

# Intraspinal Stretch Receptor Neurons Mediate Different Motor Responses Along the Body in Lamprey

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## ABSTRACT

In lampreys, stretch receptor neurons (SRNs) are located at the margins of the spinal cord and activated by longitudinal stretch in that area caused by body bending. The aim of this study was a comprehensive analysis of motor responses to bending of the lamprey body in different planes and at different rostrocaudal levels. For this purpose, *in vitro* preparation of the spinal cord isolated together with notochord was used, and responses to bending were recorded from SRNs, as well as from motoneurons innervating the dorsal (dMNs) and ventral (vMNs) parts of a myotome. It was found that SRNs were activated on the convex (stretched) side of the preparation during bending both in the yaw and in the pitch plane. By contrast, responses of motoneurons depended on the site and plane of bending. In the yaw plane, concave responses

to bending of rostral segments and convex responses to bending of mid-body segments prevailed. In the pitch plane, convex responses in dMNs and concave responses in vMNs to bending in mid-body segments prevailed. These spinal reflexes could contribute to feedback regulation of locomotor body undulations and to the control of body configuration during locomotion. After a longitudinal split of the spinal cord, only convex responses in motoneurons were present, suggesting an important role of contralateral networks in determining the type of motor response. Stimulation of the brainstem changed the type of motor response to bending, suggesting that these spinal reflexes can be modified by supraspinal signals in accordance with different motor behaviors. *J. Comp. Neurol.* 521:3847–3862, 2013.

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**INDEXING TERMS:** edge cells; proprioceptive inputs; posture; locomotion; spinal reflexes

In vertebrates, proprioceptive inputs provide sensory information about body configuration that is important for the control of different movements including locomotion. In the lamprey, muscle spindles were not found in trunk muscles, proprioceptive inputs are provided by intraspinal mechanoreceptors (the stretch receptor neurons, SRNs), also known as edge cells (Grillner et al., 1984). SRNs are located at the lateral margins of the spinal cord and are activated by longitudinal stretch in that area (Grillner et al., 1984). Since the spinal cord is tightly attached to the notochord (Fig. 1B), bending of the notochord (caused by body bending) is accompanied by stretch or compression of the marginal areas of the spinal cord. Neurons located in the marginal area of the spinal cord with morphology similar to that of SRNs in lamprey, and possibly providing sensory signals about body configuration were found in elasmobranchs, amphibians, and reptiles including snakes (Schroeder, 1986; Schroeder and Egar, 1990; Anadon et al., 1995). The general goal of this study was to analyze the SRN-mediated motor responses evoked by bending of the

lamprey body in different planes at different rostrocaudal levels.

Bending in the horizontal (yaw) plane occurs in all forms of locomotion in lamprey: fast forward swimming, slow forward swimming, backward swimming, and crawling (Williams et al., 1989; Archambault et al., 2001; Islam et al., 2006; Islam and Zelenin, 2008). Bending in the yaw plane activates SRNs on the stretched (convex) side, and inactivates those on the compressed (concave) side (Grillner et al., 1982) (Fig. 1C,D).

Like limb mechanoreceptors in mammals, SRNs affect the central pattern generator (CPG) for locomotion by exciting or inhibiting different groups of CPG

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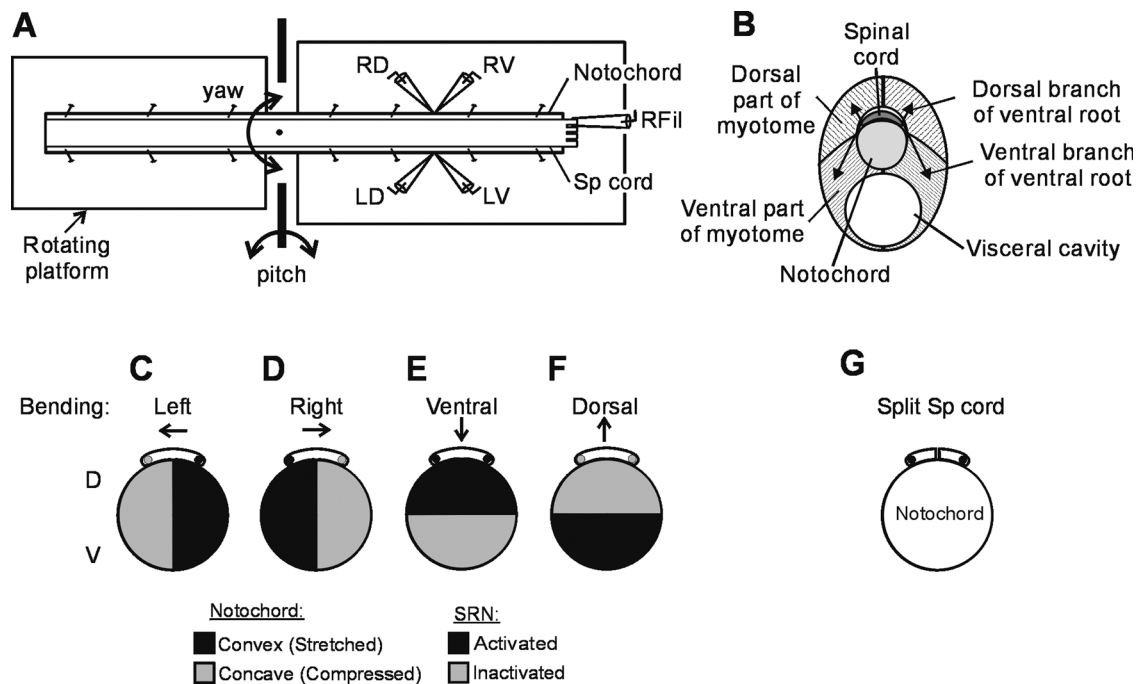
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**Figure 1.** Experimental design. **A:** A piece of the spinal cord was isolated together with the notochord and attached to two platforms. One platform was stationary and the other could be rotated in the yaw (horizontal) and pitch (sagittal) planes, causing bending of the preparation. Responses of motoneurons (innervating dorsal and ventral parts of a myotome) to bending were recorded from the dorsal (D) and ventral (V) branches of the right (R) and left (L) ventral roots in one of the segments by means of suction electrodes. The stump of the spinal cord was split into small filaments to record axons of SRNs in  $\text{Ca}^{2+}$ -free solution by means of a suction electrode (recording from the right filament, RFil, is shown). **B:** Different anatomical structures seen on the cross-section of the body. **C–G:** A scheme of the cross-sections of intact spinal cord-notochord preparations (C–F) and that split along the midline (G), with SRNs located at the margins of the spinal cord. In C–F the expected effect of bending the preparation in different planes on the activity of SRNs is shown.

neurons (Viana Di Prisco et al., 1990). Two types of SRNs have been identified—those with ipsilateral projections (IP-SRNs) and those with contralateral projections (CP-SRNs). The IP-SRNs excite ipsilateral motoneurons (MNs) and CPG interneurons, while CP-SRNs inhibit contralateral MNs, interneurons, and SRNs.

Due to connections with the CPG neurons, SRNs can affect not only the amplitude but also the rhythm of lateral locomotor undulations generated by the spinal cord (Grillner et al., 1981; Viana Di Prisco et al., 1990). It has been shown that rhythmical lateral bending of the isolated spinal cord/notochord preparation can entrain the rhythm of “fictive swimming” produced by the spinal locomotor CPG (Grillner et al., 1981; McClellan and Jang, 1993). It was shown that bending of the rostral and caudal segments produced different effects on the swim rhythm (McClellan and Sigvardt, 1988; Williams et al., 1990; Tytell and Cohen, 2008), suggesting that processing of SRNs signals is different at different rostrocaudal levels of the spinal cord, and thus spinal reflexes mediated by SRNs might be different along the extent of the spinal cord. *The first aim* of the present study was to test this hypothesis by analyzing the

responses of SRNs and MNs to bending at different sites along the lamprey body.

From simple anatomical considerations it is evident that stretching the marginal area of the spinal cord (necessary for SRNs activation) can occur not only with the notochord bending in the yaw plane (Fig. 1C,D) but also with bending in the sagittal (pitch) plane (Fig. 1E,F). One can therefore expect that SRNs will be activated with notochord bending in the pitch plane as well, and they may elicit reflex motor responses to this bending. These reflexes may potentially contribute to the maintenance of rectilinear projection of body axis in the pitch plane observed in the swimming lamprey, as well as to upward and downward maneuvers caused by body flexion in this plane (Ullén et al., 1995). However, these reflexes were not investigated previously. *The second aim* of the present study was to analyze responses of SRNs and MNs to body bending in the pitch plane.

Ventral bending in the pitch plane would be expected to activate both the right and left SRNs. There are mutual influences between the left and right segmental circuits that are mediated by CP-SRNs (Viana Di Prisco

et al., 1990), and by commissural CPG neurons (Grillner et al., 1991). The third aim of this study was to investigate the role of the contralateral networks in the generation of motor responses to body bending. For this purpose, we eliminated all crossed influences by splitting the spinal cord along the midline (Cangiano and Grillner, 2003), and compared the effects of bending in this preparation with those in the “intact” (nonsplit) spinal cord preparation.

The fourth aim was to clarify if the spinal SRNs-mediated reflex responses to bending can be regulated by supraspinal centers, as demonstrated for a number of spinal reflexes in other species (Eccles and Lundberg, 1959; Holmquist and Lundberg, 1959, 1961; Hultborn et al., 1976; Gossard et al., 1994). For this purpose, we studied the effects of electrical stimulation of different sites in the brainstem on motor responses to bending. Clear effects (including changes of the type of response) were obtained for some of the sites, suggesting that these spinal reflexes in the lamprey can be centrally regulated in accordance with different locomotor and postural tasks.

A brief account of parts of this study was published in abstract form (Hsu et al., 2012a,b).

## MATERIALS AND METHODS

Experiments were performed on the *in vitro* preparation dissected from adult lampreys (*Ichthyomyzon unicuspis*,  $N = 32$ ). All experiments were approved by the local Ethics Committee (Norra Djurförsöksetiska Nämnden). The preparation consisted of a piece of the spinal cord (15–50 segments) isolated together with the notochord; it was dissected from different rostro-caudal zones of the body. In some of the experiments ( $N = 10$ ) the spinal cord was split along its midline with the tip of a fine hypodermic needle (0.4 mm diameter; for details, see Cangiano and Grillner, 2003) (Fig. 1G).

The dissection and experiments were performed in cold (7–10°C) oxygenated Ringer’s solution containing (in mM) 91 NaCl, 2.1 KCl, 2.6 CaCl<sub>2</sub>, 1.8 MgCl<sub>2</sub>, 20 NaHCO<sub>3</sub>, and 4.0 glucose. The solution was bubbled with O<sub>2</sub> and adjusted to pH 7.4 with HCl. D-glutamate (0.5–2.0 mM) was used to increase the excitability of the spinal networks. In almost all intact spinal cord preparations it evoked “fictive locomotion” (Cohen and Wallén, 1980). In some split spinal cord preparations D-glutamate caused fast rhythmic bursting (~10 Hz) in the activity of MNs, which is consistent with previous findings (Cangiano and Grillner, 2003).

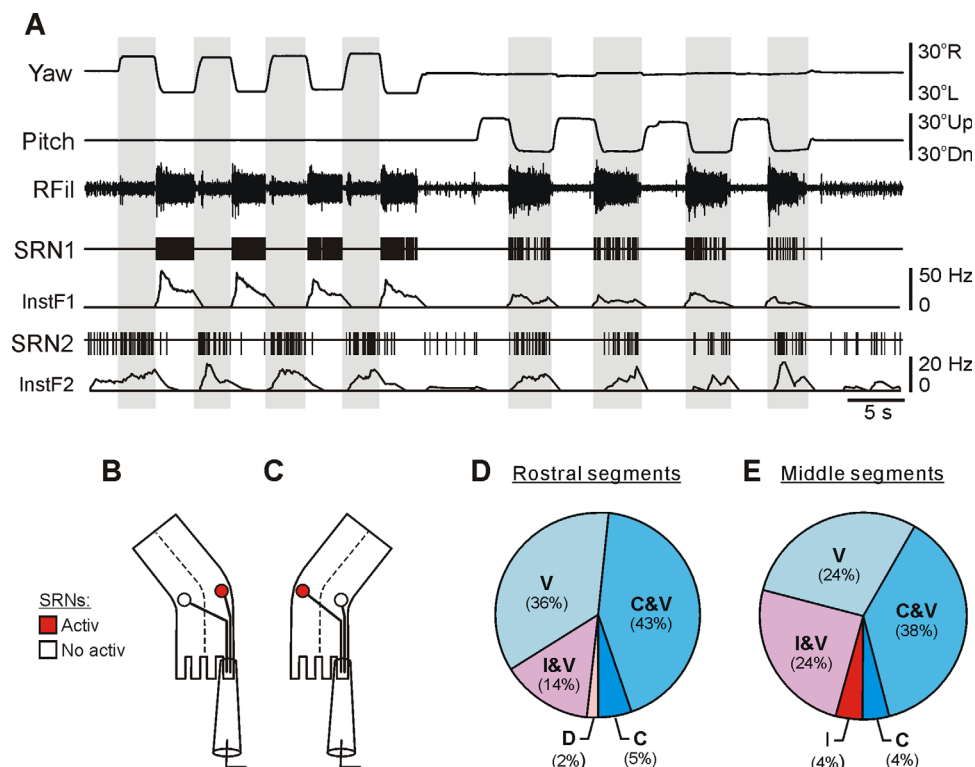
The preparation was pinned down through the notochord to the two Silgard-covered platforms positioned

in the experimental chamber (Fig. 1A). One part of the preparation was pinned to a stationary platform, and the other part to a small movable platform that could be rotated in both yaw (horizontal) and pitch (sagittal) planes, thus bending the preparation in the corresponding plane (Fig. 1A). Extra pins were used close to the bending site to keep the two parts of the preparation motionless relative to the corresponding platform. The length of the flexed area was 2–4 segments. Only the SRNs with cell body and dendrites located within this area were subjected to stretch and generated spikes (Grillner et al., 1982, 1984). The bending site was characterized by the middle point of this area.

The responses of SRNs and MNs were examined with a trapezoidal temporal pattern of the platform rotation: the peak-to-peak value was 25–40°, the transition from one position to another lasted ~1 second, and each position was maintained for ~4 seconds (Fig. 2A). The angles of rotation in the yaw and pitch planes (Fig. 1A) were measured by potentiometric transducers. In part of the experiments, bending was performed at different sites along the preparation. When the movable part of the preparation was too long to be secured on the rotating platform, the preparation was bent by hand using a thin rod, and the signals related to bending were produced manually by pressing a button. In these experiments, to perform left/right bending (in the yaw plane) the preparation was positioned with its dorsal side up. To perform dorsal/ventral bending (in the pitch plane) the preparation was positioned with its lateral side up.

Activity of MNs projecting to the dorsal and ventral parts of a myotome (dMN and vMN, respectively; Wallén et al., 1985) was recorded by means of suction electrodes from the dorsal and ventral branches of ventral roots (Fig. 1A,B) (Zelenin et al., 2001, 2003, 2007). Activity of SRNs was recorded by means of the suction electrode from their axons in thin (50–250 μm) filaments (Fig. 1A) dissected from the stump of the spinal cord (Grillner et al., 1982). Synaptic transmission was blocked by reducing the Ca<sup>2+</sup> to zero and increasing Mg<sup>2+</sup> to 4 mM in Ringer’s solution. The axons in the filament that were activated by bending the preparation in a Ca<sup>2+</sup>-free solution were considered SRNs axons (Grillner et al., 1982).

In two experiments, the brainstem-spinal cord preparation was used. The brainstem, vestibular organs, and the rostral part of the spinal cord (~45 segments) were isolated together with the cranium and notochord. The base of cranium and the rostral part of the notochord were pinned to the stationary platform, whereas the caudal part of the preparation was pinned to the rotating platform. Electrical stimulation of the brainstem



**Figure 2.** Responses in SRN axons to bending recorded in the intact spinal cord preparation under  $\text{Ca}^{2+}$ -free condition. **A:** Responses to yaw and pitch bending in segment 35 were recorded in the right filament (RFil) dissected from the segment 40. Activity of two SRNs (SRN1 and SRN2) was extracted from the mass activity. Their instantaneous frequencies (InstF1 and InstF2) are shown. **B,C:** Effect of bending in the yaw plane (to the left in B and to the right in C) on the activity of IP-SRN and CP-SRN is shown schematically. **D,E:** Proportion of SRNs responding to yaw and/or pitch bending in the rostral (10–30) segments (D), and in the mid-body (35–50) segments (E).  $N = 5$ ,  $n = 56$  in D, and  $N = 5$ ,  $n = 48$  in E. C, I, V, D: activation of SRN axons by contra-, ipsi-, ventral, and dorsal bending, respectively.

(10–20 Hz; 0.2 ms; 2–20  $\mu\text{A}$ ) was performed with a glass electrode ( $\sim 50 \mu\text{m}$ ) during bending of the caudal part of the preparation.

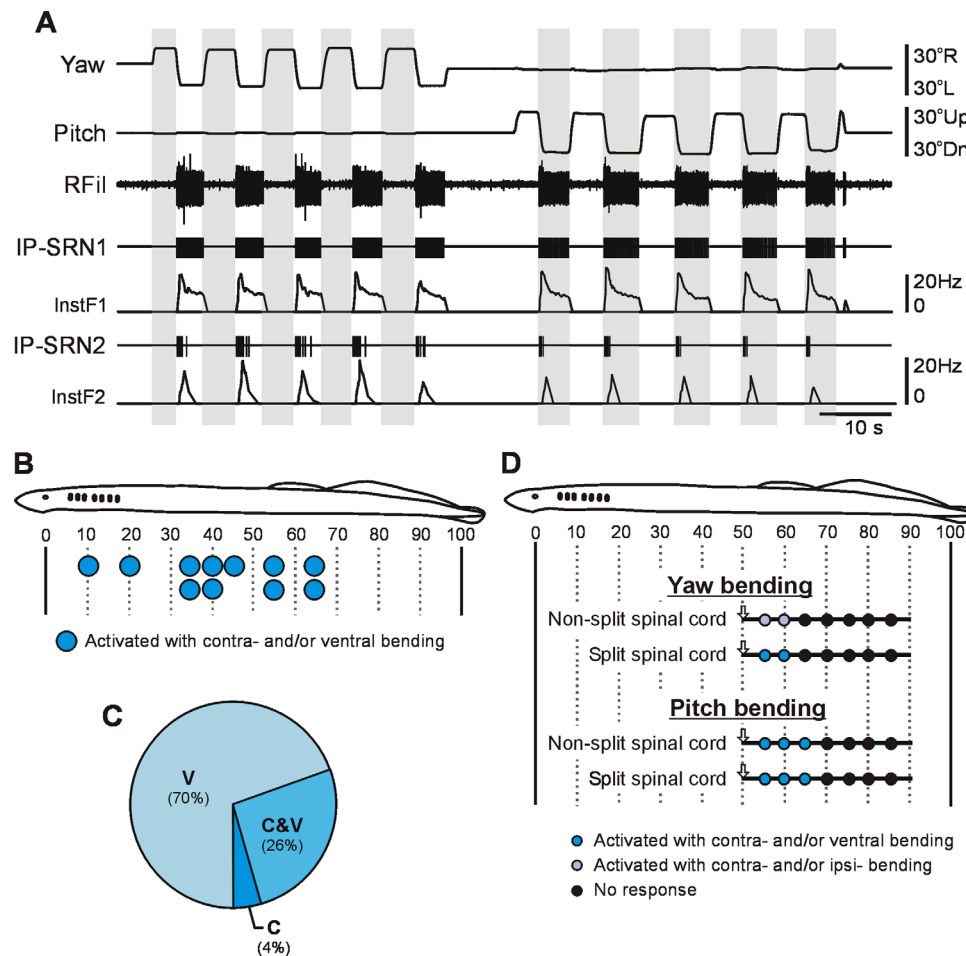
Signals from the electrodes and from the platform position sensors were amplified, digitized with a sampling frequency of 10 kHz (electrodes) and 100 Hz (sensors), displayed on the screen and saved to computer disc by means of data acquisition and analysis system (Power 1401/Spike2, Cambridge Electronic Design, Cambridge, UK). To separate activity of individual MNs from the mass activity recorded in a ventral root branch, a spike-sorting procedure was performed. The criterion for discrimination of individual neurons was the shape of the spike waveform (Zelenin, 2005). Only neurons with stable responses and consistent waveforms were included in the analysis (as individual MNs in Fig. 4B,C). Usually, from one to eight neurons could be extracted from the mass neuronal activity recorded in one branch. The same procedure and criteria were used to extract activity of individual SRNs from the mass activity in a filament.

## RESULTS

### Responses of SRNs to bending *Intact spinal cord preparation*

In five experiments on the intact (nonsplit) spinal cord preparation, responses of 104 SRNs to bending were recorded in  $\text{Ca}^{2+}$ -free solution. The majority of SRNs (80%) were recorded from the most lateral filaments. An example of responses of SRNs to bending in the yaw and in the pitch plane is shown in Figure 2A. Activities of two individual neurons (SRN1 and SRN2) were extracted from the mass activity recorded in the right filament (RFil). The neurons responded to bending in both planes. SRN1 was activated by downward bending in the pitch plane and leftward bending in the yaw plane, while SRN2 responded to downward bending in the pitch plane and rightward bending in the yaw plane. A dynamic component of the response is seen in SRN1.

Figure 2D,E shows the proportion of SRNs with different types of responses to bending in the yaw and in the pitch plane recorded in the rostral (15–30) and middle (35–50) spinal segments, respectively. One can see



**Figure 3.** Responses in SRN axons to bending recorded in the split spinal cord preparation under  $\text{Ca}^{2+}$ -free condition. **A:** Responses to yaw and pitch bending in segment 40 were recorded in the right filament (RFil) dissected from the segment 45. The activity of two SRNs (IP-SRN1 and IP-SRN2) was extracted from the mass activity. Their instantaneous frequencies (InstF1 and InstF2) are shown. **B:** Responses to bending in the yaw and pitch planes at different rostrocaudal levels were recorded in the split spinal cord preparation. Blue circles indicate the level of bending performed in individual preparations. Recordings were performed from filaments located at the distance of 5–10 segments from the bending site. **C:** Proportion of SRNs responding to yaw and/or pitch bending ( $N = 4$ ,  $n = 23$ ). Abbreviations as in Fig. 2D,E. **D:** Effect of bending at different distances from the recording site. Each line shows the rostrocaudal extent of a preparation; arrow indicates the level at which the filament was recorded; each circle indicates the site of bending and its effect. Horizontal scales in B and C indicate the number of spinal segments.

that, at both levels, almost all SRNs responded to ventral bending in the pitch plane, and about 40% responded to contralateral bending in the yaw plane. However, the proportion of SRNs with responses to ipsilateral bending was two times larger in the mid-body segments than in the rostral segments; 37% of SRNs had a distinct dynamic component of response.

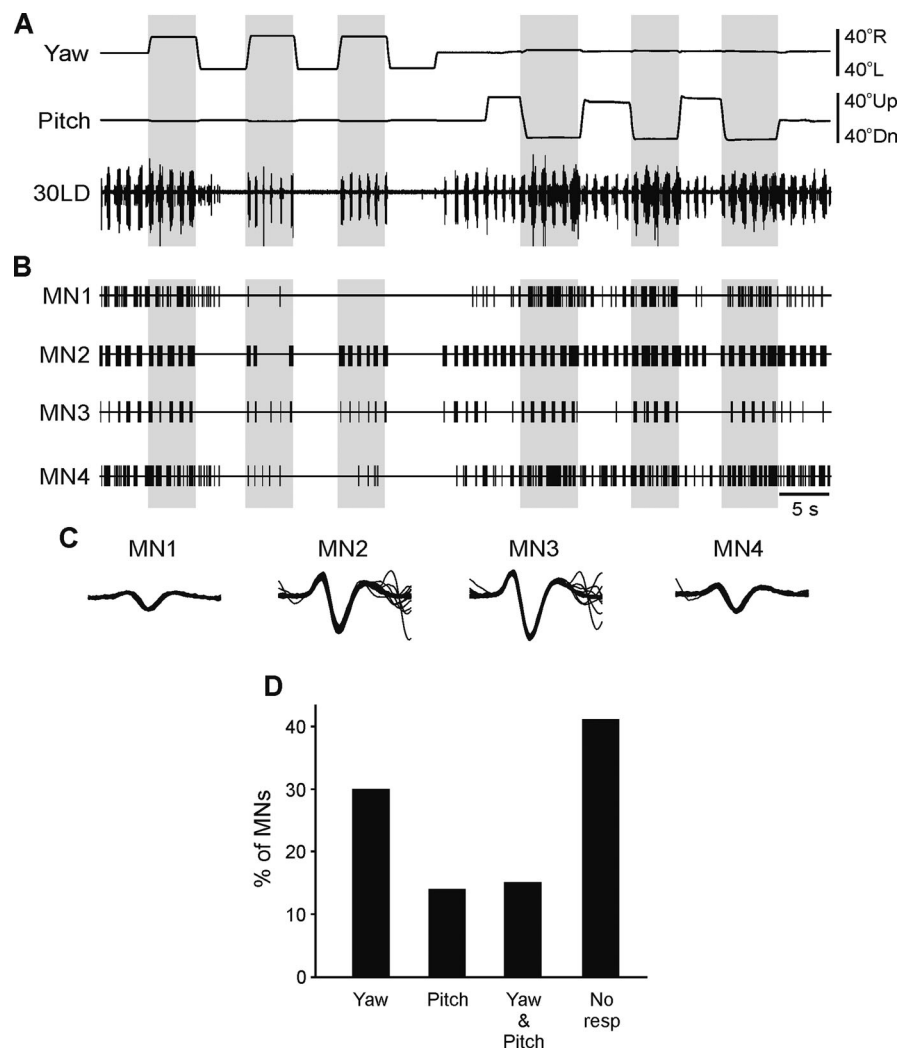
Taking into account that SRN axons recorded from the filament in the intact spinal cord preparation could belong to IP-SRNs and CP-SRNs (Fig. 2B,C) (Viana Di Prisco et al., 1990), one can suggest that axons responding to contralateral bending in the yaw plane belonged to IP-SRNs, and axons responding to ipsilateral bending belonged to CP-SRNs. To test this hypothesis,

we eliminated responses of CP-SRNs by splitting the spinal cord along its midline.

### Split spinal cord preparation

In five experiments on the split spinal cord preparation, responses of IP-SRN axons to bending were recorded from spinal filaments in  $\text{Ca}^{2+}$ -free solution (Fig. 3). In all preparations, only responses to downward bending in the pitch plane, and/or to contralateral bending in the yaw plane were observed. Bending of the preparation at different rostrocaudal levels (segments 10–65) evoked similar responses in the filaments dissected at the distance of 5–10 segments from the bending site (Fig. 3B).





**Figure 4.** Motor responses to bending in the yaw and in the pitch planes recorded in the intact spinal cord preparation. **A:** Responses in the dorsal branch of the left ventral root in segment 30 (30LD) to bending in segment 40. **B:** Spikes of individual MN1–MN4 were extracted from the mass activity of 30LD (shown in A) using a spike-sorting procedure. **C:** Superposition of spike waveforms of individual MN1–MN4. **D:** Proportion of MNs with different patterns of response to bending ( $N = 8$ ,  $n = 253$ ). To increase excitability of spinal networks, D-glutamate (0.5–2.0 mM) was used.

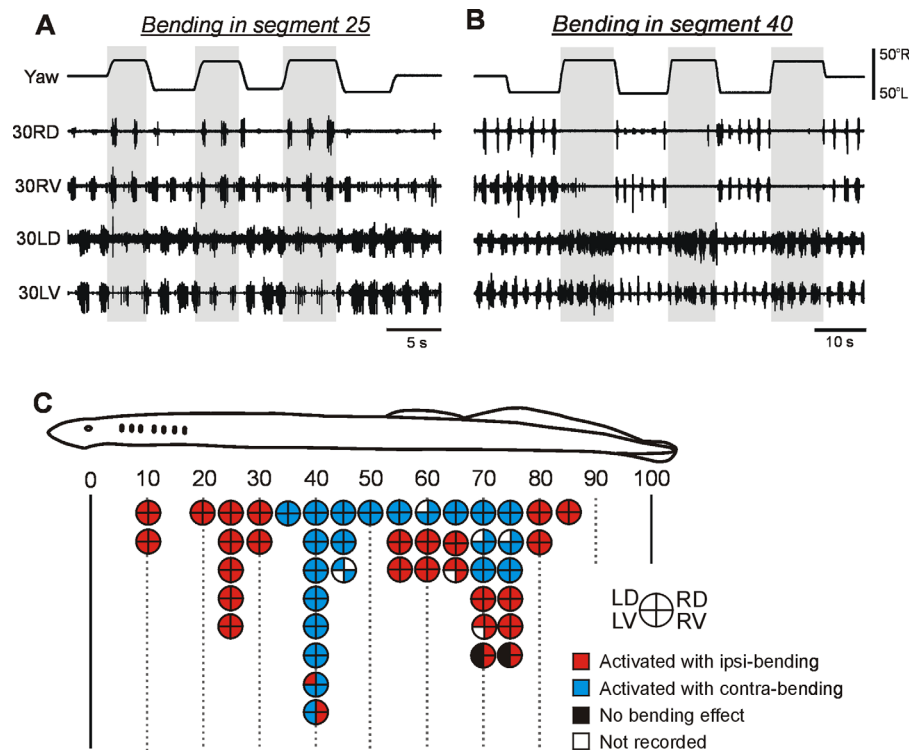
An example of recording from the filament on the right side (RFil) in segment 45 is shown in Figure 3A. The activity of two IP-SRNs (IP-SRN1 and IP-SRN2) was extracted from the mass activity. Both SRNs were activated by contralateral (leftward) bending in the yaw plane and downward bending in the pitch plane in segment 40. In both SRNs a dynamic component of the response was strongly expressed. The activity of 23 individual IP-SRNs was extracted from the mass activity of filaments recorded in four experiments. Almost all these SRNs (96%) were activated by downward bending in the pitch plane, whereas activation with contralateral bending in the yaw plane was observed only in 30% of neurons (Fig. 3C). The majority of IP-SRNs (78%) had a dynamic component of the response.

### Length of SRN axons

To estimate the length of SRN axons, in one experiment on the nonsplit spinal cord preparation and one experiment on the split spinal cord preparation we gradually increased the distance between the constant recording site and the bending site, until the response in the filament disappeared. This happened at a distance of 10 or 15 segments from the site of bending, when the preparation was bent in the yaw and in the pitch plane, respectively (Fig. 3D).

### Responses of MNs to bending Intact spinal cord preparation

Responses of MNs to bending in the yaw and pitch planes were recorded in 16 preparations. In these experiments,

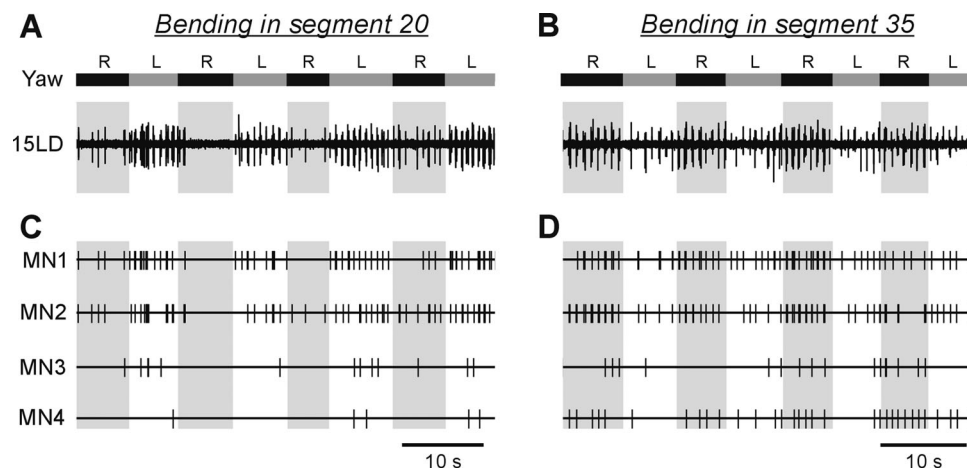


**Figure 5.** Motor responses in the intact spinal cord preparation to bending in the yaw plane at different rostrocaudal levels. **A,B:** An example of responses in the ventral and dorsal branches of right and left ventral roots of segment 30 to bending performed in segment 25 (**A**) and in segment 40 (**B**). LD, LV, RD, RV: left dorsal, left ventral, right dorsal, and right ventral parts of myotomes (or branches of the ventral roots), respectively. **C:** Summary of motor responses evoked by bending at different sites along the body ( $N = 22$ ,  $n = 47$ ). Position of each circle diagram along the horizontal axis indicates the segment in which bending was performed. Each diagram shows a cross-section of the body, with the parts of myotome that would be activated with a given pattern of activity in the ventral root branches (caused by bending in a particular segment). The distance between the sites of bending and recording was 5–10 segments. Different colors designate the type of effect. To increase excitability of spinal networks, D-glutamate (0.5–2.0 mM) was used.

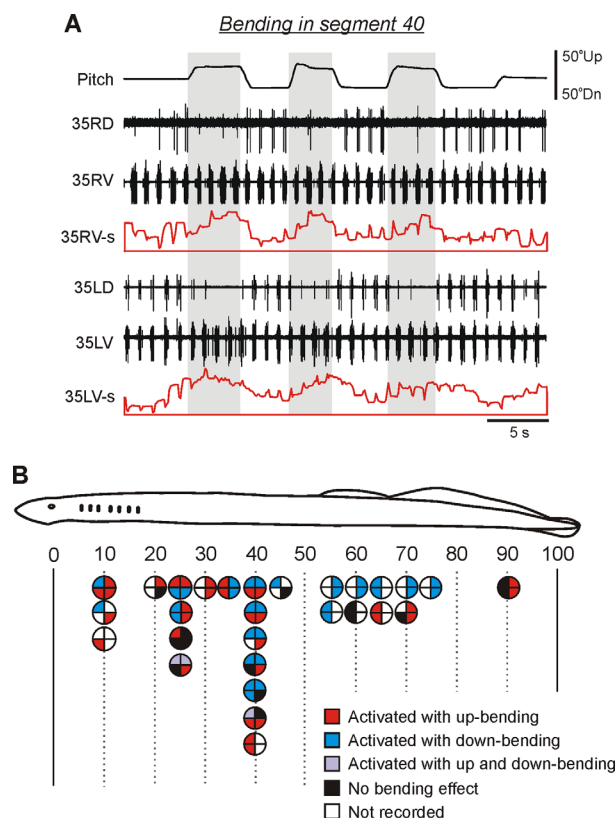
D-glutamate (0.5–2.0 mM) was used to increase the excitability of spinal networks. In almost all intact spinal cord preparations it evoked “fictive locomotion” (Cohen and Wallén, 1980). A representative example of recording is shown in Figure 4A. In this experiment, a preparation of 30 segments in length was taken from the rostral half of the body. Bending was performed in segment 40, and MNs were recorded in segment 30 from the dorsal branch of the left ventral root. Responses to bending were expressed as a modulation of the intensity of locomotor bursting. In the yaw plane, rightward bending caused an increase of mass activity in this branch, while leftward bending caused a decrease. In the pitch plane, downward bending caused an increase in activity, while upward bending caused a decrease. The activity of four individual MNs was extracted from the mass activity using the spike-sorting procedure (MNs 1–4 in Fig. 4B,C). Individual MNs responded differently: MN1 responded to bending in the pitch plane, but not to bending in the yaw plane, whereas MNs 2–4 responded to bending in both planes.

Among 253 MNs recorded in eight experiments, 30% responded to yaw-bending only, 14% to pitch-bending only, 15% to bending in any plane, and 41% did not respond to bending (Fig. 4D). The phase of the motor responses depended on the site of bending. In the example shown in Figure 5A,B, all four ventral root branches were recorded in segment 30 during yaw bending in segment 25 (Fig. 5A) and in segment 40 (Fig. 5B). With bending in segment 25, MNs in the right ventral root (in both dorsal and ventral branches) were activated by rightward bending, and those in the left ventral root were activated by leftward bending. By contrast, with bending in segment 40, MNs in the right ventral root were activated by leftward bending and those of the left ventral root by rightward bending.

Figure 5C summarizes motor responses evoked by yaw bending at different sites along the body. Responses were recorded at the distance of 5–10 segments from the bending site. Altogether, 47 sites were tested in 22 preparations. Bending in rostral segments



**Figure 6.** Responses of individual MNs (1–4 shown in C,D) extracted from the left dorsal branch of the ventral root in segment 15 (15LD) to bending (to the left, L, and to the right, R) performed in segment 20 (A,C) and in segment 35 (B,D).



**Figure 7.** Motor responses in the intact spinal cord preparation to bending in the pitch plane at different rostrocaudal levels. **A:** An example of responses in all ventral roots branches of segment 35 to bending in segment 40. The red traces (35RV-s, 35LV-s) show rectified and then smoothed (time constant 1 sec) signals in the right and in the left ventral branches. **B:** Summary of motor responses evoked by bending at different sites along the body ( $N = 18$ ,  $n = 28$ ). Designations as in Figures 3 and 5. To increase excitability of spinal networks, D-glutamate (0.5–2.0 mM) was used.

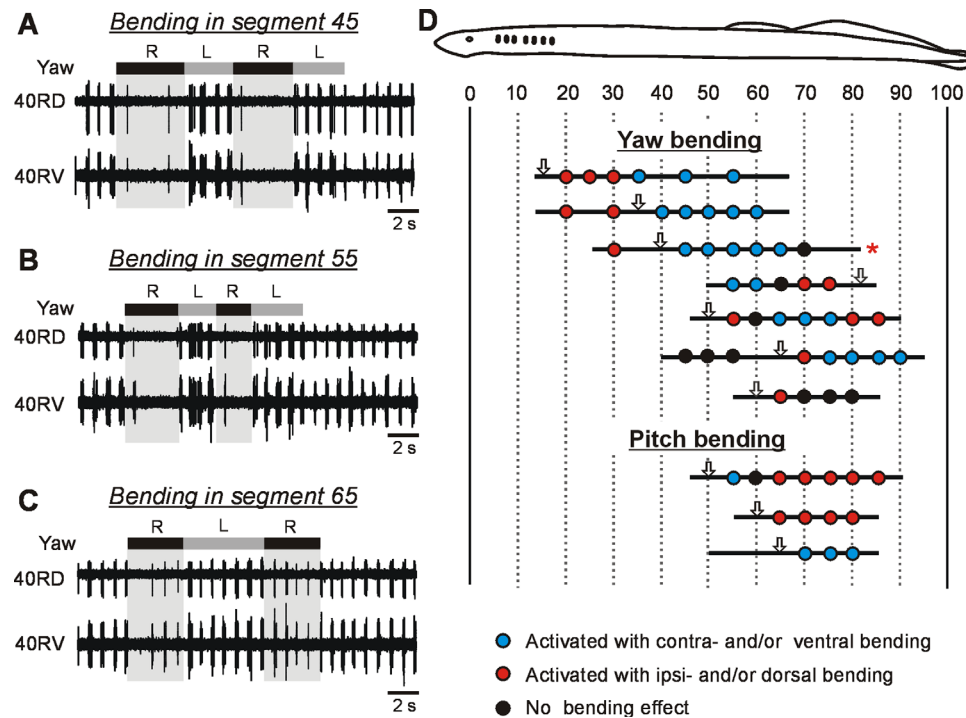
(10–30) caused activation of MNs on the concave side, while bending in mid-body segments (35–50) caused activation of MNs on the convex side. Bending in caudal segments (starting from segment 55) caused in some experiments activation of MNs on the concave side, and in some experiments on the convex side. However, responses were consistent within an experiment.

Since the phase of motor response in individual branches of the ventral root depended on the site of bending (see Fig. 5A,B), we compared responses of individual MNs to bending at different sites. As shown in Figure 6, individual MNs (1–4) in the left dorsal branch of segment 15 responded to left (ipsilateral) bending in segment 20 (Fig. 6A) and to right (contralateral) bending in segment 35 (Fig. 6B); this effect being more pronounced in MN1. Thus, the phase of response of individual MNs depended on the site of bending.

An example of responses to pitch bending in different branches of the ventral roots is shown in Figure 7A. Bending was performed in segment 40, while motor responses were recorded in segment 35. Downward bending caused activation of MNs in the dorsal branches of the right and left ventral roots, whereas upward bending caused activation of MNs in the ventral branches. This pattern of motor responses was observed in 5 out of 7 preparations tested by bending in segment 40 (Fig. 7B). However, pitch bending in other segments evoked much more diverse responses. In almost half of the preparations, the bending produced the same effect on MNs in both (dorsal and ventral) branches of a hemisegment (Fig. 7B).

In 10 experiments, we determined the maximal distance at which bending could affect the motor output. For this purpose, we gradually increased the distance from a constant recording site to the bending site, either rostrally or caudally (see Materials and Methods). Figure 8A–C shows





**Figure 8.** Distance at which bending affected segmental motor output in the intact spinal cord preparation. **A–C:** Responses in the dorsal and ventral branches of the right ventral root in segment 40 (40RD and 40RV, respectively) to bending in segment 45 (A), in segment 55 (B), and in segment 65 (C). **D:** Summary of motor responses in a given segment (indicated by arrow) to bending at different sites along the body. Each line shows the rostrocaudal extent of a preparation ( $N = 10$ ); the circles indicate the bending sites. Different colors designate the type of bending effect. Asterisk marks the preparation in which responses (shown in A–C) were recorded.

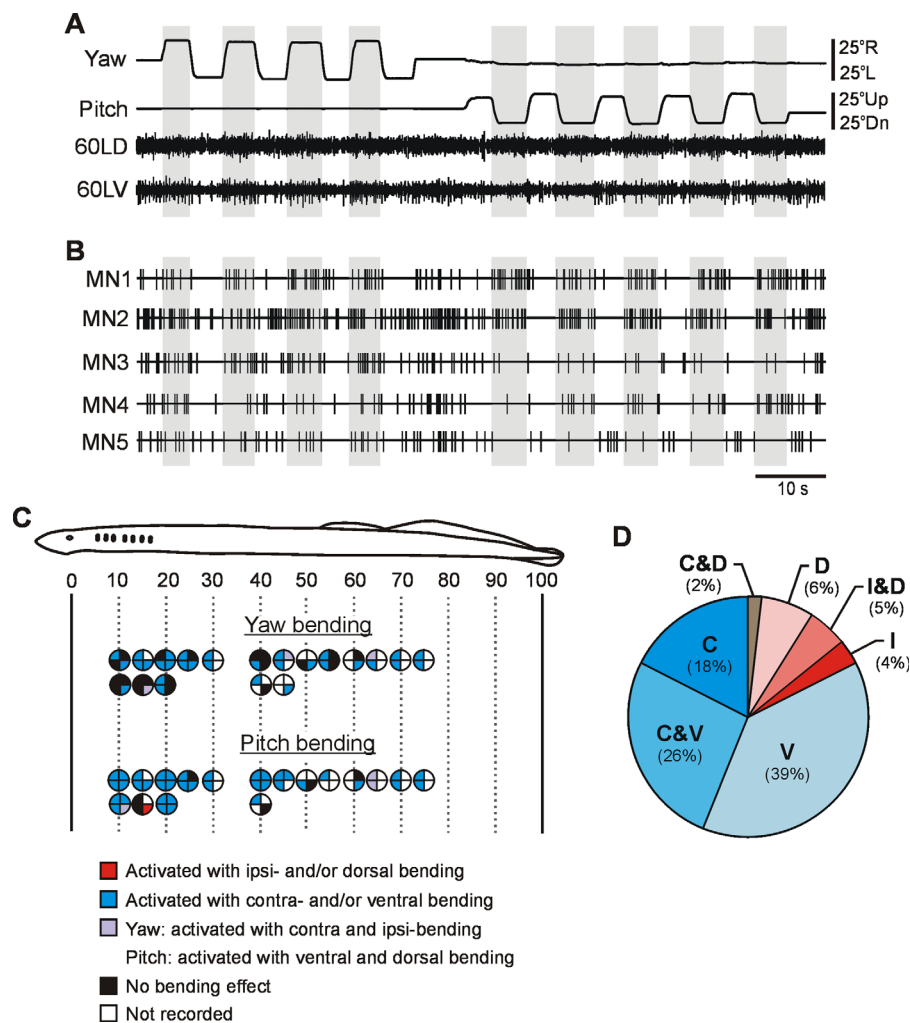
the activity of MNs recorded from the dorsal and ventral branches of the right ventral root in segment 40, whereas yaw bending was performed in segment 45 (Fig. 8A), 55 (Fig. 8B), and 65 (Fig. 8C). In all cases, MNs were excited with leftward (contralateral) bending and inhibited with opposite bending. One can also see that responses of MNs were weaker when bending was produced in segment 65 as compared to the responses caused by bending in segments 55 and 45. The responses disappeared when bending was performed in segment 70 (not illustrated). Figure 8D summarizes results of these experiments. The distance at which bending affected the motor output ranged from 20 to 40 segments. Responses of MNs in a given segment to yaw bending could change with a change of the bending site. In three experiments with yaw bending (upper lines), MNs responded to ipsi-bending in segments 20–30, and to contra-bending in segments 35–65 (Fig. 8D). The phase of response to pitch bending also could change along the responding area.

### Split spinal cord preparation

To reveal the contribution of the networks residing in the contralateral hemicord to the processing of SRN signals, in 10 experiments we recorded motor responses to

bending in the split spinal cord preparation. In these experiments, D-glutamate (0.5–2.0 mM) was used to increase the excitability of spinal networks. In some split spinal cord preparations, D-glutamate caused fast rhythmic bursting ( $\sim 10$  Hz) in the activity of MNs (Cangiano and Grillner, 2003). An example of the responses of MNs to bending in this preparation is shown in Figure 9A,B. Bending was performed in segment 65, while motor responses were recorded from the branches of the left ventral root in segment 60. Rightward (contralateral) bending in the yaw plane and downward (ventral) bending in the pitch plane caused an increase in the mass activity in both dorsal and ventral branches, while leftward (ipsilateral) and upward (dorsal) bending caused a decrease (Fig. 9A). Individual MNs (1–4) recorded from the left ventral branch responded to contralateral and/or ventral bending, but with one exception: MN5 responded to dorsal bending (Fig. 9B).

We have found that motor response in the split spinal cord preparation, in contrast to the intact one, did not depend on the site of bending (Fig. 9C). Bending at 32 out of 35 sites (in segments 10–75) caused similar responses in MNs: they were excited by contralateral and/or ventral bending. Among 57 MNs recorded in



**Figure 9.** Motor responses in the split spinal cord preparation to bending in the yaw and pitch planes. **A:** An example of responses in the dorsal and ventral branches of the left ventral root of segment 60 (60LD and 60LV, respectively) to bending in segment 65. **B:** Activity of individual motoneurons (MN1–MN5) was extracted from the mass activity of 60LV (shown in A). **C:** Summary of motor responses evoked by bending at different sites along the body ( $N = 10$ ,  $n = 34$ ). **D:** Proportion of MNs with different patterns of response to bending ( $N = 7$ ,  $n = 57$ ). Designations as in Figures 3 and 5. To increase excitability of spinal networks D-glutamate (0.5–2.0 mM) was used.

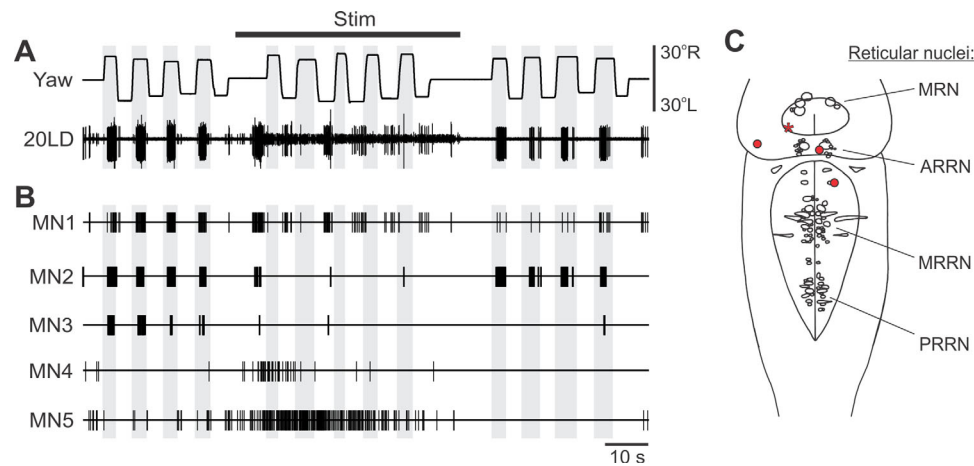
seven experiments, 83% responded to contralateral and/or ventral bending, while only 17% had opposite pattern of responses (Fig. 9D).

The striking difference between the diversity of MN responses in the nonsplit preparation and rather uniform responses in the split preparation suggests that integrity of the networks residing in the contralateral hemicord is important for determining the phase of the motor response to bending.

### Effects of brainstem stimulation on motor responses to bending

Effects of electrical stimulation of the brainstem on the motor responses evoked by bending were studied in two brainstem-spinal cord preparations. Four sites

were found (circles in Fig. 10C) that strongly affected responses to bending. One site was located on the surface of the lateral tectum, one in the middle of the caudal tectum, one in the area of the mesencephalic reticular nuclei (MRN), and one in the area of anterior rhombencephalic reticular nuclei (ARRN). The effect of electrical stimulation of the site located in the area of MRN is shown in Figure 10A. Before stimulation, responses to contralateral (rightward) bending in segment 35 were observed in the left dorsal branch of the ventral root in segment 20. When the stimulation (20 Hz; 0.2 ms; 10  $\mu$ A) was switched on, the phase of response was reversed. Contralateral responses to bending reappeared when stimulation was switched off. The activity of five individual MNs was extracted from



**Figure 10.** Gating of reflex motor responses to bending by brainstem stimulation. **A:** An example of the effects of electrical brainstem stimulation on motor responses recorded from the left dorsal branch of the ventral root of segment 20 (20LD) to bending in the yaw plane in segment 35. **B:** Activity of individual motoneurons (MN1–MN5) was extracted from the mass activity of 20LD (shown in A). **C:** Sites of brainstem stimulation (red circles) strongly affecting motor responses to bending. The site of stimulation in A is marked by an asterisk. MRN is the mesencephalic reticular nucleus; ARRn, MRRn, and PRRn are the anterior, middle, and posterior rhombencephalic reticular nuclei, respectively.

the mass activity of the dorsal branch. The effect of stimulation on the activity of individual MNs was different (Fig. 10B): the phase of the MN1 response was reversed; responses in MN2 and in MN3 were suppressed; MN4 and MN5 (which did not respond to bending) were tonically activated.

## DISCUSSION

Both animals and humans actively maintain a specific configuration of their body during locomotion (Horak and Macpherson, 1996). The present study was devoted to the control of body configuration in the swimming lamprey. This animal swims due to symmetrical body undulations in the yaw plane, while in the pitch plane its body is straight, and only the tail is deflected down (Ullén et al., 1995). Results of the present study suggest that the spinal reflex mechanisms, driven by the intraspinal SRNs may significantly contribute to the control of body configuration in both planes.

In previous studies (Grillner et al., 1982, 1984) it was shown that SRNs are sensitive to body bending in the yaw plane, and mediate reflex motor responses to this bending (Viana Di Prisco et al., 1990). However, responses of SRNs and motor neurons to body bending at different rostrocaudal levels and in different planes were not investigated. These issues were addressed in the present study.

### Sensitivity of SRNs to bending in different planes and at different rostrocaudal levels

The activity of SRNs was recorded from their axons in a filament of the spinal cord, while the synaptic

transmission was blocked to separate the cells with mechanical sensation (Grillner et al., 1982). In the intact spinal cord preparation, two types of responses to bending in the yaw plane were found in a filament: SRN axons responding to ipsilateral bending and SRN axons responding to contralateral bending (Fig. 2A). Since splitting the spinal cord along the midline abolished ipsilateral responses, while the contralateral responses were preserved (Fig. 3A,B), one can suggest that axons responding to contralateral bending belong to IP-SRNs, and axons responding to ipsilateral bending belong to CP-SRNs (Fig. 2B,C) (Viana Di Prisco et al., 1990). Since the proportion of SRN axons with ipsilateral responses to yaw bending in the intact preparation was higher in the middle segments as compared with those in the rostral ones (Fig. 2D,E), one can suggest that CP-SRNs are more numerous (or more excitable) in the middle part of the body than in the rostral segments.

We have found that SRNs are sensitive to body bending in the pitch plane as well. They were activated by ventral bending, and can thus provide sensory input for reflex motor responses in this plane.

Despite SRNs respond to bending in both planes, the central nervous system (CNS) can differentiate the actual plane of bending by comparing signals from the left and right SRNs. As shown in Figure 1C–F, leftward bending in the yaw plane activates SRNs on the right side of the spinal cord (C), while rightward bending activates those on the left side (D). In the pitch plane, ventral bending activates SRNs on both right and left sides

of the spinal cord (E), while upward bending inactivates SRNs on both sides (F).

We found that activation with ventral bending was observed in almost all SRNs, while activation with contralateral bending was observed in about half of these SRN (Fig. 2D,E). The possible explanation for this difference is a larger stretch of the marginal area of the spinal cord during pitch bending, when this area is positioned at a larger distance from the neutral plane (the border between black and gray sectors in Fig. 1C–F), which is not subjected to longitudinal stretch or compression. We also found that the basic pattern of SRN responses to yaw and pitch bending was the same when bending was performed at different sites along the body (Fig. 3B). One can thus conclude that the population of SRNs can provide information about body bending at different rostrocaudal levels and in different planes. Individual SRNs with ipsilateral projections can convey this information at a distance of up to 15 segments (Fig. 3D), which is an estimated maximal length of their axons. A similar estimate for the maximal length of axons (20 segments) was obtained in a morphological study (Tang and Selzer, 1979).

### Spinal reflexes evoked by natural stimulation of SRNs

In the present study we analyzed responses of segmental motor output to natural stimulation of SRNs, i.e., to bending in different planes and at different rostrocaudal levels.

*Bending in the yaw plane* could evoke two main types of responses (Fig. 5), with activation of MNs projecting to the convex side (“convex response”), and with activation of those projecting to the concave side (“concave response”). In both cases, similar response patterns were usually observed in the dorsal and ventral branches of a ventral root, suggesting that reflex motor responses to body bending in the yaw plane would occur in the same plane, either increasing or decreasing the value of bending. The phase of motor response depended on the site of bending: the concave responses were observed with bending in the rostral area of the spinal cord (segments 10 to 30), while the convex responses were observed with bending in the mid-body area (segments 35–50). Bending in a given area evoked motor responses (concave or convex) not only in a neighboring part (Fig. 5) but also in the remote parts of the spinal cord (up to 40 segments, Fig. 8). A given segment could respond to bending in either of these two areas, with the corresponding type of response (Fig. 5B,C). One can suppose that the reverse of the motor response is due to activation of

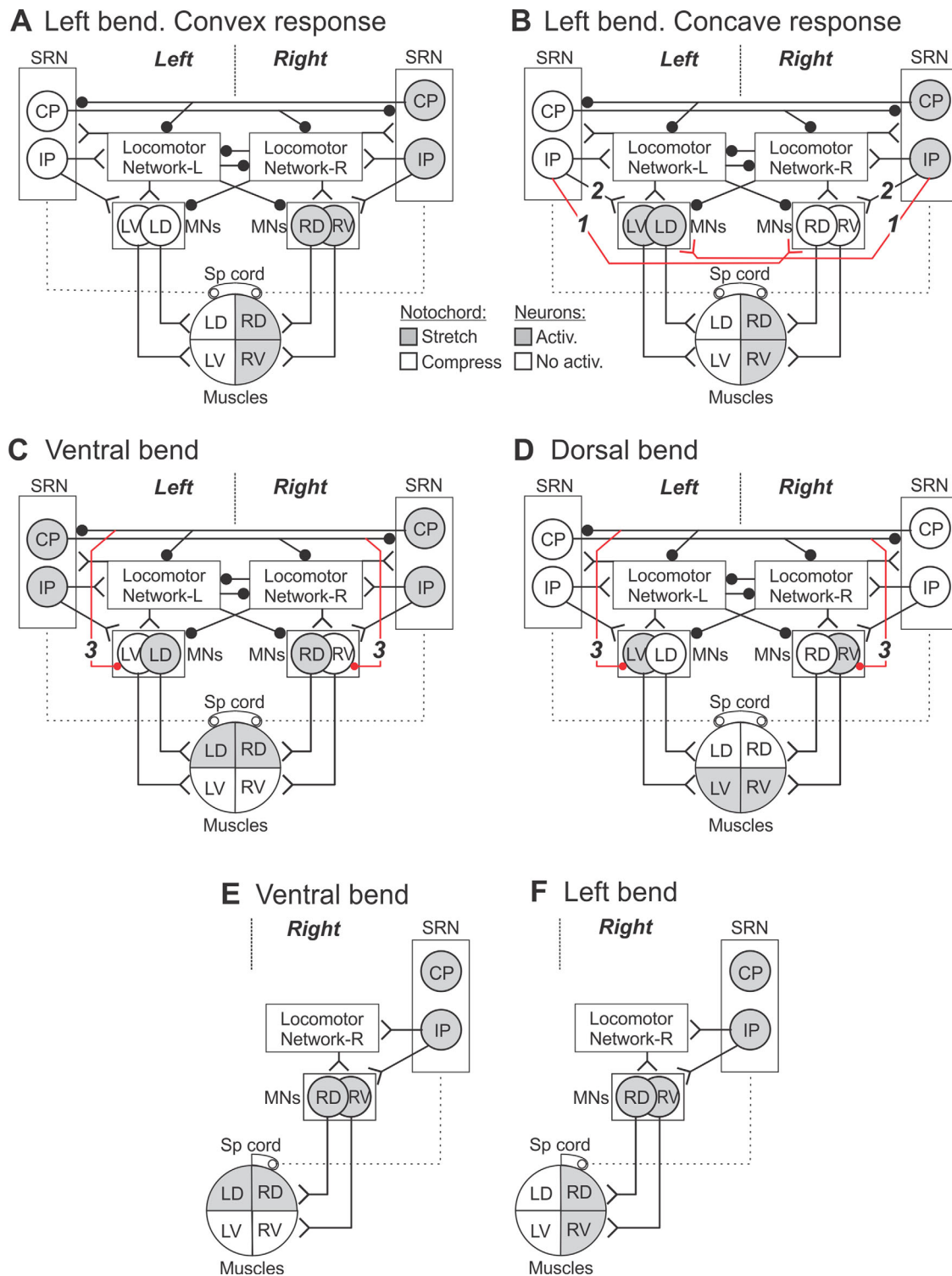
different populations of segmental MNs by bending in different segments. However, we have demonstrated that individual MNs change the phase of their response depending on the site of bending (Fig. 6). These results suggest that the organization of the spinal networks processing the signals from the rostral and mid-body SRNs is different.

Generation of convex motor responses caused by bending in the yaw plane can be explained on the basis of known connections between SRNs and neurons of the spinal networks shown in Figure 11A (Viana Di Prisco et al., 1990; Vinay et al., 1996). Lateral bending (e.g., to the left) causes stretching of notochord and spinal cord tissue on the opposite (right) side, resulting in activation of IP-SRNs and CP-SRNs on that side. The IP-SRNs excite MNs innervating the dorsal and ventral parts of the myotome (RD and RV, respectively). This monosynaptic route is supplemented by the polysynaptic route through the right locomotor network. At the same time, CP-SRNs on the stretched right side inhibit several groups of neurons on the opposite side, including SRNs.

Generation of concave motor responses, i.e., excitation of left MNs with activation of right SRNs, cannot be explained on the basis of connections shown in Figure 11A. We suggest that for generating concave responses some changes in the spinal circuits should occur (Fig. 11B). One possible change would be a new excitatory connection (1, red line) from SRNs to contralateral MNs, either mono- or polysynaptic. Another change would be abolition of the excitatory connection 2 from SRNs to ipsilateral MNs, e.g., via presynaptic inhibition from the contralateral side. Other changes in connectivity are also possible. The anatomical substrate for these changes (e.g., for crossed excitatory influences of SRNs) is not known.

The idea that concave responses are driven by the contralateral SRNs was supported by our finding that, after splitting of the spinal cord, convex responses persisted while concave responses disappeared (Figs. 9, 11F). Our hypothesis implies that the connectivity in spinal networks differs between the rostral and mid-body regions; they are shown in Fig. 11A,B, respectively. Recently, it was demonstrated that rhythmic lateral bending of the rostral and caudal regions of the spinal cord-notochord preparation produced different effects on the rhythm of fictive swimming (Tytell and Cohen, 2008), also suggesting a considerable difference between the corresponding networks in these two regions.

*Bending in the pitch plane* could evoke numerous patterns of motor responses with different combinations of activity of the four groups of MNs (Fig. 7B). The most



**Figure 11.** Hypothetical spinal circuitry generating motor responses to bending in intact (**A–D**) and split (**E,F**) spinal cord preparations. A scheme in the bottom of each panel (**A–F**) shows a cross-section of the notochord and spinal cord. The stretched area of the notochord, the SRNs activated by this stretch, and the MNs responding to this stretch (LD, LV, RD, and RV) are shown in gray. Other designations: open triangle, excitatory connection; small filled circle, inhibitory connection; black line, known connection; red line, presumed connection. See Discussion for explanations.



consistent were the responses in the mid-body region elicited by bending in segment 40: ventral and dorsal bending caused activation of MNs innervating the dorsal and ventral part of a myotome, respectively (Fig. 7A). These responses cannot be explained on the basis of known network connections. One possible change in this network, which would allow generating these responses, could be selective inhibition (either mono- or polysynaptic) of contralateral MNs innervating the ventral part of the myotome, performed by CP-SRNs (a new inhibitory connection 3, red line in Fig. 11C,D). The existence of reciprocal activation of the ventral and dorsal motoneurons under certain conditions was demonstrated in the intact spinal cord preparation (Aoki et al., 2001).

The proportion of MNs responding to pitch bending was smaller than that responding to yaw bending (29% vs. 45%, respectively; Fig. 4D) while an opposite proportion was observed in SRNs after the block of synaptic transmission (95% vs. 62% and 92% vs. 70% in the rostral and middle spinal segments, respectively; Fig. 2D,E). The most likely reason for this difference was a reciprocal inhibition between the populations of left and right SRNs (Viana Di Prisco et al., 1990) (Fig. 11A). These two populations are activated simultaneously by ventral bending, and suppress each other through crossed inhibitory connections of CP-SRNs. Evidence for this suggestion was obtained in the experiments on the split spinal cord preparation (Fig. 11E,F): the proportion of MNs responding to pitch bending in this preparation was much larger than that responding to yaw bending (78% vs. 55%, respectively; Fig. 9D). By contrast, during periodical yaw bending the left and right SRNs are active in anti-phase and, therefore, do not inhibit the opposite SRNs when their activity is high. Due to an increased input from IP-SRNs to MNs and simultaneously their release from CP-SRNs inhibition, the depth of modulation of activity of MNs caused by yaw bending is larger than that caused by pitch bending (Fig. 4A). Elimination of inhibitory contralateral influenced by splitting the spinal cord leads to some reduction in the depth of modulation of MNs caused by yaw bending (compare Figs. 4A, 9A, respectively). In addition, abolishing the connections between the left and the right parts of the network (Fig. 11E,F) results in uniform responses of MNs to pitch and yaw bending along the spinal cord: MNs respond only to contralateral bending in the yaw plane and only to ventral bending in the pitch plane (Fig. 9). These responses are generated by the hemi-cord network (Fig. 11E,F).

The responses to yaw and pitch bending in SRN axons could be recorded at the distance of up to 15

segments from the site of bending (Fig. 3D), which is presumably determined by the length of these axons. This distance was much shorter than that in MNs (40 segments). These results imply involvement of the propriospinal neurons (for review, see Buchanan, 2001) with long axons (e.g., lateral interneurons, Viana Di Prisco et al., 1990) in the transmission of SRN signals along the spinal cord, as well as in the formation of large functional synergies comprising dozens of segments.

### Functional role of SRN-mediated reflexes

The SRNs constitute the main source of proprioceptive information in the lamprey. They are signaling body bending in different planes and at different rostrocaudal levels. SRNs can affect both amplitude and frequency of locomotor undulations. It was suggested that SRNs provide feedback to the locomotor system generating lateral body undulations (Grillner et al., 1982, 1984; Viana Di Prisco et al., 1990).

During fast forward swimming, MNs are active on the convex side of the body (Williams et al., 1989; Islam et al., 2006). Taken that SRNs are also active on that side, one can suggest that the effect of SRN on locomotor undulations will depend on the type of SRN-mediated reflex responses in a given body region. In the mid-body region (with convex responses) the amplitude of undulations will increase, and in the rostral region (with concave responses) it will decrease. Taking into account that SRNs affect motor output of the remote segments of the spinal cord in the rostral and caudal directions (Fig. 8D), one can suggest that signals from both SRNs located in the rostral and in the middle body segments may contribute to a decrease of the amplitude of locomotor undulations in the rostral part of the body while increasing it in the middle part. Such rostrocaudal gradient of undulations is observed in lampreys during free fast forward swimming (Pedley and Hill, 1999; Wilbur et al., 2002; Islam et al., 2006).

The functional roles of SRNs could differ in different types of behaviors. For example, for lateral turns during swimming (Fagerstedt and Ullén, 2001) and for the posture-dependent startle behavior in larval lampreys (Currie and Carlsen, 1987; Currie, 1991), one can suggest that the SRN-mediated reflexes in rostral segments contribute to the augmentation of the bend (with concave responses), which can enhance the changes of body configuration in the yaw plane (McClellan and Grillner, 1983).

The SRN-mediated motor responses to pitch bending were less consistent than those to yaw bending, except for the mid-body region. Ventral bending in this region

caused activation of MNs innervating the dorsal parts of myotomes, and dorsal bending–activation of MNs innervating their ventral parts of myotomes (Fig. 7). This finding suggests that, in this region, any deviation from the normal body configuration in the pitch plane will cause a motor response to reduce the bend, thus contributing to maintenance of rectilinear body axis in this plane.

In the present study we also addressed the question if the reflex responses to bending (mediated by SRNs) could be regulated by supraspinal centers. For this purpose, we studied the effect of stimulation of different sites in the brainstem on the reflex responses to lateral bending. Clear effects (including reversal of responses) were obtained with stimulation of some sites in the tectum, as well as some sites located in the area of MRN and ARRN, from which reticulospinal neurons originate (Fig. 10). Previously, it was shown that stimulation of axons of I-cells (originating from ARRN) in the spinal cord produced excitatory effects on SRNs (Rovainen, 1974). One possible explanation of the inconsistency of reflex responses evoked by pitch bending in the isolated spinal cord is that, in the intact lamprey, they are under continuous control from supraspinal centers. These data suggest that spinal reflexes in the lamprey can be centrally regulated in accordance with different forms of motor behavior such as fast forward swimming, slow forward swimming, backward swimming, crawling, etc. (Archambault et al., 2001; Zelenin, 2005; Islam et al., 2006; Islam and Zelenin, 2008).

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## CONFLICT OF INTEREST

There are no conflicts of interest.

## ROLE OF AUTHORS

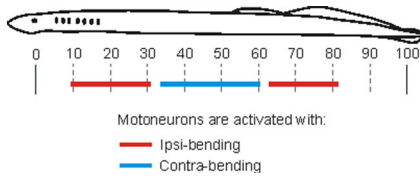
All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: L.-J.H., G.N.O., T.G.D. Acquisition of data: L.-J.H. Analysis and interpretation of data: L.-J.H., P.V.Z., G.N.O., T.G.D. Drafting of the article: L.-J.H., G.N.O., T.G.D. Critical revision of the article for important intellectual content: L.-J.H., P.V.Z., S.G., G.N.O., T.G.D. Statistical analysis: L.-J.H. Obtained funding: T.G.D., P.V.Z. Administrative, technical, and material support: L.-J.H., P.V.Z., S.G., G.N.O., T.G.D. Study supervision: T.G.D., G.N.O.

## LITERATURE CITED

- Anadón R, Molist P, Pombal MA, Rodríguez-Moldes I, Rodicio MC. 1995. Marginal cells in the spinal cord of four elasmobranchs (*Torpedo marmorata*, *T. torpedo*, *Raja undulata* and *Scyliorhinus canicula*): evidence for homology with lamprey intraspinal stretch receptor neurons. *Eur J Neurosci* 7:934–943.
- Aoki F, Wannier T, Grillner S. 2001. Slow dorsal-ventral rhythm generator in the lamprey spinal cord. *J Neurophysiol* 85:211–218.
- Archambault PS, Deliagina TG, Orlovsky GN. 2001. Non-undulatory locomotion in the lamprey. *Neuroreport* 12:1803–1807.
- Buchanan JT. 2001. Contributions of identifiable neurons and neuron classes to lamprey vertebrate neurobiology. *Prog Neurobiol* 63:441–466.
- Cangiano L, Grillner S. 2003. Fast and slow locomotor burst generation in the hemispinal cord of the lamprey. *J Neurophysiol* 89:2931–2942.
- Cohen AH, Wallén P. 1980. The neuronal correlate of locomotion in fish. “Fictive swimming” induced in an in vitro preparation of the lamprey spinal cord. *Exp Brain Res* 41:11–18.
- Currie SN. 1991. Vibration-evoked startle behavior in larval lampreys. *Brain Behav Evol* 37:260–271.
- Currie SN, Carlsen RC. 1987. Functional significance and neural basis of larval lamprey startle behaviour. *J Exp Biol* 133:121–135.
- Deliagina TG, Fagerstedt P. 2000. Responses of reticulospinal neurons in intact lamprey to vestibular and visual inputs. *J Neurophysiol* 83:864–878.
- Eccles RM, Lundberg A. 1959. Supraspinal control of interneurons mediating spinal reflexes. *J Physiol* 147:565–584.
- Fagerstedt P, Ullén F. 2001. Lateral turns in the Lamprey. I. Patterns of motoneuron activity. *J Neurophysiol* 86:2246–2256.
- Gossard J-P, Brownstone RM, Barajon I, Haltborn H. 1994. Transmission in a locomotor-related group 1b pathway from hindlimb extensor muscles in the cat. *Exp Brain Res* 98:213–228.
- Grillner S, McClellan A, Perret C. 1981. Entrainment of the spinal pattern generators for swimming by mechanosensitive elements in the lamprey spinal cord in vitro. *Brain Res* 217:380–386.
- Grillner S, McClellan A, Sigvardt K. 1982. Mechanosensitive neurons in the spinal cord of the lamprey. *Brain Res* 235:169–173.
- Grillner S, Williams T, Lagerbäck PA. 1984. The edge cell, a possible intraspinal mechanoreceptor. *Science* 223:500–503.
- Grillner S, Wallén P, Brodin L, Lansner A. 1991. Neuronal network generating locomotor behavior in lamprey: circuitry, transmitters, membrane properties, and simulation. *Annu Rev Neurosci* 14:169–199.
- Holmqvist B, Lundberg A. 1959. On the organization of the supraspinal inhibitory control of interneurons of various spinal reflexes. *Arch Ital Biol* 97:340–356.
- Holmqvist B, Lundberg A. 1961. Differential supraspinal control of synaptic actions evoked by volleys in the flexion reflex afferents in alpha motoneurons. *Acta Physiol Scand* 54(Suppl 186):1–50.
- Horak F, Macpherson J. 1996. Postural orientation and equilibrium. In: Shepard J, Rowell L, editors. *Integration of motor, circulatory, respiratory and metabolic control during exercise*. Handbook of physiology, section 12. New York: Oxford University Press. p 1–39.

- Hsu LJ, Zelenin PV, Orlovsky GN, Deliagina TG. 2012a. Motor responses to body bending in different planes elicited by intraspinal stretch receptor neurons in lamprey. *Soc Neurosci Abstr* 38:788.09.
- Hsu LJ, Zelenin PV, Orlovsky GN, Deliagina TG. 2012b. Different forms of locomotion in spinal lamprey. *FENS Forum Abstr* 6:131.18.
- Hultborn H, Illert M, Santini M. 1976. Convergence on interneurons mediating the reciprocal Ia inhibition of motoneurons. III. Effects from supraspinal pathways. *Acta Physiol Scand* 96:368–391.
- Islam SS, Zelenin PV. 2008. Modifications of locomotor pattern underlying escape behavior in the lamprey. *J Neurophysiol* 99:297–307.
- Islam SS, Zelenin PV, Orlovsky GN, Grillner S, Deliagina TG. 2006. Pattern of motor coordination underlying backward swimming in the lamprey. *J Neurophysiol* 96:451–460.
- McClellan AD, Grillner S. 1983. Initiation and sensory gating of 'fictive' swimming and withdrawal responses in an in vitro preparation of the lamprey spinal cord. *Brain Res* 269:237–250.
- McClellan AD, Jang W. 1993. Mechanosensory inputs to the central pattern generators for locomotion in the lamprey spinal cord: resetting, entrainment, and computer modeling. *J Neurophysiol* 70:2442–2454.
- McClellan AD, Sigvardt KA. 1988. Features of entrainment of spinal pattern generators for locomotor activity in the lamprey spinal cord. *J Neurosci* 8:133–145.
- Pedley TJ, Hill SJ. 1999. Large-amplitude undulatory fish swimming: fluid mechanics coupled to internal mechanics. *J Exp Biol* 202:3431–3438.
- Rovainen CM. 1974. Synaptic interactions of reticulospinal neurons and nerve cells in the spinal cord of the sea lamprey. *J Comp Neurol* 154:207–223.
- Schroeder DM. 1986. An ultrastructural study of the marginal nucleus, the intrinsic mechanoreceptor of the snake's spinal cord. *Somatosens Res* 4:127–140.
- Schroeder DM, Egar MW. 1990. Marginal neurons in the urodele spinal cord and the associated denticulate ligaments. *J Comp Neurol* 301:93–103.
- Tang D, Selzer ME. 1979. Projections of lamprey spinal neurons determined by the retrograde axonal transport of horseradish peroxidase. *J Comp Neurol* 188:629–646.
- Tytell ED, Cohen AH. 2008. Rostral versus caudal differences in mechanical entrainment of the lamprey central pattern generator for locomotion. *J Neurophysiol* 99:2408–2419.
- Ullén F, Deliagina TG, Orlovsky GN, Grillner S. 1995. Spatial orientation in the lamprey. I. Control of pitch and roll. *J Exp Biol* 198:665–673.
- Viana Di Prisco GV, Wallén P, Grillner S. 1990. Synaptic effects of intraspinal stretch receptor neurons mediating movement-related feedback during locomotion. *Brain Res* 530:161–166.
- Vinay L, Barthe JY, Grillner S. 1996. Central modulation of stretch receptor neurons during fictive locomotion in lamprey. *J Neurophysiol* 76:1224–1235.
- Wallén P, Grillner S, Feldman JL, Bergelt S. 1985. Dorsal and ventral myotome motoneurons and their input during fictive locomotion in lamprey. *J Neurosci* 5:654–661.
- Wilbur C, Vorus W, Cao Y, Currie SE. 2002. A lamprey-based undulatory vehicle. In: Ayers JW, Davis JL, Rudolph A, editors. *Neurotechnology: for biomimetic robots*. Cambridge, MA: Massachusetts Institute of Technology. p 285–286.
- Williams TL, Grillner S, Smoljaninov VV, Wallén P, Kashin S, Rossignol S. 1989. Locomotion in lamprey and trout: the relative timing of activation and movement. *J Exp Biol* 143:559–566.
- Williams TL, Sigvardt KA, Kopell N, Ermentrout GB, Remler MP. 1990. Forcing of coupled nonlinear oscillators: studies of intersegmental coordination in the lamprey locomotor central pattern generator. *J Neurophysiol* 64:862–871.
- Zelenin PV. 2005. Activity of individual reticulospinal neurons during different forms of locomotion in the lamprey. *Eur J Neurosci* 22:2271–2282.
- Zelenin PV, Grillner S, Orlovsky GN, Deliagina TG. 2001. Heterogeneity of the population of command neurons in the lamprey. *J Neurosci* 21:793–803.
- Zelenin PV, Pavlova EL, Grillner S, Orlovsky GN, Deliagina TG. 2003. Comparison of the motor effects of individual vestibule- and reticulospinal neurons on dorsal and ventral myotomes in lamprey. *J Neurophysiol* 90:3161–3167.
- Zelenin PV, Orlovsky GN, Deliagina TG. 2007. Sensory-motor transformation by individual command neurons. *J Neurosci* 27:1024–1032.

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Spinal reflex responses to body bending mediated by intraspinal stretch receptor neurons in the lamprey are different along the body. Motoneurons are activated with ipsilateral bending in the rostral part of the body, but with contralateral bending in its middle part. These spinal reflexes can contribute to the control of body configuration during locomotion.