The Journal of Experimental Biology **198**, 2581–2591 (1995)
Printed in Great Britain © The Company of Biologists Limited 1995
JEB0028

INITIATION OF LOCOMOTION BY LATERAL LINE PHOTORECEPTORS IN LAMPREY: BEHAVIOURAL AND NEUROPHYSIOLOGICAL STUDIES

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Accepted 30 August 1995

Summary

The lateral line system of lampreys includes photoreceptors distributed in the skin of the tail region. These are innervated by the trunk lateral line nerves, and the afferents terminate bilaterally in the medial octavolateral nucleus, crossing the midline through the cerebellar commissure. Stimulation of the dermal photoreceptors by tail illumination initiates locomotion. The present study was performed to characterize the response to illumination in larval and adult lampreys in detail and to elucidate the neuronal pathways responsible for the activation of locomotion. In both larval and adult quiescent lampreys, the response to unilateral illumination of the tail was found to consist of an initial turn followed by rectilinear swimming. The sign and magnitude of the turning angle were not correlated with the laterality of the optic stimulus. In mechanically restrained lampreys, spinalized at the level of segments 15–20, tail illumination evoked a complex motor response in the rostral part of the body, with switches between different patterns of coordination (turns in different directions, locomotion, and turns combined with locomotion). Thus, the response to tail illumination is not a simple reflex, but includes a behavioural choice.

Reticulospinal neurones play a crucial role in the initiation of locomotion in lampreys. The response to unilateral tail illumination in rhombencephalic reticular cells was studied with extracellular single-unit recordings. It was found that neurones in the middle and posterior rhombencephalic reticular nuclei were activated bilaterally. Tonic activity or slow bursts (<0.5 Hz) were evoked, in some cases lasting up to 60 s after the stimulation. The response remained bilateral after transection of one lateral line nerve and the cerebellar commissure. Afferents from one side can thus activate reticulospinal cells on both sides through a pathway outside the cerebellar commissure. This bilateral activation of reticulospinal neurones is presumably responsible for the activation of spinal locomotor networks, without any directional bias to the left or the right side, and for the rectilinear swimming observed in behavioural experiments.

In the caudal part of the termination area of the lateral line nerve afferents, neurones with contralateral projections were retrogradely stained with horseradish peroxidase. These neurones appear to be likely candidates for mediating the contralateral effects of the lateral line fibres.

Key words: lamprey, locomotion, initiation, behavioural choice, brainstem, reticulospinal neurones, medial octavolateral nucleus, lateral line, extraretinal photosensitivity, dermal light sense, *Lampetra fluviatilis*, *Petromyzon marinus*.

Introduction

Both larval and adult lampreys possess a dermal light sense confined to the tail region (Parker, 1905; Young, 1935). The photosensitive elements are a part of the lateral line system, which in lampreys subserves at least three distinct modalities: photoreception, mechanoreception and electroreception (Akoev and Muraveiko, 1984; Bodznick and Northcutt, 1981; Young, 1935; Ronan and Bodznick, 1991).

The skin photoreceptor cells have not been identified with certainty, but pigment-containing, multivillous cells in the epidermis of the tail skin are plausible candidates (Steven, 1950, 1951; Whitear and Lane, 1981, 1983). Photosensitive fibres are carried in the trunk lateral line nerves (Young, 1935; Ronan and Bodznick, 1991; Ullén *et al.* 1993) and enter the brain, together with mechanosensitive afferents from the body,

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through the posterior lateral line nerves. Afferents from one nerve terminate bilaterally in the medial octavolateral nucleus by crossing through the cerebellar commissure (Ronan and Bodznick, 1991).

Illumination of the tail region evokes locomotion in both larval and adult lampreys. In response to unilateral illumination of the tail, the animal either swims forwards or performs left or right turns with equal probability (Ullén *et al.* 1993, 1995*b*). Accordingly, the response to tail illumination has usually been considered to be a simple example of photokinesis, in contrast to the negative phototaxis evoked by unilateral illumination of the eye (Ullén *et al.* 1993, 1995*b*). Functionally, photokinesis in the lamprey has been interpreted as a way to make the animal move away from brightly illuminated areas, where the risk of discovery is high (Young, 1935; Ullén *et al.* 1993). For burrowing larvae, the sensitivity of the tail to light could help to ensure that movements continue until the whole body is covered with sand (Young, 1935).

The aim of the behavioural part of the present study was to characterize quantitatively the time course and the trajectory of the locomotor response to unilateral tail illumination in larval and adult lampreys. This study showed that the trajectory of swimming consists of two, essentially different, parts. The initial, short part is not linear: the animal deflects to the right or to the left from the starting position. The direction of this initial turn does not depend on which side was illuminated. After completing the initial turn (in 0.3–0.5 s), the animal swims along an almost linear trajectory for a relatively long period (typically several seconds). Thus, the response to tail illumination is not a simple reflex, but includes a behavioural choice; that is, a random choice of the sign and magnitude of the angle of the initial turn. To study this problem further, we restrained the lamprey mechanically in such a manner that the animal could not avoid a long-lasting illumination of the tail. Under these conditions, the lamprey exhibited a wide repertoire of responses, with spontaneous switches between different patterns of motor coordination, such as left and right turns, upward and downward turns, straight locomotion and locomotion combined with different turns.

In the second, electrophysiological, part of this study we addressed the problem of how an asymmetrical sensory stimulus, unilateral illumination of the tail, can evoke a response requiring symmetrical body movements, that is rectilinear swimming. Commands from the brainstem to the spinal cord are, in the lamprey, mainly transmitted by the reticulospinal (RS) system (Rovainen, 1967; Wickelgren, 1977; Brodin *et al.* 1988). The RS system is formed by neurones of four reticular nuclei (see Fig. 1C), the mesencephalic reticular nucleus (MRN) and the anterior, middle and posterior rhombencephalic nuclei (ARRN, MRRN and PRRN). In relation to locomotion, the RS system has a double function: (i) activation of the spinal locomotor mechanisms (McClellan and Grillner, 1984; Kasicki and Grillner, 1986; Ohta and Grillner, 1989) and (ii) modulation of the locomotor pattern, which is necessary for the control of posture and steering (Deliagina *et al.* 1992, 1993). To determine at which level the transformation from a unilateral to a bilateral signal takes place, responses to tail illumination in RS neurones of the different rhombencephalic reticular nuclei were recorded, both in the intact brain and after specific lesions. It was found that neurones from the MRRN and PRRN respond bilaterally to a unilateral stimulus, which implies that the transformation of a unilateral signal to bilateral activity of the RS system takes place at a pre-reticular level.

In the third, anatomical, part of this study, contralaterally projecting neurones, located in the termination area of the posterior lateral line nerve afferents (the medial octavolateral nucleus, MON), were retrogradely stained with horseradish peroxidase (HRP). These cells probably participate in the transformation of a unilateral input to bilateral activity in the RS system.

Materials and methods

Animals

Experiments were performed on larval sea lampreys (*Petromyzon marinus* L.; body length 8–15 cm), obtained from the watershed of Connecticut River, Massachusetts, USA (provided by Acme Lamprey Company), and adult river lampreys (*Lampetra fluviatilis* L., body length 25–35 cm) caught in Söderhamn, Sweden. The animals were maintained in aerated aquaria, with a 12 h:12 h light:dark cycle (white fluorescent illumination from above between 08:00 h and 20:00 h). Experiments were performed during the day or in the evening (10:00–20:00 h). All surgical procedures were performed under anaesthesia (MS-222, Sandoz).

Behavioural studies

The response to tail illumination in intact larval and adult lampreys was recorded in a shallow aquarium (80 cm×80 cm×12 cm deep). Adult lampreys usually spontaneously assumed a dorsal-side-up orientation, attached to the bottom with their sucker mouths. The tail of the resting animal was illuminated from the right or the left side with an optic guide (diameter 3 mm; 90 W white light) kept at a distance of 1–2 cm, so that a circular area of the skin (approximately 25–100 mm^2) was brightly illuminated. The stimulation was maintained until locomotion was evoked (0.4–6.5 s; see Results). The locomotor response was recorded with a video camera (25 frames s^{-1}), positioned above the aquarium. Weak, white incandescent light (20 W lamp) was used as background illumination. Each animal was tested repeatedly, with 1 min of adaption in complete darkness between the tests. The video recordings were analyzed frame by frame. In some experiments, electromyograms (EMGs) were recorded from rostral and caudal myotomes using bilateral bipolar wire electrodes (diameter 100 μm).

Quiescent larval lampreys could assume different positions on the bottom, lying with the dorsal, ventral, left or right side down. The animals were tested only when lying on the lateral

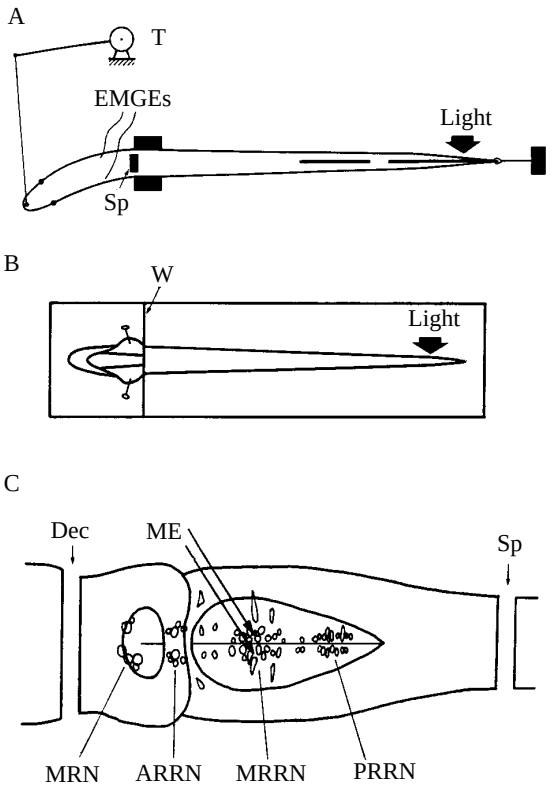

Fig. 1. Experimental arrangements. (A) Study of the response to tail illumination in the restrained spinalized animal. Black rectangles, mechanical restraints to prevent movements of the middle and posterior parts of the body; Sp, site of spinalization; T, mechanical transducer; EMGEs, electromyographical electrodes (viewed from above). (B) Study of the response in reticulospinal (RS) neurones. The semi-intact preparation was positioned in the experimental chamber, which was divided into two compartments by a wall (W). The brain was exposed and the tail was illuminated with an optic guide (Light). (C) A diagram of the brainstem of the lamprey with the levels of decerebration (Dec) and spinalization (Sp) indicated. MRN, mesencephalic reticular nucleus; ARRN, MRRN and PRRN, anterior, middle and posterior rhombencephalic reticular nuclei; ME, two microelectrodes located symmetrically in the reticular formation.

side. Illumination of the tail was performed alternately from above and from below, through the transparent glass bottom of the aquarium, and the motor response was video-recorded.

The response to tail illumination was also studied in mechanically restrained adult lampreys. To prevent the struggling movements characteristic of restrained intact lampreys, in these experiments the lamprey was spinalized just caudal to the gills, that is at the level of spinal segments 15–20, while the lateral line nerves were left intact. The experimental arrangement is shown in Fig. 1A. Movements of the middle and posterior parts of the body were prevented by the special restraints. The tail was illuminated with a 3 mm optic guide, in the same way as in the experiments on intact animals (see above). Movements of the anterior part of the body were evoked. The lateral component of these movements was recorded with a mechanical transducer. EMGs were recorded bilaterally, in some cases unilaterally, from the dorsal and ventral parts of a myotome.

Electrophysiological studies

The neuronal pathway mediating activation of locomotion by tail photoreceptor stimulation in larval lamprey was studied in a semi-intact preparation (Fig. 1B,C). The eyes and the pineal organ of the anaesthetized lamprey were removed, the brain was exposed, the animal was spinalized at the level of the second spinal segment and decerebrated (Fig. 1C). The preparation was attached with thin tungsten pins in the experimental chamber (Fig. 1B). The chamber was divided into two compartments by a plastic wall, sealed with agar. The compartment with the exposed nervous system was perfused with Ringer's solution, whereas the compartment with the intact tail part contained fresh water. A special cooling system maintained the temperature in both compartments at 10 °C. The preparation was adapted to darkness for 1–2 h. Subsequently, the tail part was illuminated unilaterally with an optic guide for 5–30 s, and the response of reticulospinal (RS) neurones was recorded extracellularly with two platinum microelectrodes following Orlovsky *et al.* (1992). Responses in the three rhombencephalic reticular nuclei (ARRN, MRRN and PRRN; Fig. 1C) were studied systematically. In the larval lamprey, large reticular neurones are clearly visible with the dissecting microscope and can be used as landmarks. Usually, two microelectrodes were positioned symmetrically, in the left and right subdivisions of a given nucleus, under visual control (Fig. 1C). The recordings were performed at different rostro-caudal levels. Besides the optical stimulation, mechanical stimulation of the tail, by pinching with forceps, was sometimes applied. In some experiments, one of the posterior lateral line nerves and the cerebellar commissure were transected.

Anatomical studies

To identify neurones which could mediate the effects of the lateral line input upon the contralateral subdivision of the RS system, two types of staining with horseradish peroxidase (HRP, Serva) were used. First, afferents of the posterior lateral line nerve (PLLN) were labelled by applying HRP to the stump of the transected PLLN, to determine the area of termination of PLLN afferents in the brainstem. Second, contralaterally projecting neurones, located in the area of termination of the PLLN, were identified. For this purpose, small crystals of HRP were applied with an insect pin (size 000) to different sites along the rhombencephalic medial reticular formation (see Fig. 8A). In both types of staining, the isolated brainstem preparation was used. After HRP application, the preparations were kept for 30–48 h in Ringer's solution at 4 °C, and subsequently fixed by immersion for 2 h in 1 % glutaraldehyde and 1 % paraformaldehyde in 0.1 mol l^{-1} phosphate buffer (pH 7.3). The preparations were processed by a modified, cobalt–nickel-intensified, diaminobenzidine method (Bourrat and Sotelo, 1986), dehydrated in acetone, embedded in Spurr's resin, and subsequently sectioned into 30 μm thick transverse

sections on a sliding microtome, after warming the block surface (West, 1972). The sections were mounted in epoxy resin and reconstructed using *camera lucida* techniques.

Results

Behavioural characterization of the response to tail illumination in larval lamprey

Forty-four responses were recorded in four animals, lying on one side of the body. In 22 cases, illumination was from above and in the other 22 cases from below. Tail illumination in all cases evoked swimming. Latencies varied between 0.4 and 6.5 s, with a mean value of 1.5±1.0 s (S.D.). Fig. 2A shows the trajectory of a typical response (the position of the head in each frame is indicated with black dots). Fig. 2B shows nine head trajectories, drawn as lines, obtained in successive tests of the same animal. Since the larva, in contrast to the adult lamprey, normally lies on its side, locomotion usually started with a righting movement, in which the head and the anterior part of the body also moved laterally, causing a deflection of the longitudinal axis of the animal from its initial position. During this righting manoeuvre, the animal always assumed the characteristic dorsal-side-up orientation. In Fig. 2A, the arrow indicates the moment and position at which this postural correction was accomplished. The initial turn was usually completed within 0.3–0.5 s, following which the animal swam along an approximately linear trajectory for a period of up to several seconds. The speed of swimming ranged from 8.5 to 12.5 cm s^{-1}, and the frequency of lateral body undulations from 1.5 to 2.5 Hz. As shown in Fig. 2B, the direction of swimming varied considerably between tests. To characterize these variations, the angle in the horizontal plane between the linear part of the trajectory and the body axis of the animal in the initial position was measured ('turning angle', Fig. 2A). Two distributions of the turning angles are shown in Fig. 2C,D for the cases with illumination from above (Fig. 2C) and from below (Fig. 2D). A positive sign for the turning angle denotes a turn towards the 'downward side', i.e. the body side on which the animal was lying in the initial position. Most turns were directed towards the 'downward side', in response to illumination both from above and from below. The bias of turning angles towards the 'downward side' was probably due to the asymmetry in sensory input, in particular tactile input, when the animal was lying on its side (see Discussion). Turning angles ranged from −30 to 60°, with a mean value of 11±20° (±S.D.). The mean of the absolute value of the turning angles was 17±16° (±S.D.), that is most turns had a relatively small amplitude. No significant difference was found between the distribution of turning angles with illumination from above and below (P<0.6, t-test). Thus, the laterality of the optic stimulus did not affect the distribution of the turning angles.

The relationship between the locomotor response to tail illumination in larval lampreys and burrowing behaviour was investigated after introducing a layer of sand (5 cm deep) onto the bottom of the aquarium. Quiescent larvae lying on the sandy bottom were illuminated from above. In all tests (N=50, four animals), tail illumination evoked swimming, and burrowing into the sand occurred only after several cycles of normal locomotion.

Behavioural characterization of the response to tail illumination in adult lamprey

In all tests (N=48; three animals), illumination of the tail evoked detachment and swimming. Latencies varied between

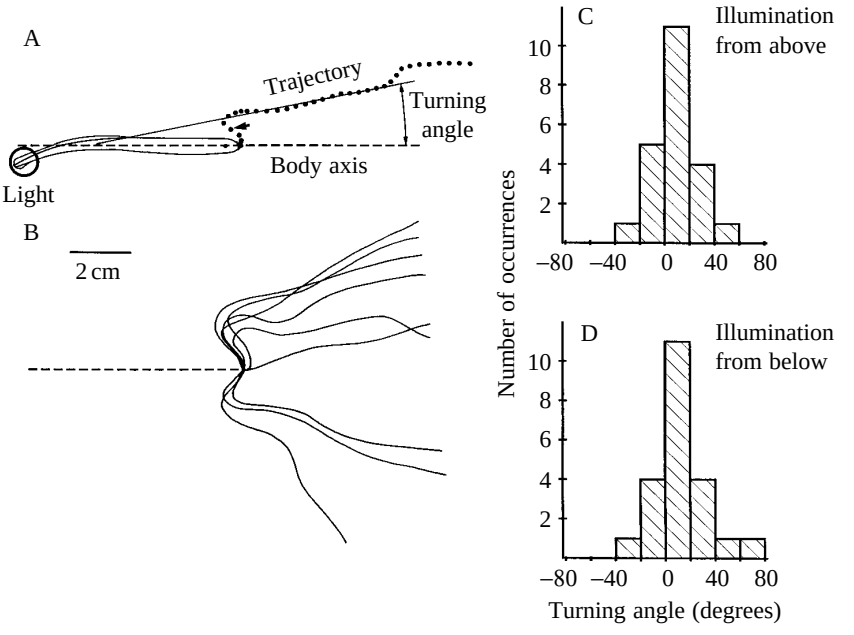

Fig. 2. Behavioural characterization of the response to tail illumination in larval lampreys. (A) A quiescent animal (viewed from above), lying with its left side down, responded with locomotion to illumination of the tail with an optic guide (Light). The trajectory is shown with black dots, indicating successive positions of the head (interval between dots is 40 ms). The turning angle of the response was defined as the angle between the trajectory of swimming and the body axis of the animal in the initial position, with a positive sign indicating a turn towards the side on which the animal was lying in the initial position. The arrow indicates the head position at which the animal assumed a dorsal-side-up orientation. (B) Trajectories of the same animal obtained in nine successive trials. (C,D) Distribution of turning angles (44 tests in four animals) with illumination from above (C) and below (D). The animal was initially resting with the left or right side of the body downwards. Most turns were directed towards the body side on which the animal was lying at the initial position. The mean turning angles (±S.D.) were 9.5±20.0° in C and 12.6±20.9° in D. The distributions of turning angles in C and D were not significantly different (P<0.06; t-test).

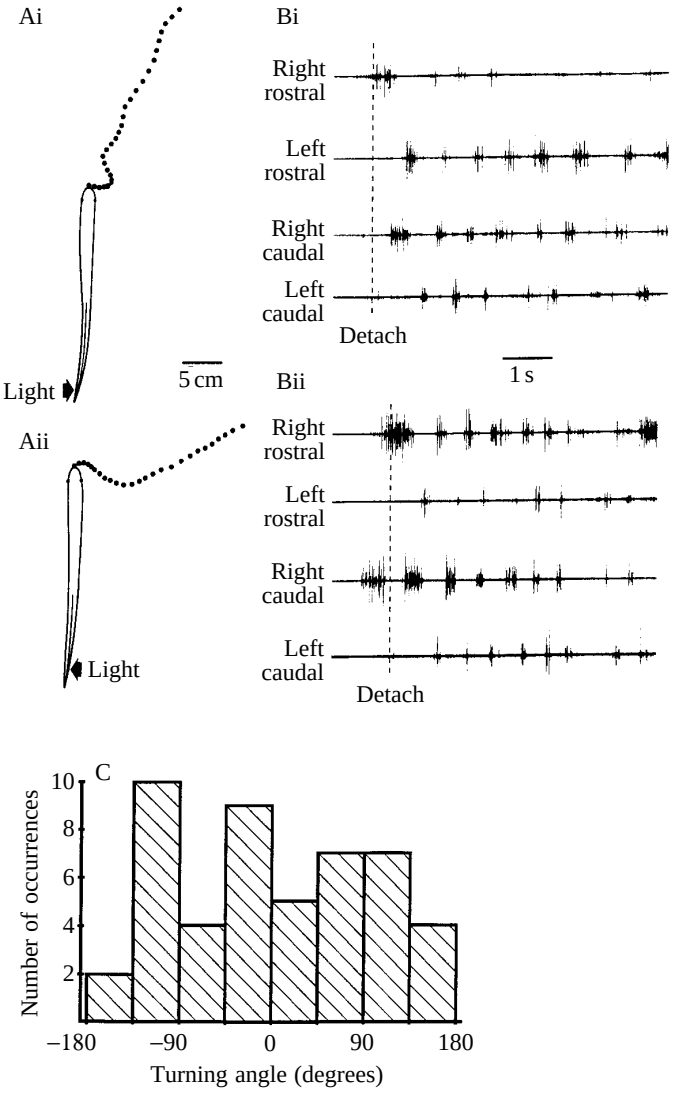

Fig. 3. Behavioural characterization of the response to tail illumination in adult lampreys. (Ai,ii) Two responses (head trajectories, 40 ms between dots) in a quiescent animal lying with the ventral side down, attached to the bottom of the aquarium with its sucker mouth. (Bi,ii) EMGs recorded bilaterally from the rostral and caudal parts of the body in the responses shown in Ai and Aii respectively. The moment of detachment from the bottom is indicated. (C) Distribution of turning angles (48 tests in three animals; a positive turning angle indicates a turn towards the illuminated side). The mean turning angle was 5.9±93 ° (±S.D.).

1.1 and 9.2 s, with a mean value of 3.8±2.2 s (±S.D.). As in larvae, the initial part of the trajectory was curved, while the rest of it was rectilinear (Fig. 3Ai,Aii). The turning angle varied considerably between tests, as illustrated in Fig. 3A,C. Stronger turns tended to be associated with a larger and longer initial EMG burst on the body side towards which the animal was turning (Fig. 3Bi,Bii). In 75 % of the cases, the EMG activity initially appeared in the caudal myotomes, on either side of the body, evoking a tail flexion which preceded detachment and locomotion (Fig. 3Bii). After the initial turn, normal locomotor EMG activity, with alternation between the left and right sides, and with a delay between the EMG bursts in rostral and caudal myotomes was observed (Fig. 3Bi,Bii). The distribution of turning angles is shown in Fig. 3C. Positive turning angles denote turns towards the illuminated side. Turning angles ranged between −160 and 170 °, with a mean value of 5.9±93 ° (S.D.). This range is much wider than that for the larvae (compare Fig. 2C,D); the mean of the absolute value of the turning angles was 80±47 ° (17±16 ° in larvae). No significant difference was found between the distributions of turning angles for the turns to the illuminated and to the contralateral side (P<0.4, t-test; Fig. 3C).

Motor responses to tail illumination in restrained animals

To be able to study the different the patterns evoked in the rostral part of the body, animals were spinalized immediately caudal to the gills (N=18), and the middle and caudal parts of the body were mechanically restrained (Fig. 1A). A continuous illumination of the tail (right side) evoked, through the lateral line nerve, a complex motor response in the anterior part of the body, with switches between different patterns of motor activity. Three basic categories of behaviour were observed; they are illustrated in Fig. 4A,B.

(1) *Lateral bending of the head* to the right or to the left (‘Right turn’ and ‘Left turn’ in Fig. 4A). The turns were always accompanied by tonic co-activation of muscles on the left and right sides, but the EMG on the side of head bending had a larger amplitude than that on the contralateral side.

(2) *Lateral rhythmical oscillations of the head* at a frequency of 1–2 Hz, with symmetrical left/right deflections from the body midline. In intact, unrestrained animals this pattern will presumably correspond to straight forward locomotion (‘Locomotion’ in Fig. 4B). This pattern was accompanied by alternating EMG bursts on the left and right sides. In addition to these alternating large bursts, smaller bursts often occurred between the main bursts (mixed bursting – such small bursts are indicated by arrows in Fig. 4B, on the right).

(3) *Lateral bending of the head, with rhythmical lateral oscillations (1–2 Hz) superimposed upon it.* In intact, unrestrained animals this pattern will presumably correspond to turns during swimming. Such a pattern is illustrated in Fig. 4B (‘Right turn and locomotion’). It was accompanied by synchronous rhythmical bursts in the left and right EMGs, but the amplitude of bursts on the side of the tonic head bending was larger than on the contralateral side. As in the case of forward locomotion, in addition to these main bursts, smaller bursts of EMG activity, occurring between the main bursts, were often seen (indicated by arrows in Fig. 4B, on the left).

Illumination of the tail in restrained, spinalized lampreys evoked not only movements of the head in the horizontal plane but also vertical head movements. These movements were not recorded, but EMG recordings from the dorsal and ventral myotomes revealed bouts of simultaneous tonic EMG activity, with either the dorsal or the ventral EMG prevailing (Fig. 4C). These bouts probably corresponded to dorsal and ventral bending of the anterior part of the body.

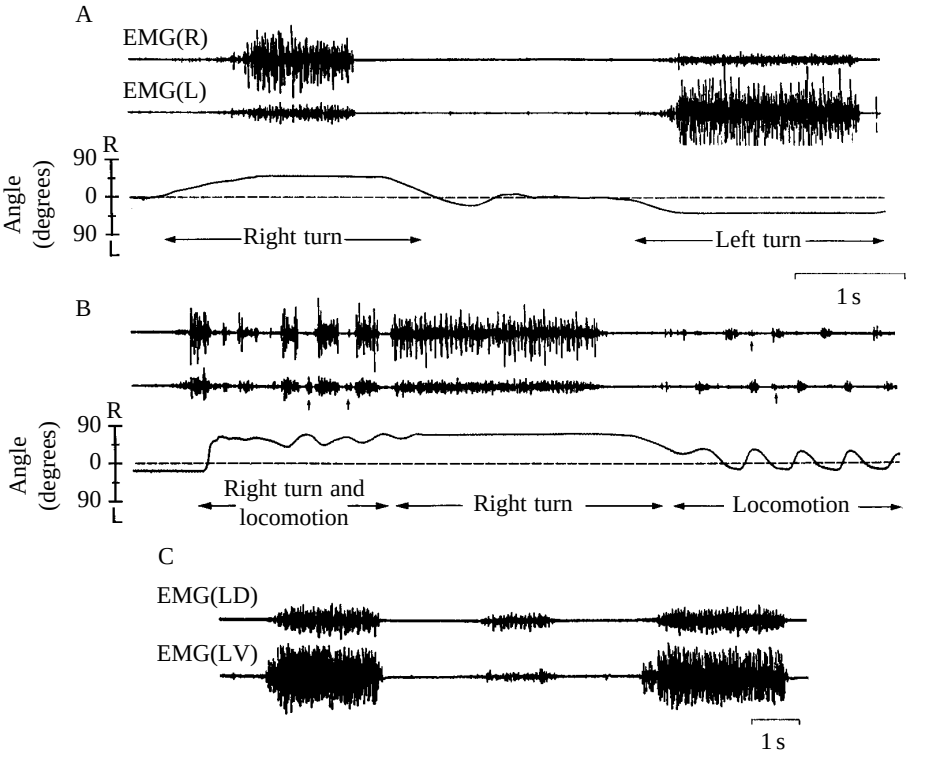

Fig. 4. Motor responses to continuous tail illumination in the spinal, restrained lamprey. (A,B) Different motor patterns appeared in succession during the tail illumination (right side). The two upper traces in A and B are EMG recordings from the right (R) and left (L) myotomes. The lower trace shows lateral head movements; the angle of lateral head bending relative to the fixed body was measured approximately with a transducer. Small arrows in B indicate additional EMG bursts between the main bursts. (C) Different patterns of EMG activity recorded from the dorsal and ventral myotomes on the left side (LD and LV).

With continuous tail illumination, spontaneous switches between different motor patterns were observed, as illustrated in Fig. 4A,B. In this particular case, a right turn was followed by a left turn (Fig. 4A). Subsequently, a right turn combined with locomotor activity occurred (Fig. 4B, left part). This pattern was followed by a motor pattern corresponding to a pure right turn (Fig. 4B, middle part), after which symmetrical locomotor activity occurred (Fig. 4B, right part). The duration of each particular motor pattern usually ranged from one to a few seconds. Fig. 4A,B also illustrates that switches between different patterns of motor activity appeared to occur randomly: the right turn in Fig. 4A was followed by a left turn, while the right turn in Fig. 4B was followed by the pattern for locomotion. The probability of transitions between different motor patterns is shown in Table 1. The total number of bouts, observed in all tests (N=34), for different motor patterns, and the estimated transition probabilities from one pattern to another are shown. The estimated transition probability, p_{AB}, for a pair of motor patterns, A and B, gives an approximate measure of the probability that a bout of pattern A would be immediately followed by a bout of pattern B, and was calculated as $p_{AB}=n_{AB}/n_A$, where n_{AB} denotes the number of observed transitions from pattern A to pattern B and n_A denotes the total number of observed bouts of pattern A (Haccou and Meelis, 1992). When a pattern occurred last in a recording, it was not counted; in this way, the sum of probabilities in each line in Table 1 will be 1. Tonic turns to the left or to the right were usually followed by a pause in the activity before a new motor pattern was initiated but, apart from this, no simple rules could be found for the sequence of motor patterns, which appeared unpredictable.

Table 1. *Transition matrix for the different patterns of motor activity evoked by tail illumination in restrained lampreys*

	Left turn	Right turn	Straight locomotion	Left turn and locomotion	Right turn and locomotion	Pause
Left turn (N=39)	–	0	0	0.13	0.13	0.74
Right turn (N=48)	0	–	0.08	0.08	0.05	0.79
Straight locomotion (N=4)	0.25	0.25	–	0	0	0.50
Left turn and locomotion (N=19)	0.21	0.43	0.05	–	0.05	0.26
Right turn and locomotion (N=20)	0.10	0.25	0.05	0.10	–	0.50
Pause (N=79)	0.37	0.35	0.04	0.13	0.11	–

The estimated transition probability, i.e. the empirically approximated probability that one pattern of motor activity will be followed immediately by another pattern, is indicated for all pairs of motor patterns.

The total number (N) of observed bouts of a given pattern is indicated on each line.

Responses of RS neurones to stimulation of the tail in larval lamprey

In animals adapted to darkness, illumination of the tail evoked activation of RS neurones in 11 out of 16 preparations. Only these experiments are considered further. Repeated stimulation before 5–10 min had passed could not usually evoke a full response.

Neurones in the MRRN (see Figs 1C, 8A) were activated most consistently and responded in repeated tests in all experiments. In all cases, a bilateral excitation was observed. A typical example of the responses recorded from two symmetrical points, in the left (L) and right (R) MRRN, is shown in Fig. 5A. Illumination of the right side of the tail elicited similar responses on both sides, with a latency of about 4 s. The latency differed slightly in different neurones recorded simultaneously by the same electrode. Some neurones stopped firing after 5–6 s, despite continuous stimulation of the tail. The response in other cells outlasted the stimulus. An additional example of a short-lasting response, with bilateral activation of MRRN neurones, is given in Fig. 6A. In some cases (approximately 30 %), stimulation of the tail evoked long-lasting responses, up to 60 s. In these responses, low-frequency rhythmical activity (approximately 0.2 Hz) was sometimes seen (Figs 6C, 7A).

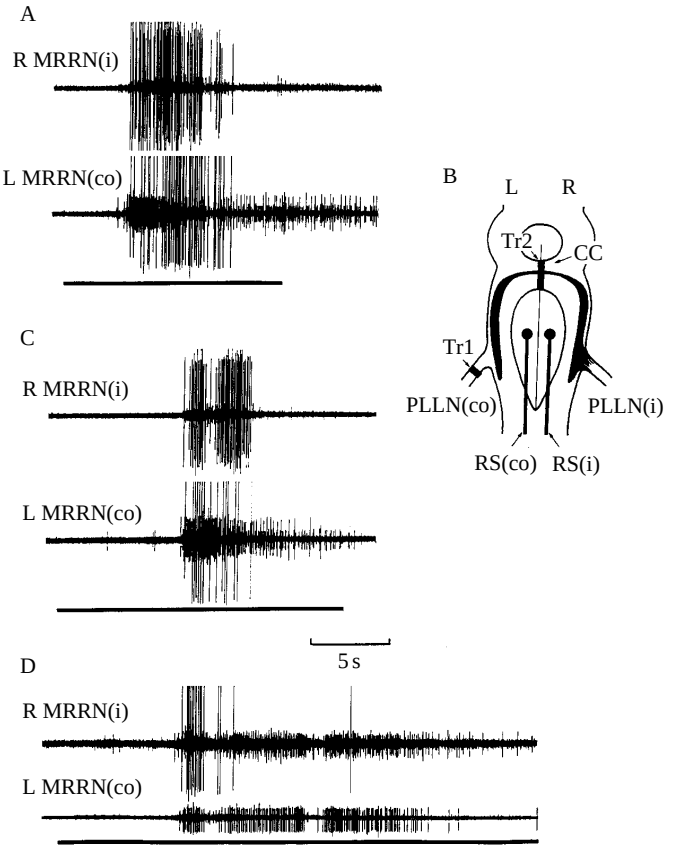

Fig. 5. Responses of lamprey reticulospinal neurones to tail illumination in the right (R; ipsilateral to stimulation, i) and left (L; contralateral, co) subdivisions of the MRRN. Application of the light stimulus is indicated by a bar below the traces. In all cases, the right side of the tail was illuminated. (A) Responses in the intact brainstem. (B) Scheme of the brainstem and the lesions employed. PLLN, posterior lateral line nerves, ipsilateral to the illuminated side of the tail (i) and contralateral to it (co); RS, reticulospinal neurones; CC, cerebellar commissure; Tr1, transection of the PLLN(co); Tr2, transection of the cerebellar commissure. (C) The response after lesion Tr1. (D) The response after lesions Tr1 and Tr2. (A, C and D are from the same experiment).

Illumination of one side of the tail will probably also activate photoreceptors on the contralateral side of the tail to some extent, as a result of the dispersion and reflection of light and because the tail of the larva is very thin and partially transparent. Each trunk lateral line nerve appears to innervate only ipsilateral receptor cells (Steven, 1951). To obtain a unilateral input from the photoreceptors, the posterior lateral line nerve on one side was transected, close to its entrance to the brainstem, in four experiments (Tr1 in Fig. 5B). In all cases, a bilateral response in the MRRN persisted (Figs 5C, 6C).

Some lateral line nerve fibres cross the midline through the cerebellar commissure and terminate on the contralateral side of the brainstem (Ronan and Bodznick, 1991). In two experiments, the cerebellar commissure was also transected (Tr2 in Fig. 5B). A bilateral response to tail illumination was still observed (Figs 5D, 6C). One can thus conclude that the contralateral response is evoked, at least partly, by the ipsilaterally projecting afferents of the lateral line nerve.

In three experiments, mechanical stimulation of the tail, by pinching with a pair of forceps, was performed while recording from the MRRN. This stimulus also evoked (through the lateral line nerve) a bilateral response in the MRRN (Fig. 6B), similar to that evoked by photostimulation (Fig. 6A). The response to mechanical stimulation persisted after transection of the posterior lateral line nerve and the cerebellar commissure (Fig. 6D), as did the response to photostimulation (Fig. 6C).

Neurones in the ARRN and PRRN also responded to photostimulation of the tail. Simultaneous recordings from the MRRN and PRRN (*N*=8) have shown that they were co-activated, as illustrated in Fig. 7A. The ARRN responded much less consistently than the MRRN and the PRRN. Fig. 7B shows a response in the ARRN, appearing simultaneously with that in the MRRN. The absence of a response in the ARRN is illustrated in Fig. 7C. Finally, Fig. 7D shows a case when a response in the ARRN appeared after the termination of activity in the MRRN.

Contralaterally projecting neurones in the medial octavolateral nucleus

The lesion experiments (Fig. 5) demonstrated that the contralateral projection of the posterior lateral line nerve (PLLN) afferents is not necessary for activation of the contralateral RS neurones. Projections of the PLLN in the brainstem were studied by HRP staining of PLLN fibres through the stump of the transected nerve (Fig. 8A, *N*=2). Stained fibres and terminals were found bilaterally in the medial and intermediate parts of the medial octavolateral

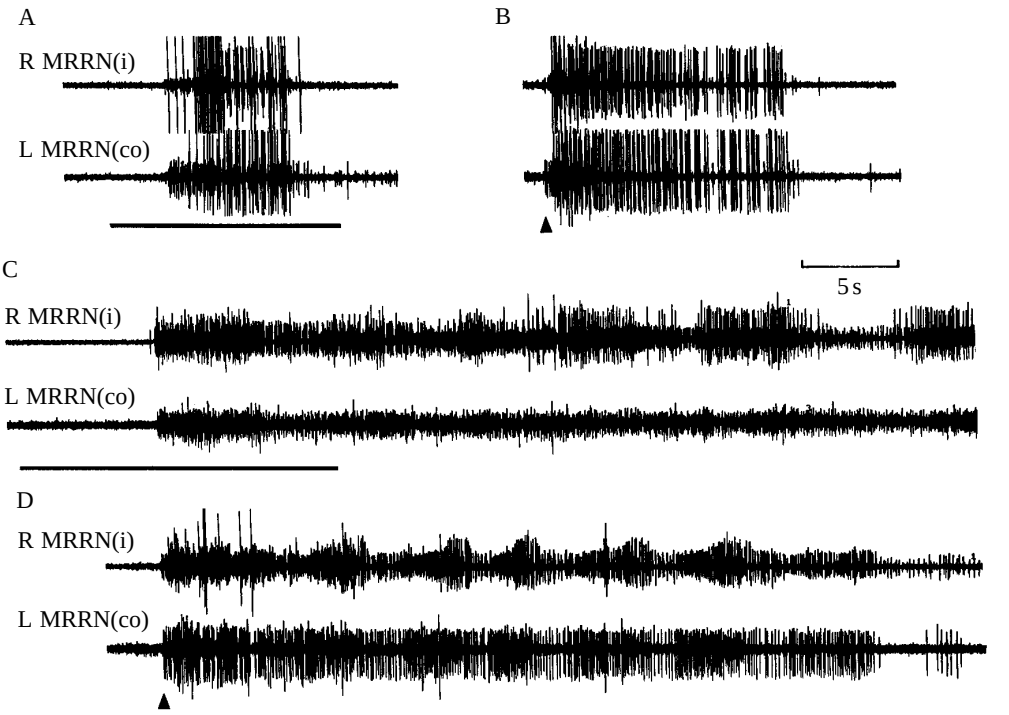

Fig. 6. Comparison of the responses of reticulospinal neurones to mechanical stimulation (pinching) and illumination of the right side of the tail, in the right (ipsilateral, i) and left (contralateral, co) subdivisions of the MRRN. Application of the light stimulus is indicated by a bar below the trace. Application of the mechanical stimulus is indicated by an arrowhead. (A,B) Responses to illumination (A) and pinching (B) in the same preparation. (C,D) Responses to illumination (C) and pinching (D) in a different preparation subjected to lesions Tr1 and Tr2 (see Fig. 5B).

nucleus (MON), located in the dorsolateral rhombencephalon, and in the cerebellar commissure (Fig. 8B,D). This confirms the findings of other investigators (Fritzsch *et al.* 1984; Koyama *et al.* 1990; Ronan and Northcutt, 1987; Ronan, 1988; Ronan and Bodznick, 1991). To investigate whether there are contralaterally projecting neurones in this area, HRP was injected into different sites of the contralateral rhombencephalic reticular formation (*N*=24) to fill commissural neurones retrogradely through their axons (eight injections in the ARRN, eight injections in the MRRN and eight injections in the PRRN; the typical sites of injection are shown in Fig. 8A). Injection of HRP in any part of the MRRN or PRRN on one side (points 3–7 in Fig. 8A) stained contralateral cell bodies in the region of the PLLN fibre terminals (Fig. 8C,E,F). Almost no crossing cells were found in the lateral part of the MON, which receives lateral line input from the head region (Ronan and Northcutt, 1987). Rostrocaudally, the commissural cells were located between the entrance of the PLLN and the root of the vagal nerve, but not in the rostral part of the MON (Fig. 8C). In contrast, HRP injection in the region of the ARRN (points 1 and 2 in Fig. 8A) did not stain cells in the MON. The diameter of the stained

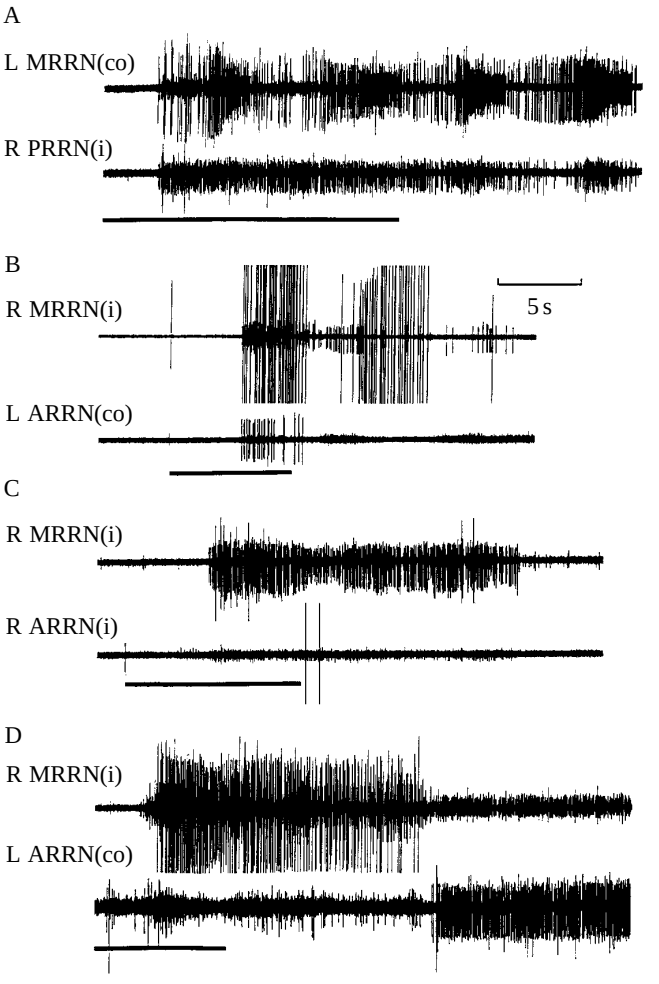

Fig. 7. Simultaneous recording of the response to tail illumination from different reticular nuclei. Application of the light stimulus is indicated by a bar below the trace. The right side was stimulated in all cases. (A) Recording from the left MRRN (contralateral, co) and the right PRRN (ipsilateral, i). (B–D) Recordings from the MRRN and ARRN. The different recordings are from different preparations. In A and D, stimulation was started 5 s before the onset of recording.

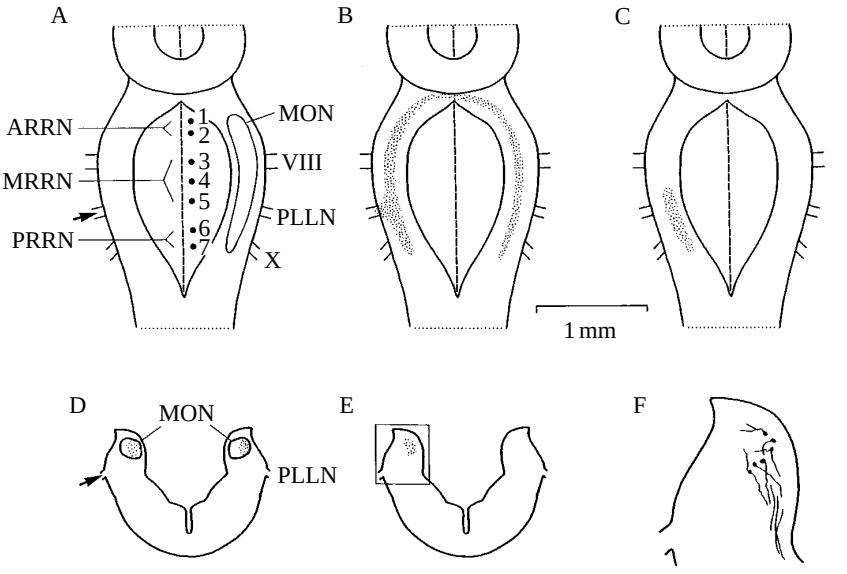

Fig. 8. Crossing neurones in the region of the medial octavolateral nucleus (MON), retrogradely stained with horseradish peroxidase (HRP). The approximate extent of the MON is indicated schematically in A (right side only) and D (both sides). (A–C) Schematic dorsal view of the medulla of a larval lamprey, with the sites of HRP application indicated in A. In different preparations, HRP was applied either to the posterior lateral line nerve (arrow in A and D; *N*=2), to label afferent terminals, or to one of seven different locations within the three rhombencephalic reticular nuclei (*N*=24), as indicated in A, to label commissural neurones within the termination area of the lateral line afferents. Afferents and terminals were found bilaterally in the MON, throughout its rostrocaudal extent, as shown by dots in B. Crossing neurones were only found in the caudal half of the nucleus, however, as indicated by dots in C. VIII, X, spinal nerves VIII and X; other abbreviations are explained in Fig. 1. (D) Schematic transverse section through the medulla, at the level of the entrance of the posterior lateral line nerve (PLLN), in a preparation where nerve afferents were labelled. The approximate extent of the MON is indicated. The locations of afferents and terminals are indicated by dots. (E) Transverse section at the level of the entrance of the PLLN, showing small crossing interneurones with cell bodies in the MON, labelled by HRP application to the contralateral posterior rhombencephalic reticular nucleus. (F) Transection through the medulla (the part shown in E, but presented at higher magnification), to show stained cells. The location of the cell bodies overlaps with the termination area of the posterior lateral line afferents (cf. D).

cells was very small (about 10 μm). These neurones are candidates for mediating the excitatory response in the contralateral RS neurones.

Discussion

Determination of the turning angle of the locomotor response

The present study has shown that the response to tail illumination in both larval and adult lampreys consists of two parts: (i) a brief initial turn, followed by (ii) rectilinear swimming. The distribution of turning angles was found to be completely independent of the laterality of the photostimulus (Figs 2C,D, 3C). This provides solid evidence to suggest that the tail photoreceptors mediate a photokinesis, that is activation of locomotion without any directional preference relative to the light source. In larval lampreys, which in the initial position were lying on their side, the distribution of turning angles was shifted towards the body side which was initially downwards, touching the bottom of the aquarium. This shift may be related to the strong thigmotactic behaviour of the larvae and may, therefore, be caused by the asymmetry of tactile inputs, owing to the unilateral contact of the body with the ground. Vestibular input will also be asymmetrical when the animal is lying on its side, but this would be expected to give body bending towards the contralateral (upward) side (Deliagina *et al.* 1992; Orlovsky *et al.* 1992). The adult lampreys, however, were initially attached to the bottom with their sucker mouths and thus oriented with their dorsal side up. In this situation, both vestibular and exteroceptive sensory inputs would be expected to be almost symmetrical. Nevertheless, these animals also exhibited both left and right turns in response to unilateral tail illumination, with a large variation in turning angle (Fig. 3C). One can therefore conclude that the motor response to tail illumination is not a simple reflex, but that it includes a behavioural choice: some parameters of the response are not determined by the stimulus, but rather they are randomly selected by the central nervous system. This is especially evident in the experiments on restrained lampreys. In these animals, stimulation of the tail evoked a complex response, with switches between different patterns of motor activity (Fig. 4; Table 1), as if different strategies were attempted sequentially to avoid tail illumination.

The EMG recordings revealed efferent patterns responsible for the different types of motor activity evoked by tail illumination in restrained animals. (i) Lateral turns were always performed with a co-activation of the left and right myotomes, with EMG activity on the side of the head deviation prevailing over contralateral EMG activity (Fig. 4A). The co-contraction of the left and right myotomes may serve to increase the stiffness of the body and to define a final degree of curvature. The neural mechanisms responsible for the generation of this pattern remain unknown. (ii) Symmetrical left/right oscillations (‘Locomotion’ in Fig. 4B) were accompanied by alternating EMG activity in the left and right myotomes. This type of activity is characteristic of rectilinear locomotion, and the spinal neuronal networks for generating this pattern have been analysed in considerable detail (Grillner *et al.* 1995). An essential component of the networks is the

reciprocal inhibition between the generator neurones in the left and right hemi-segments. This inhibition does not allow the hemi-segments to be active simultaneously but only in alternation. (iii) An essentially different efferent pattern, i.e. co-active bursting in the left and right sides, was observed when the locomotor undulations were superimposed on a lateral body flexion ('Right turn and locomotion', Fig. 4B). To generate this pattern, excitatory rather than inhibitory interactions between the networks in the hemi-segments are needed. The existence of such interactions has been demonstrated previously when the crossed inhibitory influences were partially blocked by strychnine: the alternating bursting switched to co-active bursting (Alford and Williams, 1989; Cohen and Harris-Warrick, 1983).

Activation of rectilinear locomotion

The swimming evoked by tail illumination was essentially rectilinear, except for the short initial turn (Figs 2A,B, 3Ai,Aii). During straight swimming, the spinal locomotor networks generate a bilaterally symmetrical motor pattern. For this to occur, the descending influences from the left and right subdivisions of the RS system must be equal. In electrophysiological recordings from RS neurones, it was found that even a unilateral photostimulus evoked a bilateral activation of the RS system (Fig. 5A). The bilateral response in some RS neurones is rather long, lasting up to 60 s, and it may considerably outlast the light stimulus, which corresponds well to the long-lasting period of swimming along a straight trajectory observed in the behavioural experiments.

A question of fundamental interest is where the unilateral sensory signal is transformed into a bilateral, long-lasting activity. One possibility would be that this occurs within the RS system itself. No interconnections between the left and right subdivisions of the RS system have been reported, however. Furthermore, it has been shown that some sensory inputs, such as vestibular and visual signals, can elicit a selective unilateral excitation of RS neurones, without exciting their contralateral partners (Deliagina *et al.* 1992, 1993; Orlovsky *et al.* 1992). The transformation from a unilateral to a bilateral signal therefore probably takes place at a pre-reticular level. Signals from photoreceptors and mechanoreceptors in the tail region arrive at the brainstem *via* the afferent fibres of the posterior lateral line nerve (PLLN), which has a bilateral projection in the brainstem (Fig. 8B). It is therefore possible that these bilateral projections of the afferents are necessary for a bilateral activation of the RS system by tail stimulation. However, a bilateral excitation of RS cells was also seen after transection of the contralaterally projecting afferents (Figs 5D, 6C). Neurones in the termination area of the PLLN afferents, that is the medial octavolateral nucleus (MON) (Ronan and Northcutt, 1987), seem likely candidates for mediating the bilateral activation of the RS system. It is possible that MON neurones receive the lateral line input and project further to both ipsilateral and contralateral RS neurones, thereby transforming the unilateral input into a bilateral signal, activating the RS system. The MON neurones could perform similar transformations of both photosensory and mechanosensory inputs, which both evoke similar bilateral and long-lasting activations of the RS neurones (Fig. 6). A crossing projection of MON neurones has been demonstrated in the present study by retrograde staining with horseradish peroxidase injected into the contralateral PRRN and MRRN (Fig. 8). In contrast, no projection from the MON to the ARRN was found in the present study.

Rectilinear swimming is the most common pattern of locomotion in the lamprey. It occurs in different behavioural contexts and can be evoked by different sensory stimuli (Ullén *et al.* 1995*a*,*b*). An alternative possibility for the formation of a symmetrical bilateral input to the RS neurones would be that this takes place in a higher centre, such as the mesencephalic locomotor region of higher vertebrates (Shik *et al.* 1966; Jordan, 1986), as recently reported for the lamprey by Sirota *et al.* (1994). In this case, the task of equalizing the excitatory inputs to the left and right subpopulations of RS neurones would be solved in a universal way by using the same group of neurones with bilateral projections to the RS neurones.

The spontaneous variation in turning angle in the initial part of the response to tail illumination (Figs 2B, 3Ai,Aii, 4) is a simple example of a behavioural choice, which may indicate that higher brain centres are involved in generating the response. From the recordings of the response in RS neurones, it is not possible, however, to determine whether an asymmetry, corresponding to the initial turn, is present in the RS activity. The difference in latency of individual RS neurones was about 1 s (Figs 5–7), which is comparable to the duration of the turn (0.3–0.5 s), that is the period of the presumed asymmetry in the reticulospinal influences.

We plan to address the problem concerning the site in the brain responsible for this behavioural choice by observing the response to tail illumination in animals with different lesions to the brain.

This study was supported by the Swedish Medical Research Council (grant no. 3026) and by a grant from the Spanish Ministry of Education (EX9236003383), by the Karolinska Institute Fund and by the Royal Swedish Academy of Sciences (Research Grant for Swedish–Russian scientific cooperation).

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