

RETICULOSPINAL NEURONS IN LAMPREY: TRANSMITTERS, SYNAPTIC INTERACTIONS AND THEIR ROLE DURING LOCOMOTION

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INTRODUCTION

Reticulospinal systems are represented throughout the vertebrate kingdom and are present early in phylogeny (see e.g. 43, 51, 68, 70, 73, 82, 94, 95, 100, 119, 120). The same general organisation is found in lower vertebrates, including cyclostomes (see below), elasmobranch fish (115) teleost fish (88), lungfish (99) and frog (2, 82, 126). Cyclostomes have attracted the attention of comparative neurobiologists for more than a century due to its position early in phylogeny, deviating from the main vertebrate line approximately 450 million years ago and remaining relatively unchanged (see e.g. 54). Several early investigators have studied the structure of the brain and spinal cord of the lamprey and the related hagfish (36, 50, 60, 85, 110, 116, 121). In more recent years, many of the "new" neurobiological techniques have been applied to the lamprey CNS, which is comparatively simple and well suited for in vitro studies. Fairly detailed knowledge has been accumulated on both anatomy, physiology and pharmacology of the reticulospinal systems, as well as on their role during motor behaviour. The mesencephalic and rhombencephalic reticular formation in lamprey have been shown to contain several classes of descending neurons with different morphologies and putative neurotransmitters. In this article we will review the morphology and function of reticulospinal systems in lamprey and make some comparisons with other vertebrates. We will neither consider the vestibulospinal system which projects to the rostral spinal cord (103) nor other smaller descending projections. For earlier reviews see Rovainen (102, 104, 105).

ORGANISATION OF RETICULOSPINAL NEURONS.

The central nervous system of the lamprey has many basic features in common with higher vertebrates including mammals, but contains fewer neurons and is

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devoid of myelin. The reticular formation (Fig. 1) occupies the medial regions of the brainstem and extends from the caudal part of the rhombencephalon to the rostral mesencephalon. In analogy with other lower vertebrates, the rhombencephalic (medullary) reticular formation has been subdivided into three nuclei (Fig. 1): the posterior, middle and anterior rhombencephalic reticular nuclei (p.r.r.n., m.r.r.n. and a.r.r.n. respectively; 86; cf. 90, 91). The reticular area of the mesencephalon is usually termed the mesencephalic reticular nucleus (m.r.n.; 86) and its vostral portion probably corresponds to the nucleus of the medial longitudinal fascicle in other animals. For an extensive review of the early comparative literature, see Ariens Kappers et al. (1). The four reticular nuclei all include neurons

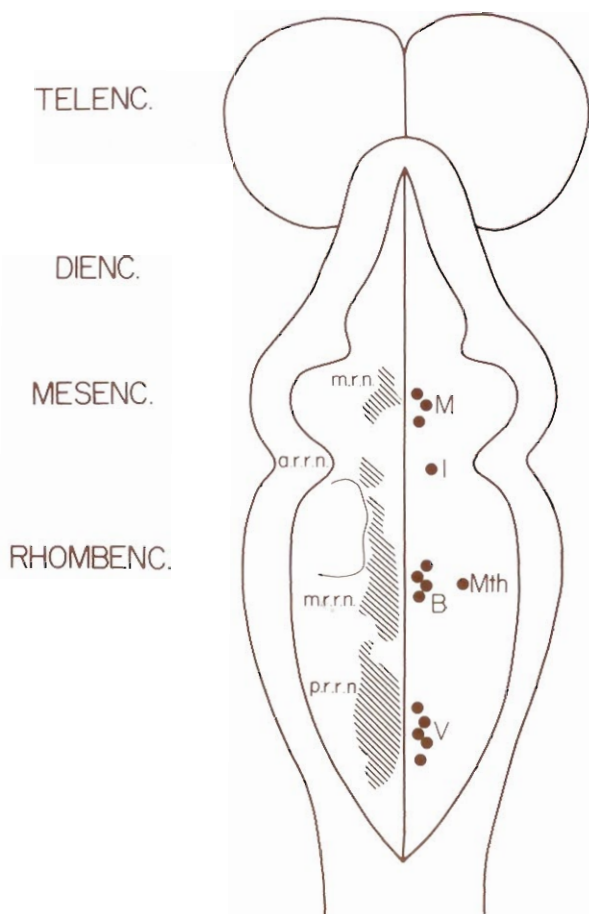


Fig. 1. — *Schematic representation of the reticular formation in lamprey.*

The reticular area (left) occupies the medial brainstem from the caudal rhombencephalon (medulla), to the rostral mesencephalon and is subdivided into the posterior (p.r.r.n.), middle (m.r.r.n.) and anterior (a.r.r.n.) rhombencephalic reticular nuclei, and the mesencephalic (m.r.n.) reticular nucleus. Within the reticular nuclei there are several large, identifiable reticulospinal neurons (right). These are termed mesencephalic (M), isthmus (I), bulbar (B) Müller cells, Mauthner cells (Mth), and posterior rhombencephalic reticulospinal cells or V-cells (V).

which project to the spinal cord (10, 106). The earlier studies of reticulospinal neurons have mainly dealt with the larger cells which are comparatively few in number (see below). With the improvement of the techniques for retrograde tracing, many small and medium-sized reticular neurons have also been found to project to the spinal cord.

In this context it is important to note that different tracer compounds may preferentially label different neuronal populations and that in the lamprey long survival times (3-6 weeks) usually are required to obtain perikaryal labeling (10 and unpublished observations). Thus, an injection of fluorescent dextran-amines (Fig. 2) (38, 39) into the rostral spinal cord (segment 10-15) gives rise to an intense fluorescence in the largest reticular cell bodies and their axons, but a less intense labeling of medium sized neurons. Small cells are only weakly stained or even unlabeled (Fig. 2A, C, D). Other compounds such as Fast blue (5) and, although less well studied, Fluoro-Gold (109), give rise to a different pattern of labeling. In this case small and medium-sized reticulospinal neurons are well stained (Fig. 2B) whereas the largest neurons, such as Müller cells (see below) hardly exhibit any fluorescence at all (10 and unpublished). Horseradish peroxidase (HRP) mainly labels medium-sized and large reticulospinal neurons (Buchanan et al., unpublished).

The number of reticulospinal neurons in lamprey has not been analysed in detail but a high spinal injection of Fast blue will label, in the order of, 150 neurons in the posterior rhombencephalic reticular nucleus on one side. The other reticular nuclei contain fewer fluorescent cells, with the lowest number in the mesencephalic reticular nucleus. A small proportion of the labeled cells in all reticular nuclei has crossed spinal projections. In addition, there is a crossed projection from the lateral aspect of the posterior rhombencephalic reticular nucleus (10 and unpublished, cf. tadpole, 98). We have not studied neurons located rostral to the mesencephalon. The morphology of single reticulospinal neurons have been studied by intracellular injection of Lucifer yellow (Fig. 2 E, F; 87; Ohta *et al.*, in preparation; Dubuc and Grillner, in preparation).

Reticulospinal neurons are markedly heterogenous in their morphology. Cell body diameters range from 5-10 μ m up to about 150 μ m for the giant Müller and Mauthner cells. The latter type of neurons are well characterized (see 74, 85, 100-102, 106, 132, 134). They occur in a fixed number and fixed positions (Figs. 1, 2D) and each of them can be identified individually from animal to animal. There are three Müller cells in the mesencephalic reticular nucleus (M1-3), one or two in the anterior rhombencephalic reticular nucleus (11,2, in the isthmial region) and four in the middle rhombencephalic reticular nucleus (B1-4, in the bulbar region). Müller cells have ipsilateral axons. The two reticulospinal Mauthner cells (Mth) are located lateral to the B cells and they project to the contralateral spinal cord. In addition, there are several large reticulospinal cells with ipsilateral axons in the posterior rhombencephalic reticular nucleus. These neurons are often referred to as vagal cells (V), since they are located medial to the vagal complex, although they have no association with the Xth nerve (121; these cells are referred to as "V" in Figs. 1 and 4).

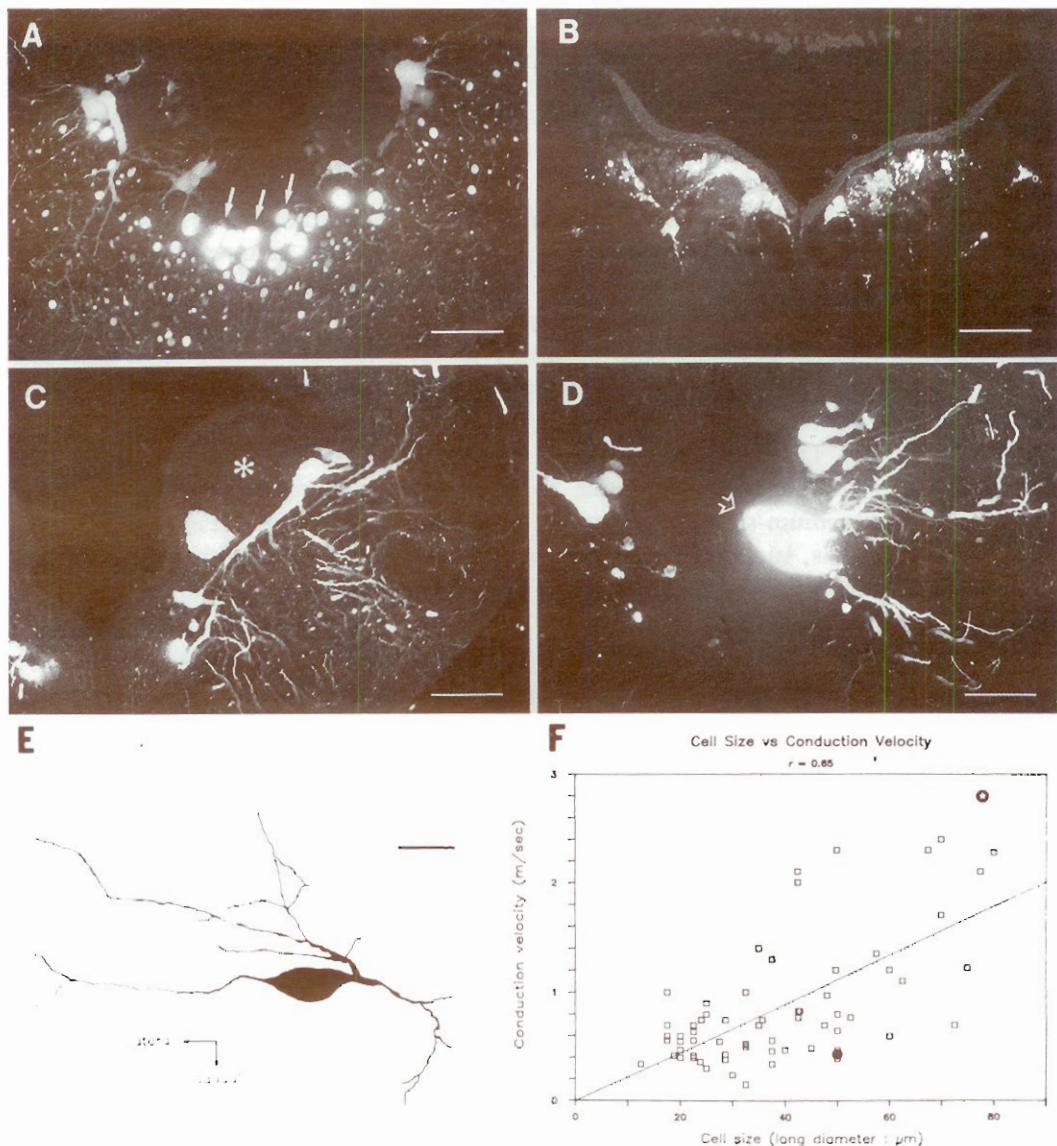


Fig. 2. — Reticulospinal neurons labeled retrogradely or by intracellular dye-injection.

A-D show labeling after injection of fluorescein-conjugated dextran-amines (FDA; A, C, D) or Fast blue (B) in the rostral spinal cord (segment 11-12). A: FDA-fluorescence in the posterior rhombencephalic reticular nucleus (p.r.r.n.). Medium-sized reticular neurons exhibit a fairly weak labeling, whereas many large axons (arrows) are intensely labeled. Small cells are virtually unlabeled. B: Fast blue labeling in the p.r.r.n. This dye preferentially labels smaller cell bodies located near the ventricular surface. Axonal labeling is lacking. C: FDA-fluorescence in the middle rhombencephalic reticular nucleus and the trigeminal motor nucleus (asterisk). D: FDA-fluorescence in the anterior rhombencephalic reticular nucleus. The isthmus Müller cell II (open arrow) is intensely labeled. Scale bars: 100 μm . E: Reconstruction of a reticulospinal neuron in the p.r.r.n. injected with Lucifer yellow via an intracellular recording electrode. The neuron had a fast-conducting spinal axon. Scale bar: 50 μm . F: Relation between longitudinal cell body diameter and axonal conduction velocity for reticulospinal neurons in the p.r.r.n. Each square represents a neuron which was dye-filled and reconstructed after the intracellular recording. The cell shown in E is indicated with a star on a spot. The filled spot indicates a 5-HT immunoreactive neuron. The coefficient of correlation was 0.65.

Reticulospinal axons descend in the lateral and ventral columns throughout the larger part of the spinal cord (Figs. 8, 9B; 105, 106). The prominent Müller axons, with diameters of 20-40 μm , are located in the ventral column. The axons of the mesencephalic Müller cells are located most medially (Fig. 9B) and those of the Müller cells in the anterior (I1,2) and middle (B1-4) rhombencephalic reticular nuclei more laterally. The larger reticulospinal axons from the posterior rhombencephalic reticular nucleus run in the dorsal part of the lateral column as do the axon of the contralateral Mauthner cell. Medium sized and small reticular axons (with diameters down to about one μm), including those with immunoreactivity to neuropeptides and 5-HT, are distributed throughout the lateral and ventral columns (Fig. 8; see below).

Several species of lampreys have been investigated and so far the species differences appear to be very small (104). The studies in our laboratory have been conducted on adult specimens of *Ichthyomyzon unicuspis* and *Lampetra fluviatilis*.

Basic physiological properties.

Reticular cells in lamprey show similar electrical properties to those of mammalian neurons, e.g. resting membrane potentials of 50-85 mV with spontaneous synaptic potentials, action potentials having overshoots of about 30 mV (87, 100, 132). The conduction velocities of reticular axons spans over a wide range as suggested by the variation in diameter (6). Müller cells conduct at a speed of 2 m/s or more (values up to 7 m/s have been reported, see 102), whereas small reticulospinal neurons may conduct as slow as 0.1 m/s. The relation between cell body diameter and conduction velocity for reticulospinal neurons in the posterior rhombencephalic nucleus is shown in Fig. 2F.

INPUT TO RETICULOSPINAL NEURONS.

A. Vestibular input.

Müller, Mauthner and posterior reticulospinal cells all respond to stimulation of the labyrinth or vestibular nerve (34, 102-104, 132, 133). The rostral Müller cells (M_{1-3} , I) receive excitation from the contralateral labyrinth and inhibition from the ipsilateral. The bulbar Müller cells and many posterior rhombencephalic reticulospinal neurons receive excitatory postsynaptic potentials (EPSPs) after stimulation of the ipsilateral anterior vestibular nerve. The latter are also excited by stimulation of the contralateral posterior vestibular nerve. The Mauthner cell receives mono- (and poly-) synaptic input from the ipsilateral anterior vestibular nerve, being the only known direct vestibulo-reticulospinal connection (102). The contralateral anterior vestibular nerve evokes polysynaptic excitatory responses. The vestibular nerves also activate a direct vestibulospinal projection to the rostral segments of the spinal cord (103).

B. Input from other cranial nerves.

Trigeminal nerve stimulation evokes both EPSPs and IPSPs in most of the large reticulospinal cells, but monosynaptic connections have not been detected (34, 100, 102, 132, 133). The optic, olfactory and lateral line organ nerves all elicit polysynaptic responses. Anatomical studies has shown that a wide afferent input is channelled from the optic and vestibular nerves and from the spinal cord in precise, stratified layers in the tectum and the adjacent torus semicircularis (see e.g. 50). From these areas there are specific connections to different brainstem regions. It is possible that some effects on reticulospinal cells caused by e.g. cranial nerve stimulation could be elicited via these areas, which are thought to be involved in the integration of sensory information of importance for equilibrium and visuo-motor control.

The synaptic responses in Müller cells following cranial nerve stimulation can show habituation during low-frequency stimulation and a potentiation following tetanic (10 Hz) stimulation (133).

C. Input from the spinal cord.

The large reticulospinal neurons receive a prominent ascending input from the spinal cord (33, 34, 102; cf. also 64, 75). The responses to stimulation of specific spinal cord fasciculi, have been studied in both Müller cells and posterior rhombencephalic reticulospinal neurons (33). As shown in Fig. 3, stimulation of the ipsilateral spinal dorsal column causes excitation of posterior reticulospinal cells whereas that of the contralateral dorsal column mainly gives inhibition. Stimulation of the lateral fasciculi causes excitation and a late inhibition may follow, particularly from stimulation of the contralateral side. The larger part of the responses are polysynaptic although some early components persist at stimulation rates of 10 Hz. The synapses appear to be within the brainstem since the responses remain unchanged when the spinal cord, but not the brainstem, is exposed to Mn ions (33, 34). As there is no evidence to suggest mutual excitation within the population of reticulospinal neurons, the excitatory effects does not appear to be caused by antidromic activation of other reticulospinal neurons. Specific spinal cord lesions have indicated that the responses are carried by the respective stimulated fasciculi. The dorsal column response is presumably due to stimulation of the central axons of sensory neurons, while the effects from the lateral tracts may be due to spinobulbar and spinothalamic cells. The spinal input to reticulospinal neurons is phasically active during locomotion and will excite and inhibit reticulospinal neurons in each locomotor cycle (64; Dubuc and Grillner, unpublished; see below).

Several groups of ascending spinal neurons have been identified electrophysiologically and by retrograde tracing (34 and unpublished). Large crossed ascending neurons (giant interneurons) which synapse directly onto some reticulospinal cells (101) are present in the caudal spinal cord. Recent immunohistochemical observations has indicated that an ascending GABAergic system is present in the lamprey spinal cord (Brodin *et al.*, unpublished, cf. rat. 37). The anatomical organisation of the ascending systems may be similar to that of teleost fish (89).

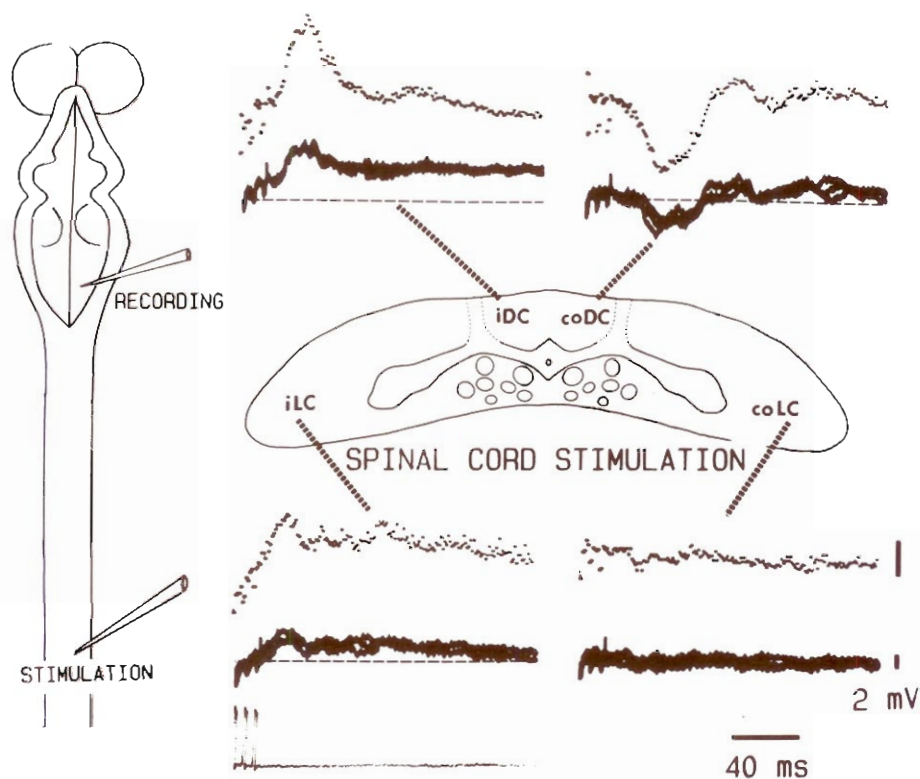


Fig. 3. — Responses of an identified posterior reticulospinal neuron to the stimulation of different spinal cord fasciculi, the ipsilateral (iDC) and contralateral (coDC) dorsal columns, and lateral columns (iLC, coLC).

The fasciculi were stimulated using glass-coated tungsten microelectrodes (3 pulses at 120 Hz, 30 μ A). Average of eight responses are illustrated on top of the superimposed raw records.

TARGET NEURONS AND TRANSMITTERS OF RETICULOSPINAL SYSTEMS.

A. Fast-conducting reticulospinal neurons utilize excitatory amino acid transmission.

1. *Target cells of fast reticulospinal neurons.* — The connectivity between large reticulospinal neurons and nerve cells in the spinal cord has been established by paired intracellular recording experiments (18, 23, 101). Most of the fast-conducting reticulospinal cells produce monosynaptic EPSPs in spinal motoneurons and/or interneurons by means of dual electrical and chemical transmission (101). As illustrated in Fig. 4, different classes of spinal neurons receive a specific reticular input. Thus, for example, motoneurons are excited by the cells M1, M3, I1, Mth, B1-4, and large posterior rhombencephalic reticulospinal cells, whereas inhibitory premotor interneurons receive input from B1-4 and posterior rhombencephalic reticulospinal cells (for details see 18, 101). The connections do not occur onto all spinal neurons within a class and there may be a selectivity in the input to

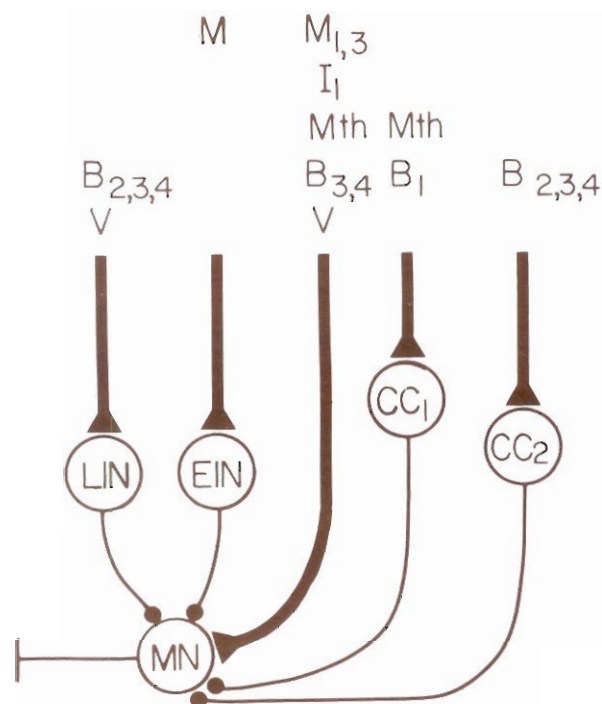


Fig. 4. — Survey over the connectivities between large reticulospinal cells and different classes of spinal neurons.

Abbreviations: M, mesencephalic Müller cells; B, bulbar Müller cells; I, isthmich Müller cell; V, posterior rhombencephalic or "vagal" cells; MN, motoneuron; EIN, excitatory premotor interneuron; LIN, lateral premotor interneuron; CC₁ and CC₂, inhibitory premotor interneurons with crossed, caudal axons. The connectivities have been defined by intracellular stimulation of the identified reticulospinal cell bodies and simultaneous recording from spinal neurons, but in the case of EIN, only mesencephalic Müller cell axons were tested. The scheme, which is based on the studies of Rovainen (101), Buchanan (18) and Buchanan and Grillner (23), gives the main connections known.

Some inputs that occur infrequently, e.g. B₁, 2 - motoneuron, are not included.

different neuronal subclasses. The synaptic strength in different connections is markedly unequal due to a difference in size of both the chemical and the electrical component of the EPSPs (19, 101). Ultrastructural evidence for a vesicular transmitter release by reticulospinal neurons has been provided by Wickelgren *et al.* (134).

Dual electrochemical transmission also occurs between fast-conducting reticulospinal neurons and motoneurons in frog (2). In contrast to lamprey, the majority of synapses are purely chemical whereas a smaller part also include electrical coupling.

2. *The chemical component of reticulospinal EPSPs is depressed by EAA receptor blockers.* — The transmitter of fast-conducting reticulospinal neurons appear to be an excitatory amino acid (EAA) such as glutamate or aspartate. These types of transmitters exert their actions via EAA receptors, which has been classified into N-methyl-D-aspartate (NMDA), kainate and quisqualate receptors (see 76, 131; for lamprey see 29; 47). Combined blockers of all three receptors (e.g. kynurenic acid, KYAC; cis-2,3-piperidine dicarboxylate, PDA) depress the entire chemical

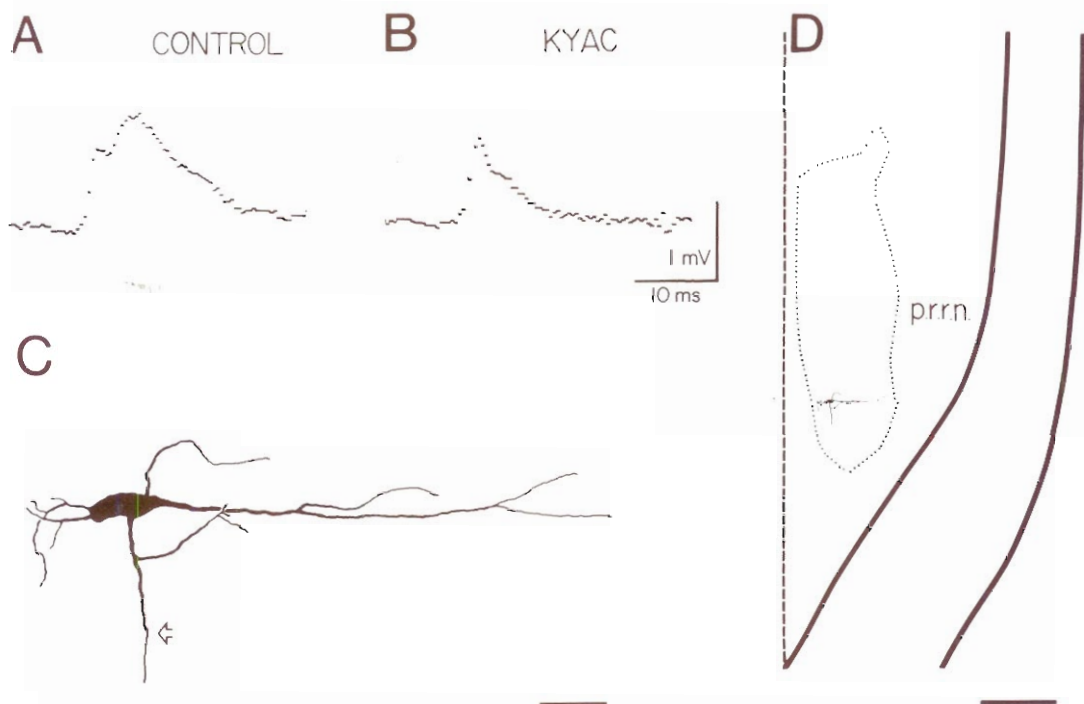


Fig. 5. — Depression of a monosynaptic reticulospinal EPSP by an excitatory amino acid (EAA) antagonist.

A: Control recording of an EPSP in a motoneuron of the rostral spinal cord, evoked by intracellular stimulation of the reticulospinal cell shown in C and D. The EPSP is composed by an early electrotonic component and a later chemical component. Repetition rate 2 Hz, the sweep was averaged 64 times. The motoneuron was identified by recording of its axonal spike in a ventral root. B: Depression of the chemical component of the EPSP after bath application of 2 mM kynurenic acid (KYAC), while the initial electrotonic component is not affected. The chemical component recovered to the control level after washout of KYAC. C: After the recordings, the reticulospinal neuron was injected with Lucifer yellow and subsequently reconstructed. Scale bar = 50 μ m. D: Schematic drawing of the caudal brainstem showing the actual size and location of the neuron within the posterior reticular nucleus (p.r.r.n.). Scale bar: 500 μ m. The conduction velocity of the reticulospinal axon was 2.3 m/s. After the pharmacological experiment fictive locomotion was induced in the preparation by spinal cord stimulation and during this activity the reticulospinal neuron was excited in phase with the ipsilateral, rostral ventral root burst and inhibited in phase with the contralateral burst.

component of reticulospinal EPSPs in motoneurons and interneurons (Figs. 5, 8 C). This depressant effect has been observed on excitation from mesencephalic, isthmic and bulbar Müller cells, the Mauthner cell, and from fast-conducting reticulospinal neurons in the posterior rhombencephalic nucleus (19, 87; Ohta *et al.*, unpublished; cf. also 29). In some of these synapses, pure NMDA antagonists (2-amino-5-phosphonovalerate, 2-APV; Mg ions) have been found to selectively depress the late phase of the chemical component (Fig. 8C), leaving the early phase unchanged.

The EPSPs produced by the large EAA neurons may thus have two chemical components following the initial electrotonic potential. The early chemical phase is probably mediated by kainate receptors (or possibly quisqualate receptors; 19,

29). These receptors are linked to conductances which display conventional properties. The late NMDA-receptor mediated phase, on the other hand, reflects an activation of a voltage-sensitive cationic (Na, Ca, K) conductance which is gated by Mg ions (76; for lamprey see 29, 41, 47, 84). This component cannot be seen at resting membrane potential, in the presence of physiological levels of Mg, but when the postsynaptic cell is depolarised by other excitatory inputs the Mg block will be relieved and the NMDA channels will contribute depolarising current, and thereby boost the responsiveness of the neuron. Another important consequence of an activation of the NMDA-conductance is that it will promote oscillatory behaviour of individual spinal neurons. These intrinsic pacemaker properties, which can be revealed by a blockade of synaptic interactions with tetrodotoxin (TTX), contribute to the generation of a rhythmic output from the spinal locomotor network (11, 13, 46, 129; see also below).

The spinal cord has an effective uptake system for excitatory amino acids. A pharmacological blockade of this system leads to an increase in the overflow of endogenous L-glutamate simultaneously with a potentiation of the spinal motor output (12, 16, 41).

3. *(3 H)-D-aspartate labeling of reticulospinal neurons.* — Selective uptake and retrograde transport of (3H)-D-aspartate is considered to be a specific method for labeling of neurons which utilize L-glutamate / L-aspartate as their transmitters (26). In a recent study in lamprey, we observed neuronal labeling in the brainstem after injection of (3H)-D-aspartate into the rostral spinal cord (15). Autoradiographic labeling was present over axons and cell bodies of Müller cells. Large posterior rhombencephalic reticulospinal neurons were heavily labeled (Fig. 6A). Small and medium-sized cell bodies containing (3H)-D-aspartate were observed in the mesencephalic as well as the anterior, middle and posterior rhombencephalic reticular nuclei (Fig. 7).

In a study in rat, spinal injections of (3H) D-aspartate gave no clear labeling of reticular neurons (107), but due to the many similarities in organization and physiological properties, it would still seem likely that the fast reticulospinal system in higher vertebrates (see 2, 94, 126) also utilize EAA neurotransmission.

B. 5-hydroxytryptamine neurons.

By combining retrograde fluorescence tracing with 5-hydroxytryptamine (5-HT) immunohistochemistry (see 114), two groups of reticulospinal 5-HT neurons have been identified. They are located in the caudal part of the posterior rhombencephalic reticular nucleus and within and medial to the anterior rhombencephalic reticular nucleus (Figs. 6B, 7; 10; cf. 4). Lesion experiments have shown that descending 5-HT fibers supply the lateral and ventromedial columns of the spinal cord (Fig. 8D; 9, 49), but not the dorsal horn (cf. 97, 113). The small diameter of the descending 5-HT axons (cf. 124) suggests that they have a slow conduction velocity which was confirmed by intracellular recording experiments, with subsequent dye injection (Lucifer Yellow) and 5-HT immunohistochemistry. The conduction velocity

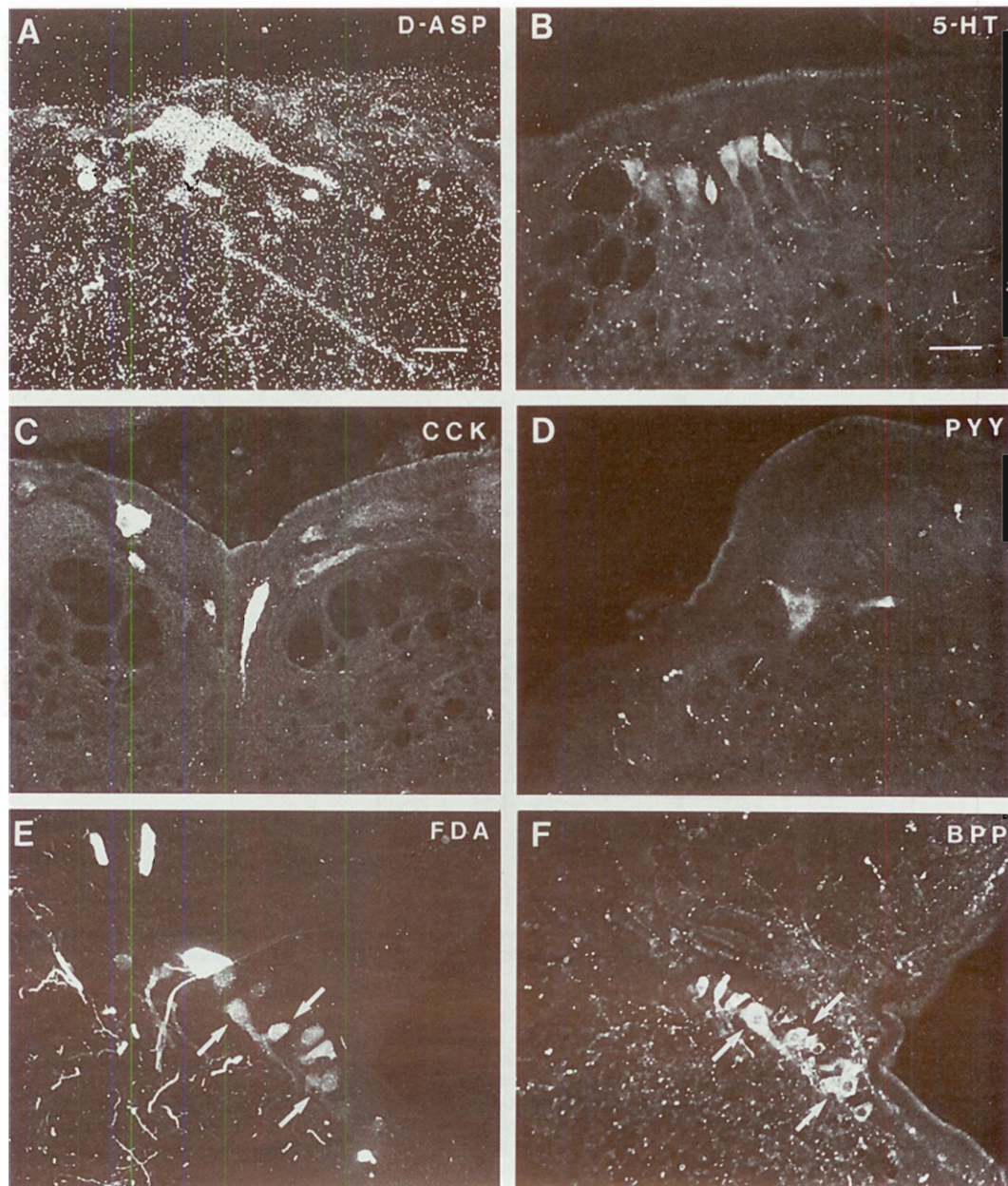


Fig. 6. — Transmitter-related markers in lamprey reticulospinal neurons.

A: (3H)-D-aspartate labeled neuron in the posterior rhombencephalic reticular nucleus (p.r.r.n.) following a spinal injection. B: 5-hydroxytryptamine (5-HT) containing reticulospinal cells in the p.r.r.n. The section was incubated with a polyclonal rabbit anti-5-HT antiserum followed by fluorescein-coupled antirabbit antibodies. The neurons also contained Fast blue fluorescence, retrogradely transported from the spinal cord. C: Cholecystikinin immunoreactive reticulospinal neurons in the p.r.r.n., detected by a monoclonal CCK antibody. D: Descending neuron in the rostral pole of the Vth nucleus showing immunofluorescence to peptide YY (PYY). This group of cells also reacted with antisera to rat and bovine pancreatic polypeptide (RPP, BPP). E: Fluorescent neurons in the anterior rhombencephalic reticular nucleus after a spinal injection of fluorescein-coupled dextran-amine (FDA). F: Immunofluorescence (rhodamine) to BPP in the same section. Reticulospinal (double-labeled) BPP-immunoreactive neurons have been indicated with arrows. Scale bars: 50 μ m.

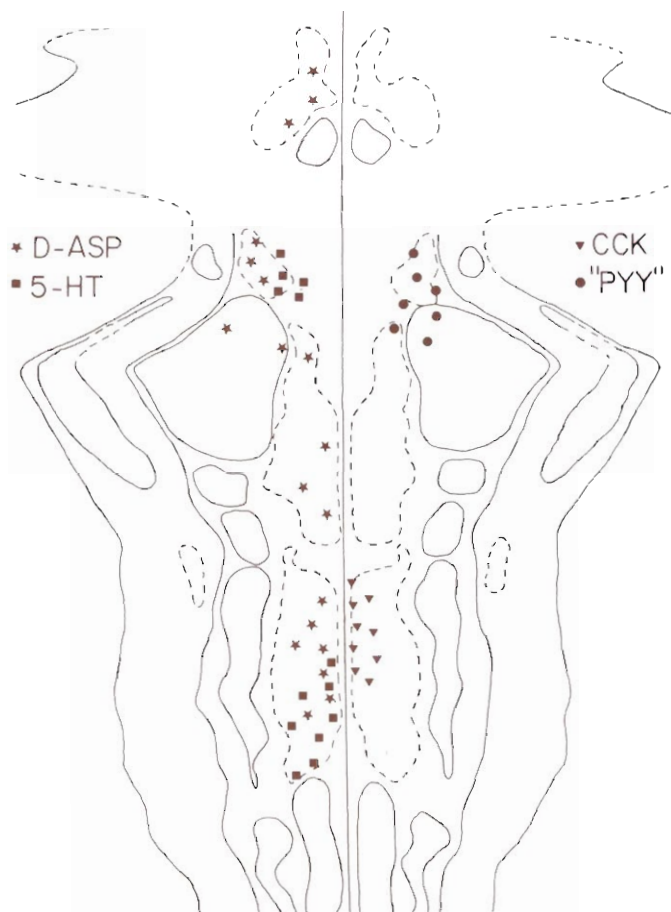


Fig. 7. — Schematic representation of reticulospinal neurons labeled by transmitter-related markers, as they project onto a topological map of the lamprey brainstem (adapted from Nieuwehuys, 86).

(3H)-D-aspartate labeled cells (stars) are present in the mesencephalic as well as the anterior, middle and posterior rhombencephalic reticular nuclei, following a spinal injection.

Groups of descending 5-HT neurons (squares) are located in the anterior and posterior rhombencephalic reticular nuclei. Cholecystikinin (CCK) immunoreactive reticulospinal cells (triangles) are distributed in the rostromedial part of the posterior rhombencephalic reticular nucleus. Descending peptide YY (PYY) immunoreactive neurons (dots) are located in the anterior rhombencephalic reticular nucleus and rostromedial part of the Vth nucleus. These cells are also recognized by antisera to rat and bovine pancreatic polypeptide. The 5-HT, CCK and "PYY" neurons indicated are all retrogradely labeled after injections of fluorescent tracers in the rostral spinal cord.

ty was found to be below 0.5 m/s (Fig. 2F; 87, and unpublished). The lamprey spinal cord also contains intrinsic 5-HT neurons which give rise to a dense plexus in the ventromedial area (49, 53, 124), as well as 5-HT neurons in spinal ganglia supplying the dorsal areas (9, 124; see below). In a study of possible target neurons for 5-HT terminals in lamprey (125), different classes of spinal neurons were injected with Lucifer yellow and subsequently processed for 5-HT immunohistochemis-

try. Close appositions of 5-HT boutons ($\leq 1 \mu\text{m}$) were observed on both motoneurons and lateral interneurons, mainly in the ventromedial spinal cord. Part of this 5-HT innervation is probably intraspinal in origin but to what an extent descending 5-HT fibers contribute is at present unknown. It is important to note that primary and second order sensory neurons have no or few close contacts with 5-HT boutons and thus there is no evidence that descending 5-HT neurons should affect sensory elements in the spinal cord. On the other hand 5-HT is contained within an afferent spinal system of small cell bodies in the spinal ganglia, which projects to the dorsal horn and dorsal column. This system in addition co-contains both calcitonin-gene related peptide- (CGRP) and bombesins-like immunoreactivities (9). There is no dense 5-HT plexus in the dorsal root entry zone as is the case for certain neuropeptides (20).

Lamprey, as well as several other lower vertebrates (4, 53, 93, 97) thus have both descending and intraspinal 5-HT systems. Many higher vertebrates appear to lack intraspinal 5-HT systems (see e.g. 27, 113) but there is indeed some evidence for intraspinal 5-HT systems in mammals (32, 69). In rat, the descending 5-HT projection of the nucleus raphe pallidus affects motor functions, whereas e.g. that of the nucleus raphe magnus affects sensory transmission (see 3, 113). It should be noted that some authors consider the raphe complex to be part of the reticular formation (see e.g. 8). Descending 5-HT projections in mammals co-contain several other putative neurotransmitters (7, 56, 59, 61, 72, 83). In lamprey such coexistence has so far not been observed.

5-hydroxytryptamine may potentiate the motor output from the lamprey spinal cord, since this amine increases the spike frequency in motoneurons and premotor interneurons in response to a depolarising pulse. This effect is due to a reduction of the post-spike afterhyperpolarisation, in turn caused by a depression of a Ca-dependent K-conductance (47, 123, 127; cf. also 55). Application of 5-HT during fictive locomotion causes a potentiation of each motor burst simultaneously with a lowering of the burst rate (48; Grillner and Wilén, unpublished). Low concentrations of 5-HT will also depress the chemical component of Müller cell EPSPs in motoneurons, very likely due to a presynaptic action of 5-HT (24). Whether this effect is limited to Müller cell synapses or not is unknown. Light microscopic observations have shown 5-HT immunoreactive terminals in the vicinity of putative Müller cell-motoneuron synapses.

C. Cholecystokinin neurons.

Several groups of cholecystokinin (CCK) immunoreactive cell bodies are present in the lamprey brain (9). Combined retrograde fluorescence tracing and CCK immunohistochemistry has shown that one group of medium-sized CCK neurons (Figs. 6C, 7), located in the rostromedial part of the posterior rhombencephalic reticular nucleus, has a spinal projection. Chronic lesion experiments have suggested that these neurons project to both the lateral and ventral columns of the spinal cord (Fig. 8E). There is no evidence for intraspinal CCK neurons in lamprey (9).

In a study combining intracellular injection of Lucifer Yellow into spinal neurons with CCK immunohistochemistry, close appositions were consistently observed between CCK boutons and motoneurons, in most instances in the ventromedial column (Ohta *et al.*, in preparation). Close contacts with giant interneurons and, in a low number, with lateral interneurons were also detected. The role of the CCK transmission is unclear but in a preliminary study, mammalian CCK-7 has been found to cause some reduction in the frequency of bursting during fictive locomotion (21).

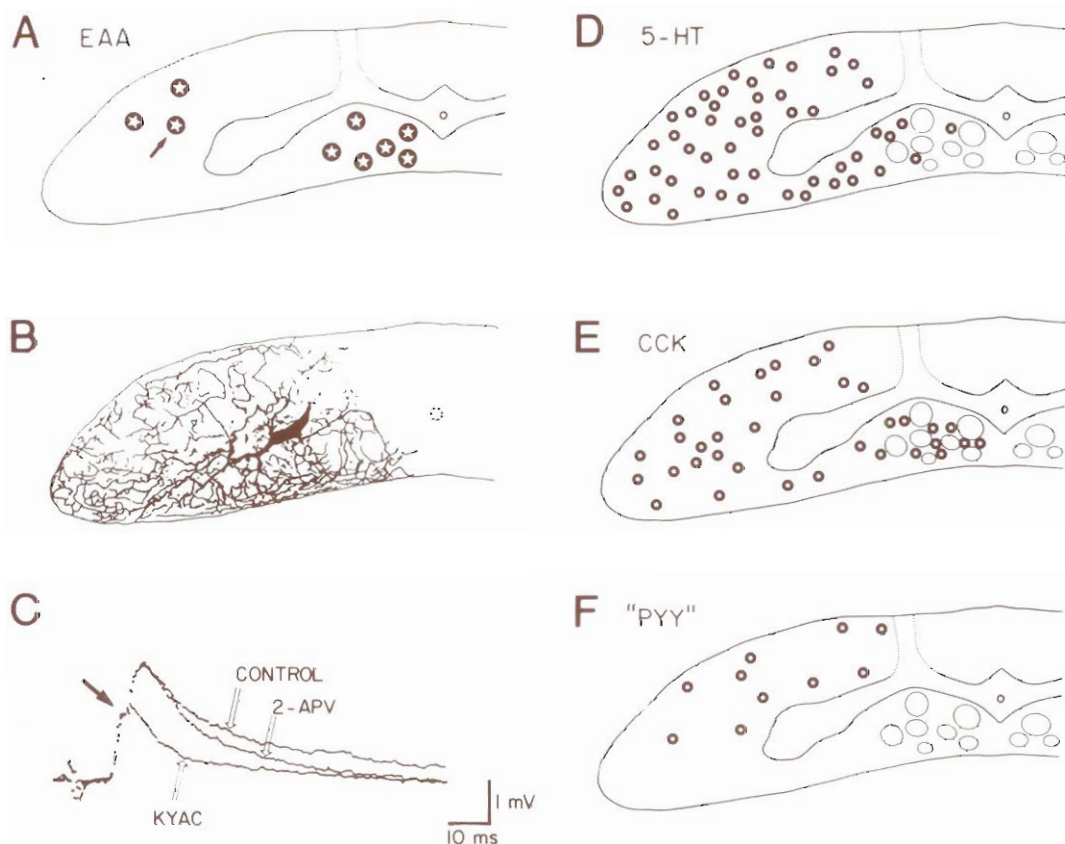


Fig. 8. — Spinal projections of different reticulospinal systems.

A: Location of large reticulospinal axons which elicit EPSPs in motoneurons/interneurons, that can be depressed by excitatory amino acid (EAA) antagonists. These include axons of mesencephalic, isthmic and bulbar Müller cells (ventromedial column), the Mauthner cell (arrow) and posterior reticulospinal ("V") neurons (lateral column). B: Dendritic arborizations of a motoneuron, which was injected with lucifer yellow and subsequently reconstructed from transverse sections. (Modified from Wallén *et al.*, 128). C: Monosynaptic EPSP in a spinal motoneuron evoked by intracellular stimulation of the contralateral Mauthner cell. The recordings were made in Mg-free physiological solution. Averaged sweeps ($n=32$) during different conditions have been superimposed. The EPSP consist of an initial electrotonic component (arrow; still present in calcium-free solution), followed by a larger chemical component. The late phase of the chemical component was selectively depressed by $100 \mu\text{M}$ 2-amino-5-phosphonovaleate (2-APV). 2 mM kynurenic acid (KYAC) depressed the entire chemical component. D-F: Distribution of reticulospinal fibers immunoreactive to 5-HT, CCK and PYY, respectively. The fibers are all lost below a chronic spinal transection (after more than 3 months of survival time).

Biochemical analyses have shown that the CCK-like peptides present in lamprey are similar, but not identical, to mammalian cholecystokinins (9, 52). Although comparative phylogenetic studies are lacking, it should be mentioned that the rat has both descending and intraspinal CCK systems, which partly co-contain other putative transmitters (37, 58, 59, 72, 122).

D. Pancreatic polypeptide-like compounds in reticulospinal neurons.

In an immunohistochemical study with antisera raised against different mammalian pancreatic polypeptides (Brodin *et al.*, in preparation), we observed labeling of a reticulospinal system. A group of cellbodies in the anterior rhombencephalic reticular nucleus, extending into the rostro-medial parts of the Vth motor nucleus (Figs. 6D, F, & 7) were recognised by antisera to peptide YY (PYY) as well as to rat (RPP) and bovine pancreatic polypeptide (BPP), but not by neuro peptide Y (NPY) antiserum. The peptide (or peptides) contained within this system is yet unidentified and will tentatively be referred to as "lamprey pancreatic polypeptide" (LPP). Spinal projections of these LPP-containing cells have been verified with retrograde tracing (Figs. 6E, F). Other, smaller LPP neurons were detected in the mesencephalic and middle rhombencephalic reticular nuclei. Spinal LPP (PYY) fibers are distributed in the dorsal part of the lateral column (Fig. 8F) and they appear to belong to descending neurons since they are all lost below a chronic spinal transection. The PYY-antiserum appeared to give selective labeling of the reticulospinal system, whereas the RPP and BPP antisera in addition recognised other spinal fibers which remained below a chronic lesion. Close appositions occur between PYY immunoreactive fibers and dendrites of Lucifer Yellow-filled motoneurons/lateral interneurons.

Antisera to NPY, as well as to avian pancreatic polypeptide (APP), give rise to a different pattern of labeling in that these antisera label a local spinal neuron system, which supply the dorsal horn (124; Brodin *et al.*, in preparation). The NPY/APP antisera also react with several groups of neurons in the brainstem, but none of these appear to project to the spinal cord.

The mammalian spinal cord show similarities in the distribution of pancreatic polypeptide immunoreactive neurons. In rat there is a small PYY-immunoreactive reticulospinal system originating from the rostral medulla (17). Furthermore there is an intrinsic spinal NPY-system mainly innervating the dorsal horn (31, 57, 108; c.f. cat, 66). It should be noted that the pancreatic polypeptide-like compounds present in the lamprey CNS exhibit a somewhat different pattern of cross-reactivity to the antisera used (cf. 71), indicating that there may be species differences in the amino acid sequences.

Reticulospinal neurotransmitters: summary.

Lampreys have several different populations of reticulospinal neurons originating from the four nuclei of the reticular formation. Based on the distribution of transmitter-related markers, tracing studies and pharmacological experiments, there

appear to be at least four different types of reticulospinal neurons with regard to their putative neurotransmitters/neuromodulators; excitatory amino acid (EAA), 5-hydroxytryptamine (5-HT), cholecystinin (CCK) and pancreatic polypeptide ("LPP") neurons. Fast-conducting reticulospinal EAA neurons, with a selective input to motoneurons and several types of premotor interneurons, are distributed in all four reticular nuclei. The posterior rhombencephalic reticular nucleus has in addition to EAA neurons, populations of 5-HT and CCK containing reticulospinal neurons, whereas the anterior rhombencephalic reticular nucleus includes groups of reticulospinal 5-HT, "LPP" and EAA cells. The connectivity of the peptide containing neurons is indicated by light-microscopic observations, which have shown that the CCK and "LPP" axons have boutons in close apposition to motoneurons and some types of interneurons. 5-HT boutons also appear to contact the same groups of neurons, but it is uncertain whether they originate from descending and/or intraspinal neurons. Coexistence of transmitters have so far not been shown in any of the reticulospinal systems in lamprey. It is as yet unclear if monoamine/peptide immunoreactive neurons also accumulate (3H)-D-aspartate.

THE RETICULOSPINAL CONTROL OF LOCOMOTION.

The different species of lamprey have an eel like body form without paired fins. They swim with alternating contractions of the two sides at any given segment. The different segments are coordinated such that rostral segments always contract before caudal ones, which results in a mechanical wave transmitted along the body (40; 130). The motor pattern underlying locomotion can be coordinated by the isolated spinal cord. The spinal locomotor networks are normally from the brainstem.

A. Initiation of locomotion from the brainstem.

The motor pattern underlying swimming can be initiated from the brainstem presumably via reticulospinal neurons. Microstimulation in the area of the posterior rhombencephalic reticular nucleus as well as in more rostral areas including mesencephalon can elicit swimming or its neural correlate in the isolated lamprey nervous system (Fig. 9A) (81). Alternating bursts of activity occur in the left and right ventral roots in a segment, with a constant phase lag between segments along the spinal cord in the rostrocaudal direction (Fig. 9C) (25; 130). This motor pattern is referred to as fictive swimming or locomotion. The stronger the stimulation of the locomotor area (e.g. from 5 to 10 μ A), the higher the rate of bursting. Thus, a simple relation exists between the brainstem control signal and the spinal motor output.

Local application of excitatory amino-acids from micropipettes in the area of the posterior and middle rhombencephalic reticular nuclei can also elicit bouts of locomotor activity (78; Brodin unpublished). This finding suggests that neuronal

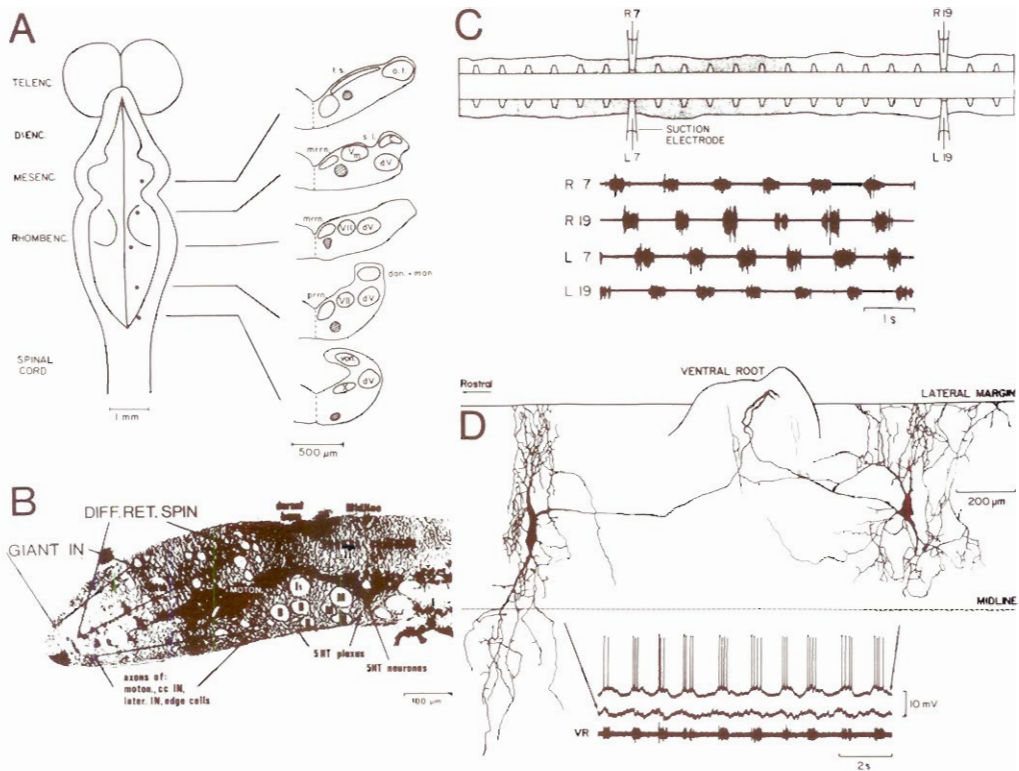


Fig. 9. — The lamprey CNS can generate fictive locomotion in vitro.

A: Schematic representation of the lamprey brainstem (dorsal view). Fictive locomotion can be evoked by low current stimulation ($1.5\text{--}3\ \mu\text{A}$) in the hatched areas shown in the transverse sections. Abbreviations: c, cerebellum; don, dorsal octavolateralis nucleus; dV, descending sensory tract of Vth nucleus; mon, medial octavolateralis nucleus; m.r.r.n., middle rhombencephalic reticular nucleus; ot, optic tectum; p.r.r.n., posterior rhombencephalic reticular nucleus; sl, sulcus limitans; ts, torus semicircularis; von, ventral octavolateralis nucleus; Vm, Vth motor nucleus; VII, VIIth sensory nucleus; X, vagal motor nucleus; (modified from Rovainen *et al.*, 104). B: The lamprey spinal cord in transverse section. The regions containing axons of different neurons are indicated by sectors (solid and dashed lines). Some of the large, identifiable Müller and Mauthner axons have been indicated. Abbreviations: diff. spin., different reticulospinal axons; giant in., giant interneuron axon; moton.-motoneurons; ccIN, CC-interneurons; later IN, lateral interneurons; Mth, Mauthner axon; I, B, M, isthmia, mesencephalic and bulbar Müller axons respectively; CC, central canal; (modified from Rovainen *et al.*, 106). C: In vitro preparation of the lamprey spinal cord. Record traces show bursting activity in the ventral roots indicated (R, right side; L, left side; numbering starts with the most rostral segment in the section of spinal cord). The solution contained $0.6\ \text{mM}$ D-glutamate (modified from Cohen and Wallén, 25). D: Two motoneurons reconstructed after injection of Lucifer yellow. The left cell is a ventral myotome type of motoneuron and the right a dorsal myotome type (note the difference in morphology). The traces show membrane potential oscillations in the motoneurons during fictive locomotion, in phase with the ventral root (VR) bursts (modified from Wallén *et al.*, 128).

cell bodies located in these areas can elicit locomotion, rather than fibers of passage, which would also have been activated by the electrical stimulation.

A substantial part of the inhibition within the brainstem, including that of reticulospinal neurons, is mediated by GABA receptors. A blockade of the GABAergic inhibition in the brainstem, by bath application of receptor blockers such as bi-

cuculline, can elicit longlasting episodes of fictive locomotion in the spinal cord. The GABA receptor blockade probably lowers the threshold for activation of a variety of neurons in the brainstem including that of reticulospinal neurons (Brodin and Grillner, unpublished). An increased activity in reticulospinal neurons will activate the locomotor circuitry in the spinal cord.

If skin areas are left innervated in brainstem/spinal cord preparations, sensory stimulation can elicit bouts of locomotor activity either by afferents acting directly on spinal cord interneurons (80) or by cranial nerves (77, 78), presumably causing a polysynaptic activation of reticulospinal neurons (see above). The descending pathways can activate the spinal circuitry eliciting locomotion along the entire spinal cord (77, 78, 81).

The descending control of locomotion in lamprey thus shares the same general principles with higher vertebrates (for reviews see: 40, 62, 78, 111, 118). In other fishes (45, 63), as well as in turtle (65) bird (117), cat (112), and monkey (35) a local stimulation of specific brainstem regions can evoke locomotion. The stronger the stimulation, the faster the animal will locomote.

B. Activity of reticulospinal neurons during fictive locomotion.

The large reticulospinal neurons in the mesencephalic as well as all three rhombencephalic reticular nuclei show marked membrane potential oscillations during fictive locomotion, which are in phase with the ipsilateral motoneurons in the rostral spinal cord (Fig. 10). The activity in the posterior and middle rhombencephalic reticular nuclei is indeed very similar to that occurring in motoneurons, whereas the activity in the mesencephalic cells also is linked to the locomotor activity but being much more variable. This pattern of activity includes the large Müller cells but also other, adjacent neurons (33, 34, 64, 87 and unpublished). In the posterior rhombencephalic nucleus, smaller neurons were also studied, some of which contained 5-HT or CCK. A proportion of the smaller reticulospinal neurons exhibited oscillations in phase with the ipsilateral ventral root during bouts of fictive swimming, whereas other showed no oscillations at all. There appeared to be no significant correlation between cellbody diameter and the occurrence of membrane potential oscillations (87 and unpublished). In some experiments the respiratory motor pattern was sampled by recordings from the vagus nerve (64). The efferents producing respiration are all located in cranial nerves (104). The respiratory motor pattern was not related to the activity of reticulospinal neurons, whereas most large reticulospinal neurons were markedly affected when locomotor activity or other types of spinal motor activity was initiated (cf. cat. 92).

The large descending neurons in all reticular nuclei utilize excitatory amino acid (EAA) transmission, with a chemical EPSP with a rapid rise time, elicited via kainate/quisqualate receptors and in the decay phase NMDA receptors may also be activated (see above). This means that during ongoing locomotor activity spinal cord interneurons will receive a continuous, phasically modulated reticulospinal input from neurons which utilize EAA transmission.

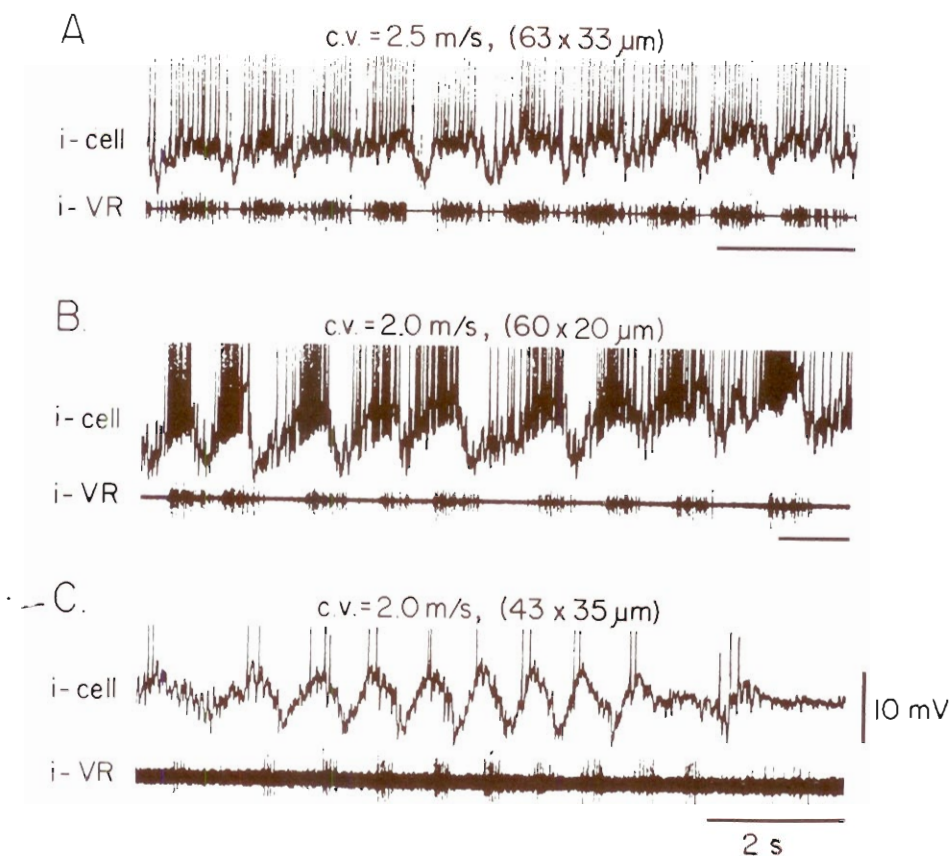


Fig. 10. — The activity of fast conducting reticulospinal neurons during fictive locomotion evoked by short-lasting electrical stimulation of the caudal spinal cord.

The cells are depolarized and spike in phase with the ipsilateral ventral root burst and they are inhibited in the interburst interval. The full spike height is not shown. The axonal conduction velocity (c.v.) was measured by recording the volley of the descending axon. After recording, the neurons were injected with Lucifer yellow and subsequently reconstructed for estimation of the cell body size.

The spinal locomotor activity, which consists of alternating excitation and inhibition in motoneurons during each swimming cycle, is generated by an interaction between inhibitory premotor interneurons (L and CC of Fig. 11), which utilize glycinergic synaptic transmission (18, 44), and excitatory interneurons, which utilize EAA transmission (23, 28; cf. 11). This spinal interneuronal circuitry (Fig. 11) can produce rhythmic burst activity provided that the excitability level of the interneurons is raised such that a significant proportion of the interneurons will produce action potentials when not actively inhibited (23, 47).

If the excitability in the spinal cord circuits is raised in a very unspecific way by adding excitatory amino acids activating kainate or NMDA receptors to the bath, well co-ordinated locomotor activity will also result. The frequency of fictive locomotion depends on the concentration of EAA and increases when the dose

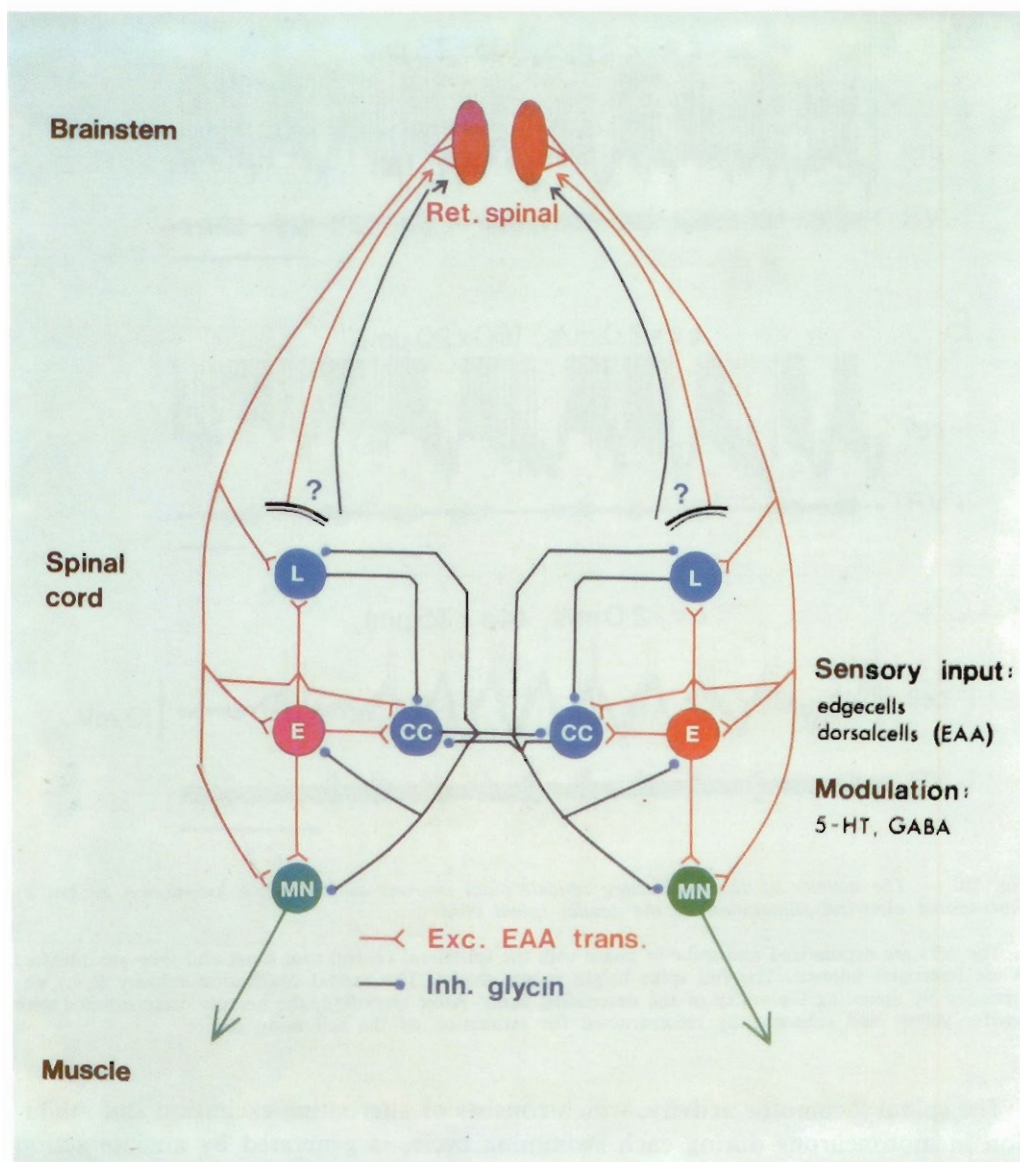


Fig. 11. — Overview of identified neurons taking part in the control and spinal pattern generation of locomotion in lamprey.

Abbreviations: EAA, excitatory amino acid; Ret. spin., fast-conducting reticulospinal EAA neuron; L, lateral interneuron, (inhibitory, glycinergic premotor interneuron); CC, CC-interneuron (inhibitory, glycinergic premotor interneuron with a crossed, caudal axon); E, excitatory premotor EAA neuron; MN, motoneuron. The spinal compartment of the network can produce rhythmic alternating activity if the excitability of the network is so high that the neurons will fire action potentials, when not actively inhibited. All interneurons on one side (E, L, CC) are depolarised at the same and the CC-interneuron which will then inhibit all contralateral neurons. The L-interneuron starts to fire action potentials in the mid-cycle, and will then inhibit the ipsilateral CC-interneuron, which will lead to a cessation of the contralateral inhibition, and to a discharge of all contralateral neurons including the contralateral CC-interneuron which will then terminate the burst on the ipsilateral side (23, 47).

of EAA is raised (14, 42, 96). The coordinated pattern of activity clearly results from an interaction between the spinal interneurons under conditions when they are tonically excited by the excitatory amino acids.

During normal conditions the excitation of the spinal networks originates from the brainstem and its reticulospinal projections. As we have seen, reticulospinal EAA neurons originate from all reticular nuclei and they are active during locomotion, but their activity is as a rule phasic (swim cycle related) and not tonic as in the case with bath applied EAA. Reticulospinal neurons project to all types of interneurons in the network (Figs. 4, 11) and to motoneurons. An increased activity in reticulospinal EAA neurons will presumably excite the interneuronal circuitry in a similar way. Although individual, large reticulospinal cells do not activate each type of interneuron and neither all interneurons of a given type, the activation of a population of reticulospinal EAA neurons will lead to an activation of all types of interneurons. In other words, we are suggesting that the summed activity in a population of reticulospinal EAA neurons from the different reticular nuclei will control the excitability level of the locomotor circuitry and thereby decide not only if the animal will swim or not, but also the speed of swimming.

It is interesting to note that the spinal locomotor networks in higher vertebrates can also be activated via EAA receptors (30, 67, 79).

C. Equilibrium control and steering control.

As an animal swims or remains in the same spot it may need to correct its orientation in relation to the surrounding world. In doing so it needs at least to differentially control the muscles located on the dorsal, ventral, right and left sides of the body, to be able to bend upwards, downwards or sideways. On anatomical grounds it has been suggested that mesencephalic reticulospinal Müller cells preferentially excite motoneurons supplying the ventral part of the myotome, whereas Müller cells in the middle rhombencephalic reticular nucleus would activate motoneurons supplying the dorsal part of the myotome (128). In keeping with this line of reasoning, it should be noted that these two groups of fast reticulospinal neurons also have different inputs from the anterior and posterior vestibular nerve, each of which can elicit differentiated types of righting responses (102, 103, 105). Moreover, certain Müller cells have specific connections to different classes of inhibitory pre-motor interneurons (18). For instance, as the motoneurons of one side is monosynaptically excited, the motoneurons of the contralateral side is inhibited (18, 103). Stimulation of single Müller cells during fictive locomotion can induce the predicted changes of the motor pattern (22).

How do we reconcile this suggested specificity, with the fact that most reticulospinal neurons will be phasically excited during locomotion and perhaps even contribute to maintaining an appropriate excitability in the locomotor network (see above). Probably there is no contradiction between these two roles. For example, if a ventro-flexion is needed during locomotion, the mesencephalic Müller cells could

be turned on more intensively in each swimming cycle (and thereby ventral myotome motoneurons), and conversely the middle rhombencephalic reticulospinal neurons could be depressed in each cycle on both left and right sides. This would result in a ventro-flexion well integrated with the ongoing locomotor movements. To elicit turning to the left or right side it would be sufficient to provide additional excitation of the reticulospinal neurons on one side and possibly to decrease the activity on the other side (cf. 40). Thus a very simple type of steering control as well as equilibrium correction could be achieved by effects acting on the spinal output by a very simple command directed to specific groups of reticulospinal neurons. It would seem logical that the most fast conducting reticulospinal neurons, that is the Müller cells, would be involved in steering, which requires very rapid corrections of the direction of swimming during demanding tasks such as attacking a prey. Little is known about which neuronal systems produce the steering and equilibrium commands, but it seems likely that tectum and the adjacent torus semicircularis are involved, with their specific afferent projections from several sensory modalities (see above). Although relevant lamprey experiments are lacking, a stimulation of tectum in dogfish is known to elicit not only locomotion but detailed turning responses related to the specific area stimulated (45).

CONCLUSIONS

It is evident that the different reticulospinal systems are very important in the control of different motor acts. We now know a fair amount about which neurons they interact with in the spinal cord and which transmitters they use, and what cellular mechanisms these transmitters act upon. Although more knowledge is required concerning the spinal mechanisms controlled, it appears that the challenge in the future will be to find out what type of brainstem neuronal connectivity is used to activate the different reticulospinal systems under various types of motor acts and patterns of behaviour. How do the basal ganglia interact with the mesencephalon and how do the tectum and torus semicircularis areas affect the motor performance. How are visual, vestibular and other afferent inputs processed in their control of behaviour, and so forth. The lamprey nervous system should remain a useful model for a further analysis of these problems.

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