestibular endorgans was performed using a computer-controlled,  
249 motorized stimulation apparatus (Turn-table and Sled; Technoshop COH@BIT, IUT de  
250 Bordeaux, University of Bordeaux). The animal was centered in the horizontal rotation and  
251 translation axes in order to provide natural activation of horizontal semicircular canals and  
252 utricles (Fig. 2; see also Lambert et al., 2008). 10 to 15-cycle sequences of sinusoidal  
253 stimulation were performed at 0.1-1.0Hz, with amplitudes of ±5-60 °/s for rotation and ±80-  
254 470 mm/s for translation, respectively. In this apparatus, T2 Vr and Ext motor nerve  
255 discharges were recorded with adjusted suction electrodes using EXT 10-2F amplifiers (NPI  
256 Electronics). Data were digitized, displayed as described for EMG recordings, and analyzed  
257 relative to the table position sinewave.

258

### 259 *Calcium imaging*

260 In CNS isolated preparations Calcium Green-1 Dextran Amine crystals (3 kD CGDA;  
261 Invitrogen) were injected unilaterally in a T2 hemi-segment (same procedure as described

262 above for neuronal tracing) in order to backfill T2-projecting lumbar neurons. The CGDA  
263 injection was performed after a partial incision of the T2 hemi-segment from the ventro-  
264 lateral surface of the cord. This partial incision ensured the labeling of lumbar INs from the  
265 thoracic lateral dendrite area but prevented damages of brainstem descending fibers in the  
266 ventro-medial cord. After 4-5h of CGDA retrograde migration, the spinal cord was book-  
267 opened from the dorsal line and flattened inside-up in the recording chamber, in order to  
268 visualize labeled neurons. Optical recording of calcium transients (Fig. 7E-F; n=5 animals) was  
269 performed with an epifluorescence microscope (Olympus BX51WI) and a high CCD camera  
270 (QImaging OptiMOS) at a 10 fps image rate (time exposure=95 ms; image interval=100 ms)  
271 under x40 water immersion objective (Olympus LUMPlanFLN). Calcium transients  
272 (fluorescence changes relative to the fluorescence baseline,  $\Delta F/F$ ) evoked in T2-projecting  
273 lumbar interneurons in response to vestibular nuclei electrical stimulation (50  $\mu$ s single  
274 pulses) were measured within ROI drawn over individual neuron somata (Fig. 7E) using  
275 MetaFluor (Meta Imaging Series 7.8; Molecular Devices). Depending on the quality of the  
276 retrograde labeling, 5-12 neurons were recorded per lumbar region at x40 and 1-3 lumbar  
277 regions were investigated per preparation. In total, 60 L1 INs projecting to ipsilateral T2  
278 segment and 91 L1 INs projecting to contralateral T2 segment (see Fig. 7E) were optically  
279 recorded in response to either LVST or TAN electrical stimulation.

280

### 281 *Data analysis and statistics*

282 Electrophysiological extracellular recordings of motor nerve discharges were analyzed  
283 offline with Spike2. To analyze the modulation of nerve activity in response to natural  
284 vestibular stimulation, discharge rates in individual nerve recordings were measured by  
285 setting an amplitude threshold to count all impulses in such multi-unit recordings. Firing  
286 rates (in spikes/s) were averaged over 10-15 stimulation cycles. Statistical analysis of  
287 electrophysiological data were performed using Prism5 (GraphPad) and OriginPro8  
288 (OriginLab Corporation), and data are given as means and standard deviation ( $\pm$ SD). Note  
289 that, as usually the case in studies requiring animal sacrifice, samples used in this study were  
290 voluntarily kept low and, consequently, a p value larger than 0.05 indicated only that the  
291 data did not give any reason to conclude that the distributions differed. Circular phase  
292 analysis was performed using Oriana2 (Kovach Computing Services). The non homogeneity  
293 of phase distributions was first tested with the Rayleigh test, and subsequent circular

294 statistical analysis was performed only on non-homogenous distributions; no distributions in  
295 this study were found homogenous. Similarity between two circular distributions was then  
296 investigated using the Watson  $U^2$  test, for which the null hypothesis is that the two  
297 distributions are identical, and a significant p value indicates rejection of the null hypothesis.  
298 Unfortunately, Oriana2 does not provide the exact p value, but the value of the  $U^2$  is given in  
299 the text for each statistical test in order to provide the more information about its  
300 significance (the lower  $U^2$ , the higher similarity between the two compared distributions).  
301 Calcium imaging data were analyzed offline; data are given as means $\pm$ SD, and statistical  
302 significance was tested using Prism5.

303

## 304 **RESULTS**

### 305 *Morphology of motoneurons innervating dorsal trunk muscles and patterns of postural* 306 *activity*

307 *Dorsalis trunci* muscles are located bilaterally along the vertebrae and consist of thin  
308 fleshy fascicles inserted dorsally between transverse and spinous processes principally,  
309 although the most anterior ones appear to link also ipsilateral ribs (Fig. 1A left). Especially,  
310 the third myomeres are inserted between thoracic vertebra T1's spinous process and T2's  
311 transverse process (Fig. 1A right). Similarly, the 4<sup>th</sup> myomeres are inserted between T2 and  
312 T3 spinous processes. In this configuration, a contraction of these specific *dorsalis* myomeres  
313 on one side would only twist the vertebral column along the body axis, with the rostral  
314 vertebra slightly rotating towards the contracted myomeres and the caudal vertebra in the  
315 opposite direction. Such vertebral twists create small amplitude rolling angles that allow  
316 trunk postural adjustments and body orientation.

317 A previous study in freely behaving juveniles has shown that the activity of the third  
318 myomere of *dorsalis trunci* muscles depends on whether the animal is swimming or not  
319 (Beyeler et al., 2008). Bilateral EMG recordings in swimming animals (Fig. 1B) exhibit  
320 rhythmic bursts that occur mainly in phase with ipsilateral *plantaris longus* bursts. In this  
321 situation, *dorsalis* activity corresponds to pro-active postural adjustments previously shown  
322 to be driven directly by the lumbar CPG for hindlimb kicking during swimming. In resting  
323 animals (the absence of swimming is corroborated by the absence of coordinated *plantaris*  
324 bursts; Fig. 1C), bilateral *dorsalis* activity occurs mainly uncoordinated, likely driven only by  
325 reflex sensory-motor circuits.

326 Morphology and dendritic organization of thoracic MNs innervating *dorsalis trunci*  
327 muscles was revealed by retrograde labeling with two distinct fluorescent dyes injected in  
328 *dorsalis* third myomere on each side of the vertebral axis (Fig. 1D<sub>1</sub>). Cell bodies of these MNs  
329 were located mainly in the second thoracic spinal segment (T2), organized in bilateral  
330 ventro-medial motor columns (Fig. 1D and E). These postural T2 MNs exhibited large somata  
331 and a particularly extended dendritic arborization that surprisingly projects long branches  
332 into the two hemi-cords (Fig. 1D<sub>2</sub>-F). Indeed, in addition to classical dendrites running into  
333 the ipsilateral hemi-segment (thereafter called “lateral dendrites”) long commissural  
334 dendrites were found to project far into the contralateral hemi-cord, ending in the same  
335 regions as the lateral dendrites of contralateral MNs. Although both dendrite types were  
336 found in a similar proportion ( $2.7\pm 0.9$  vs.  $2.6\pm 0.9$ , respectively;  $n=14$  animals; t-test,  $p=0.72$ ;  
337 Fig. 1G), lateral dendrites tended to show more ramifications ( $6.6\pm 2.9$ ) than commissural  
338 ones ( $4.5\pm 1.4$ ; t-test,  $p=0.09$ ). This particular dendritic arrangement suggests that postural  
339 T2 MNs are able to gather the same type of information from both sides of the cord,  
340 although probably to a different extent.

341

#### 342 *Dual responses of thoracic motoneurons to natural vestibular stimulation*

343 In the juvenile *Xenopus*, forward translation corresponds to the main motion  
344 component of body displacement during swimming. Backward translation can also be  
345 observed, especially in startling responses or when the animal retreats before a threat.  
346 Bilateral T2 and hindlimb Ext motor nerve activities were recorded in semi-intact  
347 preparations in response to natural stimulation of vestibular endorgans (Fig. 2). Utricular  
348 maculae, otolithic endorgans detecting head linear accelerations in all body reference plans  
349 including gravito-inertial signals (Straka and Dieringer, 2004; Lambert et al., 2008), were  
350 activated with antero-posterior horizontal translations (Fig. 2A and B<sub>1</sub>). Horizontal  
351 semicircular canal ampulae, detecting head horizontal angular accelerations (Straka and  
352 Dieringer, 2004; Lambert et al., 2008), were activated with horizontal rotations (Fig. 2A, B<sub>2</sub>  
353 and E<sub>1</sub>). In both translation and rotation conditions, series of sinusoidal movements (see  
354 Methods) were applied in order to record horizontal body plane-specific dynamic responses  
355 of, respectively, utricle-driven and canal-driven motor responses in T2 Vr and Ext motor  
356 nerves specific to the horizontal body plane.

357 T2 Vr bursts on both sides always increased simultaneously during forward linear  
358 motion, the maximum discharge rate occurring near the table mid-course between its two  
359 extreme positions (Fig. 2C<sub>1</sub>; n=5 animals). The circular distribution of phases for both left and  
360 right T2 activity was similar ( $p>0.5$ , Watson U<sup>2</sup> test:  $U^2=0.053$ ), and analysis showed that left  
361 and right peak discharges occurred with a mean phase lead of  $114\pm 15^\circ$  and  $116\pm 8^\circ$ ,  
362 respectively, relative to the maximal forward position of the motion table (Fig. 2D<sub>1</sub> left, plain  
363 light and dark blue arrows). In most trials (81%) backward linear movements bilaterally  
364 triggered simultaneous motor bursts in T2 and Ext nerves (Fig. 2B<sub>1</sub>; n=5 animals). In such  
365 cases, Ext discharges were related to small amplitude postural movements of the hindlimbs  
366 (Suppl. Movie 1) that differed fundamentally from the large amplitude locomotor kicks (see  
367 Beyeler et al., 2013). During backward translation, no T2 Vr response was ever observed  
368 alone, *i.e.* in absence of Ext burst, suggesting that backward vestibular-induced T2 Vr motor  
369 activity required the activation of the lumbar circuitry. Bilaterally simultaneous bursting  
370 activity was recorded from both Ext and T2 motor nerves, the maximum of which occurred  
371 around backward mid-course. Phase distributions were similar for bilateral Ext ( $p>0.5$ ,  
372 Watson U<sup>2</sup> test:  $U^2=0.055$ ) and bilateral T2 activity ( $p>0.2$ , Watson U<sup>2</sup> test:  $U^2=0.116$ ), but  
373 different between homolateral Ext and T2 activity ( $p<0.05$ , Watson U<sup>2</sup> test). Analysis showed  
374 respective mean phase leads relative to the maximal backward table position of  $124\pm 10^\circ$  and  
375  $123\pm 20^\circ$  for left and right Ext (Fig. 2D<sub>1</sub> left, plain light and dark orange arrows), and  $76\pm 7^\circ$   
376 and  $75\pm 15^\circ$  for left and right T2 (Fig. D<sub>1</sub> left, dashed light and dark blue arrows). Notably,  
377 none of the phase relationships was dramatically affected by changing table motion  
378 amplitude or frequency (Fig. 2D<sub>1</sub> right). Phase analysis also demonstrated that Ext discharges  
379 systematically preceded T2 bursts ( $50\pm 8^\circ$  mean phase shift), which further supported the  
380 hypothesis that backward utricle-induced lumbar network activity is mandatory to trigger  
381 concomitant vestibular-induced motor response in thoracic motor nerves.

382 Bilateral T2 Vr discharges were also modulated by horizontal sinusoidal head rotations  
383 (n=7 animals). Leftward rotations evoked motor bursts in the left T2 Vr (Fig. 2B<sub>2</sub>), the  
384 maximum discharge rate occurring around the table maximum leftward excursion (Fig. 2C<sub>2</sub>),  
385 with a mean phase shift of  $-4\pm 8^\circ$  relative to the maximal leftward position of the table  
386 (Fig. 2D<sub>2</sub>); conversely, the right side T2 Vr discharge was modulated in a similar way by  
387 rightward rotation. Thus, left and right T2 discharges showed very opposite phase  
388 distributions (Fig. 2D<sub>2</sub> left;  $p<0.001$ , Watson U<sup>2</sup> test:  $U^2=0.542$ ) but none was significantly

389 affected by table movement frequency or amplitude (Fig. 2D<sub>2</sub> right). In addition, in less than  
390 20% of the preparations exposed to horizontal rotation, T2 Vr activity demonstrated extra  
391 motor bursts coupled with ipsilateral Ext discharges during horizontal rotation, around the  
392 mid-course of the round-table in the homolateral direction (Fig. 2E<sub>1-2</sub>), as akin to what was  
393 observed as a general rule for frontal backward translation (see above). Similarly too, the  
394 circular distribution of both Ext and T2 activity on either side was not clearly affected by  
395 table rotation frequency or amplitude, and T2 Vr responses systematically followed Ext  
396 bursts with a mean phase shift of  $34 \pm 15^\circ$  (Fig. 2E<sub>3</sub>).

397 Taken together these results showed the existence of two temporally distinct  
398 vestibular-evoked reflex responses in postural thoracic MNs, suggesting the potential  
399 existence of two distinct parallel functional pathways. A first vestibulospinal command  
400 seems to activate only thoracic motor networks, without related Ext motor nerve activity,  
401 and may be related to small postural adjustments restricted to the trunk only. A second  
402 pathway activates conjointly thoracic and lumbar MNs, and could be involved in a broader  
403 vestibular-evoked control of the body balance that would necessitate the coordination of  
404 trunk and hindlimbs postural adjustments. This latter vestibular-evoked reflex suggests that  
405 the lumbar motor network could constitute a neuronal relay in some vestibulospinal  
406 pathways projecting to thoracic motor networks.

407

#### 408 *Brainstem location of vestibulospinal neurons projecting on thoraco-lumbar spinal segments*

409 Vestibulospinal neurons projecting on thoraco-lumbar spinal cord were located in the  
410 brainstem by performing retrograde labeling from spinal segments. Two sequential dye  
411 applications were made from homolateral T2, then C1 hemi-segments (see Methods), in  
412 association with anterograde labeling of the VIII<sup>th</sup> nerve anterior ramus in order to visualize  
413 its entry in the brainstem as an anatomical marker of rhombomere 4 (Fig. 3A). Cell bodies  
414 were labeled on the two sides of the brainstem, in the regions where vestibular afferents  
415 contact central secondary vestibular neurons. A first cluster of cell bodies was found  
416 ipsilateral to the injection sites, located laterally and dorsally in the alar plate, and  
417 corresponded to neurons of the lateral vestibulospinal tract (LVST; Straka et al., 2001;  
418 Fig. 3B<sub>1</sub>). A second cluster was found contralaterally, located slightly more ventrally, and  
419 corresponded to neurons of the tangential nucleus (TAN; Straka et al., 2001; Fig. 3B<sub>2</sub>). Most  
420 neurons in both vestibulospinal nuclei showed double labeling (insets in Fig. 3B). Cell

421 counting revealed that neurons projecting to thoraco-lumbar segments represented the  
422 main proportion of the labeled LVST (86±4%) and TAN (80±3%) population neurons  
423 (Fig. 3C<sub>1</sub>).

424 3D reconstruction from brainstem slice series (see Methods; Fig. 3D) demonstrated  
425 that C1- and T2-projecting vestibulospinal neurons were intermingled and distributed within  
426 the entire LVST and TAN nuclei, spanning from 200 µm rostral to 750 µm caudal to the VIII<sup>th</sup>  
427 nerve for the LVST, and from 100 µm rostral to 600 µm caudal to the VIII<sup>th</sup> nerve for the TAN  
428 (Fig. 3C<sub>2</sub>). Combination of double retrograde labeling from the first lumbar (L1) and T2  
429 segments on two other groups of juveniles (not illustrated) further showed that LVST  
430 neurons preferentially project into the lumbar spinal cord (76±8%) while TAN neurons  
431 projected more comparably into both thoracic and lumbar segments (T2: 43±5%; L1:  
432 57±5%). Altogether, these results showed a rhombomeric organization of vestibulospinal  
433 neurons in *Xenopus* that was comparable to what had been previously described in other  
434 vertebrates (Straka et al., 2001; McCall et al., 2017), and further revealed that most of the  
435 vestibulospinal neurons project to thoraco-lumbar segments without rostrocaudal  
436 somatotopic arrangement within the two brainstem nuclei.

437

#### 438 *Thoracic postural motoneurons receive direct vestibulospinal inputs*

439 Retrograde labeling from T2 segment indicated that a large part of the TAN and LVST  
440 vestibular neurons might project onto T2 postural circuitry. In normal saline, electrical  
441 stimulation of either vestibulospinal nucleus evoked compound action potentials recorded  
442 extracellularly from T2 ventral roots. Stimulation magnitude was adjusted in order to record  
443 minimal responses, which typically consisted of fast and short duration compound response  
444 (Fig. 4A-B; n=15 animals) rarely followed a few tens of milliseconds later by longer lasting  
445 bursts (not shown). In these conditions, single stimulation pulses applied in the LVST nucleus  
446 consistently evoked compound action potentials only on the ipsilateral T2 Vr with a  
447 8.1±6.9 ms delay (Fig. 4B top). These extracellular potentials persisted in high divalent cation  
448 saline, although occurring with a significantly increased delay (14.6±9.4 ms; Wilcoxon paired  
449 test, p=0.002). Single minimal stimulation pulses in the TAN consistently evoked  
450 contralateral compound action potentials in T2 Vr (delay: 10.3±2.9 ms), sometimes  
451 accompanied by compound action potentials in the ipsilateral T2 Vr (59% of the cases; delay:  
452 9.9±0.3 ms). However, whereas fast contralateral responses always persisted in high divalent

453 cation saline with a slightly but non-significant longer delay ( $12.6 \pm 2.1$  ms; Wilcoxon paired  
454 test,  $p=0.19$ ), ipsilateral responses persisted in only few preparations (<20%; e.g., Fig. 4B  
455 bottom), with a slightly increased delay ( $11.3 \pm 0.2$  ms; Mann-Whitney test,  $p=0.002$ ).

456 Compound action potentials evoked in T2 ventral roots in response to TAN and LVST  
457 electrical stimulation under high divalent cation saline suggested that T2 MNs received  
458 monosynaptic inputs from both vestibulospinal nuclei. Unilateral retrograde labeling of T2  
459 MNs was combined with anterograde labeling from the ipsilateral TAN, the contralateral  
460 TAN or the ipsilateral LVST nucleus, together with subsequent immuno-detection of the  
461 presynaptic protein synapsin (Fig. 4C-E). Because TAN and LVST neurons are partly  
462 intermingled, a hemisection at the first spinal cervical segment was performed either  
463 ipsilateral to the TAN tracer injection (to avoid LVST terminals labeling; see injection scheme  
464 in Fig. 4C and D) or contralateral to LVST tracer injection (to avoid TAN terminals labeling;  
465 see injection scheme in Fig. 4E). Sites of close apposition between LVST or TAN terminals and  
466 MN dendrites or somata that co-localized with synapsin were found on all labeled thoracic  
467 MNs. Synapsin appositions were found between terminals labeled from TAN area and  
468 commissural dendrites of ipsilateral thoracic MNs (Fig. 4C), and somata and lateral dendrites  
469 of contralateral MNs (Fig. 4D), respectively. Terminals labeled from LVST location presented  
470 synapsin apposition with both soma and lateral dendrites of ipsilateral thoracic MNs  
471 (Fig. 4E). Although the mean repartition of synapses appeared similar on somata and  
472 dendrites ( $2.0 \pm 1.0$  vs.  $2.7 \pm 1.3$ , respectively), careful analysis from three animals  
473 demonstrated heterogeneous distributions since vestibulospinal terminal/synapsin  
474 appositions were primarily distributed in the proximo-medial part of both commissural and  
475 lateral dendrites (see Fig. 6F).

476 The anatomical observation of vestibulospinal terminals onto T2 MNs (Fig. 4C-E) is  
477 consistent with T2 motor activities evoked in response to vestibular stimulation (Fig. 2 and  
478 Fig. 4A-B). Such a combination of electrophysiological and anatomical evidence of direct  
479 vestibulospinal inputs on thoracic MNs supports the idea of a direct vestibulospinal pathway  
480 to thoracic motor network that would command for specific trunk reflexive postural control  
481 in response to head movements as shown in Figure 2. In contrast, the existence of  
482 concomitant bursts in hindlimb Ext and T2 motor nerves, occurring with a different  
483 stimulus/response phase relationship than T2 responses alone, might require the  
484 involvement of another dedicated vestibulospinal circuit.



485

486 *Thoracic motoneurons receive direct inputs from ipsilateral L1 ascending interneurons*

487         The natural stimulation of vestibular endorgans triggered coordinated bursting activity  
488 in both hindlimb extensor and thoracic motor nerves (see above). These vestibular-evoked  
489 coordinated thoraco-lumbar reflex responses occurred in a stereotyped sequential manner,  
490 the lumbar Ext bursts always preceding T2 ones, which suggested some vestibulospinal  
491 commands conjointly activate both thoracic and lumbar motor networks and a foreseeable  
492 lumbar interneuronal relay to achieve such coordinated postural adjustments of the trunk  
493 and hindlimbs.

494         The existence of lumbar interneurons (INs) putatively projecting into segment T2 was  
495 investigated by performing a sequential retrograde labeling within the spinal cord, which  
496 consisted of two consecutive, 12 h-delayed injections of two distinct fluorescent dyes within  
497 a cervical hemi-segment and its ipsilateral T2 hemi-segment, respectively (see scheme on  
498 Fig. 5A<sub>1</sub>). Such an approach allowed the anatomical segregation of lumbar INs projecting  
499 only into T2 segments (single labeling) from INs projecting more rostral (double labeling)  
500 (Fig. 5A<sub>2</sub>). Notably, singly-labeled cell bodies were found on both sides of the entire lumbar  
501 cord (Fig. 5A-B and Fig. 6A) and located more medially than hindlimb MNs (identified by  
502 retrograde tracing from lumbar ventral roots; *e.g.*, Fig. 5B<sub>1</sub> and Fig. 6A). Moreover, T2  
503 projecting INs were found with a decreasing rostro-caudal gradient along the three lumbar  
504 segments (Fig. 5B<sub>2</sub>).

505         Given their location, the more dorsal group of lumbar INs are likely specialized in  
506 processing and dispatching proprioceptive sensory information towards other spinal  
507 segments (Lu et al., 2015). In contrast, we hypothesized that the more ventral group of INs  
508 could play a major role in coupling motor functions of both lumbar and thoracic segments  
509 and, thus, operate the combined vestibular activation of Ext and T2 MNs. To test this  
510 hypothesis, unilateral electrical stimulation of minimal magnitude was applied in the rostral  
511 part of L1 lumbar segment (see scheme in Fig. 5C; n=12 preparations), in the ventro-medial  
512 region where the highest density of T2-projecting INs was found. Motor responses were  
513 reliably triggered (99.8%; n=162 trials) in the ipsilateral T2 Vr, although the response delay  
514 varied a lot among preparations (mean delay of  $5.9 \pm 8.6$  ms; Fig. 5C), generally followed by  
515 delayed long-duration bursts. Perfusion of high divalent cation saline did not prevent the  
516 occurrence of the initial T2 Vr discharge, which occurred with non-significantly increased

517 delay (mean delay of  $8.1 \pm 14.8$  ms; Wilcoxon paired test,  $p=0.15$ ), whereas the secondary  
518 discharge consistently disappeared. Although we could not rule out the additional  
519 participation of polysynaptic pathways or the possibility of an antidromic activation of  
520 thoracic INS likely projecting to the lumbar cord, the persistence of the fast T2 Vr response in  
521 high divalent cation saline strongly suggested the existence of direct monosynaptic inputs  
522 between ipsilateral rostral L1 INs and T2 MNs. Unilateral retrograde labeling of T2 MNs was  
523 then performed with concomitant dye injection in ipsilateral rostral L1 ventral hemicord (see  
524 inset in Fig. 5D). Synapsin immuno-detection was performed on T2 cross sections presenting  
525 MN labeled from dorsal muscle and L1 ascending terminals. Numerous close appositions of  
526 synapsin signal and ipsilateral L1 ascending IN axons were found on every labeled T2 MN  
527 commissural and lateral dendrites (Fig. 5D). Apposition analysis suggested that L1 INs  
528 connected in seemingly comparable proportions to commissural (through axon collaterals  
529 crossing the midline at the thoracic level; not shown) and lateral dendrites ( $2.60 \pm 0.89$  vs.  
530  $2.58 \pm 1.08$ ;  $n=5$  animals; t-test,  $p=0.95$ ), although the distribution of L1 INs putative synapses  
531 was differing between the two dendritic regions (Fig. 6F). Thus, our electrophysiological and  
532 anatomical results highlighted direct excitatory connections between rostral L1 ascending  
533 INs and T2 MNs that could robustly couple lumbar Ext and axial MN motor activities during  
534 vestibular-controlled postural adjustments.

535         It is notable that only the stimulation of the rostral L1 segment triggered exclusively  
536 ipsilateral lumbar-evoked T2 motor response. These T2 responses were often accompanied  
537 by a fast motor burst on hindlimb motor nerves, followed or not by repeated rhythmic-like  
538 bursts (*e.g.*, Fig. 6C-E). Yet, the electrical stimulation of more caudal lumbar regions (not  
539 illustrated) could also trigger responses in T2 and hindlimb motor nerves from either the  
540 same or opposite side of the cord, and synapsin appositions suggesting direct synaptic  
541 contacts were sometimes observed between contralateral INs and T2 MN soma/lateral  
542 dendrites (*e.g.*, Fig. 6B). Nevertheless, such synapsin appositions were more rarely identified  
543 anatomically, and T2 motor responses to electrical stimulation of more caudal lumbar  
544 regions were much more labile than rostral L1-evoked ones. In addition, more than half of  
545 them were prevented in high divalent cation saline (not illustrated). Hence, T2-projecting INs  
546 located in the different lumbar segments could participate in coordinating the discharges of  
547 both hindlimb extensor and trunk MNs in response to vestibular signals. However, such a  
548 coordinating function would require lumbar INs to receive vestibular inputs at first.

549

550 *T2-projecting lumbar interneurons receive vestibular inputs*

551 Anterograde labeling of vestibular terminals projecting to lumbar segments was  
552 performed by dye injection in either vestibulospinal area, while retrograde labeling of T2-  
553 projecting lumbar INs was obtained from unilateral dye injection in a T2 hemi-segment.  
554 Synapsin immunodetection was then realized on fixed L1 spinal cord slices to detect putative  
555 synaptic contacts from either ipsilateral LVST (Fig. 7A-B) or contralateral TAN vestibulospinal  
556 fibers and ascending L1 INs (Fig. 7C-D). Several close appositions were found between  
557 vestibulospinal terminals of either nucleus and L1 IN somata and identified proximal  
558 dendrites that co-localized with synapsin. Careful apposition counting performed on four  
559 distinct animals showed that 56% of the labeled L1 INs received LVST synapses and 68%  
560 received TAN synapses, with a respective mean number of synaptic contacts of  $2.1 \pm 0.2$  and  
561  $2.2 \pm 0.2$ . These observations suggested that ascending lumbar INs could receive synaptic  
562 inputs from vestibulospinal axons of the two nuclei.

563 Optical recordings were undertaken to investigate the potential activation of T2-  
564 projecting L1 INs by central vestibular inputs (Fig. 7E-F). Crystals of Calcium-Green Dextran  
565 Amine (CGDA) were applied laterally in a T2 hemi-segment to fill retrogradely ascending L1  
566 INs on both sides (see scheme in Fig. 7E). Calcium transient activity from such backfilled L1  
567 INs and extracellular motor nerve activity from T2 Vr were recorded simultaneously in  
568 response to either ipsi- or contralateral vestibular nuclei electrical stimulation. Single pulse  
569 stimulation of a LVST nucleus bilaterally evoked calcium transient in T2-projecting L1 INs that  
570 was coupled to the vestibular-evoked burst discharge in the T2 Vr motor nerve (Fig. 7F<sub>1</sub>,  
571 upper traces). 100% of recorded neurons (60 ipsilaterally and 91 contralaterally projecting L1  
572 INs; n=4 preparations) responded to LVST stimulation as exemplified in Fig. 7F<sub>2</sub> (left traces)  
573 with a  $\Delta F/F$  peak of  $2.4 \pm 2.1\%$  and  $4.0\% \pm 3.2\%$  (Fig. 7F<sub>3</sub>) for ipsi- and contralaterally T2-  
574 projecting L1 INs, respectively. The same pattern of response was observed after TAN  
575 electrical stimulation in 100% of T2-projecting neurons L1 INs on the two sides (e.g., Fig. 7F<sub>1</sub>,  
576 bottom traces; Fig. 7F<sub>2</sub>, right traces) with a  $\Delta F/F$  peak of  $2.6 \pm 1.6\%$  and  $3.4\% \pm 1.7\%$  (Fig. 7F<sub>3</sub>)  
577 for ipsi- and contralaterally T2-projecting L1 INs, respectively. No statistical differences were  
578 found between either groups (Kruskal-Wallis test,  $p=0.15$ ). Sometimes, a single pulse (either  
579 in LVST or TAN) elicited repeated rhythmic-like transients, consecutive to the first optical  
580 response, over several seconds (3-10s) in all recorded neurons (Fig. 7F<sub>2</sub>, see black arrow on

581 left traces), suggesting a possible involvement in the generation and/or maintaining of  
582 locomotor-like activity (as also suggested above by the observation of rhythmic bursts on  
583 hindlimb motor nerves in response to L1 IN electrical stimulation). These optical recordings  
584 thus demonstrated that T2-projecting lumbar INs are responsive to vestibulospinal inputs  
585 and, thus, might be able to relay vestibulospinal commands to postural thoracic MNs.

586

## 587 **DISCUSSION**

588 This study reports anatomical and physiological evidence that the thoracic MNs  
589 responsible for *dorsalis trunci* 3<sup>rd</sup> myomere postural activity receive a dual command from  
590 brainstem vestibular nuclei at rest, one conveyed by direct descending vestibulo-thoracic  
591 pathways, the other mediated by yet undescribed vestibulo-lumbo-thoracic pathways  
592 involving a lumbar interneuronal relay (Fig. 8). We propose that the former pathway is  
593 involved in the treatment of sensorimotor signals resulting exclusively in postural  
594 adjustments of the trunk while the latter is rather engaged in the balance of the entire body  
595 by coordinating trunk and hindlimbs reflexive movements.

596 Due to their peculiar dendritic arbor, notably characterized by long commissural  
597 dendrites, thoracic postural MNs on both sides are likely allowed to gather information from  
598 the same sources, even though those sources project unilaterally in the spinal cord.  
599 Comparable anatomo-functional organization was found in the lateral line system of teleost  
600 fishes, where octavolateralis efferent neurons integrate bilateral sensory inputs and their  
601 dendrites extent both ipsi- and contralaterally (Roberts and Meredith, 1989). MNs with  
602 dendrites crossing the ventral commissure have been observed in many vertebrate species,  
603 including lampreys (Wallen et al., 1985), mudpuppies (Jovanovic and Burke, 2004), terrestrial  
604 frogs (Szekely, 1976; Erulkar and Soller, 1980; Bacskai et al., 2010), turtles (McDonagh et al.,  
605 2002), rodents (Rose and Collins, 1985; Allan and Greer, 1997; Tarras-Wahlberg and Rekling,  
606 2009), and cats (Light and Metz, 1978; Cameron et al., 1983). However, commissural  
607 dendrites were not found in all motoneuronal populations (Ulfhake and Cullheim, 1981;  
608 Ulfhake and Kellerth, 1981; Rosenthal and Cruce, 1985). Crossing dendrites may be specific  
609 to axial MNs, the ventro-medial position of which allowing them to extend dendrites  
610 towards their contralateral counterparts. Initially, commissural dendrites in the adult frog  
611 were proposed to play a major role in the reciprocal activation of dorsal muscles on the two  
612 sides, each side exciting the other (Szekely, 1976), although there was no proof of such

613 reciprocal excitation. In contrast such central MN-MN co-excitation exists in the larval  
614 zebrafish, although it remains homolateral and involves central axon collaterals (Menelaou  
615 and McLean, 2012; Bello-Rojas et al, 2019). The present study in *Xenopus* juvenile rather  
616 suggests that crossing dendrites allow MNs on both sides to integrate the same sets of  
617 inputs, whether descending from the brainstem or ascending from the lumbar spinal cord,  
618 the result of which is a coordinated activation (perhaps synchronization) of bilateral dorsal  
619 muscles.

620 Thoracic MNs appear to play a particularly pivotal role in the spinal control of posture.  
621 Indeed, in addition to lumbar swimming CPG-generated postural commands (Beyeler et al.,  
622 2008) both direct and lumbar-relayed vestibular commands converge onto T2 MNs. In  
623 normal saline, minimal electrical stimulation of brainstem vestibular nuclei consistently  
624 elicited fast T2 ventral root responses that mostly persisted in high divalent cation saline.  
625 While LVST stimulation activated only ipsilateral T2 MNs, TAN minimal stimulation triggered  
626 responses in both ipsi- and contralateral T2 motor nerves. However, only TAN-evoked  
627 contralateral responses always persisted under high calcium/high magnesium saline, which  
628 could suggest TAN-triggered ipsilateral response relies totally on local interneuronal relay.  
629 But, because motor bursts on both sides occurred with roughly the same delay and possible  
630 synaptic contacts were observed between TAN projections and ipsilateral T2 MNs, it was  
631 more likely that T2 motor responses on the two sides resulted from monosynaptic  
632 vestibulospinal inputs. Due to the existence of long motoneuronal dendrites laying in the  
633 two hemicords, it is highly conceivable that fibers from a given TAN nucleus activate  
634 contralateral MNs through synaptic contacts on lateral dendrites and ipsilateral MNs  
635 through contacts on commissural dendrites. High divalent cation saline is used to conserve  
636 only monosynaptic responses because it stabilizes neuronal membranes and, hence, limits  
637 signal propagation along inactive neurites (Berry and Pentreath, 1976). Given that TAN  
638 synapses were found farther from the soma on commissural than lateral dendrites,  
639 perfusion of high divalent cation saline should impede more TAN signal transmission along  
640 commissural dendrites and, as a consequence, prevent MNs to reach firing threshold in  
641 response to ipsilateral TAN minimal stimulation. In support to this assumption that high  
642 divalent cation saline disturbed signal transmission along MN dendrites was the concomitant  
643 observation of an increased delay of LVST-evoked T2 compound action potentials and the

644 tendency, although non-significant in our experiments, of TAN-evoked bursting responses to  
645 occur also with an increased delay in the contralateral T2 motor nerve.

646 Postural T2 MNs are driven by the lumbar circuitry during locomotion, subjugating the  
647 dynamic control of posture to the hindlimb kicking command (Beyeler et al., 2008). In this  
648 context, each Ext burst is accompanied by a concomitant motor burst in the ipsilateral T2  
649 ventral root during fictive swimming, and similar coordination is observed between back and  
650 Ext muscle activity during actual swimming. Here, we describe an ascending lumbo-thoracic  
651 connection that involves lumbar INs making possible connections onto T2 postural MN  
652 dendrites. Minimal stimulation of these INs evoked motor responses in T2 ventral roots that  
653 for the most part persisted in high divalent cation saline, confirming the existence of  
654 functional synapses with T2 MNs. Often, lumbar IN stimulation simultaneously triggered  
655 responses on hindlimb motor nerves (Fig. 6C-E). Taken together, these results suggest that  
656 such lumbar INs may be responsible for the strict coupling between hindlimb and dorsal  
657 MNs that was observed during swimming (Beyeler et al., 2008). Beyond, our results also  
658 demonstrate T2-projecting lumbar INs to respond to vestibular nuclei activation, as  
659 previously reported for other spinal INs (Miller et al., 2009; Kasumacic et al., 2015). In  
660 addition, we show that coordinated discharges of both hindlimb Ext and postural T2 MNs are  
661 produced in response to passive head motion, with Ext activity systematically preceding T2  
662 discharge by 150-200 ms (Fig. 2). Taken together, our results suggest that ascending lumbar  
663 INs dispatch a part of the passive head movement-induced vestibulospinal commands  
664 towards hindlimb and thoracic postural MNs. As a consequence, in addition to its essential  
665 function during swimming the lumbo-thoracic circuitry appears to play a major role in the  
666 control of posture in response to passive motion too. Our results further suggest that the  
667 dual vestibular-driven thoracic motor response, either independent or coupled to vestibular-  
668 driven hindlimb extensor motor activity, reflects a differential central sensory-motor  
669 processing of head motion signals arising from inner ear sensory organs. Such a functional  
670 organization of sensory-motor transformation was already proposed for vestibulo-ocular  
671 pathways (Straka et al., 2009; Beraneck and Straka, 2011; Straka et al., 2014). All neuronal  
672 relays of the vestibulo-ocular arc, from vestibular afferents to extraocular MNs, demonstrate  
673 neural integrative properties that tune vestibulo-ocular pathways in parallel frequency-  
674 tuned channels. Such a temporal treatment leads to transform head acceleration sensory  
675 signals into motor output signals with different levels of integration, like velocity and

676 position (Straka et al., 2009, 2014). In our experiments, during natural translation or rotation  
677 of the head in the horizontal plane (Fig. 2) the peaks of all motor responses always occurred  
678 when the stimulus acceleration changed, i.e. either when the table approached extreme  
679 positions or around turn-table/sled velocity peaks between two maximal positions.  
680 Therefore, we hypothesize that the dual vestibular-driven thoracic response could also result  
681 from a differential transformation of the acceleration input signals in some position- or  
682 velocity-related motor output signals, involved in either a trunk-restricted postural  
683 adjustment or a whole-body balance control, respectively.

684 In mammals, projections from vestibulospinal neurons on one side of the brainstem  
685 are organized in two main tracts, one projecting medially in the two sides of the spinal cord  
686 and the other lateral, projecting exclusively ipsilaterally (see Shinoda et al., 2006). In the  
687 larval frog in contrast, medial projections originate only from contralateral second order  
688 vestibular neurons (Straka et al., 2001). We described here two similar pathways in the  
689 juvenile *Xenopus*, which originated respectively from TAN and LSVT nuclei. Such  
690 vestibulospinal nuclear organization appears to be a common feature in early vertebrates  
691 and prefigures vestibular nuclei organization in higher vertebrates (Straka and Baker, 2003).  
692 However, whereas medial projections classically stop within the cervical spinal segments  
693 while lateral projections run through the entire spinal cord (*e.g.* cat: Kuse et al., 1999;  
694 Kushiro et al., 2008; mouse: Kasumacic et al., 2010, 2015; primate: Boyle and Johanson,  
695 2003; terrestrial frog: Fanardjian et al., 1999) we report here long projections running down  
696 to the lumbar segments from both groups of vestibulospinal nuclei. Indeed, only a small  
697 amount of vestibulospinal neurons stop their course into the cervical cord, and TAN neurons  
698 project almost equally into the thoracic and lumbar segments while the majority of LVST  
699 neurons project into the lumbar cord. Long ago, vestibulospinal projections were associated  
700 with their functional effects on animal postural control (see Keshner and Cohen, 1989),  
701 medial cervical projections being mostly dedicated to neck control and head stabilization  
702 (Boyle, 1993) and lateral projections mainly controlling axial and limb muscles for body  
703 postural adjustment and antigravity extensor tone (Cottingham and Pfaff, 1987; Ali et al.,  
704 2003). In juvenile *Xenopus*, we found a relatively small proportion of vestibulospinal neurons  
705 projecting only in cervical segments. This might be explained by the absence of head  
706 movement with respect to the trunk and by the thinness of forelimbs compared to the body  
707 size, which excludes any significant forelimb-mediated postural adjustment. In contrast,

708 thoracic and lumbar segments were the favorite targets of vestibulospinal neurons, where  
709 they could activate directly axial and extensor MNs respectively, the two main postural  
710 effectors in the juvenile *Xenopus*. Direct connections between vestibulospinal fibers and  
711 spinal MNs were already described in various species, at various spinal levels (Grillner et al.,  
712 1970; Magherini et al., 1974; Wilson et al., 1970). As a totally unexpected result, we  
713 described two distinct pathways to convey vestibular signals to thoracic MNs. Indeed, we  
714 found that direct vestibular inputs reached T2 MNs directly, whereas indirect vestibular  
715 information visibly involved a relay in the lumbar spinal cord from where it activated  
716 simultaneously T2 MNs through ascending INs and hindlimb Ext MNs. The existence of such  
717 two distinct pathways may be explained by the gross morphology of the two major postural  
718 effectors in the juvenile *Xenopus* and their likely involvement in two distinct types of  
719 postural responses. When activated alone, axial back muscles allow only a small amplitude  
720 and precise buoyancy control which would require only accurate positional information. In  
721 contrast, involving the hindlimbs mediates larger postural adjustments that occur in  
722 response to stronger disequilibrium, the strength of which would be more reliably encoded  
723 as disturbance velocity. Simultaneously, and as shown previously during swimming (Beyeler  
724 et al., 2008), adequate trunk stiffening would be generated during hindlimb extension in  
725 order to stabilize the whole body. Thus, in the juvenile *Xenopus* the vestibulospinal system is  
726 organized in such a way that the main postural effectors are in direct link with precise  
727 vestibulospinal projections, and maybe vestibular sensitivities, in order to generate the most  
728 efficient postural adjustment.

729 In most animal models, postural muscles are also involved in locomotor movements. In  
730 terrestrial quadrupeds for instance, the vertebral column is long and flexible, and virtually all  
731 back muscles are also engaged in propulsion during walking and running (Alexander et al.,  
732 1985; Muybridge, 1957). In the post-metamorphosis *Xenopus*, on the contrary, the vertebral  
733 column is short (8 vertebra, from atlas to urostyle), and its stiffness does not allow the trunk  
734 to produce any lateral undulation or antero-posterior flexion/extension that could  
735 participate in propelling the animal. The anatomical arrangement of *dorsalis trunci*  
736 myomeres suggests that contracting unilaterally the 3<sup>rd</sup> and/or 4<sup>th</sup> myomere results in a  
737 slight twist of the vertebral column towards the side of the contracted muscles, so creating a  
738 small amplitude rolling angle between anterior (skull/scapula) and posterior (ilio-  
739 sacrum/urostyle) more rigid bony ensembles. A previous modelization study demonstrated



740 that the degree to which this part of the column is twisted has strong impact on the animal  
741 static posture, and suggested that controlling such a twist may be one essential function of  
742 the dynamic control of posture during swimming (Beyeler et al., 2013). The present  
743 description of the convergence of direct and lumbar-relayed vestibulospinal influence on T2  
744 MNs strongly reinforces the idea of a general role of *dorsalis trunci* muscles in postural  
745 control, both during swimming and in response to passive motion. In addition, the  
746 conjunctural *dorsalis* and hindlimb extensor MNs activation in response to vestibular head-  
747 motion signals implicates lumbar INs that likely also participate in coupling these two motor  
748 entities during swimming. This further suggests that proactive and reflex postural responses  
749 share common propriospinal pathways in vertebrates.

750

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894

895 **Additional Information**

896 Competing Interests

897 The authors declare no competing interests.

898 Author contributions

899 All experiments were performed at the Institut de Neurosciences Cognitives et  
900 Intégratives d'Aquitaine. All persons who participated in this work are listed as authors  
901 and all authors have significantly contributed to this work: Conception: FML and DLR;  
902 data acquisition and analysis: AOB, RG, LC, EC, FML and DLR; drafting and revising  
903 critically: AOB, FML and DLR. All authors approved the final version of the manuscript  
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916

917 **FIGURE LEGENDS**

918 **Figure 1. Dorsal postural system of *Xenopus laevis* trunk. A.** Dorsal (left) and lateral (right)  
919 views of *dorsalis trunci* third myomere (3rd myo; light green) insertion (arrowheads in  
920 bottom right image) between thoracic vertebra (Vert.) 1 and 2. Bottom right schematic  
921 illustrate better the myomere insertion on vertebra. Scale bars=3 mm for left and top right  
922 images. Curved arrows in right panel illustrate the vertebral leftward twist induced by the  
923 left 3<sup>rd</sup> myomere contraction. T1 Vr to T3 Vr=thoracic ventral roots 1 to 3. Dotted green lines  
924 delineate vertebra. OC=otic capsule; C=caudal; D=dorsal; R=rostral. **B-C.** In vivo EMG  
925 recordings from *dorsalis trunci* (dt) and *plantaris longus* (pl) on left (le) and right (ri) sides  
926 during **(B)** and in the absence **(C)** of active swimming. **D.** Confocal images orthogonal  
927 projection of a T2 cross-section after dextran (Dex.) retrograde labeling of *dorsalis* 3<sup>rd</sup>  
928 myomere MNs on left (AD 647; cyan) and right (AD 568; magenta) sides **(D<sub>1</sub>)**, and left side  
929 MNs extraction illustrating *dorsalis* MNs lateral (Lat.) and commissural (Com.) dendritic  
930 morphology **(D<sub>2</sub>)**. **E.** Confocal images orthogonal projection of MNs from a whole-mount  
931 open-book T2 spinal segment. **F.** Schematic representation of a T2 MN. c.c=central canal. **G.**  
932 Quantification of primary and secondary dendrite branching in MN lateral and commissural  
933 dendrites (Lat. d. and Com. d., respectively). Error bars are SDs. ns=non-significant.

934 **Figure 2. Thoracic and lumbar motor responses to vestibular endorgans natural**  
935 **stimulation. A.** Schematic of the semi-intact preparation used to record bilateral ventral root  
936 activity from the second thoracic spinal segment (le T2 Vr, dark blue, and ri T2 Vr, light blue)  
937 and bilateral hindlimb extensor motor nerves (le Ext Vr, red, and ri Ext Vr, orange), in  
938 response to angular (green) and linear (purple) head displacements and the respective  
939 activation of intact semi-circular canals (green) and utricles (purple). Black dot in the  
940 brainstem indicates the center of rotation. **B-C.** Examples of motor responses evoked by a 1-  
941 Hz sinusoidal forward/backward translation **(B<sub>1</sub>)** and a 0.5-Hz sinusoidal 30° left-right  
942 rotation **(C<sub>1</sub>)** in the horizontal plane, and corresponding mean discharge rates **(B<sub>2</sub> and C<sub>2</sub>)**  
943 respectively) in response to sinusoidal variations of the stimulating motion table horizontal  
944 position (continuous sinusoids). Broken sinusoids indicate stimulus instantaneous  
945 acceleration, as derived from motion table position. Alternate pink and white rectangles in  
946 **B<sub>1</sub> and C<sub>1</sub>** highlight the sinusoidal stimulus alternating phases. **D.** Phase analysis from all  
947 experiments in horizontal translation **(D<sub>1</sub>)** and horizontal rotation **(D<sub>2</sub>)** configurations for all

948 recorded motor nerves. Left panels: circular diagrams illustrating the mean distribution  
949 vector for each nerve activity. Right panels: mean phase relationship compared to the  
950 maximal frontal ( $D_1$ ) and maximal lateral ( $D_2$ ) table position for T2 and Ext activities as a  
951 function of stimulus frequency. **E.** Thoracic and lumbar motor responses to horizontal  
952 angular rotation. Examples of motor bursts ( $E_1$ ) and corresponding mean discharge rate ( $E_2$ )  
953 in response to 25-Hz, 30° sinusoidal horizontal rotation of the motion table (continuous  
954 sinusoids). Broken sinusoids indicate the stimulus instantaneous acceleration, as derived  
955 from motion table position.  $E_3$ . Circular phase analysis from all experiments for all recorded  
956 motor nerves. Top panel: circular diagram illustrating the mean distribution vector for each  
957 nerve activity. Bottom panel: mean phase relationship compared to the maximal lateral  
958 position of the stimulating motion table for T2 and Ext activities as a function of stimulus  
959 frequency. Error bars are SDs.

960 **Figure 3. Brainstem spatial distribution of vestibulospinal neurons projecting in cervical**  
961 **and thoraco-lumbar spinal cord.** **A.** Scheme of isolated (brainstem-spinal cord) CNS  
962 preparation illustrating the sequential, homolateral injection of dextran dyes in the 2<sup>nd</sup>  
963 thoracic segment (RDA in T2, red) and 1<sup>st</sup> cervical segment (AD 647 in C1, cyan). Dyes are  
964 retrogradely transported to the cell bodies (dots in the hindbrain, Hb) of neurons that  
965 project in injected spinal cord (SC) segments. A third dye injection in ipsilateral cranial nerve  
966 eight (VIIIth N., green) allowed antero-posterior localization in the brainstem. **B.** Confocal  
967 images orthogonal projection of a hindbrain cross-section showing LVST ( $B_1$ ) and TAN ( $B_2$ )  
968 vestibulospinal neurons labeled from cervical (cyan) and thoraco-lumbar (red) segments  
969 (scale bar=100 $\mu$ m). Insets **a** and **b** show single labeled neurons from thoraco-lumbar (red)  
970 and from cervical (blue), as well as double labeled neurons in both LVST (**a**) and TAN (**b**)  
971 nuclei (merged channels images; scale bar=20 $\mu$ m). IVth=fourth ventricle. **C.** Ratio ( $C_1$ ) and  
972 rostro-caudal repartition ( $C_2$ ) of LVST and TAN neurons projecting (proj.) respectively to  
973 cervical (C1, blue) and thoraco-lumbar (T2, red) spinal cord. Error bars are SDs **D.** 3D  
974 reconstruction from confocal image stacks showing spatial distribution of LVST and TAN  
975 vestibulospinal neurons retrogradely labeled from C1 (blue) and T2 (red) segments, (scale  
976 bar=500 $\mu$ m). D=dorsal, L=lateral, C=caudal.

977 **Figure 4. Electrophysiological and anatomical characterization of direct vestibular inputs to**  
978 **thoracic motoneurons.** **A.** Left: CNS scheme illustrating LVST site stimulation and right T2



979 ventral root recording (ri T2 Vr). Right: Superimposed examples of discharge evoked in a T2  
980 ventral root by minimal electrical stimulation of the ipsilateral LVST nucleus. **B.** Left: LVST  
981 and TAN minimal stimulation-evoked responses in ipsilateral and contralateral T2 ventral  
982 roots respectively, in control saline (black), high divalent cation saline (high Ca<sup>2+</sup>/Mg<sup>2+</sup>, red)  
983 and during wash (gray). Mean responses are illustrated: raw traces were firstly aligned on  
984 stimulation artifact initiation; thereafter, stimulation artifacts were truncated, and raw  
985 traces were averaged. Little arrows on mean traces point to the initiation of the mean  
986 compound burst in control and high divalent cation solution, respectively. Right: Box charts  
987 of delays from all preparations in control and under high divalent cation saline for LVST-  
988 evoked ipsilateral T2 responses (top) and TAN-evoked ipsilateral (ipsi) and contralateral  
989 (contra) T2 responses (bottom). Error bars are 95% confidence intervals. **C.** Left panel: CNS  
990 scheme depicting retrograde labeling of *dorsalis trunci* (d.t.) MNs (AD 647, magenta),  
991 ipsilateral TAN neurons anterograde labeling (RDA, cyan), and cervical spinal cord  
992 hemisection (C1 hsct; see Methods). Middle panel: Confocal images orthogonal projection of  
993 a T2 cross-section illustrating retrogradely labeled T2 motoneurons (magenta) and  
994 anterogradely labeled vestibulospinal fibers (cyan). Scale bar=100µm. Right panel:  
995 magnification of square area (a) in middle panel, illustrating vestibulospinal fiber terminals  
996 (vest.fib), T2 MN commissural dendrites, and synapsin (Syn.) immunofluorescence labeling.  
997 Merged image illustrating fluorescent signals close apposition is shown at the bottom right.  
998 Scale bar=20µm. **D-E.** Protocol schematic and merged magnifications for contralateral TAN  
999 and ipsilateral LVT nucleus, respectively. Greater magnifications of areas "a" are shown  
1000 below as separated channels to better illustrate apposition zones. Scale bar=20µm;  
1001 com. d.=commissural dendrites; lat. d.=lateral dendrites.

1002 **Figure 5. Electrophysiological and anatomical characterization of L1 interneuronal**  
1003 **ascending inputs to thoracic motoneurons.** **A.** CNS scheme illustrating injection sites for  
1004 dextran (Dex.) retrograde labeling of lumbar ascending interneurons from the 1<sup>st</sup> cervical  
1005 (RDA, orange) and 2<sup>nd</sup> thoracic segments (AD 647, cyan), and cross section scheme of the 1<sup>st</sup>  
1006 lumbar segment. Insets show ipsilateral and dorsal (**a**), and contralateral and ventral (**b**)  
1007 lumbar ascending interneurons projecting to T2 hemi-segment (**A<sub>1</sub>**), as well as interneurons  
1008 projecting to C1 hemi-segment (**A<sub>2</sub>**, orange). **A<sub>1</sub>** and **A<sub>2</sub>** from two distinct preparations. **B.** 3D  
1009 reconstruction of lumbar spinal segment from confocal image stacks depicting spatial

1010 organization of interneurons projecting into the 2<sup>nd</sup> thoracic segment (blue dots) in  
1011 transversal (**B<sub>1</sub>**) and dorso-lateral (**B<sub>2</sub>**) views. Retrograde labeling of L1 MNs (light brown)  
1012 allowed rostro-caudal localization within the lumbar cord. L1 Vr=L1 ventral root. Scale  
1013 bars=100µm. **C.** Top left: CNS scheme showing L1 electrical stimulation site and T2 ventral  
1014 root (T2 Vr) recording. Top right: Typical example of recordings in ipsilateral (top) and  
1015 contralateral (bottom) T2Vr in response to L1 IN minimal stimulation in control saline (black),  
1016 high divalent cation saline (red) and during wash (gray). Bottom: Box charts of responses  
1017 delays from all preparations in control and under high divalent cation saline illustrating the  
1018 large variation in delays of L1 IN-evoked ipsilateral T2 responses. Error bars are 95%  
1019 confidence intervals. **D.** Confocal images orthogonal projection of a 2<sup>nd</sup> thoracic cross-  
1020 section illustrating retrogradely labeled *dorsalis* MNs (AD 647, magenta) and lumbo-thoracic  
1021 fibers ascending from ipsilateral L1 hemi-segment (asc. ipsi. LIN fib.; RDA, cyan). Merged  
1022 magnifications on the right illustrate fluorescent signal close appositions (arrowheads) on  
1023 MNs lateral dendrites (lat. d.) together with synapsin immunofluorescence (green). Inset in  
1024 left image depict neuronal labeling protocol.

1025 **Figure 6. Variety of lumbar INs and IN stimulation-evoked motor responses.** **A.** Confocal  
1026 images orthogonal projection from a 2<sup>nd</sup> lumbar segment cross-section showing ascending  
1027 ipsilateral and contralateral INs (aIIIN and aCIN, respectively; RDA, cyan) retrogradely labeled  
1028 from left T2 hemi-segment together with L2 motoneurons and afferent fibers (L2 MN and L2  
1029 af, respectively; AD 488, orange) labeled from L2 ventral root (L2 Vr). **B.** Retrograde labeling  
1030 of T2 *dorsalis* MNs (AD 647, magenta) and anterograde labeling (RDA, cyan) of contralateral  
1031 L1 IN terminals coupled with synapsin immuno-detection (green). Arrowheads point to close  
1032 apposition between the three fluorescent signals, near the MN soma and on lateral  
1033 dendrites (lat. d.). **C.** CNS scheme illustrating the electrical stimulation of L1 INs (stim) while  
1034 recording simultaneously from hindlimb flexor (Flex) and Extensor (Ext) nerves and from the  
1035 2<sup>nd</sup> thoracic ventral roots (T2 Vr) on both sides (le=left; ri=right). **D.** Example of motor nerves  
1036 early responses to the minimal stimulation left L1 INs in control (black), high divalent cation  
1037 (red) and wash (gray) conditions. **E.** Example of rhythmic locomotor-like bursting activity  
1038 recorded from most of the motor nerves in response to a left L1 IN single electrical  
1039 stimulation. **F.** Schematic summary of the mean distribution on T2 postural MN soma, lateral  
1040 (lat.) and commissural (com.) dendrites of putative synaptic contacts from LVST (black bars),

1041 TAN (grey bars) and L1 IN (empty bars) terminals. Error bars are SDs. dist.=distal;  
1042 med.=medial; prox.=proximal.

1043 **Figure 7. LVST inputs onto T2-projecting L1 interneurons.** **A.** CNS scheme showing injection  
1044 sites for LVST fibers anterograde labeling (RDA, magenta) and ipsilateral L1 INs retrograde  
1045 labeling from contralateral T2 hemi-segment (AD647, cyan). Spinal hemisection was  
1046 performed at C1 level contralaterally to the injected LVST. **B.** Confocal images orthogonal  
1047 projection of a L1 cross-section illustrating T2-projecting lumbar INs (T2 proj. IN; cyan) and  
1048 LVST terminals (vest.fib; magenta). Scale bar=100 $\mu$ m. Insets **a** and **b** illustrate two examples  
1049 of fluorescent signal appositions (arrowheads) together with synapsin immunofluorescence  
1050 (green) on IN somata and dendrites; scale bar=20 $\mu$ m. **C-D.** Same arrangement as A for  
1051 contralateral TAN-L1 INs synaptic contacts. **E.** Scheme of the isolated brainstem-spinal cord  
1052 preparation used to record LVST stimulation-evoked calcium transients ( $\Delta F/F$ ) from L1 INs  
1053 (orange) retrogradely loaded from a T2 hemi-segment with Calcium Green Dextran Amine  
1054 (CGDA<sup>+</sup>), and examples of CGDA-labeled INs. **F.** Representative examples of calcium  
1055 transients recorded from 11 L1 INs in response to LVST and TAN stimulation (single pulse =  
1056 50  $\mu$ s). **F<sub>1</sub>.** Superimposed responses illustrating the similarity of calcium response in all  
1057 recorded INs to LVST (top) and TAN (bottom) stimulation. **F<sub>2</sub>.** Averaged calcium transients  
1058 recorded from the same 11 distinct L1 INs projecting into contralateral T2 segments in  
1059 response to repeated ipsilateral LVST (left) and contralateral TAN (right) stimulation. Arrow  
1060 on left panel indicates rhythmic-like bursting response to the first LVST stimulation. **F<sub>3</sub>.**  $\Delta F/F$   
1061 means for each preparations (top; error bars are SDs), and box charts (bottom; error bars are  
1062 95% confidence intervals) illustrating for all preparations the distribution of calcium  
1063 response amplitudes for ipsilateral (black) and contralateral (grey) L1 INs to both LVST and  
1064 TAN electrical stimulation. ns=non-significant.

1065 **Figure 8. Direct and indirect vestibulo-thoracic pathways involved in *dorsalis* motoneurons**  
1066 **activation.** **A-B.** Summary of postural responses during forward (**A<sub>1</sub>**) and backward (**A<sub>2</sub>**) linear  
1067 translations, and during rightward (**B<sub>1</sub>**) and leftward (**B<sub>2</sub>**) angular rotations. Dark colors: T2  
1068 response alone; light colors: coupled T2 and Extensor motor responses. **C.** Schematic  
1069 organization of direct and lumbar-relayed LVST and TAN pathways to T2 *dorsalis* MNs.  
1070 Bs=brainstem; Lumbar Sc=lumbar spinal cord; iIN=ipsilateral interneuron; cIN=contralateral  
1071 interneuron.

1072 **Supplemental movie 1. Hindlimb reflex movements induced by forward/backward**  
1073 **repetitive natural stimulation of vestibular endorgans.** Sliding bar on the left part of the  
1074 movie indicates sled motion. During each backward displacement, observe the small  
1075 amplitude paw movements that correspond to electrophysiological motor bursts recorded  
1076 from *plantaris longus* motor nerves in the same condition (e.g, Fig. 2B1). Such movements,  
1077 corresponding to postural adjustments, have amplitude much smaller than full hindlimb  
1078 extension characteristic of swim kicking (see Beyeler et al, 2008, 2013).