Differential Effects of the Reticulospinal System on Locomotion in Lamprey

T. WANNIER, T. G. DELIAGINA, G. N. ORLOVSKY, AND S. GRILLNER

The Nobel Institute for Neurophysiology, Department of Neuroscience, Karolinska Institute, S-17177 Stockholm, Sweden; and A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119 899, Russia

Wannier, T., T. G. Deliagina, G. N. Orlovsky, and S. Grillner. Differential effects of the reticulospinal system on locomotion in lamprey. J. Neurophysiol. 80: 103–112, 1998. Specific effects of stimulating different parts of the reticulospinal (RS) system on the spinal locomotor pattern are described in lamprey. In the in vitro brain stem and spinal cord preparation, microstimulation in different areas of the reticular formation was performed by ejecting a small amount of D-glutamate from a micropipette. These areas were distributed over the four reticular nuclei of the brain stem: the mesencephalic reticular nucleus (MRN) and the anterior, middle and posterior rhombencephalic reticular nuclei (ARRN, MRRN, and PRRN, respectively). To prevent synaptic spread of excitation within the brain stem, the synaptic transmission was blocked by using a low Ca$^{2+}$, high Mn$^{2+}$ physiological saline in the brain stem pool. “Fictive” locomotion was evoked by applying N-methyl-D-aspartate (NMDA) to the spinal cord. Rhythmic discharges of motoneurons were recorded bilaterally in the midbody area, from the ventral roots that had been subdivided in dorsal and ventral branches, supplying the dorsal and ventral part of the myotome, respectively. Two major effects of brain stem stimulation were elicited: a change in the frequency of the locomotory rhythm and an induction of asymmetry (left/right, dorsal/ventral) in the segmental motor output. Approximately 50% of the stimulated sites evoked a change in locomotor frequency. In the PRRN almost all effective sites evoked an increase in frequency (10–50%). In the other nuclei, increase and decrease (10–30%) were observed equally frequently. Most of the stimulated sites (50–80%) in any reticular nucleus evoked asymmetry in the segmental motor output. Distortion of the segmental output symmetry was classified into eight categories by comparing the intensity of locomotor bursts in the dorsal and ventral branches of the two ventral roots, ipsilateral and contralateral to the stimulated side. These categories differed in the direction of the body flexion, which would be evoked during normal swimming: ipsilateral (I), contralateral (C), dorsal (D), ventral (V), ipsilateral and dorsal (ID), ipsilateral and ventral (IV), contralateral and dorsal (CD), and contralateral and ventral (CV). The different categories were not equally represented in each nucleus and across the nuclei. The most pronounced categories for each nucleus were as follow. In MRN: I (33%); ARRN: C (44%); MRRN: rostral part, I (36%) and caudal part, CV (42%); and PRRN: rostral part, I (40%) and caudal part, IV (35%). Other categories were also present but less common in each nucleus. To examine if the effects of brain stem stimulation were uniform along the spinal cord, recordings were performed from distal parts of the cord. Stimulation of a given point in the brain stem produced similar pattern of effects in 59% of cases and different patterns in 41% of cases. The main conclusion of the present study is that the proportion of RS neurons with different influences on the spinal locomotor network differs significantly among different parts of the reticulospinal formation of the lamprey. The specificity of RS influences may represent a basis for modifications of the segmental locomotor output necessary for the control of equilibrium and steering during locomotion.

INTRODUCTION

The reticulospinal (RS) system plays a predominant role in the control of posture and locomotion in all vertebrates. This paper describes the specificity in organization of the RS projections in the lamprey, a phylogenetically ancient vertebrate. The lamprey is extensively used as a model system for studying the basic neural mechanisms controlling motor behavior (see Grillner et al. 1995; Wallén et al. 1992). In the lamprey the RS system is the main descending system projecting from the brain to all parts of the spinal cord. The vestibulospinal system is much less developed, projecting only to the anterior part of the spinal cord (Ronan 1989; Rovainen 1979). The RS system is formed by neurons (~2,500 cells) located in the following four reticular nuclei of the brain stem: the mesencephalic reticular nucleus (MRN) and the anterior, middle, and posterior rhombencephalic reticular nuclei (ARRN, MRRN, and PRRN, respectively) (Bussières 1994; Nieuwenhuys 1972; Ronan 1989; Swain et al. 1993).

A general characteristic of RS neurons is that they receive synaptic input from a number of different sources including the different brain stem centers, the cranial nerves, and the spinal cord (Deliagina et al. 1993; Di Prisco et al. 1995; Dubuc et al. 1993; Rovainen 1967, 1979; Wickelgren 1977). Although neurons in all nuclei may respond with a mixture of excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) to electrical stimulation of a variety of cranial nerves, a clear specificity can be revealed by using natural stimuli. In response to natural vestibular stimulation (tilt), most RS neurons exhibit both static and dynamic reactions in specific spatial zones (Deliagina et al. 1992a) because of input from specific groups of vestibular afferents (Deliagina et al. 1992b). Asymmetrical visual input (illumination of one eye or tonic electrical stimulation of the optic nerve) evokes excitation of ipsilateral and inhibition of contralateral RS neurons (Deliagina et al. 1993). Many RS neurons exhibit rhythmic modulation in response to “effence copy” signals arising from the spinal cord during locomotion (Kasicki et al. 1989; Vinay and Grillner 1992). RS neurons exert both excitatory and inhibitory action on different types of spinal neurons (Ohta and Grillner 1989; Rovainen 1974; Wannier et al. 1995).
Evidence strongly suggests a glutamatergic excitatory action of RS neurons on their targets throughout the whole extent of the spinal cord that results in activation of segmental locomotor networks and, therefore, initiation of swimming (Grillner et al. 1995; Ohta and Grillner 1989). Much less is known, however, about the specificity of RS influence on the spinal cord. Such a specificity is expected on the basis of behavioral studies. When swimming, the lamprey performs numerous maneuvers related to steering and equilibrium control. For example, the lamprey is able to perform flexions in limited or extended areas of the trunk, either in the sagittal or horizontal plane or in both planes simultaneously. It can also perform body twisting. The changes in body configuration are superimposed on undulatory locomotor movements (McClellan 1984; McClellan and Hagevik 1997; Ullén et al. 1995a,b). These observations suggest that the RS system of the lamprey does not only exert general effects on the spinal cord, leading to initiation and maintenance of locomotor activity, but also specific local effects, such as the induction of local body flexions in different planes. Rovainen (1967) was the first to demonstrate that large RS neurons (Müller cells) can evoke specific changes in the body shape in a quiescent animal.

The goal of the present study was to investigate the specificity of the influences of the RS system on the spinal network when it generates locomotor activity. For this purpose we used the isolated brain stem–spinal cord preparation. N-methyl-d-aspartate (NMDA) was applied to the spinal cord to evoke “fictive” locomotion (Grillner et al. 1981). Small groups of RS neurons in the brain stem were excited by local applications of d-glutamate (d-Glu), and their effects on the segmental motor output were studied. The main focus was the MRRN and PRRN that contain the vast majority of RS neurons (Bussières 1994).

The present study shows that activation of small groups of RS neurons in different parts of the brain stem reticular formation elicits a variety of specific influences on the locomotor pattern. This specificity of RS descending influences may present a basis for modifications of segmental locomotor outputs necessary for equilibrium and steering control.

**METHODS**

**Surgery**

Adult lampreys (*ichthyomyzon unicuspis*, *n* = 16) were anesthetized with tricaine methane sulphonate (MS-222; 200 mg/l water: Sandoz). The brain stem and spinal cord (~50–60 segments; i.e., ~1/3 of the whole body length) with the basal cranium and notochord attached were dissected in cold, oxygenated Ringer solution containing (in mM) 91 NaCl, 2.1 KCl, 2.6 CaCl₂, 1.8 MgCl₂, 20 NaHCO₃, and 4 glucose, pH 7.4. The choroid plexus over the brain ventricle. This point corresponded approximately to the middle part of the brainstem reticular nuclei: the tectal and cerebellar commissures were transected along the midline. The preparation was pinned down in a silicone elastomer (Sylgard)-lined chamber (Fig. 1A) and an agar barrier was built at a level of 15–20 segments caudal to the brain stem to form two pools, one for the brain stem and anterior part of the spinal cord and the other for the remaining part of the spinal cord. Cold oxygenated Ringer solution was continuously perfused through both pools. In most experiments the dorsal and ventral branches of a ventral root that supply the like parts of a myotome (Tretjakoff 1927) were exposed on both sides in one of the midbody segments (30–40).

**Microstimulation**

All four reticular nuclei of the brain stem (MRN, ARRN, MRRN, and PRRN; Fig. 1B) were stimulated in the present study. To evoke activation of small groups of RS neurons, d-Glu (1 mM) was pressure ejected from a micropipette (~10-µm tip diameter). The pressure pulse lasted 15 s. The pressure was adjusted before insertion of the micropipette into the brain stem so that a droplet (100-µm diam; i.e., ~5 µl) appeared at the tip of the micropipette. In preliminary experiments, such a stimulus could activate cells at a distance of up to 100 µm from the ejection site. Stimulation by d-Glu produces local excitation of cell bodies and dendrites but does not affect axons crossing the stimulated area (Goodchild et al. 1982). To prevent polysynaptic activation of neurons located outside the stimulated area, synaptic transmission in the brain stem was blocked by perfusing it with a solution in which the Ca²⁺ concentration was reduced to 0.26 mM in the presence of 2 mM Mn²⁺ or, in some cases, with a Ca²⁺-free solution (Ca²⁺ was replaced by Mn²⁺). The tip of the micropipette was positioned at a depth of 170 µm under the surface of the brain stem and at a distance of 100 µm from the midline, in a zone occupied by cell bodies and numerous dendrites of RS neurons (Martin 1979).

**Elicitation of locomotor activity**

Rhythmic activity in the spinal cord (fictive locomotion) was evoked by adding NMDA to the Ringer solution in the caudal pool (Grillner et al. 1981). The optimal NMDA concentration (0.1–0.15 mM) was established in preliminary experiments (see RESULTS). The activity of motoneurons was recorded by means of glass pipettes (10- to 20-µm tip diameter) positioned either on the whole ventral root or on one of the main branches innervating either the dorsal or ventral myotome. Brain stem stimulation with the parameters described above did not evoke activity in the ventral rootlets in a quiescent preparation, but had pronounced effects during fictive locomotion.

**Experimental protocol**

After fictive locomotion was evoked, different sites in the brain stem reticular formation were stimulated in succession (with 2- to 5-min time intervals). They were positioned along the straight line crossing the centers of the reticular nuclei. No more than 18 sites were stimulated on one side of the brain stem in a single animal (Fig. 1B). Point 1 was reached through the bottom of the third ventricle. This point corresponded approximately to the middle part of the MRN (for anatomy of reticular nuclei see Nieuwenhuys 1972; Ronan 1989; Swain et al. 1993). The remaining points (2–18) were evenly distributed along the floor of the fourth ventricle; they approximately corresponded to the following areas of the reticular formation: points 2–4 were in the ARRN, points 5–10 in the MRRN, points 11–15 in the compact part of the PRRN, and points 16–18 in the caudal, diffuse part of the PRRN. The estimated error in positioning the stimulating pipette in relation to the reticular nuclei was 100 µm in the rostro-caudal direction and 50 µm in the latero-medial direction.

Responses to stimulation of each of the points were recorded from the dorsal and ventral branches of a ventral root bilaterally in one of the midbody segments (30–40). Each recording lasted 1 min, consisting of 30 s before stimulation, 15 s during, and 15 s after. Such mapping of the brain stem was performed in 11 experiments. In a different series of experiments (*n* = 5), two pairs of recording electrodes were positioned at different rostro-
caudal levels (distance ~20 segments) to compare the effects of stimulation in two different parts along the spinal cord.

Processing of data

The effects of the stimulation on the locomotor frequency and on the symmetry of the segmental motor output were examined. To evaluate a change of the locomotor frequency, the mean frequency was measured for 10 cycles preceding stimulation and for 10 cycles during stimulation, with the latter value divided by the former. In some cases the stimulation produced a complete suppression of the locomotor bursts in one or two ventral root branches, which lasted for a few cycles. Because in the lamprey the locomotor rhythm is common for all segments, we used the branches with a clear locomotory rhythm for measuring the frequency. In a schematic example shown in Fig. 1C, the locomotory rhythm was best expressed in the contralateral ventral branch (cv). To evaluate spontaneous fluctuations of the locomotor frequency, a similar procedure was performed for two adjacent intervals (10 cycles each) preceding stimulation.

To evaluate a distortion of symmetry in the segmental motor output caused by brain stem stimulation, we compared the bursting activity before (10 cycles) and during stimulation (5–10 cycles) for each of the two (dorsal and ventral) branches of the ipsilateral and contralateral ventral roots. We considered the effect of stimulation as excitatory in the following cases: 1) an increase of the efferent burst duration, 2) an increase of the intraburst discharge rate, and 3) a tonic activation of the motoneurons. We considered the effect of stimulation as inhibitory in the following cases: 1) a decrease of the locomotor burst duration, 2) a decrease of the intraburst discharge rate, and 3) the disappearance of bursts in some cycles. Figure 1C schematically illustrates the case when stimulation resulted in an increase in burst duration in the ipsilateral dorsal branch (id), in tonic activation of motoneurons in the ipsilateral ventral branch (iv), and in inhibition of bursting in the contralateral dorsal branch (cd), but produced no effect on the cv.

The next step in the analysis was to group the patterns of activity...
in the four branches that resulted in a similar net effect on the motor output. Figure 1D shows three patterns (I–3) that will all result in an ipsilateral flexion of the body. This motor effect is shown by a vector (I-flexion) in Fig. 1H. Figure 1E shows five patterns (I–5) that will result in a simultaneous ipsilateral and ventral flexion; this motor effect is shown by a vector (IV-flexion) in Fig. 1I. With this type of analysis, we classified all distortions of axial symmetry of the segmental motor output in the following eight categories, according to the net motor effect (direction of body flexion) that will be induced: ipsilateral (I), contralateral (C), dorsal (D), ventral (V), ipsilateral and dorsal (ID), ipsilateral and ventral (IV), contralateral and dorsal (CD), and contralateral and ventral (CV). All these categories produce postural effects (a change of the body shape superimposed on the lateral locomotor undulations). Some patterns, however, do not produce a net postural effect (Fig. 1, F1–F3) but they may affect the degree of activation of the myotomes during locomotion. Finally, Fig. 1, G1 and G2, shows patterns affecting the plane of the locomotor body undulations.

**RESULTS**

**Efficiency of brain stem stimulation depends on degree of locomotor activity**

Our standard stimulation exerted a larger effect on the ongoing efferent motor pattern when the NMDA concentration was lower and the locomotory rhythm was slower (Brodin et al. 1985). Figure 2 illustrates the effects of stimulation at different NMDA concentrations (tested in 7 experiments). In this experiment, one site in the MRN was stimulated and the responses in the ipsilateral and contralateral ventral roots were recorded. At low NMDA concentrations (0.05 mM), the locomotor frequency was ~0.8 Hz. Stimulation of the MRN strongly activated ipsilateral motoneurons and produced a complete inhibition of contralateral ones. At 0.1 mM the frequency increased to 1–1.2 Hz, whereas the excitatory and inhibitory effects were reduced. At 0.2 mM, the frequency increased further while small excitatory and inhibitory effects could still be seen. Finally, at 0.4 mM the frequency reached 2.4 Hz and the stimulation produced no clear effect. A possible explanation for this finding is that the relative contribution of the descending RS input to the activity of segmental motoneurons decreases at higher locomotor frequencies as compared with the drive exerted by bath-applied NMDA to the segmental rhythm generating network. In the following experiments we usually used the NMDA concentration of 0.15 mM, which evoked a moderate level of locomotor activity (1–1.5 Hz) while allowing the effects of brain stem stimulation to be seen.

**Effect of stimulation on the locomotor frequency**

Measurements were taken in 8 of 11 experiments for the effect of stimulation on the locomotor frequency in mapping the brain stem for 155 stimulated sites. In the absence of brain stem stimulation, the locomotor frequency was relatively stable. In most experiments the mean value of the relationship of frequencies in two adjacent sections of a recording (see METHODS) was close to unity with SD of ±2–3%. We therefore assume that deviations of frequency of >5% (2 SD) were induced by the stimulation. Such deviations were observed with stimulation in ∼50% of sites in all reticular nuclei. Figure 3 shows the distribution of the effects of stimulation (as a percent of the change in locomotor frequency) for all stimulated points in the four nuclei.

In the MRN and ARRN (Fig. 3, A and B) these deviations were in the range of ±5 to ±15%; sites that both speeded the rhythm up and slowed it down were encountered. For the MRRN (Fig. 3C) the result was generally similar, but a few points evoked larger changes of frequency (up to 35%); the number of points speeding up or slowing down the rhythm were almost equal. In PRRN (Fig. 3D), most of the efficient points evoked an increase of frequency (from 15–25% to 40–50%). However, in this nucleus some points were found [7 of 77 (9%)] that produced a very different effect, that is a complete termination of the locomotor activity similar to that shown in Fig. 2A, with tonic activation of motoneurons in some branches of the ventral roots and inhibition of motoneuron activity in other branches. The increase in the variance of the distribution of frequency changes during stimulation as compared with control was statistically significant for the ARRN, MRRN, and PRRN (F = 19.7; P < 0.0001, t-test). The effect of stimulation was consistent in repeated tests.

The excitatory points (eliciting an increase of frequency for >5%) constituted 18% of all stimulated points in the ARRN, 31% in the MRRN, and 55% in the PRRN. The inhibitory points constituted 27, 28, and 5%, correspondingly. For the MRN, no statistically significant conclusion can be drawn because of the limited number of observations obtained (n = 8).

**Effect of stimulation on the symmetry of segmental output**

The influence of stimulation on the symmetry of the segmental output (dorsal/ventral, left/right) was analyzed in 11 experiments, in which 165 sites were stimulated. Of these sites, 107 (65%) had an effect on the segmental output (83% in MRN, 47% in ARRN, 71% in MRRN, and 63% in
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The linear increase of the CNOS value before stimulation indicates the stability of the rhythmic locomotor pattern. The stimulation evoked a change in the slope of CNOS curves; an increase in the slope (which reflected an increase of the mean frequency caused by a higher intraburst discharge rate or larger burst proportion in the cycle) corresponded to an excitatory effect, whereas a decrease of the slope corresponded to an inhibitory effect. After termination of the stimulus, the slope returned to its initial value. The initial effect was followed by an inhibition of the cv branch. As demonstrated in Fig. 4A, the effects may outlast the stimulus. According to the criteria described in METHODS, these patterns were classified as eliciting a ventral body flexion (V-flexion) followed by IV-flexion (Fig. 4A, diagrams on the right). In addition to visual inspection of the recordings, the cumulative number of spikes (CNOS) was calculated for the dorsal and ventral branches of the left and right ventral roots (by means of the IDL program, Research Systems) and plotted as a function of time. The threshold for spike discrimination was set slightly above the noise level to count spikes of different amplitude. Figure 4, B–E shows four examples of CNOS versus time plots. Stimulation was performed in the MRRN (Fig. 4, B and C) and PRRN (Fig. 4, D and E). The linear increase of the CNOS value before stimulation indicates the stability of the rhythmic locomotor pattern. The stimulation evoked a change in the slope of CNOS curves; an increase in the slope (which reflected an increase of the mean frequency caused by a higher intraburst discharge rate or larger burst proportion in the cycle) corresponded to an excitatory effect, whereas a decrease of the slope corresponded to an inhibitory effect. After termination of the stimulus, the slope returned to its initial value. In Fig. 4B, the stimulation evoked an increase of activity in the iv branch and a decrease in the cv and cd branches; in the id branch, an initial decrease was followed...
The delayed effects of stimulation were then considered and their vectors were added to the corresponding vectors of the initial effect (Fig. 5, right column). Although the delayed effect in each particular case may differ from the initial effect, the polar plots in the right column do not show any substantial change of the angular distribution of vectors as compared with the initial effect (left column). This is especially true for the MRRN and PRRN, where we have more observations (41 and 52 points) than for the ARRN (9 points).

Do different parts of a particular reticular nucleus produce similar or different postural effects? To answer this question, the MRRN was divided into a rostral part (Fig. 1B, sites 6 and 7) and a caudal part (sites 8 and 9). Similarly, the PRRN was divided into a rostral part (sites 11–15) and a caudal part (sites 16–18). We did not subdivide the reticular nuclei further because there are no reliable anatomic landmarks to accurately define the position of the stimulating pipette in relation to specific areas of the nucleus. Figure 6 (left column) shows that the distribution of vectors for the rostral and caudal parts of each nucleus are essentially different. In the rostral MRRN, the I-vector was the most pronounced (Fig. 6A), whereas in the caudal MRRN it was

by an increase of activity. The initial effect of stimulation was thus classified as IV-flexion, followed by I-flexion (as shown by the vectors in diagrams below the CNOS plot in Fig. 4B). Delayed effects of the stimulation, which differed from the initial ones, were observed in 52 of 108 stimulated points (48%). They could be accounted for by diffusion and thus the gradual spread of the excitatory action of D-Glu on neighboring RS neurons that may differ in their action from the initially excited neurons. Similarly, the vectors for the cases presented in Fig. 4, C–E, are shown in diagrams below the corresponding CNOS plots. The effect of stimulation of each particular site was consistent in all cases (~15% of the total numbers of sites) when the stimulation was performed repeatedly with a time interval of >5 min.

Summation of the vectors was performed separately for each of the eight categories (see METHODS) for all effective sites across each of the reticular nuclei. The polar plots in the left column in Fig. 5 represent the histograms (the angular distributions of vectors) for each nucleus; only the initial effects of the stimulation were considered. The most pronounced effects for each nucleus are as follows: in MRN, I (33%); in ARRN, C (44%), CV (23%), and ID (22%); in MRRN, CV (30%), I (22%), and C (22%); in PRRN, I (31%) and IV (19%).

The delayed effects of stimulation were then considered and their vectors were added to the corresponding vectors of the initial effect (Fig. 5, right column). Although the delayed effect in each particular case may differ from the initial effect, the polar plots in the right column do not show any substantial change of the angular distribution of vectors as compared with the initial effect (left column). This is especially true for the MRRN and PRRN, where we have more observations (41 and 52 points) than for the ARRN (9 points).

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the CV-vector (Fig. 6B). In the rostral PRRN the I-vector was the most pronounced (Fig. 6C), whereas in the caudal PRRN it was the IV-vector (Fig. 6D). Adding the vectors for the delayed and the initial effects did not substantially change the distributions (Fig. 6, right column). Thus the rostral and caudal divisions in each nucleus (MRRN and PRRN) differ in the postural effects that they can produce.

Besides the postural effects reported here (Figs. 5 and 6), in a few cases we observed a pattern in which similar change occurred in all branches (as shown schematically in Fig. 1F) or a pattern affecting the plane of the locomotor body undulations (Fig. 1G; data not shown).

Effect of stimulation on the segmental output in different areas along the spinal cord

In five experiments we simultaneously investigated responses in two segments at different rostro-caudal levels (segments 25–33 and 50–55). The recordings were performed either from the whole ventral root or from the dorsal and ventral branches. Figure 7 illustrates the effects elicited from different stimulated sites. In Fig. 7, A–C, recordings were performed from the whole ventral roots 25 and 54 on the right side of the spinal cord (shown schematically in Fig. 7E). Stimulation of the right MRRN (Fig. 7A) evoked excitation in the VR25(R), but produced almost no effect in VR54(R). Stimulation of the right PRRN (Fig. 7B) evoked inhibition in VR25(R) but almost no effect in VR54(R). Finally, stimulation of the left PRRN evoked excitation in VR25(R) and inhibition in VR54(R).

In Fig. 7D, recordings were performed from the dorsal and ventral branches of a ventral root in two segments (33 and 56) on the same side, and the ipsilateral PRRN was stimulated (shown schematically in Fig. 7F). Stimulation produced opposite effects at the two rostro-caudal levels: in segment 33, the dorsal branch was initially inhibited and then activated, whereas the ventral branch was inhibited; in segment 56, the dorsal branch was inhibited and the ventral branch was activated.

In this series of experiments, 7 sites in MRRN and 10 sites in PRRN were effective when stimulated. The effects on two remote segments were similar in 10 cases (59%) and different in 7 cases (41%).

Discussion

Effect of brain stem stimulation on RS neurons

A goal of the present study was to reveal specificity in the influences of the RS system on spinal locomotor mechanisms in the lamprey. By utilizing intracellular stimulation, Rovainen (1967) demonstrated that larger RS neurons (Müller cells) can evoke specific changes in the body shape in a quiescent animal. In in vitro experiments, intracellular stimulation of the Müller cells could evoke considerable changes in the pattern of fictive locomotion (Buchanan and Cohen 1982). However, large cells represent only a small population of RS neurons. Anatomic studies have shown that the RS system of the lamprey is formed by ~2,500 neurons, the overwhelming majority of which are small cells (10- to 20-μm diam) (Bussières 1994). Intracellular stimulation of such small neurons presents a difficult problem, and extracellular microstimulation seems to be a more suitable method for studying the specificity of RS influences on spinal networks.

Cell bodies of RS neurons in the lamprey are located close to the midline and also close to the bottom of the ventricles. They form two “columns,” one on each side of the brain stem, with a width of 200–300 μm and a rostro-caudal extension of ~5 mm in the adult lamprey (Fig. 1B). Descending axons of RS neurons from more rostral areas traverse more caudal areas of the reticular formation (Nieuwenhuys 1972). With such a structure, extracellular electrical microstimulation of the reticular formation can hardly be used because, in addition to the cell bodies located close to the electrode, the axons of neurons located rostrally to the electrode will also be stimulated. Therefore in this study we have used pharmacological microstimulation of the reticular formation by ejecting a small bolus of D-Glu from the micropipette.
D-Glu produces a local excitation of cell bodies and dendrites, but does not affect axons crossing the stimulated area (Goodchild et al. 1982). The amount of D-Glu (see Methods) was chosen to evoke a noticeable effect on the locomotor pattern generated by the spinal cord. This amount appeared to activate neurons at the distance of up to 100 μm from the pipette (Wannier, unpublished observation). One can estimate that such a stimulus may excite up to 20–40 RS neurons in the region of their maximal density (PRRN) (Bussières 1994).

We have found that the effect of stimulation on the symmetry of segmental output could change during the course of a single D-Glu ejection (see Fig. 4A). Such changes were observed in ~50% of the stimulated sites. The most likely explanation of this finding is that at the beginning of ejection, the cells close to the pipette were excited. Later, D-Glu reached and excited more remote cells, which may produce different effects on the segmental output. This finding strongly suggests that RS neurons with different and possibly opposite influences on the spinal cord are intermingled in the reticular nuclei. However, by averaging we managed to reveal a substantial difference between the reticular nuclei in the distribution of cells exerting different influences on the locomotor activity generated by the spinal cord. We distinguished two major types of influences: 1) influences on the locomotory rhythm and 2) influences on the symmetry of the segmental motor output.

**Activation of the locomotor system**

Initiation and maintenance of locomotor activity is a well-established function of the RS system in all vertebrates including the lamprey (Drew 1991; Jordan 1991; Ohta and Grillner 1989). The evidence strongly suggests that this is achieved in the lamprey primarily through a glutamatergic population of RS neurons affecting segmental locomotor networks throughout the spinal cord (Brodin et al. 1985; Grillner et al. 1981, 1995; McClellan and Grillner 1984; Ohta and Grillner 1989). Glutamatergic RS neurons are present in all reticular nuclei (Brodin et al. 1988), which raises the question as to whether all the nuclei participate in the initiation of locomotion and in the modulation of its intensity. The present study has shown that microstimulation of certain sites in any of the four reticular nuclei resulted in an increase in the locomotor frequency generated by the spinal cord (Fig. 3). These sites constituted 18% of the total number of stimulated sites in the ARRN, 31% in the MRRN, and 55% in the PRRN. Thus it seems likely that the excitatory locomotor function is distributed unevenly among the reticular nuclei with its maximum in the PRRN (data for the MRN is not statistically significant).

Stimulation of certain sites could result in a decrease of locomotor frequency. The inhibitory sites constituted 27% in the ARRN, 28% in the MRRN, and 5% in the PRRN. This finding suggests that the intensity of locomotion can be determined not only by the intensity of excitatory inflow to the spinal network but also by the intensity of inhibitory inflow. Inhibitory RS neurons presumably contribute to the inhibitory effect (Wannier et al. 1995).

The RS system activates the segmental locomotor networks by supplying them with a tonic excitatory inflow. On the other hand, we have shown that the RS system can exert a very specific action on the segmental motor output, resulting in a distortion of its symmetry and the bending of the body in different planes, effects that are necessary for the control of equilibrium and steering. The question arises as to whether the activation of locomotor mechanisms and the control of equilibrium and steering are performed by the same or different RS neurons.

We have found that stimulation of practically all sites in the reticular formation that affected the locomotor frequency also produced asymmetrical effects on the segmental output. The absence of sites with only symmetrical excitatory effects suggests that the two functions (the activation of locomotor mechanisms and the control of posture and steering) are not completely separated at the level of the RS neurons. This suggestion is supported by the finding that stimulation of individual Müller cells affected both the locomotory rhythm and the symmetry of segmental output (Buchanan and Cohen 1982). This suggestion is further supported by the fact that the majority of RS neurons in all the reticular nuclei receive specific, powerful excitatory inputs from the contralateral labyrinth and ipsilateral eye (Deliagina et al. 1992a,b; Ulleén et al. 1996) and are therefore involved in equilibrium and steering control. Because of the vestibular input, any rapid change in spatial orientation results in the activation of numerous RS neurons. This change has two consequences: correction of posture and speeding up of the locomotory rhythm (Orlovsky et al. 1992).

For symmetrical activation of the segmental output, which is a necessary condition for eliciting rectilinear locomotion, a special system supplying the subpopulations of RS neurons responsible for turns in different planes with equal excitatory inputs is needed. In many species, this role is played by the mesencephalic locomotor region; stimulation of this area evokes symmetrical activation of the locomotor mechanisms on the left and right sides (Shik et al. 1966; Shik and Orlovsky 1976). In the lamprey a presumed analogue of the mesencephalic locomotor region has been described (Sirota et al. 1995). A second area is located in the thalamus (El Manira et al. 1997). An effect of the presumed system equalizing excitatory inputs to the left and to the right subpopulations of RS neurons was observed in the avoidance reaction of the lamprey. The illumination of the tail dermal photoreceptors evokes a rectilinear locomotion, and this stimulus produces bilateral activation of MRRN and PRRN neurons even with unilateral sensory input (Deliagina et al. 1995).

**Control of equilibrium and steering**

Modifications of the body shape, superimposed on locomotor undulations, is a basis for the control of equilibrium and steering in the swimming lamprey (Ekeberg 1993; Ulleén et al. 1995b). These modifications are caused by four classes of segmental motoneurons that control four corresponding classes of myotomes—the dorsal and ventral ones on the left and right sides of the body (Wallén et al. 1985). Activity of a particular class of motoneurons will evoke body flexion in the plane intermediate between the sagittal and horizontal planes of the animal. For a horizontal turn of the lamprey simultaneous activation of two classes of motoneurons (ipsilateral dorsal and ipsilateral ventral) is needed; for a vertical
Turns in the horizontal plane need a lateral asymmetry (left/right) in the segmental output locomotor pattern (Ekeberg 1993; McClellan 1984; McClellan and Hagevik 1997; Wallén et al. 1994). We found that a considerable portion of the sites throughout all the reticular nuclei could produce such an asymmetry when stimulated, with a resulting vector directed either ipsilaterally or contralaterally. Therefore the lamprey could, in principle, perform a horizontal turn by exciting unilaterally one or a few different groups of RS neurons. The anterior parts of the MRRN and PRRN (Fig. 6, A1 and C1) seem to be the most efficient in eliciting C- and ID-flexions. The rostral part of the MRRN evoked predominantly I-flexion, whereas its caudal part evoked CV-flexion. Finally, the rostral part of the PRRN evoked predominantly I-flexion, whereas its caudal part evoked IV-flexion. These findings indicate that there exists within the reticular formation of the lamprey a certain topographical organization such that different patterns of movement are represented in different but overlapping regions of the brain stem, like previously shown for the cat (Drew 1991).

Turns in the sagittal plane can be performed by a flexion in the anterior part of the body that propagates caudally (Ekeberg 1993; Wallén et al. 1994). A horizontal turn in the swimming lamprey starts with a lateral flexion of the anterior part of the body that propagates caudally (Ekeberg 1993; Wallén et al. 1994). A horizontal turn in the swimming lamprey starts with a lateral flexion of the anterior part of the body that propagates caudally (Ekeberg 1993; Wallén et al. 1994). A horizontal turn in the swimming lamprey starts with a lateral flexion of the anterior part of the body that propagates caudally (Ekeberg 1993; Wallén et al. 1994).

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