

## The pattern of motor coordination underlying the roll in the lamprey

Pavel V. Zelenin\*, Sten Grillner, Grigori N. Orlovsky and Tatiana G. Deliagina

*The Nobel Institute for Neurophysiology, Department of Neuroscience, Karolinska Institute, SE-171 77, Stockholm, Sweden*

\*Author for correspondence (e-mail: Pavel.Zelenin@neuro.ki.se)

Accepted 14 April 2003

### Summary

The lamprey swims by caudally directed lateral undulations of its body. During swimming the animal is oriented with its dorsal side up, and any deviation from this posture (roll tilt) elicits a corrective motor response aimed at restoring the normal orientation. Video recording was used to study the kinematic pattern of the response to a 90° roll tilt imposed in the intact lamprey. The corrective responses were associated with specific modifications of the swimming movements. The plane of locomotor undulations deviated from the normal, i.e. frontal plane in one direction at the beginning of the rotation and in the opposite direction at its end. A similar motor pattern was also observed in the anterior part of

the body of lampreys in which the spinal cord had been transected in the mid-body area, when performing postural corrections. It could also be observed during roll turns performed by lampreys after a rostral hemisection of the spinal cord. We argue that these modifications of the locomotor pattern generate the moments of force necessary for initiation and termination of the corrective roll turn. Possible neuronal mechanisms causing the corrective movements are discussed.

Key words: motor coordination, roll turn, kinematics, postural reflex, spinal cord damage, reticulospinal system, lamprey, *Lampetra fluviatilis*, *Ichthyomyzon unicuspis*.

### Introduction

The lamprey (Cyclostome) has been extensively used as a model for studying different aspects of motor control. The lamprey swims by lateral undulations of its body that are propagated in the rostro-caudal direction. The spinal mechanisms generating these undulatory movements have been analyzed in considerable detail at the network and cellular levels (for reviews, see Grillner et al., 1995, 2000). When swimming, the lamprey maintains orientation of its body in the transverse plane (normally dorsal side up), and in the sagittal plane (usually horizontal). These two postural control systems are driven by vestibular input (de Burlet and Versteegh, 1930; Ullén et al., 1995a).

To elucidate the postural mechanisms in lamprey, both electrophysiological and behavioral experiments have been carried out. The electrophysiological experiments have shown that the main route for transmitting corrective motor commands from the brain to the spinal cord is *via* the reticulospinal (RS) pathways (Rovainen, 1967; Nieuwenhuys, 1972; Brodin et al., 1988; Bussièrès, 1994). Specific subgroups of RS neurons are activated by the vestibular input when the body orientation in space is changed in the transverse or sagittal plane (Orlovsky et al., 1992; Deliagina et al., 1992a,b, 1993, 2000; Deliagina and Fagerstedt, 2000; Pavlova and Deliagina, 2002). The activity of these subgroups of RS neurons mediates the commands that, *via* spinal mechanisms, elicit postural corrective responses (Deliagina et al., 1993, 2002; Deliagina, 1997; Pavlova and Deliagina, 2002).

The behavioral experiments were primarily aimed at understanding the motor patterns underlying postural corrections (Ullén et al., 1995a,b). These experiments showed that body flexion in the sagittal plane is used by the lamprey to change the body orientation in this plane. In contrast, the motor patterns leading to roll turns (turns in the transverse plane) have remained unclear for a number of reasons. First, the dorsal-side-up orientation is so well stabilized that deviations from this orientation and the corrective responses are difficult to observe. Second, there were no convenient ways to perturb this posture. Third, the lamprey does not have paired fins, which are used for tilt compensation in fish (Gray, 1968; Blake, 1983; Timerick et al., 1990). The lamprey thus has to rely upon the body, tail and dorsal fin movements to control its orientation in space. Monitoring these movements is technically difficult and direct experimental data are lacking, but a few hypothetical motor patterns have been discussed (Ullén et al., 1995a), such as ‘body twisting’ (around the longitudinal axis), bending of the dorsal fin and asymmetric lateral movements of the ventrally bent tail.

The aim of the present study was to determine how the lamprey performs roll turn movements. To this end, we positioned the lamprey on its lateral side and video recorded the sequence of movements during restoration of the normal orientation. This motor pattern was compared to the pattern seen during undisturbed swimming. The main finding is that, during the actual turn, the lateral locomotor undulations are

coordinated with specific dorso-ventral flexions. We argue that these modifications of the locomotor pattern generate the moments of force necessary for initiation and termination of the corrective roll turn. This interpretation was further supported by monitoring the motor pattern during roll turns in lampreys subjected to spinal cord hemisection or complete transection. The results are discussed in the context of our current knowledge of neuronal postural mechanisms.

### Materials and methods

Experiments were performed on 12 adult lampreys (*Lampetra fluviatilis* L.,  $N=9$ , and *Ichthyomyzon unicuspis* L.,  $N=3$ , body length 25–30 cm). No differences were observed in the postural behavior between the two species, and data for all animals are presented together. All experiments were

approved by the local ethical committee (Norra Djurförsöksetiska Nämnden). Free swimming of the lampreys was examined in a large aquarium (70 cm long, 35 cm wide and 35 cm deep). Animals were observed from the side, as well as from above using a mirror (Fig. 1A). White markers were attached to the skin along the dorsal midline in some cases for more precise assessment of the roll tilt angle. Lampreys were either released freely into the water, or from a hand-held position. In the latter case, the animals were positioned by hand with their right, left or dorsal side up prior to release. The movements of the animal were recorded with a video camera ( $25 \text{ frames s}^{-1}$ ) and analyzed frame by frame.

For the analysis, we chose only the episodes in which an animal swam approximately parallel (within  $30^\circ$ ) to the long axis of the aquarium. For each frame, the outlines of the lamprey were manually traced from the video screen. To obtain a top view, the image of the animal seen in the mirror (Fig. 1A) was flipped vertically and normalized so that the longitudinal lengths of the side and top views were equal (the resulting top view presented in Fig. 1B is the view that an observer would get looking at the aquarium from above). Both the side and top views were used to characterize the body shape. This was done only for the frames in which the animal was oriented with its dorsal, ventral, right or left side facing upwards, or very close to these orientations. When the lamprey was oriented in that way, body flexions in the frontal and sagittal planes of the animal could be directly assessed from the front and top views. For these frames, we measured the degree of lateral and dorso-ventral flexions at different points along the body, with the side and top views of the body outlines traversed by 21 parallel, equidistant, straight lines (Fig. 1B). In this and subsequent figures, the abscissa  $x$

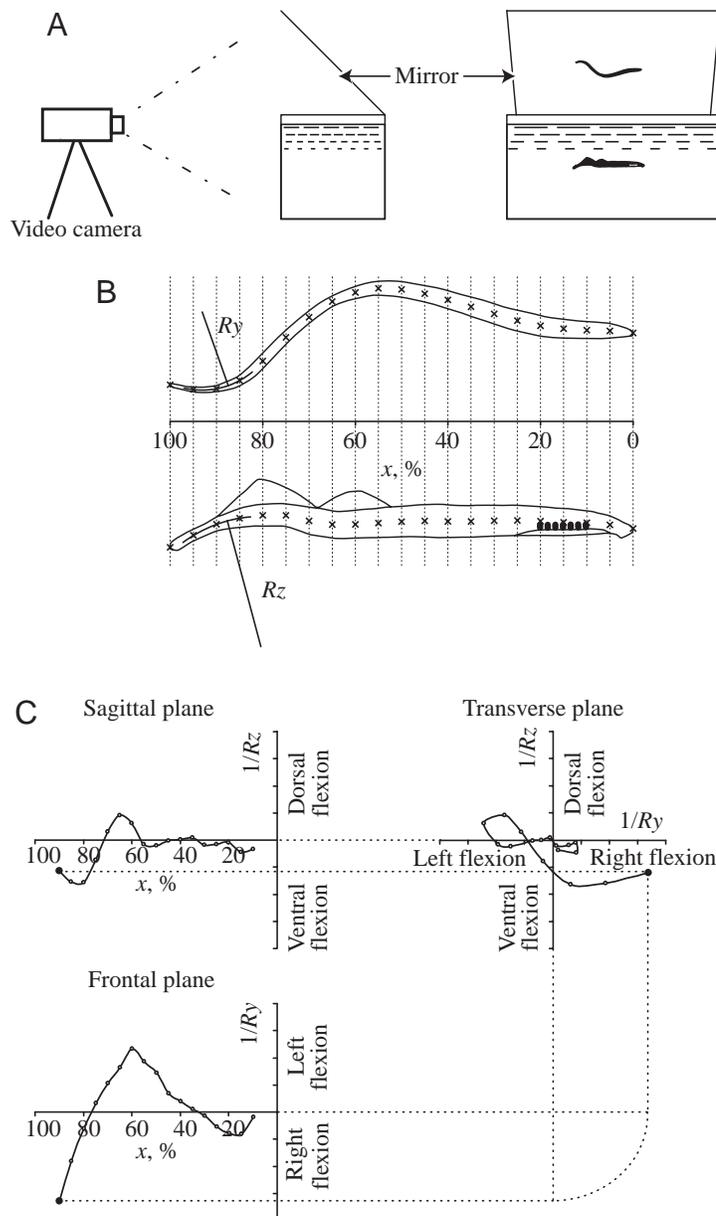


Fig. 1. (A) Experimental arrangement. The side view and view from above, seen in the mirror, of the swimming lamprey were video recorded. (B) Processing of video images. The body outlines in the frontal (top) and sagittal (bottom) planes obtained from the frame in which the animal was positioned with its back up. The position of the gills is shown in the side view. The body midline is marked by 21 circles located equidistantly along the 'body length' axis ( $x$ ), where anterior = 0% and posterior = 100%. The body shape was approximated by circles drawn through each of three neighboring midline points. The arcs of the circles for one of the points are shown together with their radii,  $R_y$  and  $R_z$ , for the frontal and sagittal planes, respectively. (C) The inverse of the circle radius characterizes the curvature at a given point of the body. The curvature was plotted in coordinates  $x$  ('body length') versus  $1/R_z$  (curvature in the sagittal plane),  $x$  ('body length') versus  $1/R_y$  (curvature in the frontal plane), and  $1/R_y$  (curvature in the frontal plane) versus  $1/R_z$  (curvature in the sagittal plane). The last plot provides information about coordination of flexions in the sagittal and frontal plane. In these and all following plots, the units for the  $x$  axis are percent of body length, while those for the  $1/R_y$  and  $1/R_z$  axes are arbitrary units inverse to the  $x$  units. The scales for  $1/R_y$  and  $1/R_z$  axes are always the same.

represents the percentage length of the body projection. We will call this dimension 'body length' for short, though it differs from the real body length because of the non-parallel orientation of the lamprey and the body flexions. Midpoints between the points of intersection of the straight lines with the body outline (crosses in Fig. 1B) were considered to represent the body shape. For each point, a circle was drawn through it and its closest neighbors (illustrated in Fig. 1B for one of the points located near the tail). In this and following figures,  $R_y$  is the radius of this circle for lateral flexions and  $R_z$  that for dorso-ventral flexions. The inverse values ( $1/R_y$  and  $1/R_z$ ) were taken as characteristics of the body flexions (curvature) at each point along the body. The left two panels in Fig. 1C show the curvature at different points of the body calculated for the body shape shown in Fig. 1B. The upper panel represents body flexions in the sagittal plane; the lower one in the frontal plane. From these two presentations, the body shape was also presented using the coordinates  $(1/R_y)-(1/R_z)$ . This coordinate system illustrates coordination between the flexions in the frontal and sagittal planes (we will call this 'the body flexions in the transverse plane', Fig. 1C, right panel). These three curvature plots illustrate the net result of the activity of the muscles that bend the body in the frontal and sagittal planes in different points along the body longitudinal axis.

In six lampreys, a load (4–10 g) was sutured to the back in the gill region to destabilize the normal dorsal-side-up orientation.

In some animals, one of the following lesions of the spinal cord was performed under general MS222 anaesthesia (Sandoz): (i) complete transection at the level immediately rostral to the anterior dorsal fin ( $N=3$ ), (ii) complete transection between the dorsal fins ( $N=3$ ), or (iii) left hemisection at a level of the third gill ( $N=6$ ) (the seven gills are shown in Fig. 1B; the hemisection level is indicated in Fig. 6A, frame 1). Each animal was tested 1 day after surgery. At the end of the experimental series, the animals were killed with an overdose of MS-222. *Post mortem* investigation showed that, in all cases, spinalization or hemisection was complete.

## Results

### *Undisturbed swimming of intact lampreys*

To detect modifications of the locomotor pattern during corrective roll turns, we first examined undisturbed horizontal swimming. Fig. 2 presents the body outlines and body curvature data in the sagittal, frontal and transverse planes for nine consecutive frames taken during undisturbed swimming. Undisturbed swimming is characterized by periodic, lateral undulations of the body propagated in the rostral-caudal direction (Fig. 2B). The amplitudes of the undulations and body curvature in the frontal plane increase caudally (Fig. 2D). In contrast, the shape of the body in the sagittal plane is almost straight, with only minor bending in the tail region (Fig. 2A). Correspondingly, the curvature data points in Fig. 2C are located close to the  $x$ -axis. Taken together, these large lateral and small dorso-ventral flexions lead to movements of the body

confined almost entirely to the frontal plane so that, in  $1/R_y-1/R_z$  coordinates, the data points are distributed close to the  $1/R_y$ -axis (Fig. 2E).

A similar body shape was observed in all the 58 episodes of swimming analyzed (six animals). The body was almost straight in the sagittal plane; dorso-ventral flexions were rare and were not correlated with the locomotor lateral flexions (for example, see frames 4 and 6 in Fig. 2). A minor tendency for the tail to be bent ventrally was seen (of the 113 frames taken from 10 episodes of swimming, the tail was bent ventrally in 51 cases, bent dorsally in 12 cases, and almost straight in the rest).

### *Restoration of the dorsal-side-up orientation by intact lampreys*

When released from the experimenter's hand, the animals were oriented with their right or left side up. They started to swim immediately and – at the same time – performed the corrective roll movement, which led to rapid (within 0.5 s) restoration of the dorsal-side-up position, and continued swimming with a normal locomotor pattern.

An example of the corrective movement is presented in Fig. 3. Immediately after release, the animal was situated with its right side up and its white belly facing the observer (Fig. 3A,B, frames 1 and 2). The roll angle in frames 1 and 2 did not change (white 'belly' appeared symmetric). In frame 3, however, the belly appeared asymmetric due to a change of the roll angle. The roll movement thus started between frames 2 and 3. Consider the body shape in frame 2. Due to the swim undulations, the body was bent to the right in the rostral and caudal parts and to the left in the middle part (Fig. 3A, frame 2, and the curvature plot in Fig. 3D). At the same time, in the top view one can see the right side of the animal with the body flexed ventrally in the rostral and caudal parts, and with dorsal flexion in the middle part (Fig. 3B, frame 2). This is also seen in the curvature plot (Fig. 3C). The combination of the flexions in the frontal and sagittal planes formed an oblique pattern in the transverse plane with prevailing left-dorsal↔right-ventral flexions (Fig. 3E). This pattern differed strongly from that observed in normal swimming (Fig. 2E).

In all cases (69 episodes in six animals) a similar body shape was observed at the beginning of the corrective roll movement, i.e. a flexion of the up-facing lateral side combined with ventral flexion, or a flexion of the down-facing lateral side combined with dorsal flexion. In other words, initiation of the corrective roll turn was always associated with an oblique plane of locomotor undulations, the deviation from the frontal plane being in the direction of body rotation (Fig. 7Ciii).

There was no correlation between the phase of the locomotor cycle and the beginning of the roll corrective movement. For 33 episodes of the movement that started from the left-side-up position, the rostral part of the body was flexed to the right in 18 cases and to the left in 15 cases. Similarly, for 36 episodes of turning from the right-side-up position, the rostral part of the animal was flexed to the right in 17 cases and to the left in 19 cases.

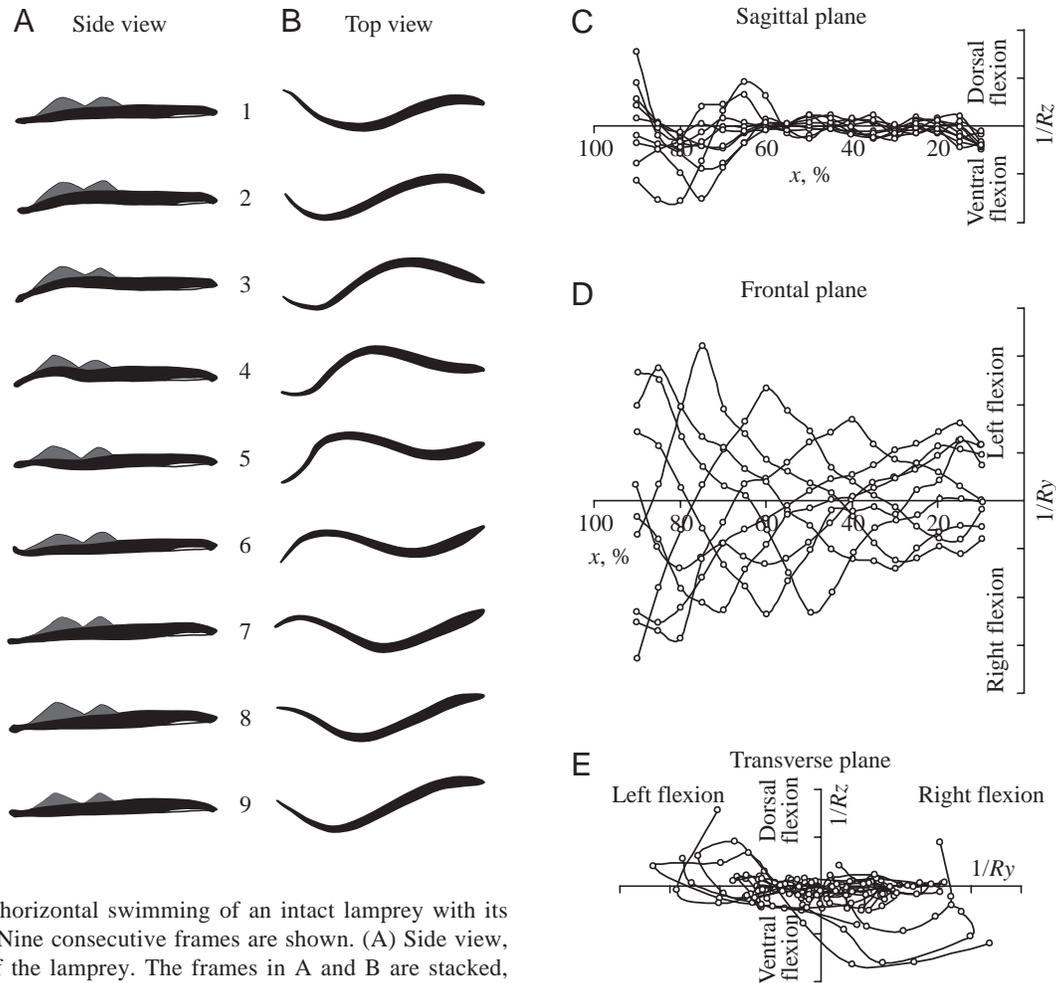


Fig. 2. Normal horizontal swimming of an intact lamprey with its dorsal side up. Nine consecutive frames are shown. (A) Side view, (B) top view of the lamprey. The frames in A and B are stacked, with no forward movement indicated. (C) Body flexions in the sagittal plane. (D) Body flexions in the frontal plane. (E) The curvature data presented in the transverse plane. Data points corresponding to the same frame are connected with lines. The points and lines for all 9 frames are superimposed.

As the animal approached its normal, dorsal-side-up position, the relationships between the flexions in the frontal and sagittal planes changed. Consider two frames, 13 and 18 (Fig. 3A,B). In frame 13, the lamprey was close to the dorsal-side-up orientation; however, rotation still continued, as judged by the displacement of the dorsal midline markers in the sequential frames 13–17. The top view shows that, due to the swimming undulations, the body was bent to the right in the rostral and caudal parts, and to the left in the middle part (Fig. 3A, frame 13). This is well reflected in the curvature plot (Fig. 3D). The side view, as well as the curvature plot, shows that the body was flexed dorsally in the rostral and caudal parts, and ventrally in the middle part (Fig. 3B,C). The combination of flexions in the frontal and sagittal planes formed an oblique pattern in the transverse plane, with prevailing right-dorsal $\leftrightarrow$ left-ventral flexions (Fig. 3E). This pattern strongly differed from the right-ventral $\leftrightarrow$ left-dorsal flexions seen in the beginning of the turn (compare curves for frames 2 and 13 in Fig. 3E). The same pattern of flexions was observed in all cases when a lamprey was approaching the dorsal-side-up position. As a result, the plane of locomotor undulations was oblique

and deviated from the frontal plane in a direction opposite to that of the body rotation (Fig. 7Civ). Similarly to the beginning of the corrective roll movement, an animal could approach the dorsal-side-up position at any phase of the locomotor cycle.

Soon after the animal had restored the dorsal-side-up position and its rotation stopped, the body was straightened in the sagittal plane, except for a small ventral flexion in the tail part, as shown in frame 18 and corresponding curvature plots (Fig. 3). This flexion pattern was observed at the end of the corrective movement in all cases, except for six cases when an animal changed the swim trajectory and started a lateral, up or down turn. This pattern was similar to that observed during undisturbed swimming (Fig. 2).

The corrective roll movements were quick and took  $0.43 \pm 0.22$  s (mean  $\pm$  s.d.), or  $86 \pm 17\%$  of the duration of one locomotor cycle ( $0.50 \pm 0.10$  s). Usually, while performing the corrective roll turn, the animal moved forward and covered a distance approximately equal to its body length. In four episodes, however, the roll turn was performed by an animal swimming against a wall of the aquarium, that is, without forward progression. In these cases, the body shape, sequence

and timing of the events were practically the same as during normal roll turn.

It is unlikely that the corrective roll movements were caused by movements of the ventrally bent tail as suggested earlier (Ullén et al., 1995a). The tail was bent almost as often dorsally or ventrally at the beginning of the roll movements (33 against 43 cases, correspondingly), as well as during the deceleration period (41 against 37 cases). We also did not observe any twisting of the body, at least when it was positioned with its lateral or dorsal side facing upwards, and such twisting would have easily been noticed.

*Swimming of intact lampreys with a destabilizing load*

A load attached to the back of an animal produced a moment of force rotating the animal towards the dorsal-side-down position. Inactive animals with a load, when released into water, turned from the dorsal-side-up to the ventral-side-up position within approximately 1 s. During swimming, the animals with a load were often able to maintain the normal dorsal-side-up orientation. However, the load hampered the animals' corrective attempts considerably. They needed more efforts to perform this behavioural task and sometimes failed for short time periods. Specifically, the animals could roll alternatively from right to left during the dorsal-side-up swimming, or swim with a constant tilt to the left or right, or in the worst case, swim with the ventral side up.

The most interesting situation was swimming with a constant 90° tilt to the right or to the left. We observed 37 such episodes in six animals, each lasted for 0.24–0.88 s ( $0.48 \pm 0.19$  s, mean  $\pm$  s.d.). One of the episodes – swimming with the left side up – is presented in Fig. 4. It lasted for 0.32 s, and was preceded and followed by swimming with the normal dorsal-side-up orientation. As the angle of the roll tilt did not change during this episode, the destabilizing moment of force produced by the load must have been balanced by the moment of force produced by the swimming body movements. Let us consider these movements. During the entire episode (that is in all eight frames), the flexions in the frontal and sagittal planes formed an oblique pattern in the transverse plane with a prevailing right-dorsal  $\leftrightarrow$  left-ventral

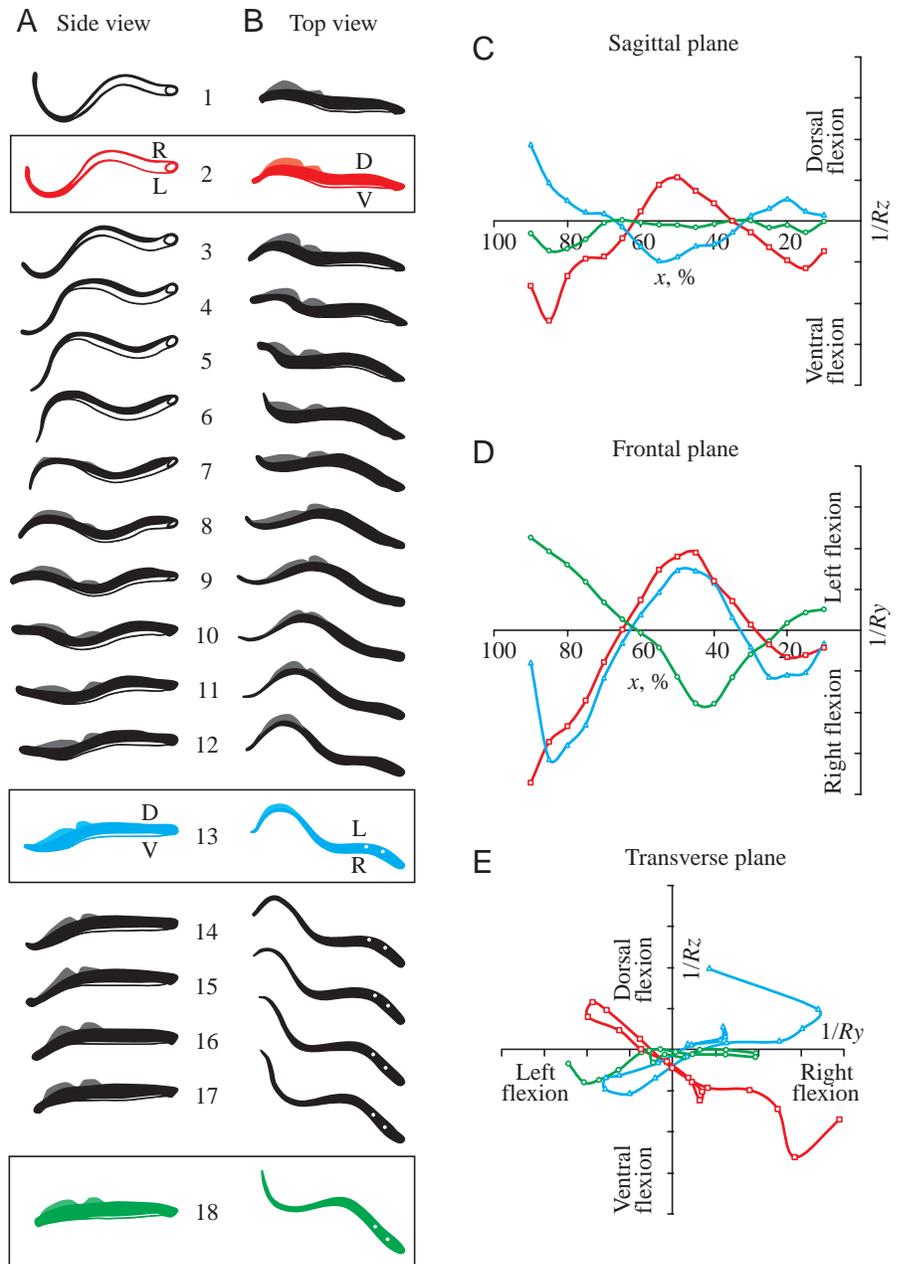


Fig. 3. Corrective roll turn performed by an intact animal. (A,B) The side and top views for 18 consecutive frames. (C–E) Curvature plots for frames 2 (red), 13 (blue) and 17 (green) are analogous to those in Fig. 2. In frame 2, the animal is positioned with its right side up and starts rolling to the right. In frame 13, the animal has almost reached the dorsal-side-up position. In frame 18, the animal is positioned with its dorsal side up and its body is straightened in the sagittal plane; this shape is similar to that during normal swimming. Note that in E all data points for frame 2 are situated in right ventral and left dorsal quadrants and that most of the data points for frame 13 are located in left ventral and right dorsal quadrants. On the other hand most of the data points for frame 18 are located close to the abscissa, as seen during undisturbed swimming. R, right; L, left; D, dorsal; V, ventral sides of the body. White dots in frames 13–18 are the white markers attached to the skin along the dorsal midline.

flexion, as illustrated for frames 2, 5 and 8 (Fig. 4C). This oblique pattern was similar to that observed in intact animals just before the beginning of the left roll corrective movement.

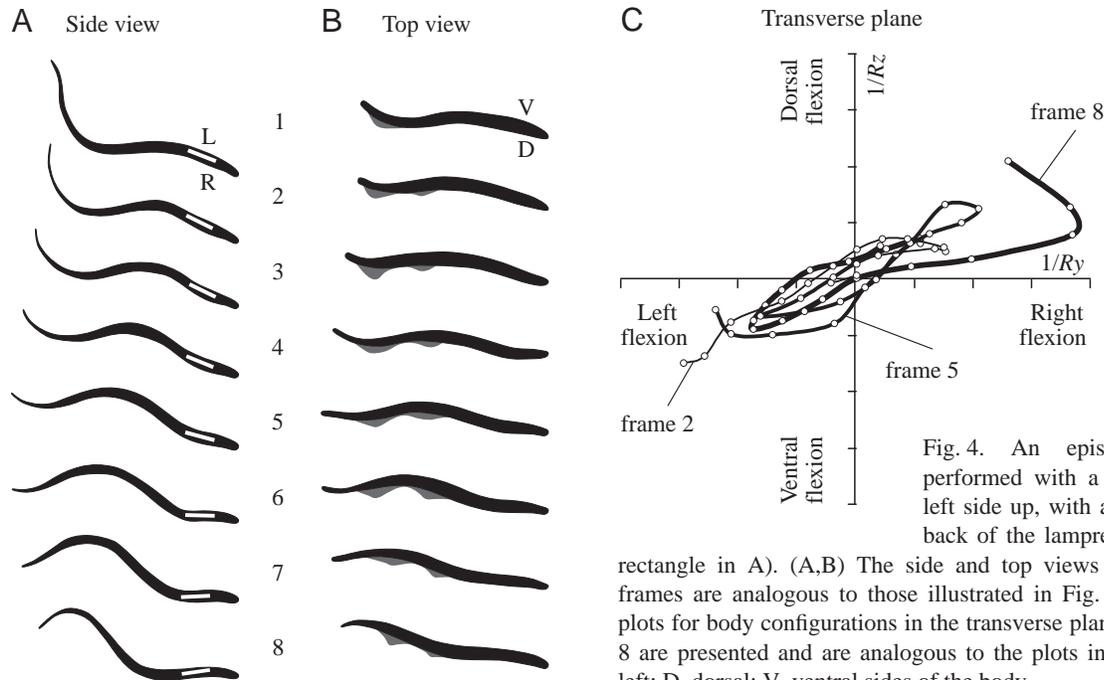


Fig. 4. An episode of swimming performed with a stable orientation of left side up, with a load attached to the back of the lamprey (shown as a white rectangle in A). (A,B) The side and top views in eight consecutive frames are analogous to those illustrated in Fig. 2. (C) The curvature plots for body configurations in the transverse plane for frames 2, 5 and 8 are presented and are analogous to the plots in Fig. 3E. R, right; L, left; D, dorsal; V, ventral sides of the body.

In all 37 cases of 'lateral-side-up' swimming with a load, the plane of flexions remained constant throughout the episodes. It was right-dorsal $\leftrightarrow$ left-ventral when the lamprey swam with its left side up (21 cases). A similar pattern was observed in the beginning of the left corrective roll turn performed by intact lampreys without a load. Correspondingly, the plane of flexions was left-dorsal $\leftrightarrow$ right-ventral when the 'loaded' lamprey swam with its right side up (16 cases). A similar plane was observed in the beginning of the right corrective roll turn (Fig. 3).

#### *Restoration of the dorsal-side-up orientation by spinalized lampreys*

Lampreys, spinalized rostrally to the first or the second dorsal fin, were able to perform a corrective roll turn with the pattern of body flexion similar to that observed in intact animals. Fig. 5 shows an example of restoration of the dorsal-side-up orientation. Initially, the animal was positioned with its left side up (frame 1). The rostral part of the body was straightened in the sagittal plane. Immediately afterwards, the body started rolling to the left, as judged from the movement of the dorsal midline marker (frames 2–5). This roll occurred along with an increasing dorsal flexion combined with a right flexion in the rostral part of the body. These combined flexions are seen in frame 4 and in the corresponding curvature plot (Fig. 5C, solid line). In frame 8, the dorsal-side-up position had almost been reached (Fig. 5A,B, frame 8). At this moment, left-dorsal $\leftrightarrow$ right-ventral flexions were seen in the rostral part of the body (Fig. 5D, solid line) instead of the right dorsal ones seen in the beginning of the movement. Soon afterwards, dorso-ventral flexions disappeared, and the animal swam rectilinearly with its dorsal

side up and with only a minor dorso-ventral flexion (Fig. 5A,B,E, frame 12).

Thus, in the above example, the shape of the rostral part of the body of a spinalized lamprey performing a corrective roll turn was similar to that of an intact lamprey. This relates to the beginning, middle and end of the turn. The same was true for the majority of corrective roll movements (67 of all 71 episodes in six spinalized animals). The entire corrective roll movements took  $0.51 \pm 0.20$  s, or  $96 \pm 25\%$  of the locomotor cycle duration ( $0.53 \pm 0.24$  s).

Contrary to the rostral part, the flexions in the frontal and sagittal planes were not always coordinated in the part of the body caudal to the site of spinalization. This is seen, for example, in Fig. 5C–E (broken lines). Since the caudal part of the spinal cord was separated from the head, these flexions probably resulted from the passive spread of undulatory movements from the rostral to the caudal part of the body.

In contrast to the intact lampreys, spinalized lampreys often twisted their body in the gill region during the corrective roll turn. The twisting was directed to the side of the turn at its beginning, and to the opposite side at its end. Twisting was more often observed in the animals with the spinalization rostral to dorsal fins (15 of 19 cases) than those with the spinalization between dorsal fins (7 of 13 cases). We estimate the maximal angle of twisting to be approximately  $30^\circ$ .

#### *Roll turns in lampreys with hemisection of the spinal cord*

When swimming, lampreys subjected to a hemisection of the spinal cord either remained in an abnormal position or were rolling continuously. This is illustrated in Fig. 6 for an animal with left hemisection in the rostral spinal cord (arrow in frame 1). In the beginning (Fig. 6A,B, frame 1), the animal was

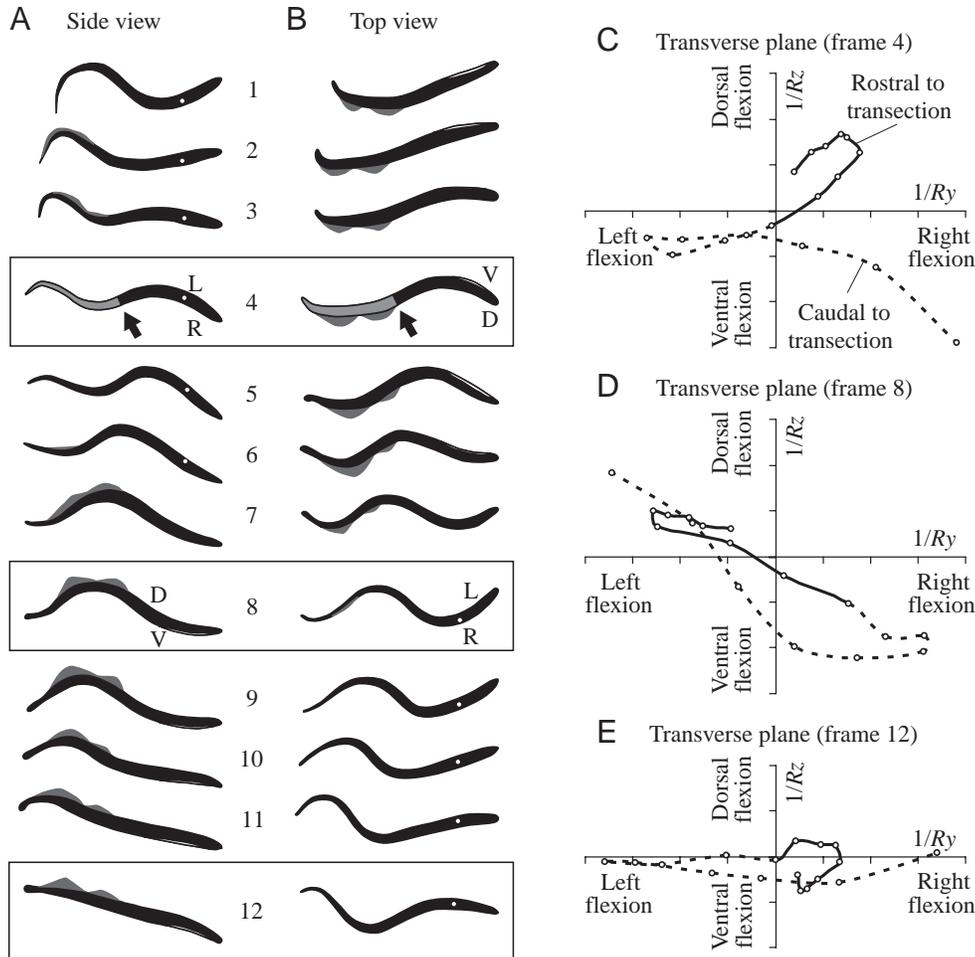


Fig. 5. An example of corrective roll turn performed by a spinalized animal. (A,B) The side and top views in twelve consecutive frames are analogous to those in Fig. 2. (C–E) The curvature plots in the transverse plane for frames 4, 8 and 12; they are analogous to frames 3, 13 and 18 in Fig. 3: rolling accelerates, rolling decelerates, body is straightened. In frame 4, the level of spinalization is indicated with arrows; the part of the body caudal to the spinalization site is shown in grey. The curvature graph for the body part rostral to the spinalization level is shown with solid lines, while that for the part caudal to the spinalization is shown with broken lines. R, right; L, left; D, dorsal; V, ventral sides of the body. White dots in A and B are the white markers attached to the skin along the dorsal midline.

positioned with its ventral side up. After 1.2 s (Fig. 6A,B, frame 30) it reached the left-side-up position and after that it did not roll further. This 270° roll turn was performed in three consecutive stages; the beginning and the end of each stage are designated as ‘start’ and ‘stop’ in Fig. 6. Between frames 1 and 9, the animal rolled from the ventral-side-up to the right-side-up position. Between frames 12 and 19, the animal rolled from the right-side-up to the dorsal-side-up position. Finally, between frames 25 and 30, the animal rolled from the dorsal-side-up to the left-side-up position. At the beginning of each consecutive turn (frames 1, 12 and 25), the body was flexed both in the frontal and sagittal plane, attaining the oblique pattern of flexions (left-dorsal↔right-ventral, Fig. 6C, red lines) similar to that observed in intact animals just before the beginning of the right roll (Fig. 3E, frame 2). When rolling decelerated and stopped (frames 9 and 19), the body flexions occurred along a direction different to that seen in the beginning of each small turn (right-dorsal↔left-ventral, Fig. 6E, blue lines). A similar body shape was observed when an intact animal reached the dorsal-side-up position (Fig. 3E, frame 13). Between the periods when the animal was turning, the roll angle did not change. Instead, transformation of the body shape took place (Fig. 6A,B, periods between frames 9 and 12, as well as between frames 19 and 25). The pattern of coordination between lateral and dorso-ventral flexions gradually changed

from the right-dorsal↔left-ventral flexions to the left-dorsal↔right-ventral flexions. At the moment the animal had reached the left-side-up orientation, it started a left turn in the horizontal plane because of the ventral body flexion. After completing the turn, it continued to swim with its left side up, with a body shape similar to that observed during dorsal-side-up swimming of the intact lamprey, i.e. with lateral locomotor undulations and only minor flexions in the sagittal plane, which were not correlated with the lateral flexions (not illustrated).

Such rolling directed to the side contralateral to the hemisection was observed in all tests (139 episodes in 6 animals). Most often (96 out of 139 cases), the rolling stopped near a position with the lesioned side facing upwards and this position persisted for at least several seconds. The position was quasi-stable: if the tilt to the right increased the animal did not reduce the tilt angle but instead rolled to the right all the way around to reach the quasi-stable position from the left. This quasi-stable angle varied between approximately 45° tilt to the right and 90° tilt to the right. In the remaining cases (43 out of 139 episodes), an animal performed one or more full roll turns before attaching to the aquarium wall or bottom. Both the continuous rolling and the roll turns towards the quasi-stable position were always performed in steps: turn – stop – turn – stop etc., as in the case presented in Fig. 6. The number of the stops and their angles could vary widely among different episodes.

## Discussion

### *Kinematics of roll turn in the lamprey*

In the present study we have found that a lamprey, positioned on its lateral side and then released, rapidly (in approximately 0.5 s) assumes the normal, dorsal-side-up orientation. This postural corrective roll turn includes an initial phase when the

body rotation starts and accelerates, and a final phase when the rotation decelerates and stops. Evidently, the forces responsible for the turn are generated in these two phases.

Our main result is that the postural corrections are incorporated into the locomotor undulatory pattern, which is modified in a characteristic way. In the absence of postural

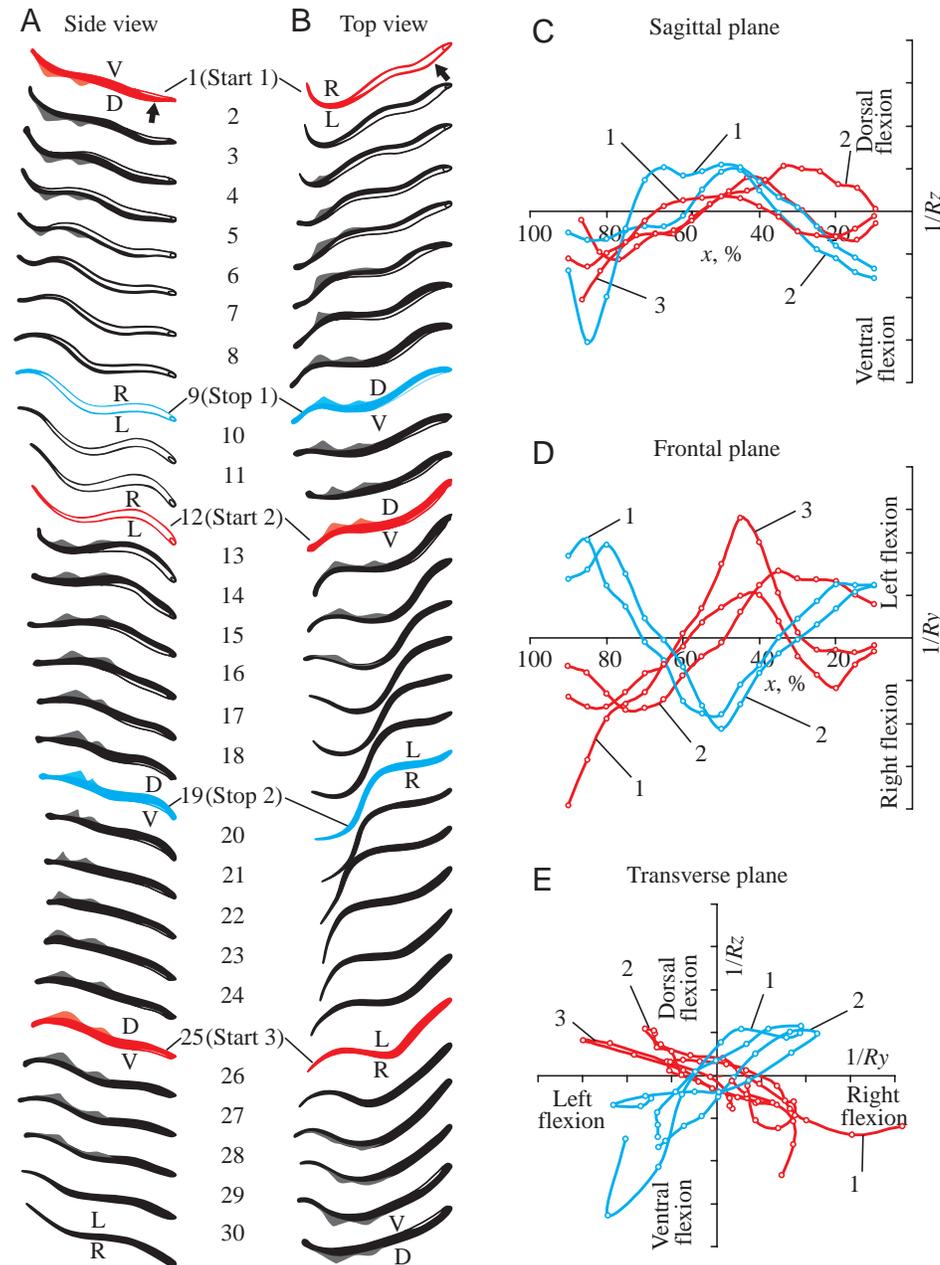


Fig. 6. An example of roll turn performed by a lamprey with left hemisection of the spinal cord. (A,B) Side and top views for 30 consecutive frames. The level of hemisection is indicated in frame 1 (arrows). (C-E) The curvature plots for selected frames analogous to those used in Fig. 2. The roll turn is performed in steps. In frames 1, 12 and 25, the animal starts rolling to the right (start 1, 2 and 3). In frames 9, 19 the rolling decelerates (stop 1, 2). Data for the start moments are shown in red, and for the stop moments in blue. Note that in E most of the data points for the start moments are located in right ventral and left dorsal quadrants, and most of those for stop moments are located in left ventral and right dorsal quadrants, i.e. similar to Fig. 3E. R, right; L, left; D, dorsal; V, ventral sides of the body.

corrections, the locomotor undulations occur only in the frontal plane of the animal (Fig. 2). In the initial and final phases of the roll turn, however, the plane of the body flexions is deviated to the side of the corrective turn or to the opposite side, respectively (Fig. 3). Upon termination of the turn, the locomotor undulations again occur in the frontal plane. These deviations of the plane of body flexions were associated with postural corrections not only in intact animals, but also in spinalized animals, in which they were limited to the body region rostral to the lesion (Fig. 5). In lampreys with a hemisectioned spinal cord, similar patterns of flexions were also observed at each 'step' of rolling (Fig. 6). Since the same type of corrections occur under these three conditions, it is suggested that these patterns of body flexions are responsible for the generation of the moments of force necessary for the roll turns. The hypothesis that the oblique plane of locomotor undulations causes a moment of force rotating the body in the transverse plane is directly supported by the experiments in which lampreys swam with a destabilizing load (Fig. 4).

It is not quite clear, however, how the oblique body undulations produce a moment of force. The most likely hypothesis is that this happens because of the non-circular shape of the transverse section of the lamprey's body. If the shape was a circle, the effect of lateral and oblique body flexions would not differ. In fact the body is slightly flattened (ellipsoidal). That is why during lateral flexions, the body cross-section moves through water along the short axis of the ellipse, whereas during oblique flexions there is an angle between the short axis and the velocity vector. If one supposes that the viscous forces tend to orient an ellipse perpendicular

to a water flow then the oblique movements will cause the moment of force rotating the animal in the transverse plane. This question awaits an appropriate hydrodynamic analysis.

Several mechanisms for the generation of roll turns have been suggested, for example ‘elliptical’ body movements, body twisting, deflections of the dorsal fins, and asymmetrical right–left movements of the tail if bent ventrally (Ullén et al., 1995a; Ekeberg and Grillner, 1999). In the present experiments, ‘elliptical’ movements were never observed. The spinalized lampreys, although unable to use their dorsal fins and tail, could still perform roll turns, thus the fins are not required. In contrast to intact animals, spinalized lampreys did often display body twisting, but were nevertheless able to perform the roll turn without any noticeable twisting. Finally, in our experiments, the tail was not consistently bent dorsally or ventrally during the roll turn, so the mechanism of the asymmetric tail beats seems not to be employed. We cannot rule out the possibility, however, that some of these mechanisms are used for postural correction in response to minor disturbances. Although these mechanisms do not seem to be of prime importance, they may serve as complementary ones.

*Possible nervous mechanisms of roll turns in the lamprey*

Body flexions in the lamprey are caused by contractions of the dorsal and/or ventral parts of a myotome on one or two sides, each part being innervated by a separate motoneuron pool (Tretjakoff, 1927; Rovainen, 1979; Wallén et al., 1985; Wannier et al., 1998). During normal horizontal swimming, motoneurons innervating the dorsal and ventral parts of myotomes on the same side are co-activated by the spinal locomotor network that leads to body flexions in the frontal plane (Grillner et al., 1995). However, independent control of dorsal and ventral parts of myotomes permits deviation of the plane of body flexion.

The reticulospinal (RS) system is primarily responsible for mediating postural corrections. Individual RS neurons have very selective effects and can enhance, reduce, or produce no effect on the output of the motoneurons innervating any one of the four myotome quadrants (Zelenin et al., 2001). Moreover, RS neurons exert the same effect on the segmental motor output along the whole extent of their axons, which can reach the most caudal level. The effects of an RS neuron upon different quadrants are combined in different patterns. Some of the neurons produce symmetrical effects. For example, an RS neuron can excite the ventral motoneuron pools on two sides, while another can inhibit dorsal and ventral pools on one side. Such neurons, when active, will flex the body in the sagittal or frontal plane, respectively. Many RS neurons, however, produce asymmetrical effects. For example, they may excite the ventral motoneuron pools on one side and inhibit the ventral motoneuron pools on the other side. These RS neurons, when active, will affect the motor output so that the plane of locomotor undulations will be inclined.

Tilting the lamprey from the dorsal-side-up position to the right or to the left leads to activation of many RS neurons on the side contralateral to the tilt, due to vestibular input

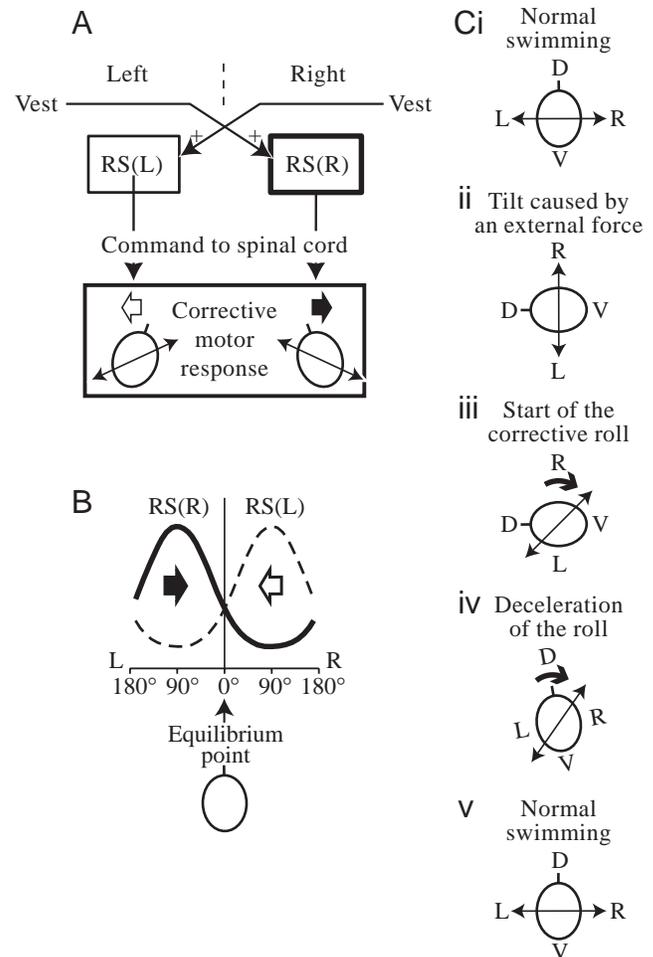


Fig. 7. Conceptual model of the roll postural system. (A) Right and left groups of reticulospinal (RS) neurons, RS(R) and RS(L), receive inputs from the labyrinths (La); they affect the spinal networks to evoke oblique body flexions (double-headed arrows) that lead to rolling of the lamprey. (B) Operation of the system. The curves represent the activity in RS(R) (thick line) and RS(L) (thin line) as a function of roll angle. Vestibular input causes activation of RS(R) and RS(L) with the contralateral tilt. Directions of rolling caused by RS(R) and RS(L) are indicated by the black and white arrows, respectively. The system has an equilibrium point at 0° (dorsal-side-up orientation). (C) Sequence of events during restoration of normal orientation by the lamprey, following an imposed 90° tilt to the left (see text for details). Thick arrows in iii and iv indicate the direction of the roll turn. R, right; L, left; D, dorsal; V, ventral sides of the body.

(Deliagina et al., 1992a,b; Deliagina and Fagerstedt, 2000; Zelenin et al., 2000). These results suggest that the activated RS neurons produce the postural corrections, i.e. the rotation of the animal in the direction opposite to the initial tilt (Deliagina, 1997; Deliagina et al., 1993). A conceptual model of the neuronal roll control system, based on this interpretation, is shown in Fig. 7A. Due to vestibular input, the activity of the left and right subgroups of RS neurons, RS(L) and RS(R), is position-dependent with a peak at approximately 90° of contralateral roll tilt (Fig. 7B), and motion-dependent, being excited by contralateral roll movement and inhibited by the

ipsilateral roll (not illustrated). Each subgroup of RS neurons is presumed to induce ipsilateral roll turns of the lamprey *via* spinal mechanisms (Fig. 7A,B, black and white arrows). This system stabilizes the orientation of the body in space with equal activation of the two subgroups (equilibrium point in Fig. 7B).

The data obtained in the present study and in the study of descending influences of individual RS neurons (Zelenin et al., 2001) allows us to refine the hypothesis for how the RS neurons elicit a roll turn. We suggest that subgroups RS(L) and RS(R) consist of (or include) neurons with asymmetrical spinal patterns, as described above. When activated, these neurons cause the plane of locomotor undulations to incline to the left or to the right, correspondingly (double-headed arrows indicate the plane of the undulations in Fig. 7A). Such undulations initiate and terminate the corrective roll.

The sequence of events occurring during a postural correction is schematically shown in Fig. 7C. Initially the lamprey swims normally, with its dorsal side up (Ci). In this position, the activities of RS(L) and RS(R) are equal to each other, and there is no deviation of the plane of locomotor undulations taking place in the frontal plane. When the lamprey is tilted to the left by an external force (Cii), the RS(R) group is activated, which causes deviation of the plane of undulations to the right, and the corrective turn of the animal to the right begins (Ciii). Rotation of the animal to the right dynamically inhibits the RS(R) group and excites the RS(L) group (Deliagina and Fagerstedt, 2000). When activated, RS(L) neurons cause deviation of the plane of undulations to the left and deceleration of turning (Civ) until it stops. This occurs at the normal position (Cv), where the activities of RS(L) and RS(R) again become equal to each other, and the locomotor undulations then proceed in the frontal plane.

In the initial version of the model it was suggested that the RS neurons causing roll to the left and to the right are located on the corresponding side of the brainstem. Since most RS neurons project to the spinal cord ipsilaterally, one could expect that a rostral hemisection of the spinal cord would lead to the abolition of the commands for roll to the side of the hemisection and that the animal would roll continuously to the opposite side. This prediction of the model has been confirmed only in part: the animal was rolling, but the roll was periodically interrupted (Fig. 6). A possible explanation for this finding is that RS neurons with opposite effects on rolling are present in both left and right populations, although the neurons eliciting the ipsilateral roll dominate.

We thank Dr Smitt for critical review of the manuscript. This work was supported by the Swedish Research Council (NT), the Swedish Medical Research Council (Grant no. 11554), Royal Swedish Academy of Sciences, and Gösta Fraenckels Foundation.

## References

- Blake, R. W. (1983). *Fish Locomotion*. Cambridge: Cambridge University Press.
- Brodin, L., Grillner, S., Dubuc, R., Ohta, Y., Kasicki, S. and Hokfelt, T. (1988). Reticulospinal neurons in lamprey – transmitters, synaptic interactions and their role during locomotion. *Arc. Ital. Biol.* **126**, 317-345.
- Bussi eres, N. (1994). Les Systemes Descendants chez la Lamproie. Etude Anatomique et Fonctionnelle. Montreal: University of Montreal.
- de Burlet, H. M. and Versteegh, C. (1930).  ber Bau und Funktion des Petromyzonlabyrinthes. *Acta oto-laryngol.* (Suppl.) **13**, 5-58.
- Deliagina, T. G., Orlovsky, G. N., Grillner, S. and Wall n, P. (1992a). Vestibular control of swimming in lamprey. 2. Characteristics of spatial sensitivity of reticulospinal neurons. *Exp. Brain Res.* **90**, 489-498.
- Deliagina, T. G., Orlovsky, G. N., Grillner, S. and Wall n, P. (1992b). Vestibular control of swimming in lamprey. 3. Activity of vestibular afferents – convergence of vestibular inputs on reticulospinal neurons. *Exp. Brain Res.* **90**, 499-507.
- Deliagina, T. G., Grillner, S., Orlovsky, G. N. and Ull n, F. (1993). Visual input affects the response to roll in reticulospinal neurons of the lamprey. *Exp. Brain Res.* **95**, 421-428.
- Deliagina, T. G. (1997). Vestibular compensation in lampreys: Impairment and recovery of equilibrium control during locomotion. *J. Exp. Biol.* **200**, 1459-1471.
- Deliagina, T. G. and Fagerstedt, P. (2000). Responses of reticulospinal neurons in intact lamprey to vestibular and visual inputs. *J. Neurophysiol.* **83**, 864-878.
- Deliagina, T., Zelenin, P., Fagerstedt, P., Grillner, S. and Orlovsky, G. (2000). Activity of reticulospinal neurons during locomotion in the freely behaving lamprey. *J. Neurophysiol.* **83**, 853-863.
- Deliagina, T. G., Zelenin, P. V. and Orlovsky, G. N. (2002). Encoding and decoding of reticulospinal commands. *Brain Res. Rev.* **40**, 166-177.
- Ekeberg,  . and Grillner, S. (1999). Simulations of neuromuscular control in lamprey swimming. *Phil. Trans. R. Soc. Lond. B* **354**, 895-902.
- Gray, J. (1968). *Animal locomotion*. London: Weinfeld & Nicolson.
- Grillner, S., Deliagina, T., Ekeberg,  ., el Manira, A., Hill, R. H., Lansner, A., Orlovsky, G. N. and Wall n, P. (1995). Neural networks controlling locomotion and body orientation in lamprey. *Trends Neurosci.* **18**, 270-279.
- Grillner, S., Cangiano, L., Hu, G. Y., Thompson, R., Hill, R. and Wall n, P. (2000). The intrinsic function of a motor system – from ion channels to networks and behavior. *Brain Res.* **886**, 224-236.
- Nieuwenhuys, R. (1972). Topological analysis of the brain stem of the lamprey *Lampetra fluviatilis*. *J. Comp. Neurol.* **145**, 165-177.
- Orlovsky, G. N., Deliagina, T. G. and Wall n, P. (1992). Vestibular control of swimming in lamprey. I. Responses of reticulospinal neurons to roll and pitch. *Exp. Brain Res.* **90**, 479-488.
- Pavlova, E. L. and Deliagina, T. G. (2002). Responses of reticulospinal neurons in intact lamprey to pitch tilt. *J. Neurophysiol.* **88**, 1136-1146.
- Rovainen, C. M. (1967). Physiological and anatomical studies on large neurons of central nervous system of the sea lamprey (*Petromyzon marinus*). I. Muller and Mautner cells. *J. Neurophysiol.* **30**, 1000-1023.
- Rovainen, C. M. (1979). Electrophysiology of vestibulospinal and vestibulo-reticulo-spinal systems in lampreys. *J. Neurophysiol.* **42**, 745-766.
- Timerick, S. J. B., Paul, D. H. and Roberts, B. L. (1990). Dynamic characteristics of vestibular-driven compensatory fin movements of the dogfish. *Brain Res.* **516**, 318-321.
- Tretjakoff, D. (1927). Das nervensystem des flussnevnages. *Z. Wiss. Zool.* **129**, 359-452.
- Ull n, F., Deliagina, T. G., Orlovsky, G. N. and Grillner, S. (1995a). Spatial orientation in the lamprey. I. Control of pitch and roll. *J. Exp. Biol.* **198**, 665-673.
- Ull n, F., Deliagina, T. G., Orlovsky, G. N. and Grillner, S. (1995b). Spatial orientation in the lamprey. II. Visual influence on orientation during locomotion and in the attached state. *J. Exp. Biol.* **198**, 675-681.
- Wall n, P., Grillner, S., Feldman, J. L. and Bergelt, S. (1985). Dorsal and ventral myotome motoneurons and their input during fictive locomotion in lamprey. *J. Neurosci.* **5**, 654-661.
- Wannier, T., Deliagina, T. G., Orlovsky, G. N. and Grillner, S. (1998). Differential effects of reticulospinal system on locomotion in lamprey. *J. Neurophysiol.* **80**, 103-112.
- Zelenin, P. V., Deliagina, T. G., Grillner, S. and Orlovsky, G. N. (2000). Postural control in the lamprey: A study with a neuro-mechanical model. *J. Neurophysiol.* **84**, 2880-2887.
- Zelenin, P. V., Grillner, S., Orlovsky, G. N. and Deliagina, T. G. (2001). Heterogeneity of the population of command neurons in the lamprey. *J. Neurosci.* **21**, 7793-7803.