JPn Neuroscience
The Journal of Physiology

Supraspinal control of spinal reflex responses to body bending during different behaviours in lampreys

Li-Ju Hsu, Pavel V. Zelenin, Grigori N. Orlovsky and Tatiana G. Deliagina

Department of Neuroscience, Karolinska Institute, SE-17177, Stockholm, Sweden

Key points

- Spinal reflexes are substantial components of the motor control system in all vertebrates and centrally driven reflex modifications are essential to many behaviours, but little is known about the neuronal mechanisms underlying these modifications.
- To study this issue, we took advantage of an *in vitro* brainstem–spinal cord preparation of the lamprey (a lower vertebrate), in which spinal reflex responses to spinal cord bending (caused by signals from spinal stretch receptor neurons) can be evoked during different types of fictive behaviour.
- Our results demonstrate that reflexes observed during fast forward swimming are reversed during escape behaviours, with the reflex reversal presumably caused by supraspinal commands transmitted by a population of reticulospinal neurons.
- NMDA receptors are involved in the formation of these commands, which are addressed primarily to the ipsilateral spinal networks.
- In the present study the neuronal mechanisms underlying reflex reversal have been characterized for the first time.

Abstract Spinal reflexes can be modified during different motor behaviours. However, our knowledge about the neuronal mechanisms underlying these modifications in vertebrates is scarce. In the lamprey, a lower vertebrate, body bending causes activation of intraspinal stretch receptor neurons (SRNs) resulting in spinal reflexes: activation of motoneurons (MNs) with bending towards either the contralateral or ipsilateral side (a convex or concave response, respectively). The present study had two main aims: (i) to investigate how these spinal reflexes are modified during different motor behaviours, and (ii) to reveal reticulospinal neurons (RSNs) transmitting commands for the reflex modification. For this purpose in *in vitro* brainstem–spinal cord preparation, RSNs and reflex responses to bending were recorded during different fictive behaviours evoked by supraspinal commands. We found that during fast forward swimming MNs exhibited convex responses. By contrast, during escape behaviours, MNs exhibited concave responses. We found RSNs that were activated during both stimulation causing reflex reversal without initiation of any specific behaviour, and stimulation causing reflex reversal during escape behaviour. We suggest that these RSNs transmit commands for the reflex modification. Application of the NMDA antagonist (AP-5) to the brainstem significantly decreased the reversed reflex, suggesting involvement of NMDA receptors in the formation of these commands. Longitudinal split of the spinal cord did not abolish the reflex reversal caused by supraspinal commands, suggesting an important role for ipsilateral networks in determining this type of motor response. This is the first study to reveal the neuronal mechanisms underlying supraspinal control of reflex reversal.

(Received 27 April 2016; accepted after revision 22 August 2016; first published online 2 September 2016)
Corresponding author T. G. Deliagina: Department of Neuroscience, Karolinska Institute, SE-17177, Stockholm, Sweden. Email: tatiana.deliagina@ki.se

DOI: 10.1113/JP272714

Abbreviations AP-5, 2-amino-5-phosphonopentanoate; ARRN, anterior rhombencephalic reticular nucleus; BS, backward swimming; CPG, central pattern generator; FFS, fast forward swimming; MLR, mesencephalic locomotor region; MN, motoneuron; MRN, mesencephalic reticular nucleus; MRRN, middle rhombencephalic reticular nucleus; PRRN, posterior rhombencephalic reticular nucleus; RSN, reticulospinal neuron; SFS, slow forward swimming; SRN, stretch receptor neuron; VR, ventral root.

Introduction

In different species, both vertebrate and invertebrate, sensory input from the mechanoreceptors of locomotor organs (limbs in bipeds and quadrupeds, trunk in fish, etc.) is important for generation of various types of movements and for their adaptation to environmental conditions (Forssberg *et al.* 1977; Grillner, 1981; Orlovsky *et al.* 1999; Pearson, 2008; Gervasio *et al.* 2013). At rest, these signals can evoke a number of spinal reflexes, which can be substantially modified (up to a complete reversal) with the initiation of a particular motor behaviour (Pearson & Collins, 1993; Büschges & El Manira, 1998; Hellekes *et al.* 2012).

Different motor behaviours, e.g. different types of locomotion in vertebrates are selected by supraspinal centres (Armstrong, 1986; Orlovsky *et al.* 1999; Deliagina *et al.* 2002; Zelenin, 2005, 2011). Do these centres also participate in the modification of spinal reflexes observed in different motor behaviours? Unfortunately, our understanding of the role of supraspinal centres in the modification of spinal reflexes in higher vertebrates is scarce, because of the complexity of neural circuits and movement patterns. The lamprey (a lower vertebrate) is a simple model system to address this issue. First, supraspinal commands in lampreys are transmitted mainly by the reticulospinal neurons (RSNs) (Ronan, 1989), whose functional role is similar to that in higher vertebrates (Le Ray *et al.* 2011). Second, locomotor movements in lampreys (lateral body undulations) are simple compared to movements of the multi-joint limb in bipeds and quadrupeds. Third, the proprioceptive input signalling the lamprey body configuration is provided by stretch receptor neurons (SRNs, also known as edge cells), which are located at the margins of the spinal cord (Grillner *et al.* 1984). This allows us to activate selectively the sensory input from one group of sensory neurons *in vitro*.

SRNs respond to stretch at the margin of the spinal cord. Since the spinal cord is tightly attached to the dorsal aspect of the notochord, bending of the body and the notochord will cause stretch at the margin of the spinal cord resulting in activation of SRNs (Grillner *et al.* 1984). Recently, in an *in vitro* spinal cord preparation, we have found two types of spinal reflex responses to passive lateral bending, with activation of motoneurons (MNs) either on the convex side or on the concave side (Hsu *et al.* 2013*a*).

Lateral bending of the body occurs in almost all types of lamprey behaviour. Fast forward swimming (FFS) is the main type of lamprey locomotion that is used for long-distance migrations. Escape behaviours, e.g. slow forward swimming (SFS), backward swimming (BS) and lateral turns, are used for avoiding obstacles and getting rid of other threats. SFS and BS are characterized by larger body undulations and slower locomotor rhythm than FFS (Islam *et al.* 2006, 2008). The first aim of the present study was to clarify if the spinal reflex responses mediated by SRNs are modified to fit different behaviours, and to reveal RSNs that may modify these responses. In this study, by using the lamprey *in vitro* brainstem–spinal cord preparation, we activated the central mechanisms underlying different behaviours, and thus evoked fictive FFS and different types of fictive escape behaviour (Hsu *et al.* 2013*b*). We have shown that the reflex responses evoked by bending during FFS are reversed during escape behaviour. We have found that RSNs, presumably transmitting commands for the reflex reversal, are located mainly in the middle rhombencephalic reticular nucleus (MRRN).

During locomotion in mammals, supraspinal centres are affected by two types of signals from the spinal cord – those coming from a central pattern generator (CPG, the 'efference copy'), and those coming from limb mechanoreceptors (afferent feedback) (Orlovsky *et al.* 1999). The second aim of this study was to clarify if RSNs in lampreys receive sensory feedback from the spinal SRNs. We have found that most RSNs responded to bending of the spinal cord, suggesting that they receive feedback from SRNs.

A brief account of parts of this study has been published in abstract form (Hsu *et al.* 2013*b*, 2015).

Methods

Ethical approval

Experiments were performed on the *in vitro* preparation dissected from adult lampreys *Ichthyomyzon unicuspis* ($N = 41$) and *Petromyzon marinus* ($N = 15$) obtained commercially (Acme Lamprey Company, Maine, USA). All experiments were conducted in accordance with NIH guidelines and were approved by the local ethical committee (Norra Djurförsöksetiska Nämden) in Stockholm.

The animals were anaesthetized by immersion in tricaine methansulfonate solution (MS-222; 100 mg l^{-1}) until non-responsive to tactile stimulation including tail pinch. The dissection and the experiments were performed in cold (7–10°C) oxygenated Ringer solution containing (in mM) 91 NaCl, 2.1 KCl, 2.6 $CaCl_2$, 1.8 $MgCl_2$,

20 $NaHCO_3$, and 4.0 glucose. The solution was bubbled with O_2 and pH was adjusted to 7.4.

The preparation consisted of the brainstem and the spinal cord (40–80 segments in length) isolated together with the cranium and the notochord (Fig. 1). In eight experiments, the spinal cord was split along its midline (starting from its second segment) with the tip of a fine hypodermic needle 0.4 mm diameter (for details see Cangiano & Grillner, 2003).

Experimental design

The preparation was pinned down to two Silgard-covered platforms positioned in the experimental chamber (Fig. 1). The rostral part of the preparation was pinned to a stationary platform, and the caudal part (~5 segments in length), to a small movable platform that could be rotated in the yaw plane, thus causing right/left bending of the preparation. The preparation was bent periodically, to the right and to the left, with a trapezoidal temporal pattern that had the following characteristics – the peak to peak value was 60–120°, the transition from one position to another lasted ~1 s, and each position was maintained for ~4 s (Fig. 4). The angles of the bend (Fig. 1) were measured by potentiometric transducers. In some 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 produces manually by pressing a button. These methods for bending the preparation were similar to those described in our previous study (Hsu *et al.* 2013*a*), which demonstrated that bending activated SRNs causing spinal reflexes.

In some experiments, to be able to apply drugs separately to the spinal cord or to the brainstem (see below), the experimental chamber was divided into the brainstem and spinal cord pools by means of a barrier positioned and sealed with agar at the level of segments 2–6 (Fig. 1).

In some experiments, responses of MNs and RSNs to bending were recorded in the preparation exhibiting different 'fictive' motor behaviours, which were evoked by continuous electrical stimulation of specific sites in the preparation using commercially available varnish-insulated tungsten microelectrodes (4–6 MΩ, FHC). It has been shown that different types of escape behaviour in the intact animal can be evoked by tactile stimulation of the skin in the head and gill region (Fagerstedt & Ullén, 2001; Islam *et al.* 2006), which is innervated by the trigeminal nerve. In the present study, motor patterns of different types of escape behaviour, i.e. fictive SFS, BS, and lateral turns, were evoked by stimulation of the trigeminal nerve (Fagerstedt & Ullén, 2001; Hsu *et al.* 2013*b*). Stimulation of different sites of the nerve with the same parameters could evoke different types of behaviour. Both SFS and BS can be evoked by either unilateral or bilateral continuous stimulation of the trigeminal nerve with the following parameters: pulse frequency, 3 Hz; pulse duration, 2 ms; current, 20–50 μA. The lateral turn was evoked by unilateral stimulation with a slightly different range of parameters (3–10 Hz; 2 ms; 10–300 μA). Direction of the turns was either ipsilateral or contralateral to the stimulation site.

In seven experiments, FFS was evoked by stimulation of the mesencephalic locomotor region (MLR) (2.5–10 Hz; 2 ms; 1–100 μA). A search for the effective site in MLR was similar to that described by Sirota and colleagues

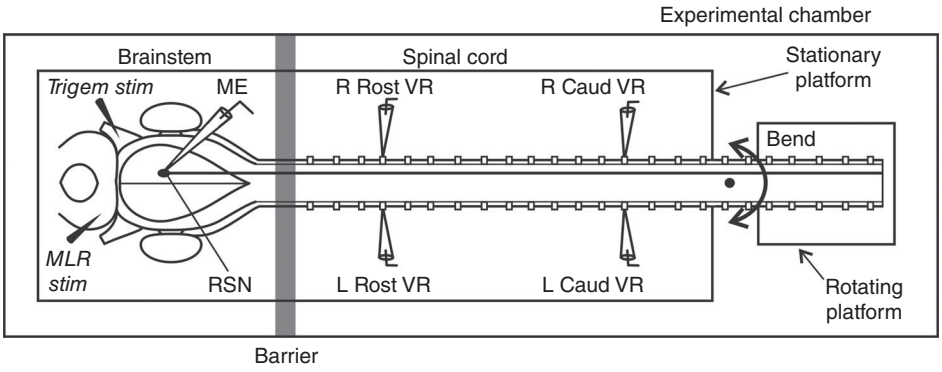

Figure 1. Experimental design
Brainstem and spinal cord were isolated together with the cranium and the notochord. The preparation was attached to two platforms positioned in the experimental chamber. One of the platforms could be rotated in the horizontal plane, causing right or left bending of the caudal part of the preparation. The chamber was partitioned into two pools by an agar barrier. The rostral pool contained the brainstem; the caudal pool contained the spinal cord. Electrical stimulation of the MLR (MLR stim) or the trigeminal nerve (Trigem stim) was used to evoke FFS or different types of escape behaviour, respectively. During these behaviours, responses of MNs to bending were recorded bilaterally from the VRs at two rostro-caudal levels (Rost and Caud VRs) by using suction electrodes. In some experiments, RSNs were recorded intracellularly with a microelectrode (ME).

(2000). In short, MLR was explored by lowering the stimulating electrode into the brain tissue, systematically from the medial to the lateral caudal mesencephalon. In six experiments, FFS was evoked by adding D-glutamate (0.5–2.0 mM) to the oxygenated saline in the pool with the spinal cord.

In eight experiments, with the aim to evoke reflex reversal, different sites in the MRRN were stimulated by means of varnish-insulated tungsten microelectrode (4–6 MΩ, FHC). Continuous monopolar stimulation through one microelectrode was used (5–10 Hz; 2 ms; 0.5–5 μA). MRRN was chosen since the majority of RSNs activated during reflex reversal were located there (see Results).

To examine contribution of the NMDA receptors to formation of the supraspinal commands causing reflex reversal, in five experiments, 2-amino-5-phosphonopentanoate (AP-5), dissolved in the oxygenated saline (200–300 μM), was applied either in the pool with the brainstem ($N = 3$) or locally in the area of MRRN ($N = 2$). For local application, the AP-5 solution was ejected from a micropipette using pressure pulses of different durations (10–100 ms). Fast green was added into the solution so that the area of AP-5 local application could be visually controlled. After each drug application, a washout period was allowed for recovery, which was manifested by restoration of the amplitude of the reversed reflex response.

Data recordings and analysis

Activity of MNs was recorded in the ventral roots (VRs) to monitor the fictive behaviours and the motor responses to bending during these behaviours. VRs were recorded at two different rostrocaudal levels using suction electrodes (Fig. 1). In some experiments, less than four VRs were recorded. As shown in our previous study (Hsu *et al.* 2013*a*), the effect of bending at a specific site decays with the increase of the distance between the bending and recording site. So, in the present study, the sites of VR recording depended on the site of preparation bending. When the bending was performed in caudal segments (Nos 35–55), VRs were recorded from segment Nos 15–25 and Nos 30–50, and called 'rostral' and 'caudal' VRs, respectively. When the bending was performed in rostral segments (Nos 15–25), 'rostral' VRs were recorded from segment Nos 5–10 and 'caudal' VRs from segment Nos 10–15. Usually bending was performed 5–10 segments caudally in relation to 'caudal' VRs (Fig. 1).

In 24 experiments, RSNs were recorded intracellularly (Fig. 1). In the lamprey, large reticulospinal neurons located in the anterior, middle and posterior rhombencephalic reticular nuclei (ARRN, MRRN and PRRN, respectively), as well as in the mesencephalic reticular nucleus (MRN), are clearly seen on the bottom of the fourth and third ventricles, respectively (Rovainen, 1978; Fig. 5*D*), and can be impaled under visual inspection with intracellular microelectrode (El Manira *et al.* 1997; Viana Di Prisco *et al.* 2000). In the present study, RSNs were impaled under visual guidance with sharp glass microelectrodes (3 M potassium acetate, 20–70 MΩ). RSNs were recorded in different reticular nuclei (Fig. 5*D*). To record the RSNs in MRN and in ARRN, in some experiments the optic tectum was cut along the midline for better access to these cells. The activity of individual RSNs was recorded during reflex reversal (evoked by the trigeminal nerve stimulation; see Results). Responses of RSNs to bending were also examined.

Signals from the recording electrodes and from the platform position sensor were amplified, digitized with a sampling frequency of 10 kHz (electrodes) and 100 Hz (sensor), 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).

Responses of individual RSNs and VRs to bending during each type of behaviour were recorded in 2–4 trials. During each trial 3–5 cycles of bending cycles were applied. To characterize VR responses, signals recorded from VRs were rectified and smoothed with time constant 0.5 s. The mean values of smoothed signal (mean VR activity) during the ipsilateral bend and during the contralateral bend were compared. If the mean VR activity was stronger with the contralateral bend, the response was classified as 'convex', and if it was stronger with the ipsilateral bend, the response was classified as 'concave'. The relative number of different VR responses in each type of behaviour was calculated. To characterize the value of VR responses, the coefficient of modulation was calculated: $K_{MOD} = 1 - A_{min}/A_{max}$, where A_{min} and A_{max} are the minimal and maximal mean VR activity in the bending cycle, respectively. To estimate the effect of AP-5 on the value of the concave response, the A_{ipsi}/A_{contra} ratio (where A_{contra} and A_{ipsi} are VR activity during contralateral and ipsilateral bend, respectively) was calculated before and during AP-5 application. The responses of RSNs to bending were classified as 'convex' or 'concave' if neurons were activated (or depolarized) with contralateral or ipsilateral bending, respectively.

All quantitative data in this study are presented as means ± SEM. Student's paired t test was used to characterize the statistical significance when comparing different means; the significance level was set as $P = 0.05$.

Results

Reflex responses to bending during different behaviours

Fast forward swimming. We examined reflex responses to bending during fast forward swimming (FFS) evoked

either by stimulation of the MLR or by bath application of D-glutamate to the spinal cord (see Methods). Figure 2*A–C* shows two examples of fictive FFS evoked by MLR stimulation. As one can see in Fig. 2*A*, the locomotor bursts were regular, with alternating activity in the right and left VRs. On each side, bursts in the rostral VRs occurred earlier than those in the ipsilateral caudal VRs, indicating a head-to-tail propagation of locomotor waves. The cycle duration (0.68 ± 0.05 s) and the burst duration (0.21 ± 0.01 s) (number of preparations $N = 7$, number of cycles $n = 42$) were similar to those observed in other studies of MLR-evoked FFS (e.g. Sirota *et al.* 2000).

Responses of VRs to bending were expressed as a modulation of the intensity of locomotor bursts, i.e. a change in their amplitude and/or duration. As one can see in Fig. 2*B*, when bending was performed in the caudal segment (No. 50), both rostral (No. 15) and caudal (No. 40) VRs on the left side were activated by right bending, while the right VRs were activated by left bending. Such 'convex' responses of both rostral (No. 10) and caudal (No. 15) VRs were also observed when bending was performed in the rostral segment (No. 20, Fig. 2*C*). As one can see in Table 1, bending in both rostral and caudal segments during FFS evoked by MLR stimulation caused mainly convex responses in the VRs. The averaged coefficient of modulation of VRs exhibiting convex response during MLR-evoked FFS was 0.33 ± 0.02 (Table 3).

We also evoked FFS by bath application of D-glutamate to the spinal cord. As during FFS evoked by MLR stimulation, bending in the caudal segments activated MNs on the convex side (Fig. 2*D*): the left VRs were activated by right bending, while the right VRs were activated by left bending. However, the response was different during bending in the rostral segments (Fig. 2*E*): the left VRs were activated by left bending, while the right VRs were activated by right bending. As one can see in Table 1, during FFS caused by D-glutamate, bending in the caudal segments caused mainly convex and bending in the rostral segments only concave responses in the VRs. The averaged coefficient of modulation of VRs during convex and concave responses were similar (Table 3).

Escape behaviours. We studied reflex responses to bending during different types of fictive escape behaviour, i.e. slow forward swimming, backward swimming and lateral turns.

Slow forward swimming (SFS). Figure 3*A* shows an example of fictive SFS evoked by bilateral stimulation of trigeminal nerves. As during FFS, locomotor bursts were regular and with left/right alternation; the rostral VRs were activated earlier than the ipsilateral caudal ones. However, the cycle duration and burst duration were 3.88 ± 0.17 s and 1.08 ± 0.06 s ($N = 6$, $n = 48$), respectively, which were much longer than those observed during FFS. Similar differences between SFS and FFS motor patterns were observed in intact lampreys (Islam & Zelenin, 2008). Bending affected the amplitude of locomotor bursts, as well as the locomotor rhythm. During SFS, right bending activated right MNs and inhibited left ones, while left bending activated left MNs and inhibited right ones (Fig. 3*A*). Bending during SFS caused mainly concave responses in the VRs (Table 2). The averaged coefficient of modulation of VRs exhibiting concave response during SFS was 0.53 ± 0.03 (Table 3).

Backward swimming (BS). Figure 3*B* shows an example of fictive BS evoked by unilateral trigeminal nerve stimulation. Locomotor bursts were regular, with right and left alternation. Caudal MNs were activated earlier than the rostral ones, indicating a tail-to-head wave propagation. The cycle duration (2.22 ± 0.17 s) and the burst proportion (0.35 ± 0.02) ($N = 5$, $n = 29$) were similar to those observed in intact lamprey during BS (Islam *et al.* 2006). Bending during BS evoked a concave response in caudal VRs. They were active during ipsilateral bends and inhibited during contralateral ones. Bending also interrupted the locomotor rhythm (Fig. 3*B*). Such interruptions were observed in the majority of preparations (3 out of 5). As one can see in Table 2, bending during BS evoked mainly concave responses in caudal VRs, while responses in the rostral VRs were absent in the majority of cases. The value of the concave response during BS was similar to that observed during SFS (Table 3).

Lateral turns. Lateral turns were characterized by strong asymmetry in tonic activation of left and right MNs, and could be evoked by unilateral stimulation of the trigeminal nerve. In Fig. 3*C*, stimulation of the right trigeminal nerve, first evoked a brief burst of activity in all recorded ventral roots (such brief bursts were observed in some trials). Later, stronger tonic activation in the left MNs than that in the right MNs developed, indicating a contralateral turn. Right bends inhibited left MNs and activated right MNs (concave responses). During ipsi- and contralateral turns, the majority of responses in the caudal VRs were concave (Table 2). The value of these responses was similar to those observed during SFS and BS (Table 3).

Responses of RSNs to trigeminal nerve stimulation causing reflex reversal

We found that even stimulation of the trigeminal nerve, which did not evoke any type of fictive motor behaviour, caused reversal of reflex response to bending. In the example shown in Fig. 4*A* and *B*, the MNs in the left VR were activated by right bends in the control (Fig. 4*A*). During trigeminal nerve stimulation, they were activated by left bends instead (Fig. 4*B*), indicating that the convex response was changed into a concave response. A similar

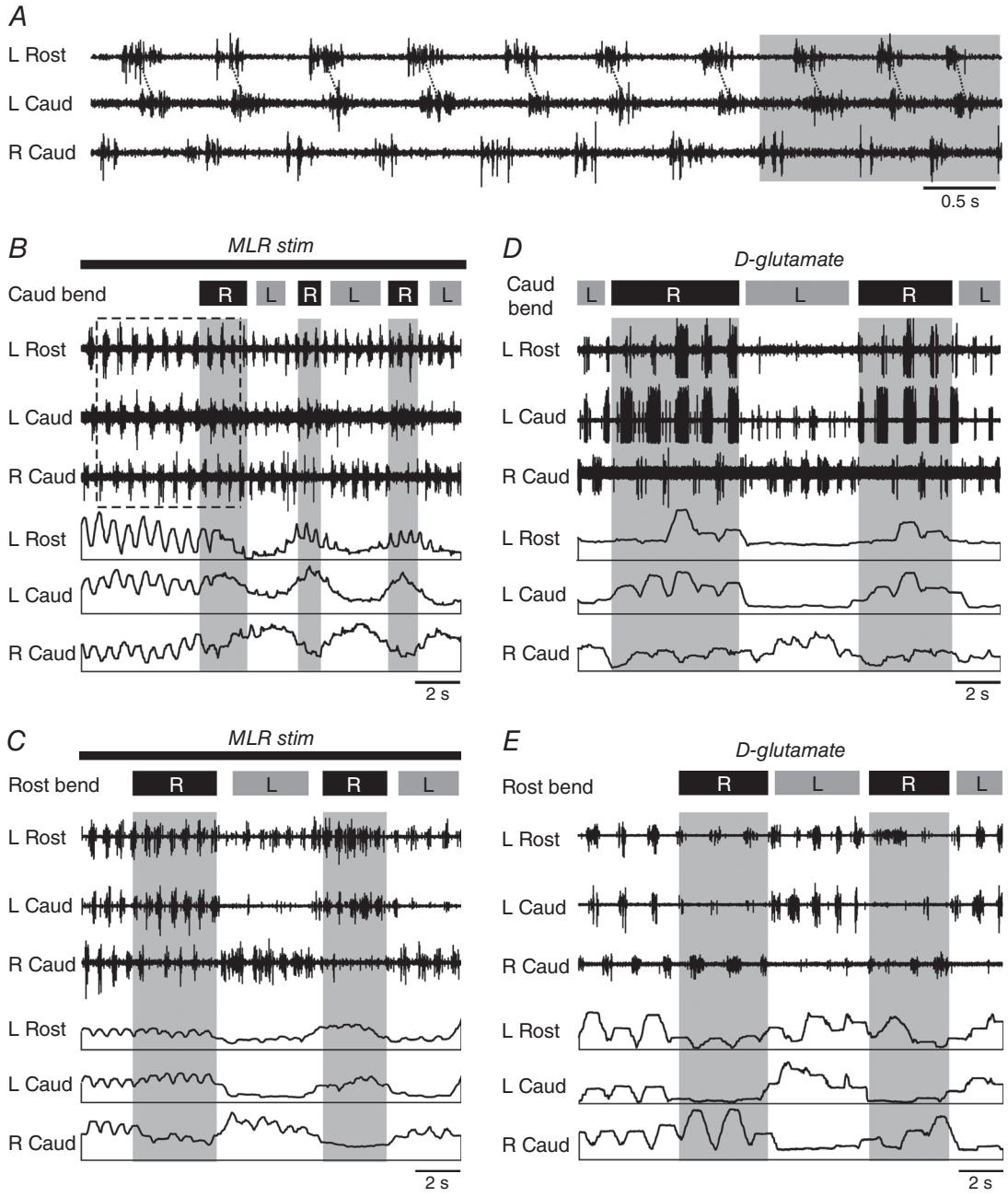

Figure 2. Reflex responses to bending during fast forward swimming
A, an example of MLR-evoked FFS *(*a part of the recording delimited by dashed line in *B* is shown with higher time resolution). Dotted lines between top traces show rostrocaudal phase lags. *B–E*, responses of caudal (Caud) and rostral (Rost) VRs to bending during FFS caused by MLR stimulation (*B* and *C*) and D-glutamate application to the spinal cord (*D* and *E*). Bending was performed in either caudal (~segment No. 50, Caud bend in *B* and *D*) or rostral (~segment No. 20, Rost bend in *C* and *E*) parts of the spinal cord. Three upper traces are VRs recordings and the lower three traces are the same recordings after rectification and smoothing (time constant 0.5 s). The upper bar in *B* and *C* indicates the duration of stimulation. R and L are right and left, respectively. Note that since in *D* (L Rost and L Caud) the amplitude of the recorded spikes varied over a very large range, to see clearly the beginning and the end of the bursts (which were formed by action potentials of small amplitude), as well as small amplitude bursts caused by bending, we used an amplification that clipped the spikes of the largest amplitude. The clipping threshold was set approximately 10 times higher than the amplitude of smallest spikes. Similar clipping was performed in Figs 4*B*, 6 and 10*C*.

Table 1. Summary of ventral root responses to bending during fast forward swimming

Initiation	Bending	CV	CC	NR	*n* (cycles)	*n* (VRs)	*N*
MLR	Caudal	96	0	4	92	10	3
	Rostral	86	14	0	54	12	4
D-Glu	Caudal	89	7	4	93	23	6
	Rostral	0	100	0	87	12	4

Percentage of convex (CV), concave (CC) or no responses (NR) to bending exhibited by VRs during FFS caused by MLR stimulation or by D-glutamate (D-Glu) application. *N*, *n* (cycles) *and n* (VRs) are number of preparations, number of bending cycles and number of VRs, respectively. Responses to caudal bending (performed at segment Nos 35–50) were recorded in the rostral (Nos 15–25) and caudal (Nos 30–40) VRs. Responses to rostral bending (performed at segment Nos 15–25) were recorded in the rostral (Nos 5–10) and caudal (Nos 10–15) VRs.

result was observed in all experiments ($N = 16$). Reversal of the reflex responses to bending during trigeminal nerve stimulation was observed in different segments, from No. 15 to No. 65 (Fig. 4*C*). This finding suggests the existence of a specific population of supraspinal neurons transmitting commands for the reflex reversal, which could be activated separately, or in combination with other populations of supraspinal neurons initiating a specific type of escape behaviour.

In the lamprey, supraspinal commands are transmitted mainly by reticulospinal pathways (Ronan, 1989). Signals from the trigeminal nerve affect RSNs bilaterally (Rovainen, 1967; Wickelgren, 1977; Viana Di Prisco *et al.* 1995). To reveal the neurons transmitting commands for reflex reversal, we recorded intracellular responses of RSNs to stimulation of ipsi- and contralateral trigeminal nerve with the parameters, which did not evoke any type of fictive motor behaviour, but caused reflex reversal.

According to the neuronal responses to stimulation, three groups of RSNs could be distinguished. Group 1 neurons were activated by stimulation of any trigeminal nerve. An example of a Group 1 neuron is shown in Fig. 5*A*. Group 2 neurons were activated by stimulation of the contralateral nerve only, as shown by the neuron in Fig. 5*B*. Finally, Group 3 neurons were not activated by stimulation of either nerve, as shown by the neuron in Fig. 5*C*. Among 99 neurons recorded in 22 experiments,

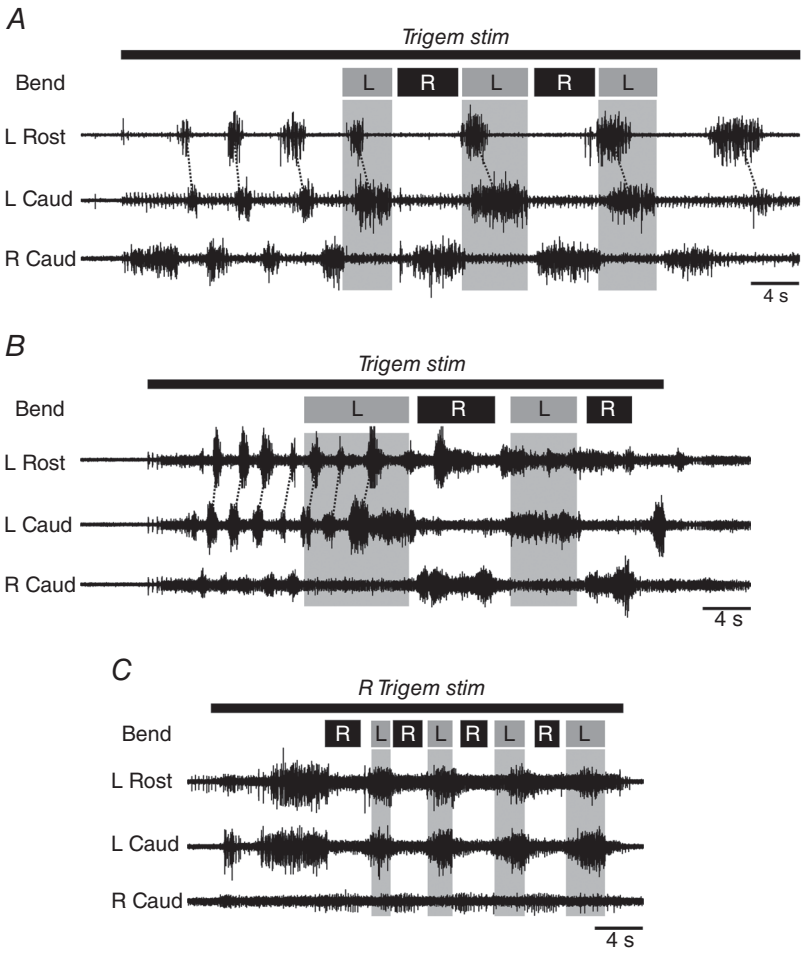

Figure 3. Reflex responses to bending during different types of escape behaviour
A–C, examples of VR responses to bending during SFS (*A*), BS (*B*), and contralateral turn (*C*) caused by stimulation of different sites of the trigeminal nerve. In *A, B* and *C* bending was performed at segments No. 50, No. 55 and No. 50, respectively. Rostral and caudal VRs were recorded in segment Nos 20, 25 and 25 and Nos 40, 50 and 40 in *A*, *B* and *C*, respectively. Rostrocaudal phase lags in *A* and caudorostral phase lags in *B* are shown by dotted lines between traces. Other designations as in Fig. 2.

Table 2. Summary of ventral root responses to bending during different types of escape behaviour

		Rostral VRs					Caudal VRs				
Behaviour	*N*	CV	CC	NR	*n* (cycles)	*n* (VRs)	CV	CC	NR	*n* (cycles)	*n* (VRs)
SFS	6	6	59	35	68	8	0	100	0	104	12
BS	5	17	0	83	18	6	0	80	20	30	10
IT	9	15	31	54	58	9	0	79	21	78	13
CT	15	6	12	82	130	18	0	83	17	192	26

Percentage of different responses to bending exhibited by VRs during slow forward swimming (SFS), backward swimming (BS), ipsilateral turn (IT), and contralateral turn (CT). Responses to bending (performed at segment Nos 35–55) were recorded in the rostral (Nos 15–25) and caudal (Nos 30–50) VRs. Other designations as in Table 1.

Table 3. Values of main ventral root responses to bending during different types of behaviour

Behaviours	CV	CC	*N*	*n* (cycles)	*n* (VRs)
FFS (MLR)	0.33 ± 0.02	—	6	134	18
FFS (D-glu)	0.40 ± 0.04	0.44 ± 0.04	6,4	82,87	20,12
SFS	—	0.53 ± 0.03	6	144	18
BS	—	0.53 ± 0.08	4	24	8
IT	—	0.46 ± 0.04	8	80	15
CT	—	0.52 ± 0.03	12	175	28

The values represent the averaged coefficient of modulation (mean ± SEM) of VRs that exhibited the main type of response to bending during a particular behaviour. FFS (MLR), fast forward swimming evoked by MLR stimulation. FFS (D-glu), fast forward swimming evoked by D-glutamate. Two values of *n* (cycles) and *n* (VRs) for FFS(D-glu) correspond to CV and CC responses caused by bending at the caudal and rostral segments, respectively. Other designations as in Tables 1 and 2.

28% were Group 1 neurons, 28% Group 2 neurons, and 44% Group 3 neurons. The majority of RSNs recorded in MRN, in ARRN, and in PRRN were not activated by trigeminal nerve stimulation (Group 3 neurons, Gr3 in Fig. 5*E*). By contrast, the majority of RSNs recorded in MRRN were activated by stimulation of any trigeminal nerve (Group 1 neurons) or of the contralateral trigeminal nerve (Group 2 neurons) (Gr1 and Gr2, respectively, in

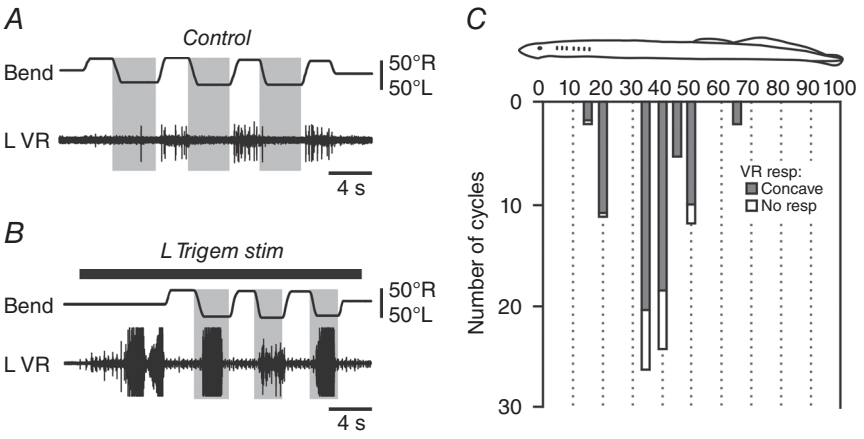

Figure 4. Reversal of reflex responses to bending during trigeminal nerve stimulation, which did not evoke any specific behaviour
A and *B*, an example of reflex reversal in a preparation with intact spinal cord: the left VR responded to right bending before stimulation (*A*, Control), and to left bending during trigeminal nerve stimulation (*B*). *C*, VR responses to bending at different rostrocaudal levels during trigeminal nerve stimulation ($N = 16$, n (VRs) $= 25$, where N and n (VRs) are number of preparations and number of recorded VRs, respectively). The bar position indicates the site of recording. Bending at different segments (from No. 20 to No. 70) was performed. Responses in VRs located 5–10 segments rostrally to the bending site were recorded. Horizontal scale indicates the spinal segment number.

Fig. 5*E*). As one can see in Fig. 5*F*, Group 1 neurons were more numerous in the rostral part and Group 2 neurons in the caudal part of MRRN (the border between these parts is indicated by the dashed lines in Fig. 5*D* and *G*).

Intracellular stimulation of the individual Group 1 or Group 2 neurons did not evoke the reflex reversal, suggesting that the commands for reflex reversal are transmitted by a population of RSNs. Since the majority of Group 1 and Group 2 neurons were located in MRRN, to activate the population of these neurons we stimulated electrically different sites within MRRN (shown in Fig. 5*G*) in eight preparations. Stimulation of most sites (indicated by red circles in Fig. 5*G*) evoked reflex reversal. An example of reflex reversal caused by stimulation of MRRN is shown in Fig. 5*H* and *I*. Reflex reversal could be also evoked by MRRN stimulation when a Ca^{2+}-free

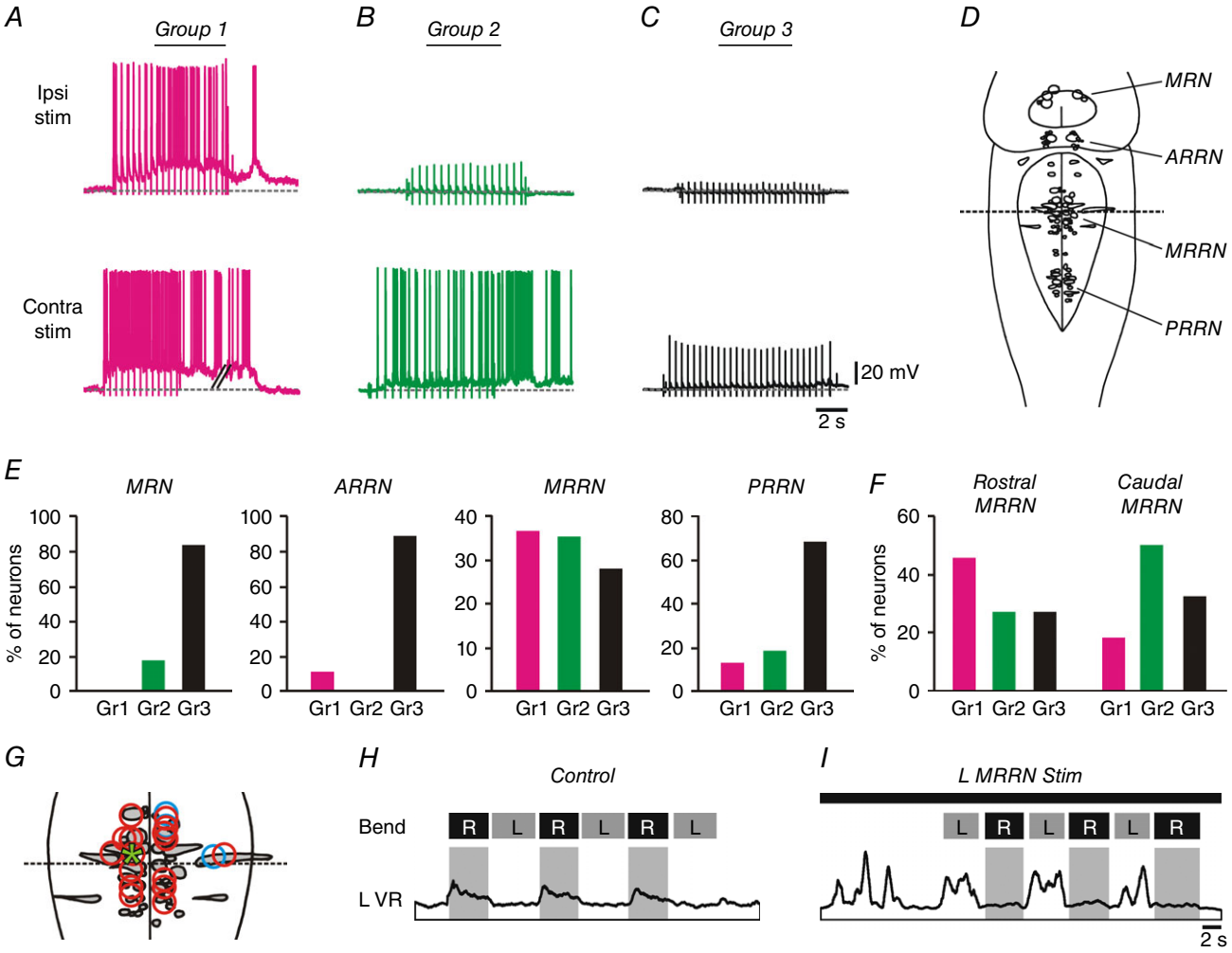

Figure 5. Classification of reticulospinal neurons according to their response to stimulation of ipsilateral and contralateral trigeminal nerve, with parameters causing reflex reversal but no specific behaviour
A, an example of a Group 1 neuron activated by stimulation of the ipsilateral (upper panel) and contralateral (lower panel) trigeminal nerve. *B*, an example of a Group 2 neuron activated by stimulation of the contralateral trigeminal nerve only. *C*, an example of Group 3 neuron that was not activated by stimulation of either nerve. In *A*–*C*, the duration of stimulation is indicated by stimulation artifacts. *D*, schematic drawing of the brainstem dorsal view with four reticular nuclei: mesencephalic reticular nucleus (MRN), anterior, middle, and posterior rhombencephalic nuclei (ARRN, MRRN and PRRN, respectively). The dashed line separates the rostral and caudal parts of MRRN. *E* and *F*, relative number of Group 1, 2 and 3 neurons recorded in different reticular nuclei (*E*, MRN, $n = 6$; ARRN, $n = 9$; MRRN, $n = 68$; PRRN, $n = 16$) and in rostral ($n = 39$) and caudal ($n = 29$) parts of MRRN (*F*). *G*, sites in MRRN, which were stimulated during bending. Sites eliciting the reflex reversal in eight experiments are indicated by red circles. Three sites indicated by blue circles did not evoked reflex reversal in two experiments. The dashed line separates the rostral and caudal parts of MRRN. *H* and *I*, electrical stimulation of a site in left MRRN (indicated by a green asterisk in *G*) caused reversal of the reflex response to bending. Bending caused the convex response before stimulation (*H*, Control) and the concave response during stimulation (*I*). Bending was performed at segment No. 45 and the VRs of segment No. 40 were recorded.

Table 4. Group 1 and Group 2 neurons recorded during different types of escape behaviour

RSNs	SFS	BS	CT	IT
Group 1	3	2	4	3
Group 2	3	1	2	0

Values are presented as number of recorded neurons. Other designations as in Table 2.

solution was applied in the pool with the brainstem (not illustrated).

Some of the Group 1 and Group 2 neurons were recorded during one of the types of escape behaviour (Table 4). All tested neurons were activated during initiation and remained active during escape behaviour. Figure 6 shows activity of a Group 1 neuron during BS. This neuron was activated immediately at the onset of trigeminal nerve stimulation, before BS started. The neuron was active until the end of stimulation and termination of BS. During this period, the reflexes to bending were reversed: one can see concave responses to bending in the caudal VRs.

During trigeminal nerve stimulation, a long-lasting depolarizing plateau was observed in some Group 1 and Group 2 neurons, like in the neuron shown in Fig 5*A*. The depolarizing plateau in RSNs in response to trigeminal nerve stimulation was described earlier (Viana Di Prisco *et al.* 1997, 2000) and is known to be due to activation of NMDA receptors. To determine if NMDA receptors contribute to formation of reticulospinal commands for reflex reversal, AP-5 (the NMDA antagonist) was applied either locally ($N = 2$), close to the RSNs in MRRN (see Methods), or bath-applied in the pool with the brainstem ($N = 3$). The effect of AP-5 application is illustrated in Fig. 7*A*–*C*. In controls (Fig. 7*A*), the concave response was observed during trigeminal nerve stimulation: the right VR was activated by right bending. After AP-5 application, the magnitude of the response was substantially decreased (Fig. 7*B*). It recovered after AP-5 washout (Fig. 7*C*). Figure 7*D* summarizes the effects of bath- and locally applied AP-5. A_{ipsi}/A_{contra} ratio of VR activity during bending (see Methods for explanation) was significantly reduced in the presence of AP-5.

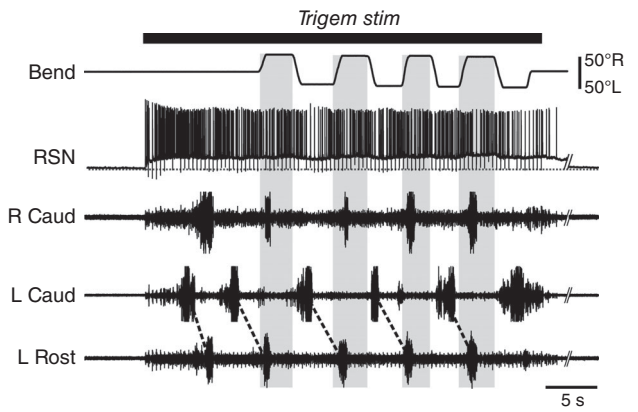

Figure 6. Activity of Group 1 neuron during backward swimming
Bending was performed at segment No. 50; rostral and caudal VRs were recorded in segments No. 25 and No. 40, respectively. The dashed line indicates the temporal sequence of activity in the left rostral and caudal VRs. Note that bending during BS causes concave responses in caudal VRs.

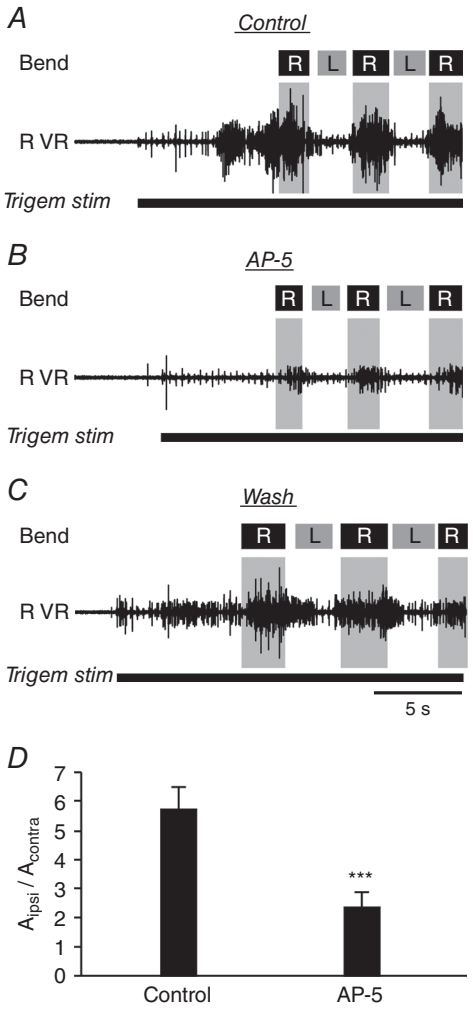

Figure 7. Effects of AP-5 application on reflex reversal
A–*C*, concave responses to body bending during trigeminal nerve stimulation (*A,* Control) substantially decreased during bath perfusion of AP-5 in the brainstem pool (*B*, AP-5) and recovered after AP-5 washout (*C*, Wash). Bending was performed at segment No. 40 and VRs were recorded in segment No. 35. The bars indicate duration of trigeminal nerve stimulation. *D*, mean value of A_{ipsi}/A_{contra} ratio (see Methods) before (Control) and during AP-5 application ($N = 5$, n (VRs) $= 10$, n (cycles) $= 30$, $P = 0.0007$). Bending was performed at segment Nos 40–45; VRs were recorded in segment Nos 30–35.

Effect of the spinal cord split on reflex reversal

To reveal the contribution of the networks residing in the ipsilateral (in relation to the SRNs) half of the spinal cord to the reflex reversal, the split spinal cord preparation (see Methods) was used in eight experiments. Bath application of D-glutamate (0.5-2.0 mM) was used to increase the excitability of spinal networks. Figure 8 *A* and *B* shows responses to bending in the left VR before and during trigeminal nerve stimulation, respectively. The concave response could still be evoked during stimulation of either the ipsilateral (Fig. 8*B*) or contralateral (not shown) trigeminal nerve. Among 23 recorded VRs, 66% had concave responses, 10% had convex responses, and 24% did not respond to bending during trigeminal nerve stimulation.

Responses of RSNs to bending

To find out if RSNs receive sensory feedback from SRNs, responses of RSNs to bending of the preparation were examined. Figure 9*A* and *B* shows examples of responding neurons. In Fig. 9*A*, the left RSN was depolarized and fired action potentials during left bends, and hyperpolarized during right bends, thus exhibiting a concave response. In Fig. 9*B*, the left RSN was depolarized by the right bend, and hyperpolarized by the left bend, exhibiting a convex response. There were also RSNs responding to the dynamic, but not the static, part of the bending in any direction. Figure 9*D* shows the relative number of neurons with different types of response to bending in the whole population of recorded RSNs (All) and in the neurons recorded in different reticular nuclei. Among 96 RSNs recorded in 20 experiments, 79% of RSNs responded to bending. The proportion of neurons showing a convex response was larger than those showing a concave response in the population of all tested neurons (All), and separately in MRN and in MRRN. The number of neurons in these two groups was equal in ARRN and PRRN.

The hyperpolarization of neurons caused by bending could be reversed if the neuron was injected with a negative current (Fig. 9*B* and *C*), indicating an inhibitory component in the sensory feedback from SRNs to RSNs. We found that 32% of responding RSNs received inhibitory inputs from SRNs (Fig. 9*E*). The relative number of neurons with inhibitory SRN inputs was similar in the groups of neurons with convex and concave responses.

Among RSNs activated during reflex reversal (Groups 1 and 2), about half of the neurons responded to bending (Fig. 10*A* and *B*). The minority of these neurons had concave responses. The majority of Group 1 neurons had convex responses, and the number of Group 2 neurons with convex and dynamic responses was equal. More than half of Group 1 and Group 2 neurons responding to bending before reflex reversal lost their response to bending during reflex reversal and were activated tonically as illustrated in Fig. 10*C*. Twenty-two per cent of Group 1 neurons and 13% of Group 2 neurons responded to bending before and also during reflex reversal. In these neurons, the phase of response was the same before and during reflex reversal (Fig. 10*D*). All these neurons had convex responses to bending (Fig. 10*A* and *B*).

A proportion of Group 1 (44%) and Group 2 (47%) neurons responded to bending neither before nor during reflex reversal (Fig. 10*A* and *B*). We have not found RSNs that did not respond to bending before reflex reversal but responded during reflex reversal.

Discussion

Spinal reflexes are substantial components of the motor control system in all vertebrates, and centrally driven reflex modifications are essential to many behaviours. However, little is known about the neuronal mechanisms underlying these modifications. This issue is difficult to address in mammals due to the complexity of their motor system. In the present study we used the lamprey as

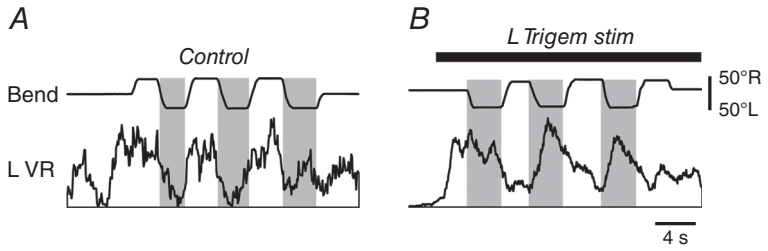

Figure 8. Reversal of reflex responses to bending in preparation with longitudinal split of the spinal cord
A and *B*, an example of reflex reversal in preparation with longitudinal split of the spinal cord: the left VR responded to right bend before stimulation (*A*, Control), and to left bend during trigeminal nerve stimulation (*B*). Signals recorded from the left VR were rectified and smoothed with time constant 0.5 s. Bending was performed at segment No. 45 and the VR of segment No. 40 was recorded.

an animal model. Organization of its CNS is similar to that in other vertebrates (Rovainen, 1979) but it has orders of magnitude fewer nerve cells than higher vertebrates. In addition, the lamprey has a wide repertoire of motor behaviours (Islam *et al.* 2006; Islam & Zelenin, 2008). To study the neuronal mechanisms underlying modifications of spinal reflexes in the context of different motor behaviours, we took advantage of an *in vitro* brainstem–spinal cord preparation of the lamprey in which spinal reflex responses to spinal cord bending (caused by signals from intraspinal SRNs) could be evoked during different types of fictive behaviours.

Functional roles of SRN-mediated reflexes during different behaviours

State-dependent reflex reversal has been observed in both vertebrates and invertebrates. In cats, inputs from Ib afferents from extensor muscles inhibit the homonymous MNs at rest, but excite the same MNs during locomotion (Pearson & Collins, 1993). In stick insect front legs, such state-dependent reflex reversal was observed during forward, but not backward walking (Hellekes *et al.* 2012), suggesting that the reflex reversal is task specific. The present study has shown that the SRN-mediated reflex response to body bending observed during FFS was reversed during escape behaviour.

Lampreys use FFS for long-distance migrations. For high velocity body progression during FFS, MNs on the convex side of undulating body are activated (Fig. 11*A*; Islam *et al.* 2006) in order to slow down the current body bending, and to initiate bending to the other side. Given that SRNs are also activated on the convex side (Islam *et al.* 2006) and during FFS cause excitation of the ipsilateral MNs (convex response to bending), we suggest that the SRN-mediated reflex responses amplify the motor output, and thus promote the generation of undulations.

Escape behaviour is characterized by large body undulations, which are less efficient in body progression but advantageous in getting rid of threats. To generate large body undulations, MNs on the concave side of the body are activated (Fig. 11*B*; Islam *et al.* 2006, 2008). We have found that passive bending activated MNs on the concave side during escape behaviour (SFS, BS and lateral turns), suggesting that the SRN-mediated reflex responses contribute to augmentation of the body undulation amplitude during escape (Fig. 11*B*). Thus reversal of the SRN-mediated reflex response to body bending is aimed at reinforcement of movements generated in each specific behaviour.

We found that during MLR-evoked FFS, bending at any rostro-caudal level evoked convex responses. However, during FFS evoked by application of D-glutamate to the spinal cord, bending at rostral segments evoked concave

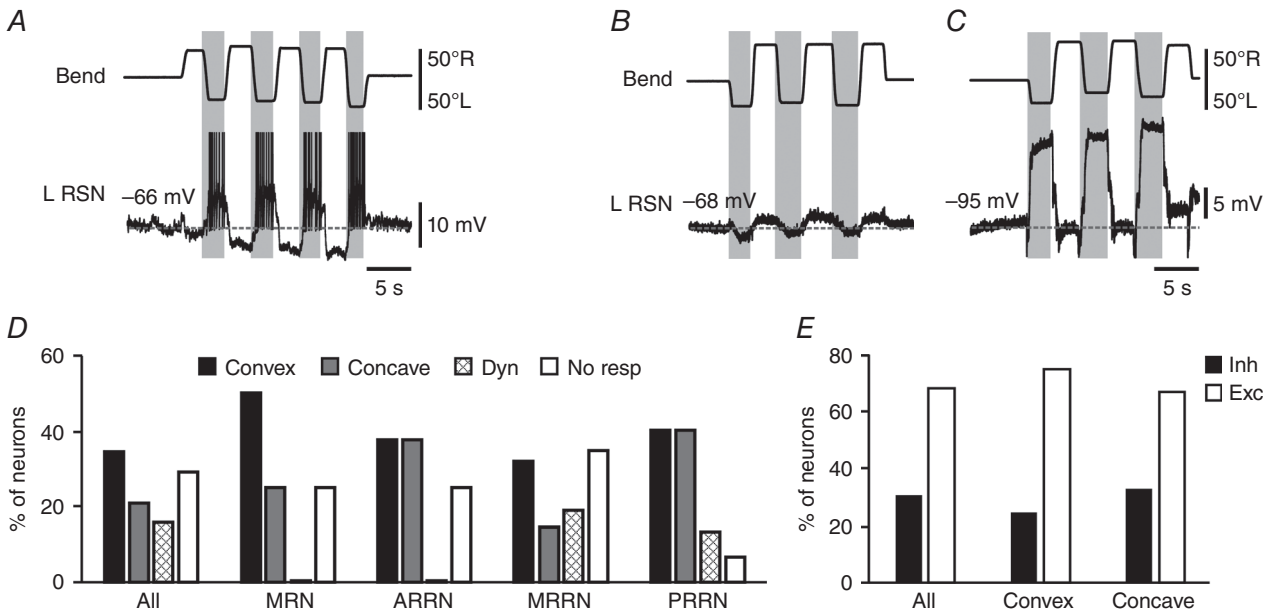

Figure 9. Responses of reticulospinal neurons to bending
A, an example of the left RSN with a concave response to bending (segment No. 50). *B* and *C*, responses of a left RSN to bending (No. 50) before (*B*) and during (*C*) intracellular injection of a negative current. Note the reverse of hyperpolarization (in *C*) indicating inhibitory input from SRNs. *D*, relative number of RSNs with different responses to bending recorded in all reticular nuclei (All), and separately in MRN ($n = 4$), ARRN ($n = 8$), MRRN ($n = 69$), and PRRN ($n = 15$). Dyn, response to the dynamic part of bending in any direction. *E*, relative number of RSNs receiving only excitatory inputs (Exc) from SRNs and those receiving inhibitory inputs (Inh) ($n = 19$).

responses. A similar result was obtained in the isolated spinal cord (Hsu *et al.* 2013*a*). This finding suggests that, first, networks underlying concave responses to bending in the rostral segments could be selectively activated and thus contribute to the behaviours like steering during FFS that require large amplitude bending in the rostral part of the body (Fagerstedt & Ullén, 2001; Saitoh *et al.* 2007; Kozlov *et al.* 2014). Second, the spinal networks, activated by spinal cord stimulation and by MLR stimulation, differ to some extent. It seems that stimulation of MLR selectively activates spinal network generating FFS (Sirota *et al.* 2000). By contrast, application of D-glutamate to the spinal cord activates all neurons containing glutamate receptors, both those involved in generation of FFS and those that may have other functions. Most likely some of the latter are responsible for the concave responses to bending observed in the rostral VRs during D-glutamate-evoked FFS. Recently, some difference in spinal networks activated during locomotion evoked by MLR and by spinal cord stimulation was demonstrated in mammals (Musienko *et al.* 2012).

RSNs transmitting commands for reflex reversal

Numerous studies on vertebrates have shown that commands from supraspinal centres could affect the magnitude of spinal reflex responses evoked by somatosensory signals (Holmqvist & Lundberg, 1959; Bretzner & Drew, 2005; Hsu *et al.* 2012). In invertebrates, signals from a higher level of CNS could also change the sign of the segmental reflex responses, resulting in reflex reversal (Mu & Ritzmann, 2008). However, neurons transmitting commands for reflex reversal were not studied.

In the present study, a population of RSNs, presumably transmitting commands for the reversal of SRN-mediated spinal reflexes in the lamprey has been revealed. This

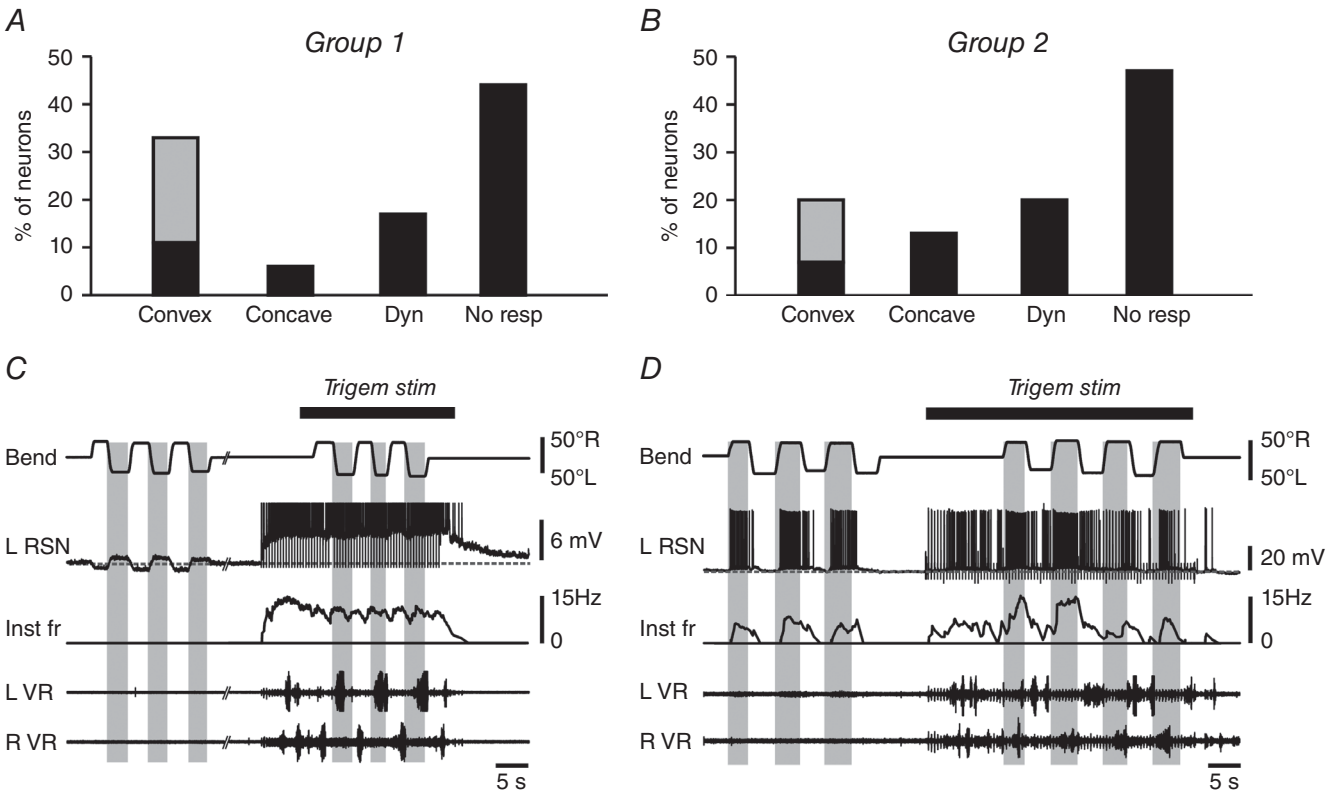

Figure 10. Responses of Group 1 and Group 2 neurons to bending
A and *B*, effect of trigeminal nerve stimulation (causing reflex reversal) on response of Group 1 (*A*) and Group 2 (*B*) neurons to bending. Bars show relative number of neurons with a particular type of response to bending (*A*, $N = 11$, $n = 18$; *B*, $N = 14$, $n = 15$). Grey part of the bar indicates the relative number of neurons, in which convex response to bending remained during trigeminal nerve stimulation. Black bars indicate the relative number of neurons, in which responses to bending disappeared or remained absent during trigeminal nerve stimulation. *C*, an example of a Group 1 neuron, which was affected by bending before, but not during reflex reversal caused by trigeminal nerve stimulation. *D*, an example of a Group 1 neuron, which was activated by left bending before as well as during reflex reversal caused by the trigeminal nerve stimulation. In *B* and *D*, the instantaneous frequency (Inst fr) of the recorded RSNs is also shown. Bending was performed at segment No. 55 and VRs were recorded in segment No. 50.

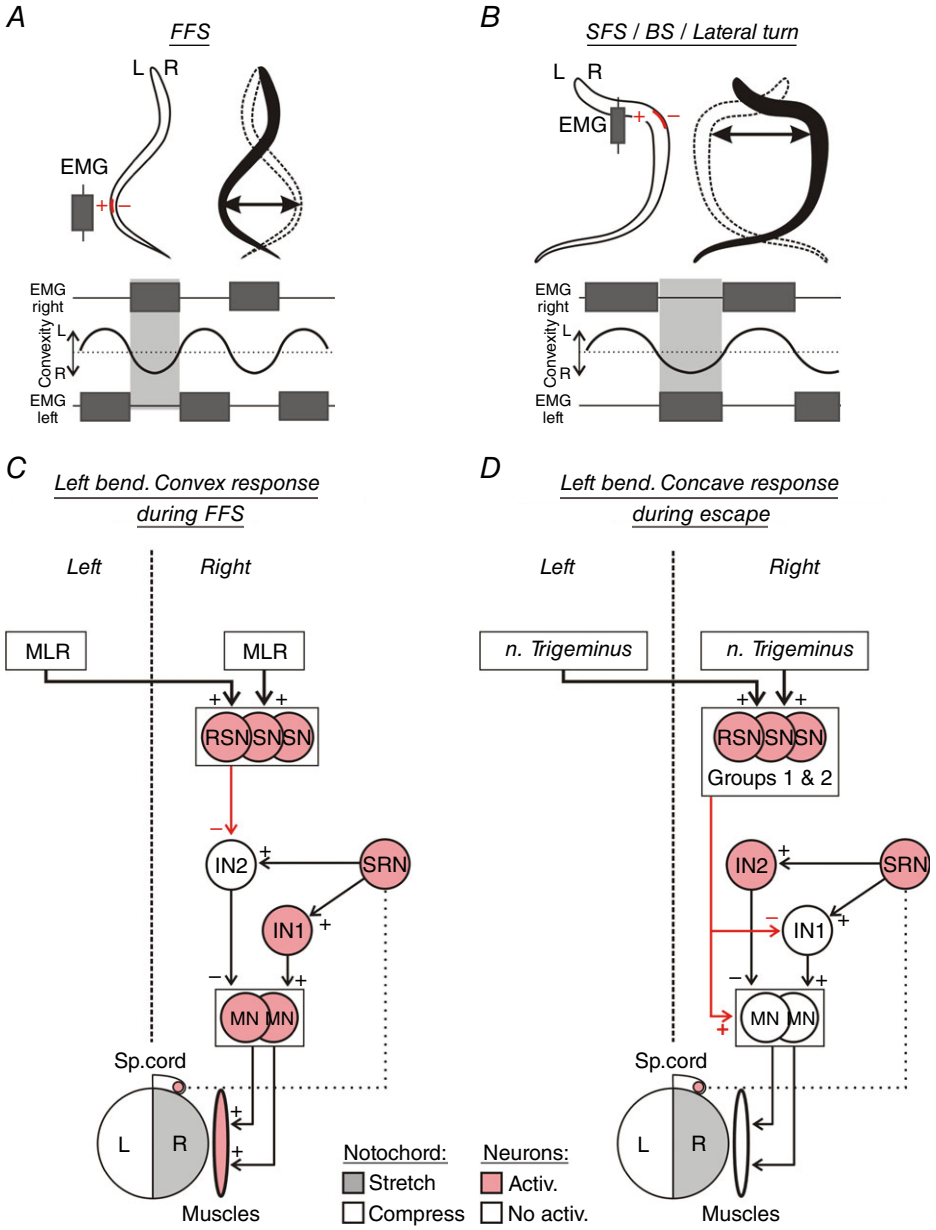

Figure 11. Functional role of the SRN-mediated spinal reflexes in different motor behaviours and a hypothesis about neuronal mechanisms underlying SRN-mediated spinal reflex reversal
A and *B*, functional role of the SRN-mediated spinal reflexes in different motor behaviours. The upper panels show the schematic body configuration of lamprey during FFS (*A*) and escape (*B*). The red line in the lamprey outline indicates SRNs activated by bending. The symbols + and − indicate activation and inhibition of MNs on the corresponding side, respectively. The lower panels show the schematic correlation of body undulation and muscle activity during FFS (*A*) and escape behaviours (*B*). During FFS (*A*), the convex response counteracts the body bend, and contributes to initiation of bending in the opposite direction, thus promoting the generation of undulations. During escape behaviours (*B*), the concave response increases the undulation amplitude, which is essential for escape behaviours. *C* and *D*, changes in a hypothetical circuitry underlying reflex responses to bending during FFS (*C*) and during escape behaviour (*D*). The stretched area of the notochord is shown in grey. Activated neurons and muscles are shown in pink, inactivated in white. Red arrows indicate supraspinal influence on spinal interneurons. Abbreviations: MLR, mesencephalic locomotor region, RSN, reticulospinal neuron, SRN, stretch receptor neuron, IN, interneuron, MN, motoneurons. *C*, stimulation of MLR, which evokes FFS, activates a specific population of RSNs. They inhibit IN2s, and thus bending to the left, which activates right SRNs, results in activation of ipsilateral MNs (convex response). *D*, stimulation of trigeminal nerves, which evokes escape behaviour, activates Group 1 and Group 2 RSNs. They inhibit IN1s and activate ipsilateral MNs. Therefore bending to the left results in inhibition of right MNs (via IN2s) and bending to the right in their disinhibition (concave response).

suggestion is based on the following findings. (i) Neurons of this population are activated by trigeminal nerve stimulation causing reflex reversal, without the presence of any specific behaviour. (ii) Electrical stimulation of different sites of MRRN, where the majority of neurons of this population are located, evoked reflex reversal. (iii) Neurons of this population are activated during initiation and remain active during different types of escape behaviour requiring reflex reversal.

Stimulation of different sites of the trigeminal nerve caused either reflex reversal alone or together with a particular type of escape behaviour (SFS, BS, lateral turns). This finding suggests the existence of a specific population of supraspinal neurons transmitting commands for reflex reversal, which could be activated separately, or in combination with other populations of supraspinal neurons initiating a specific type of escape behaviour. Co-activation of RSNs with different functions during FFS was demonstrated earlier (Zelenin, 2011).

The Group 1 and Group 2 neurons revealed in the present study were activated by trigeminal nerve stimulation causing reflex reversal alone or together with a particular type of escape behaviour, and thus most likely form the population of RSNs transmitting supraspinal commands for reflex reversal in the context of different types of escape behaviour. Group 3 neurons were not activated by trigeminal nerve stimulation causing reflex reversal alone. However, in some neurons the stimulation evoked small EPSPs. One can suggest that these neurons are responsible for initiation of specific types of escape behaviour, and that during trigeminal nerve stimulation causing reflex reversal alone the behaviour is absent since the neurons are not recruited in the activity. One also cannot rule out the possibility that these neurons belong to RSNs transmitting commands for reflex reversal but have a higher threshold for activation. However, our results have shown that their contribution is not critical for reflex reversal.

We found that the majority of Group 1 and Group 2 neurons were located in MRRN. Stimulation of different sites within MRRN (even in Ca^{2+}-free solution blocking synaptic transmission) evoked reflex reversal, suggesting that the activated RSNs transmitted commands for reflex reversal. Bilateral activation of RSNs located in MRRN during contralateral turns has been demonstrated (Fagersteadt *et al.* 2001). Most likely, a part of these RSNs are Group 1 and Group 2 neurons transmitting commands for reflex reversal.

We found that NMDA receptors are involved in the formation of supraspinal commands causing reflex reversal, since blocking NMDA receptors by bath application of AP-5 to the brainstem or by local application to MRRN (where the majority of Group 1 and Group 2 neurons are located) caused a significant decrease in the value of reversed responses to bending. NMDA receptors can mediate synaptic transmission from trigeminal afferents to RSNs at different locations – either at the sensory relay neurons or at the RSNs (Viana Di Prisco *et al.* 1995, 2005). A proportion of Group 1 and Group 2 neurons exhibited a long-lasting depolarizing plateau, which is known to be NMDA dependent (Viana Di Prisco *et al.* 1997). Earlier, it was suggested that the NMDA-mediated plateau in RSNs located in MRRN contributes to the maintenance of bouts of escape behaviours caused by trigeminal nerve stimulation (Viana Di Prisco *et al.* 2000). Thus, one can assume that both the population of RSNs initiating the escape behaviour and the population of RSNs causing reflex reversal during escape behaviour contain neurons exhibiting plateau properties.

Reflex reversal caused by trigeminal nerve stimulation persisted after splitting the spinal cord along its midline, suggesting that modifications in the unilateral spinal network cause the reflex reversal. Figure 11*C* and *D* shows a hypothetical circuit underlying the reversal of SRN-mediated reflexes. We suggest that two groups of spinal interneurons, IN1s and IN2s, receive excitatory inputs from the ipsilateral SRNs, and excite and inhibit ipsilateral MNs, respectively. Some IN1s are the interneurons of the CPG for FFS (Viana Di Prisco *et al.* 1990). The existence of IN2s was not demonstrated. Stimulation of MLR, which evokes FFS, activates a specific population of RSNs (Brocard & Dubuc, 2003; Brocard *et al.* 2010; Orlovsky *et al.* 1999). They inhibit IN2s and consequently bending to the left, which activates right SRNs, results in activation of ipsilateral MNs (convex response). Stimulation of trigeminal nerves, which evokes escape behaviour, activates Group 1 and Group 2 RSNs. They inhibit IN1s and activate ipsilateral MNs. Monosynaptic projections of RSNs to ipsilateral MNs have been demonstrated in lamprey (Buchanan *et al.* 1987). Consequently bending to the left results in inhibition of ipsilateral MNs and bending to the right in their disinhibition (concave response).

Proposed inhibition of IN2s and IN1s caused by specific populations of RSNs in the context of FFS (Fig. 11*C*) and escape behaviours (Fig. 11*D*), respectively, is most likely to be at least disynaptic (though there is some evidence for the existence of inhibitory RSNs; Wannier *et al.* 1995; Holstege, 1991; Perrins *et al.* 2002). Spinal interneurons mediating this inhibition could either directly inhibit IN1s and IN2s or cause presynaptic inhibition of SRN axons projecting to IN1s and IN2s. In mammals, presynaptic inhibition is produced by spinal interneurons with GABAergic axo-axonic synapses on primary afferent terminals (Eccles *et al.* 1962; Rudomin & Schmidt, 1999). In lampreys, the existence of such axo-axonic synapses between spinal interneurons and SRNs has not been shown; however, it has been demonstrated that SRNs are inhibited by GABA (Christenson *et al.* 1991; Vinay *et al.* 1996).

Previously we have shown that concave responses to bending in rostral VRs (observed in *in vitro* spinal cord preparations during FFS evoked by D-glutamate) were reversed to convex responses after longitudinal splitting the spinal cord (Hsu *et al.* 2013*a*). Thus, modifications in the unilateral spinal network underlying the generation of concave responses to bending in rostral VRs could be also be caused by signals transmitted from the contralateral hemicord by commissural spinal interneurons.

RSNs receive sensory feedback from SRNs signalling body configuration

During locomotion in mammals, supraspinal centres are affected by two types of signals from the spinal cord coming from the CPG (the 'efference copy'), and from limb mechanoreceptors (afferent feedback) (Orlovsky *et al.* 1999). These two types of signals were also found in lampreys. As shown by Kasicki & Grillner (1986), RSNs receive efference copy signals from the locomotor CPG via spinobulbar neurons. We have shown that RSNs also receive sensory input from intraspinal SRNs, signalling body configuration. These findings suggest that the basic principle of locomotor control, with two types of ascending signals is conserved in evolution. Input from SRNs to RSNs in lampreys is analogous to input from the dorsal spinocerebellar tract in mammals, which supplies the cerebellum with proprioceptive information (Orlovsky *et al.* 1999).

RSNs in all reticular nuclei receive inputs from SRNs, similarly to a wide distribution of inputs from the locomotor CPG (Kasicki *et al.* 1989). Inputs from SRNs to RSNs are highly diverse: there are both ipsi- and contralateral inputs; there are also both excitatory and inhibitory inputs. These results suggest that SRN signals are processed in the spinal cord before reaching RSNs.

About half of Group 1 and Group 2 RSNs received SRN inputs. During reflex reversal, the activity of some of these neurons was not affected by signals from SRNs, suggesting that their function was a tonic action on the reflex pathways underlying the reversal (Fig. 10*C*). The activity of some Group 1 and Group 2 neurons was still modulated by signals from SRNs during reflex reversal, suggesting that these neurons could modulate the efficacy of reversed reflexes in accordance with the current body configuration. We never found RSNs receiving SRN input only during reflex reversal, suggesting that RSNs do not receive feedback from the reflex pathways responsible for the reversal (e.g. inputs from IN2 in Fig. 11*D*).

Conclusions

This study has demonstrated that, during different types of behaviour in the lamprey, the spinal reflex responses to body bending mediated by intraspinal SRNs can be significantly modified (up to a complete reflex reversal) by supraspinal commands. These modifications of reflexes are aimed at reinforcement of the movements generated in each specific behaviour. Our study has revealed, for the first time, the population of RSNs transmitting commands for reflex reversal, and demonstrated the involvement of NMDA receptors in the formation of these commands, and a crucial role for the unilateral spinal networks in determining the type of SRN-mediated reflex response to bending. This study has also demonstrated how the same receptors can be used in numerous types of behaviour due to specific processing of their signals. In addition, we have found that RSNs receive sensory feedback from SRNs signalling body configuration. Similar feedback from limb afferents to RSNs also exists in mammals, suggesting similarities in functional organization of motor control in lower and higher vertebrates.

References

Armstrong DM (1986). Supraspinal contributions to the initiation and control of locomotion in the cat. *Prog Neurobiol* **26**, 273–361.

Bretzner F & Drew T (2005). Motor cortical modulation of cutaneous reflex responses in the hindlimb of the intact cat. *J Neurophysiol* **94**, 673–687.

Brocard F & Dubuc R (2003). Differential contribution of reticulospinal cells to the control of locomotion induced by the mesencephalic locomotor region. *J Neurophysiol* **90**, 1714–1727.

Brocard F, Ryczko D, Fénelon K, Hatem R, Gonzales D, Auclair F & Dubuc R (2010). The transformation of a unilateral locomotor command into a symmetrical bilateral activation in the brainstem. *J Neurosci* **30**, 523–533.

Buchanan JT, Brodin L, Dale N & Grillner S (1987). Reticulospinal neurones activate excitatory amino acid receptors. *Brain Res* **408**, 321–325.

Büschges A & El Manira A (1998). Sensory pathways and their modulation in the control of locomotion. *Curr Opin Neurobiol* **8**, 733–739.

Cangiano L & Grillner S (2003). Fast and slow locomotor burst generation in the hemispinal cord of the lamprey. *J Neurophysiol* **89**, 2931–2942.

Christenson J, Alford S, Grillner S & Hökfelt T (1991). Co-localized GABA and somatostatin use different ionic mechanisms to hyperpolarize target neurons in the lamprey spinal cord. *Neurosci Lett* **134**, 93–97.

Deliagina TG, Zelenin PV & Orlovsky GN (2002). Encoding and decoding of reticulospinal commands. *Brain Res Brain Res Rev* **40**, 166–177.

Eccles JC, Schmidt RF & Willis WD (1962). Presynaptic inhibition of the spinal monosynaptic reflex pathway. *J Physiol* **161**, 282–297.

El Manira A, Pombal MA & Grillner S (1997). Diencephalic projection to reticulospinal neurons involved in the initiation of locomotion in adult lampreys *Lampetra fluviatilis*. *J Comp Neurol* **389**, 603–616.

Fagerstedt P, Orlovsky GN, Deliagina TG, Grillner S & Ullén F (2001). Lateral turns in the lamprey. II. Activity of reticulospinal neurons during the generation of fictive turns. *J Neurophysiol* **86**, 2257–2265.

Fagerstedt P & Ullén F (2001). Lateral turns in the lamprey. I. Patterns of motoneuron activity. *J Neurophysiol* **86**, 2246–2256.

Forssberg H, Grillner S & Rossignol S (1977). Phasic gain control of reflexes from the dorsum of the paw during spinal locomotion. *Brain Res* **132**, 121–139.

Gervasio S, Farina D, Sinkær T & Mrachacz-Kersting N (2013). Crossed reflex reversal during human locomotion. *J Neurophysiol* **109**, 2335–2344.

Grillner S (1981). Control of locomotion in bipeds, tetrapods and fish. In *Handbook of Physiology. The Nervous System. Motor Control*, pp. 1179–1236. American Physiological Society, Bethesda, MD, USA.

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

Hellekes K, Blincow E, Hoffmann J & Büschges A (2012). Control of reflex reversal in stick insect walking: effects of intersegmental signals, changes in direction, and optomotor-induced turning. *J Neurophysiol* **107**, 239–249.

Holmqvist B & Lundberg A (1959). On the organization of the supraspinal inhibitory control of interneurones of various spinal reflexes. *Arch Ital Biol* **97**, 340–356.

Holstege JC (1991). Ultrastructural evidence for GABAergic brainstem projections to spinal motoneurons in the rat. *J Neurosci* **11**, 159–167.

Hsu LJ, Zelenin PV, Grillner S, Orlovsky GN & Deliagina TG (2013*a*). Intraspinal stretch receptor neurons mediate different motor responses along the body in lamprey. *J Comp Neurol* **521**, 3847–3862.

Hsu LJ, Zelenin PV, Orlovsky GN & Deliagina TG (2012). Effects of galvanic vestibular stimulation on postural limb reflexes and neurons of spinal postural network. *J Neurophysiol* **108**, 300–313.

Hsu LJ, Zelenin PV, Orlovsky GN & Deliagina TG (2013*b*). Central regulation of spinal reflex responses to body bending mediated by intraspinal stretch receptor neurons in lamprey. *Soc Neurosci Abstr* **39**, 782.20.

Hsu LJ, Zelenin PV, Orlovsky GN & Deliagina TG (2015). Reticulospinal neurons transmitting commands for modification of spinal reflex responses to body bending during escape behaviors in lampreys. *Soc Neurosci Abstr* **41**, 798.09.

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.

Kasicki S & Grillner S (1986). Müller cells and other reticulospinal neurones are phasically active during fictive locomotion in the isolated nervous system of the lamprey. *Neurosci Lett* **69**, 239–243.

Kasicki S, Grillner S, Ohta Y, Dubuc R & Brodin L (1989). Phasic modulation of reticulospinal neurones during fictive locomotion and other types of spinal motor activity in lamprey. *Brain Res* **484**, 203–216.

Kozlov AK, Kardamakis AA, Hellgren Kotaleski J & Grillner S (2014). Gating of steering signals through phasic modulation of reticulospinal neurons during locomotion. *Proc Natl Acad Sci USA* **111**, 3591–3596.

Le Ray D, Juvin L, Ryczko D & Dubuc R (2011). Chapter 4 – supraspinal control of locomotion: the mesencephalic locomotor region. *Prog Brain Res* **188**, 51–70.

Mu L & Ritzmann RE (2008). Interaction between descending input and thoracic reflexes for joint coordination in cockroach: I. descending influence on thoracic sensory reflexes. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* **194**, 283–298.

Musienko PE, Zelenin PV, Lyalka VF, Gerasimenko YP, Orlovsky GN & Deliagina TG (2012). Spinal and supraspinal control of the direction of stepping during locomotion. *J Neurosci* **32**, 17442–17453.

Orlovsky GN, Deliagina TG & Grillner S (1999). *Neuronal Control of Locomotion. From Mollusc to Man*. Oxford University Press, Oxford.

Pearson KG (2008). Role of sensory feedback in the control of stance duration in walking cats. *Brain Res Rev* **57**, 222–227.

Pearson KG & Collins DF (1993). Reversal of the influence of group Ib afferents from plantaris on activity in medial gastrocnemius muscle during locomotor activity. *J Neurophysiol* **70**, 1009–1017.

Perrins R, Walford A & Roberts A (2002). Sensory activation and role of inhibitory reticulospinal neurons that stop swimming in hatchling frog tadpoles. *J Neurosci* **22**, 4229–4240.

Ronan M (1989). Origins of the descending spinal projections in petromyzontid and myxinoid agnathans. *J Comp Neurol* **281**, 54–68.

Rovainen CM (1967). Physiological and anatomical studies on large neurons of central nervous system of the sea lamprey (*Petromyzon marinus*). I. Müller and Mauthner cells. *J Neurophysiol* **30**, 1000–1023.

Rovainen CM (1978). Müller and Mauthner cells, and other identified reticulospinal neurons in the lamprey. In *Neurobiology of Mauthner Cell*, eds Faber DS & Korn H, pp. 245–269. Raven, New York.

Rovainen CM (1979). Neurobiology of lampreys. *Physiol Rev* **59**, 1007–1077.

Rudomin P & Schmidt RF (1999). Presynaptic inhibition in the vertebrate spinal cord revisited. *Exp Brain Res* **129**, 1–37.

Saitoh K, Ménard A & Grillner S (2007). Tectal control of locomotion, steering, and eye movements in lamprey. *J Neurophysiol* **97**, 3093–3108.

Sirota MG, Viana Di Prisco G & Dubuc R (2000). Stimulation of the mesencephalic locomotor region elicits controlled swimming in semi-intact lampreys. *Eur J Neurosci* **12**, 4081–4092.

Viana Di Prisco G, Boutin T, Petropoulos D, Brocard F & Dubuc R (2005). The trigeminal sensory relay to reticulospinal neurones in lampreys. *Neuroscience* **131**, 535–546.

Viana Di Prisco G, Ohta Y, Bongianni F, Grillner S & Dubuc R (1995). Trigeminal inputs to reticulospinal neurones in lampreys are mediated by excitatory and inhibitory amino acids. *Brain Res* **695**, 76–80.

Viana Di Prisco G, Pearlstein E, Le Ray D, Robitaille R & Dubuc R (2000). A cellular mechanism for the transformation of a sensory input into a motor command. *J Neurosci* **20**, 8169–8176.

Viana Di Prisco G, Pearlstein E, Robitaille R & Dubuc R (1997). Role of sensory-evoked NMDA plateau potentials in the initiation of locomotion. *Science* **278**, 1122–1125.

Viana Di Prisco G, 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.

Wannier T, Orlovsky G & Grillner S (1995). Reticulospinal neurones provide monosynaptic glycinergic inhibition of spinal neurones in lamprey. *Neuroreport* **6**, 1597–1600.

Wickelgren WO (1977). Physiological and anatomical characteristics of reticulospinal neurones in lamprey. *J Physiol* **270**, 89–114.

Zelenin PV (2005). Activity of individual reticulospinal neurons during different forms of locomotion in the lamprey. *Eur J Neurosci* **22**, 2271–2282.

Zelenin PV (2011). Reticulospinal neurons controlling forward and backward swimming in the lamprey. *J Neurophysiol* **105**, 1361–1371.

Additional information

Competing interests

The authors declare no conflict of interest.

Author contributions

L.-J.H. and T.G.D. designed experiments; L.-J.H. performed experiments and analysed data; L.-J.H., P.V.Z., G.N.O., and T.G.D. interpreted data and wrote the paper. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

This work was supported by the Swedish Research Council (No.11554 to T.G.D. and No.21076 to P.V.Z.), and by a governmental scholarship from the Ministry of Education in Taiwan (No. 1022112031) to L.-J.H.

Acknowledgements

We thank Tommy Nord for excellent assistance in the setup construction.