Cell Tissue Res (1992) 269:481–493

Cell & Tissue Research

Cytoarchitectural organization of the electromotor system in the electric catfish (*Malapterurus electricus*)

Thomas Schikorski, Norbert Braun, and Herbert Zimmermann

Zoologisches Institut der Johann Wolfgang Goethe-Universität, Postfach 111932, Siesmayerstrasse 70, W-6000 Frankfurt am Main 11, Federal Republic of Germany

Received March 10, 1992 / Accepted May 21, 1992

Summary. The cytoarchitectural organization of the electromotor system of the electric catfish (*Malapterurus electricus*) was investigated in order to obtain insight into the neuronal reorganization accompanying the functional transition of a presumptive previous motor system to an electromotor system eliciting electric organ discharge. The electric catfish possesses two giant electromotoneurons situated within the rostral spinal cord. Intracellular dye injections have revealed the enormous extension of the dendritic tree of electromotoneurons. About 50 primary dendrites span the entire lateral funicle and intermediate grey matter, and reveal an extensive contralateral projection. The giant dendritic tree (1.2 mm in rostrocaudal direction) presumably receives inputs from all ascending and descending pathways of the spinal cord. Electromotoneurons and motoneurons receive the same type of fibre inputs, and electromotoneurons and interneurons are connected through common presynaptic elements. The innervation pattern of the electromotoneurons and spinal motoneurons is similar. Synaptic terminals with round synaptic vesicles often reveal chemical contacts and gap junctions. Furthermore, dendrites of the two electromotoneurons form juxtapositions (ephapses) with each other and also with spinal interneurons. Our results suggest that the two electromotoneurons are homologous to median (primary) spinal motoneurons and are the central structures of the electromotor system within the central nervous system of the electric catfish. A high capability of information processing can be attributed to the giant dendritic trees from functional considerations. This presumably enables the electromotoneurons to elicit an electric organ discharge in different behavioural contexts with a minimum of functional reorganization.

Key words: Cytoarchitecture – Dendritic organization – Motoneurons – Motor control – Spinal cord – Electric fish, *Malapterurus electricus* (Teleostei)

Correspondence to: H. Zimmermann

The two electric organs of the strongly electric catfish (*Malapterurus electricus*) are each innervated by a single giant electromotoneuron (Bilharz 1857; Fritsch 1887). The somata of the two electromotoneurons are situated in the cervical spinal cord between the second and third ventral root. Their axons leave the cord ventro-medially, giving rise to two single axons, the electromotor nerves. Since the somata can have a diameter of up to 0.2 mm, the electromotoneurons of the electric catfish represent a rare example of identifiable neurons within the vertebrate central nervous system.

Different types of electric organ discharge are observed depending on the behavioural context (Bauer 1968; Belbenoit et al. 1979). Long sequences of repetetive spikes (volley) lasting several seconds are used for stunning prey. The electric organ can be discharged several times depending on the size of the prey and the success of previous volleys (Bauer 1968). One volley can be composed of up to 900 individual discharges. Short volleys of normally 4 spikes are used for defence. The number of discharges can reach up to 50 per volley depending on the behavioural context.

The two electromotoneurons receive synaptic inputs from both brain and spinal cord, and are electrotonically coupled (Bennett et al. 1967). The pattern of synaptic innervation has been partially analysed in previous ultrastructural investigations (Bennett et al. 1967; Janetzko et al. 1987). Electromotoneurons carry two major types of synapses. One contains flat electron-lucent vesicles, reveals no presynaptic dense projections (type I; Uchizono 1974) and might be inhibitory. The other forms a mixed chemical and electrical contact. It contains round electron-lucent vesicles and to a lesser extent dense-core vesicles (type II). In addition to a chemical type of synaptic contact (with pre- and postsynaptic densities) these synapses contain gap junctions.

The phylogenetic origin of the electric organ of the electric catfish has long been debated. The intimate connection of the electric organ to the skin has prompted earlier investigators to suggest an origin from epidermal glandular cells (Fritsch 1883, 1887). However, the obser-

vation of Maurer (1913) that the shoulder girdle exhibits a small deficiency in the muscular wall and the demonstration of a proliferation zone of electroplaque cells near the shoulder girdle (Johnels 1957) imply a muscular origin. The functional transition from a motoneuron to an electromotoneuron eliciting electric organ discharge must have been accompanied by neuronal reorganization.

The present paper is part of a study to elucidate the mechanism of control of the electric organ discharge in the electric catfish and the type of functional and structural alterations that have occurred during the formation of this new effector system. Our preliminary results have suggested that the brain of the electric catfish resembles closely that of other siluriform fish. Furthermore, there is no indication of the existence of specialized premotor systems that could, like the pacemaker nuclei in weakly electric fish, produce the pattern of electric organ discharge (Dye and Meyer 1986). This has prompted us to study, in greater detail, the neuronal cytoarchitecture of the cervical spinal cord within the range of the giant electromotoneurons. The study includes a detailed analysis of the dendritic organization of the electromotoneuron and a comparison with that of other spinal motoneurons. Some of the results have been published in abstract form (Schikorski and Zimmermann 1989; Braun et al. 1990; Schikorski et al. 1990).

Materials and methods

Animals, anaesthesia, and fixation

Electric catfish (*Malapterurus electricus*) of either sex, between 8 cm an 16 cm in length, were obtained from local fish dealers. They were kept in de-ionized water (200 µS) at 22–26° C. Although the literature suggests that a "large mouth" species of *Malapterurus electricus* may be distinguished from a "narrow mouth" species (Poll and Gosse 1969; Mahy 1970), we did not further identify our specimens. Fish (21 animals) were anaesthezised with 0.05% tricaine methane sulphonate (Sigma, Deisenhofen, FRG). During all experimental procedures anaesthesia was maintained by superfusing the gills with water containing 0.015%–0.025% of the anaesthetic.

For fixation, animals were perfused via the arterial trunk first with 20 ml saline (pH 7.2) containing heparin (1 mg/ml, Sigma) and tricaine (0.005%) and then with fixative (90 ml) as indicated for the different procedures. After perfusion, the brain was removed and immersed in the same fixative for 2–14 h at 4° C.

General anatomy

Brains were fixed with 2.5% glutaraldehyde (Plano, Marburg, FRG) in 100 mM phosphate buffer (pH 7.4). After fixation, the tissue was washed several times with phosphate buffer, embedded in agar and cut transversally or horizontally using a Vibratome. Sections were osmicated (1% OsO_4), dehydrated and embedded in Epon. Semi-thin sections (2.5 µm) were cut from the embedded Vibratome sections and stained with toluidine blue.

Dye injection

Anaesthetized fish were paralysed by an intramuscular injection of 10 µl gallamine (0.03%, Sigma). To prevent electric organ discharges, electric nerves were cut at the site where they enter the electric organ at the ventral side of the body.

For intracellular dye injection, the electromotoneuron was identified by its electrically evoked large extracellular field potentials (Bennett et al. 1967). Glass microcapillaries were used as recording electrodes and were filled with 8% horseradish peroxidase (HRP, Sigma Type VI) in 0.2 M KCl or with cobaltic lysine (Görcs et al. 1979). After successful penetration, the substances were iontophoretically injected into the electromotoneuron using 1 Hz current pulses of +10 to +30 nA of 500 ms duration (up to 2.5 h). Before perfusion, the dye was allowed to diffuse for 1–2 h.

Following HRP-injection, the brain was fixed with 2.5% glutaraldehyde in 100 mM phosphate buffer as described above. After being washed, the Vibratome slices (100 µm) were cut and placed in phosphate buffer. Peroxidase was visualized with 0.05% diaminobenzidine (Sigma) and 0.01% hydrogen peroxide (Fluka, Neu-Ulm, FRG). Sections were then embedded in glycerol-gelatine, viewed with a light microscope and photographed. Subsequently coverslips were removed, and after several rinses with phosphate buffer, the slices were osmicated, dehydrated, immersed in 2% uranyl acetate and embedded in Epon. Semithin sections obtained from embedded slices were stained with toluidine blue. After light-microscopical analysis they were further embedded in Epon for ultrathin sectioning.

Following cobaltic-lysine labelling, animals were perfused with 100 mM phosphate buffer and 0.02% ammonium sulphide (Merck, Darmstadt, FRG) for the precipitation of cobalt ions. This was followed by perfusion with ammonium-sulphide-free phosphate buffer and finally by perfusion with 4% paraformaldehyde (Merck) and 0.5%–1% glutaraldehyde in 100 mM phosphate buffer (pH 7.4). The cobalt precipitate was visualized in Vibratome sections by silver intensification according to Gallyas (1979) and the sections were fixed with 0.5% sodium thiosulphate. The subsequent steps were identical to those described for the HRP injection procedure.

Electron microscopy

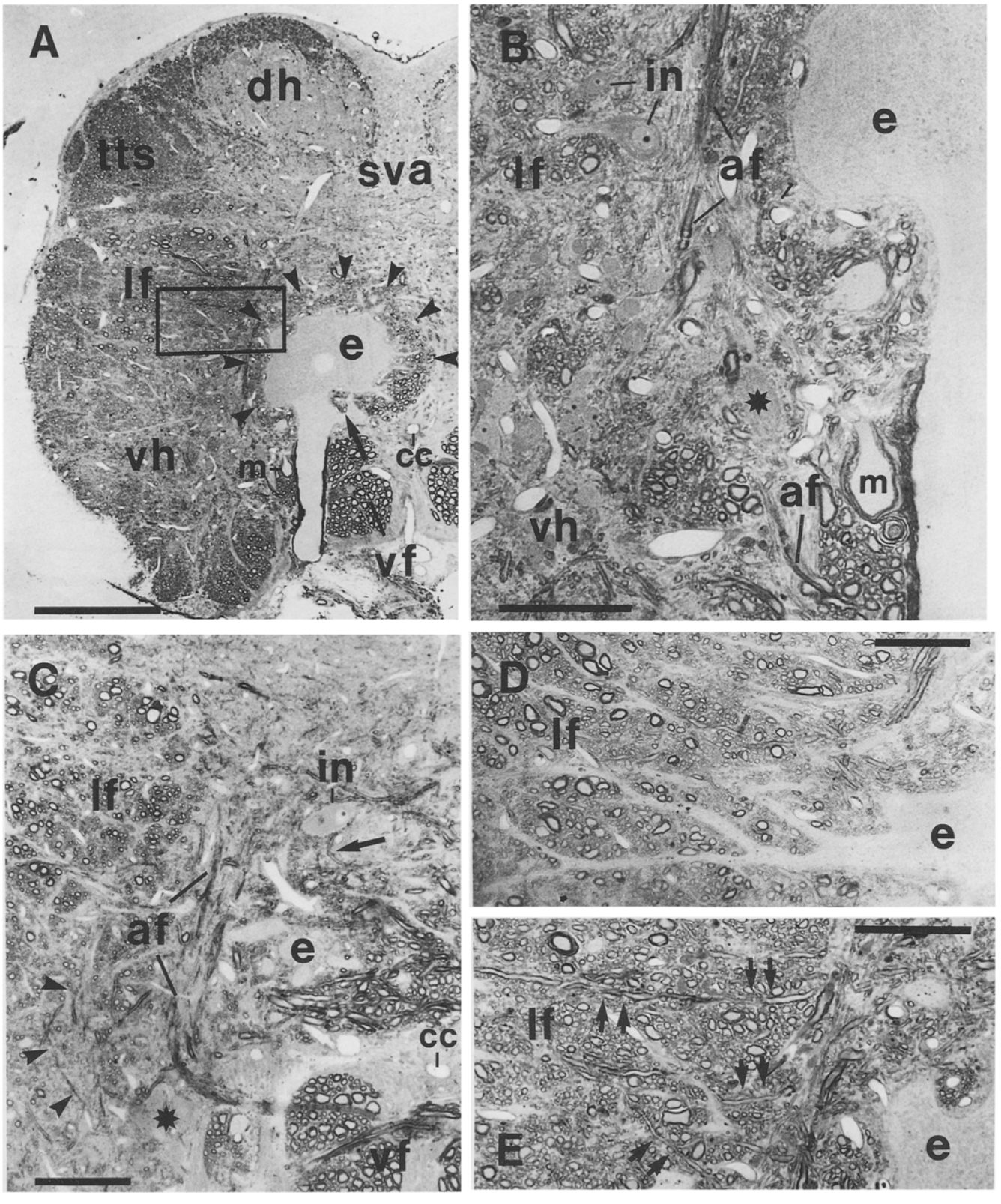
Ultra-thin sections (Ultracut, Reichert and Jung) were stained with 1% uranyl acetate and lead citrate according to Reynolds (1963). Electron micrographs were taken using a Zeiss EM-902 electron microscope.

Results

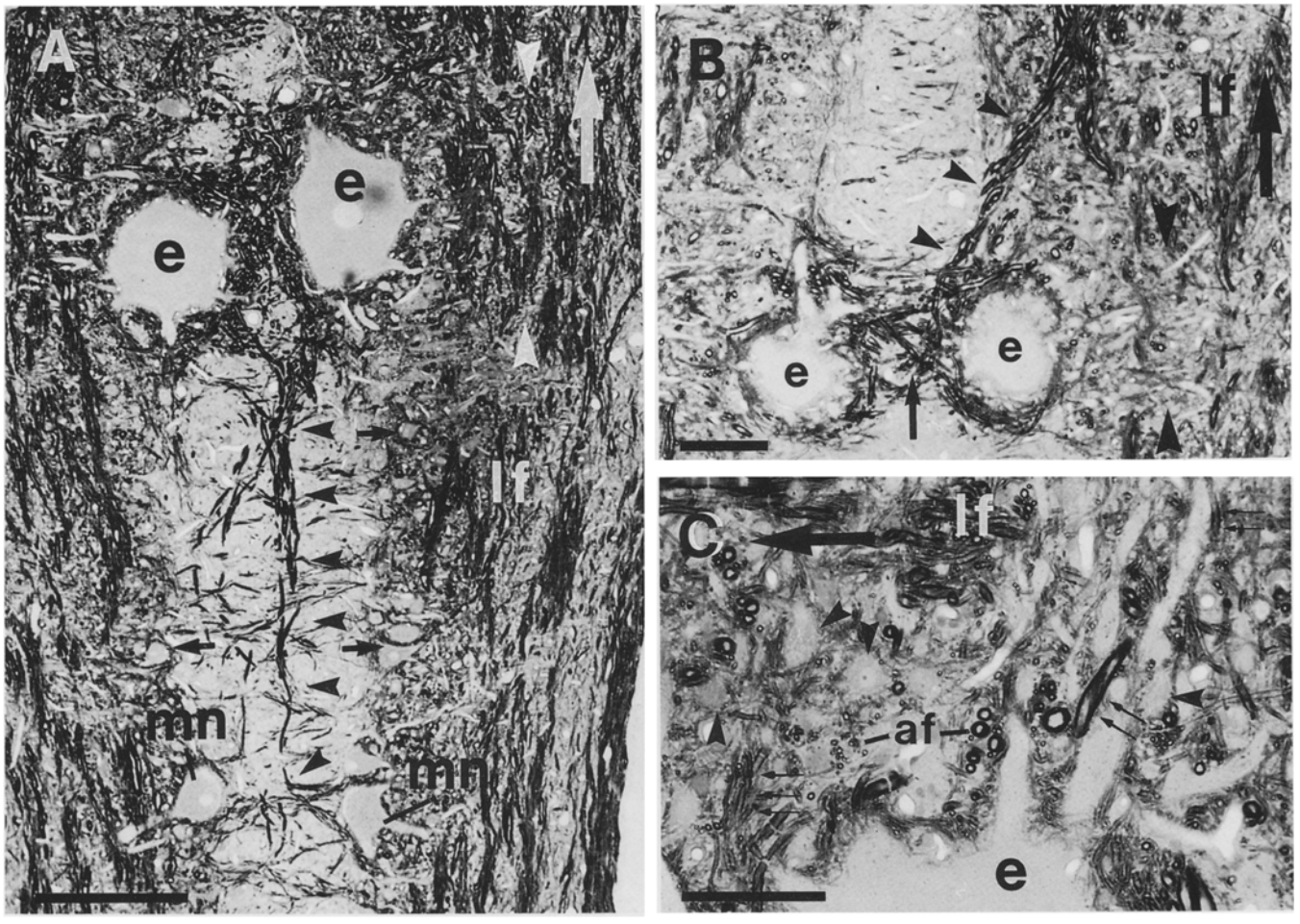
General anatomy

Electromotoneurons. The large somata of the two electromotoneurons are situated close to the sagittal plane of the cervical spinal cord, between the second and third

Fig. 1 A–E. Transverse semi-thin sections of the rostral spinal cord of the electric catfish. **A** An overview at low magnification reveals the relative size of the giant soma of the electromotoneuron (*e*). It is located dorsal to the central canal (*cc*) and is surrounded by a net of myelinated fibres (*arrowheads*). Its ventrally proceeding axon is situated medial to the Mauthner axon (*m*). The *arrow* marks a perforation of the soma of the electromotoneuron. ×110. **B** Aspect of **A** at higher magnification revealing the position of lateral interneurons (*in*, one possessing a lateral dendrite) in close contact with arcuate fibres (*af*) running in a dorsal direction adjacent to the fibre net and of a motoneuron (*star*). The Mauthner axon (*m*) forms a collateral. ×470. **C** Detail of the main course of ar-



cuate fibres (*af*) and of an additional, more laterally located course (*arrowheads*). Transverse section caudal to the electromotoneuron revealing only its dendritic trunks (*e*). A large interneuron (*in*) dorsal to the electromotoneuron in close proximity to the fibre net is contacted by a myelinated fibre (*arrow*). The *star* marks a group of motoneurons lateral to the electromotoneuron. ×170. **D** Ramification of lateral dendrites of the electromotoneuron within the lateral funicle. Dendritic processes are often in apparent contact with fibres and proceed through individual tracts within the funicle. The approximate position of the detailed view is given in the *rectangle* in **A**. ×330. **E** Centripetal projection of fibres from the lateral funicle (*arrows*) to the fibre net as observed along the entire extension of the funicle. Position as for **D**. ×410. *dh* Dorsal horn; *lf* lateral funicle; *sva* sensory visceral area; *tts* tractus spinalis trigemini; *vf* ventral funicle; *vh* ventral horn. *Bars*: 200 μm in **A**; 50 μm in **B**; 100 μm in **C**; 50 μm in **D**, **E**



Figs. 2A–C. Horizontal sections of the rostral spinal cord dorsal to the central canal. The dorsal direction is marked by *large arrows*. **A** A pair of large motoneurons (*mn*) is located within the next segment of the spinal cord caudal to the electromotoneurons (*e*). Fibres originating from the fibre net (*small arrowheads*) surrounding the electromotoneurons proceed to the motoneurons and participate in the formation of their fibre net. *Small arrows* additional smaller motoneurons; *large arrowheads* dendritic projections of electromotoneurons into the lateral funicle (*lf*). ×100. **B** Section slightly dorsal to the axon hillock of the electromotoneuron (*e*) depicting the left bundle of myelinated fibres (*small arrowheads*), entering the fibre net and surrounding the somata. Some of the fibres cross the midline (*arrow*). *Large arrowheads* dendritic projections of electromotoneurons into the lateral funicle. ×120. **C** Lateral column of interneurons (*arrowheads*). Arcuate fibres (*af*, transversally cut) run in close apposition to interneurons (*arrowheads*) and dendrites of the electromotoneuron (*e*). Fibres projecting from the lateral funicle proceed parallel to the dendrites of the electromotoneuron (*small arrows*). ×400. *Bars*: 200 μm in **A**; 100 μm in **B**; 50 μm in **C**

ventral spinal root (Figs. 1A, 2A). They are separated by bundles of myelinated fibres. In larger animals the somata are sometimes perforated several times by bundles of axons and by capillaries (Fig. 1A). Even within these perforations, the surface of the soma is densely occupied by synapses. The axon originates at the level of the central canal, which is located ventral to the electromotoneurons. The axon passes straight in a ventral direction, medial to the Mauthner axon (Fig. 1A, B), and leaves the spinal cord with the third ventral root. Numerous primary dendrites arise from the soma (Figs. 3A, 4). The lateral dendrites enter the lateral funicle, and close associations can be observed between dendrites and axons of the funicle (Figs. 1D, 2A–C). Medial dendrites cross the midline and project contralaterally into the intermediate grey matter (Figs. 3A, 4). In addition, dorsally directed dendrites and the rostral and caudal dendrites arborize within this intermediate neuropile. Myelinated fibres form a dense net around the cell bodies of the electromotoneurons (Figs. 1A, 2A, B). It has its largest extension median to the somata (~65 μm). Laterally, the fibre net is only ~20 μm thick. The fibre net dorsal to the somata is ~55 μm wide and continues into the intermediate neuropile. Only dendrites of the electromotoneurons are found in this extensive fibre net.

Fibres of the lateral funicle. The fibre net around electromotoneurons is formed by fibres originating from the lateral funicle. Fibres enter the fibre net in a centripetal direction along the entire dorso-ventral extension of the funicle (Figs. 1E, 2C). Individual fibres differ in diameter and therefore probably represent axons and their collaterals. They often run in close proximity and in parallel to the electromotoneuron dendrites (Fig. 2C). A prominent rostral fibre tract arriving at a level slightly dorsal to the axon hillocks of the electromotoneurons

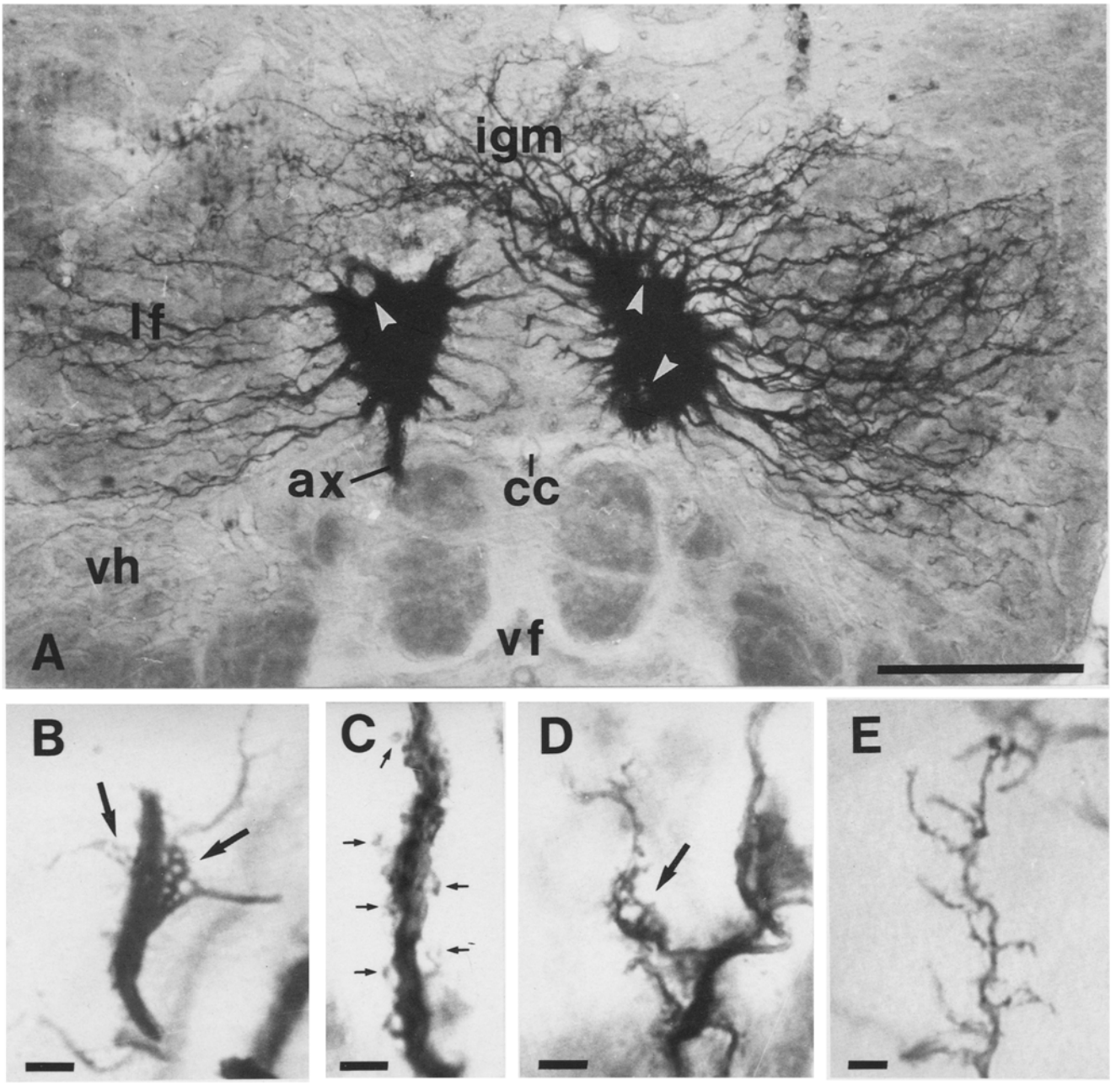


Fig. 3A–E. Aspects of cobaltic-lysine- (**A**) and HRP-filled (**B–E**) electromotoneurons. **A** 100-μm-thick transverse section through the somata of the electromotoneurons depicting a large number of long dendrites running in lateral, medial, dorsal and contralateral directions. *Arrowheads* Perforations of the cell soma; *ax* axon of electromotoneuron; *cc* central canal; *igm* intermediate grey matter; *lf* lateral funicle; *vf* ventral funicle; *vh* ventral horn. ×160. **B** Dendritic perforations (*arrows*) often occur in clusters resulting in a net-like structure. ×1500. **C** Aspect of a single dendrite showing spines (*arrows*) with long spine stems. ×1500. **D** Fine dendrites ensheathing cellular structures, which are possibly interneurons and dendrites. ×1500. **E** The distal arborization of a dendritic terminal. ×1200. *Bars*: 200 μm in **A**; 5 μm in **B–E**

continues to the median side of their somata (Fig. 2B). This tract gives rise to a large part of the fibre net surrounding the cell bodies. Axons orginating from this fibre net also continue beyond the electromotoneurons in a caudomedial direction and form a small median tract (Fig. 2A). Interestingly, the next segment of the spinal cord contains another pair of large motoneurons (Fig. 2A). Again, their somata are surrounded by a network of fibres that are derived from the median tract and from the lateral funicle. Such fibre nets are often observed around larger motoneurons (Fig. 2A) and large interneurons (Fig. 1C), and may not be detectable around small neurons.

Fibres of the ventral funicle. Fibres of the ventral funicle, like those of the median longitudinal fascicle, originate from the brain stem and descend into the spinal cord. Even in the brain stem, arcuate fibres form a prominent

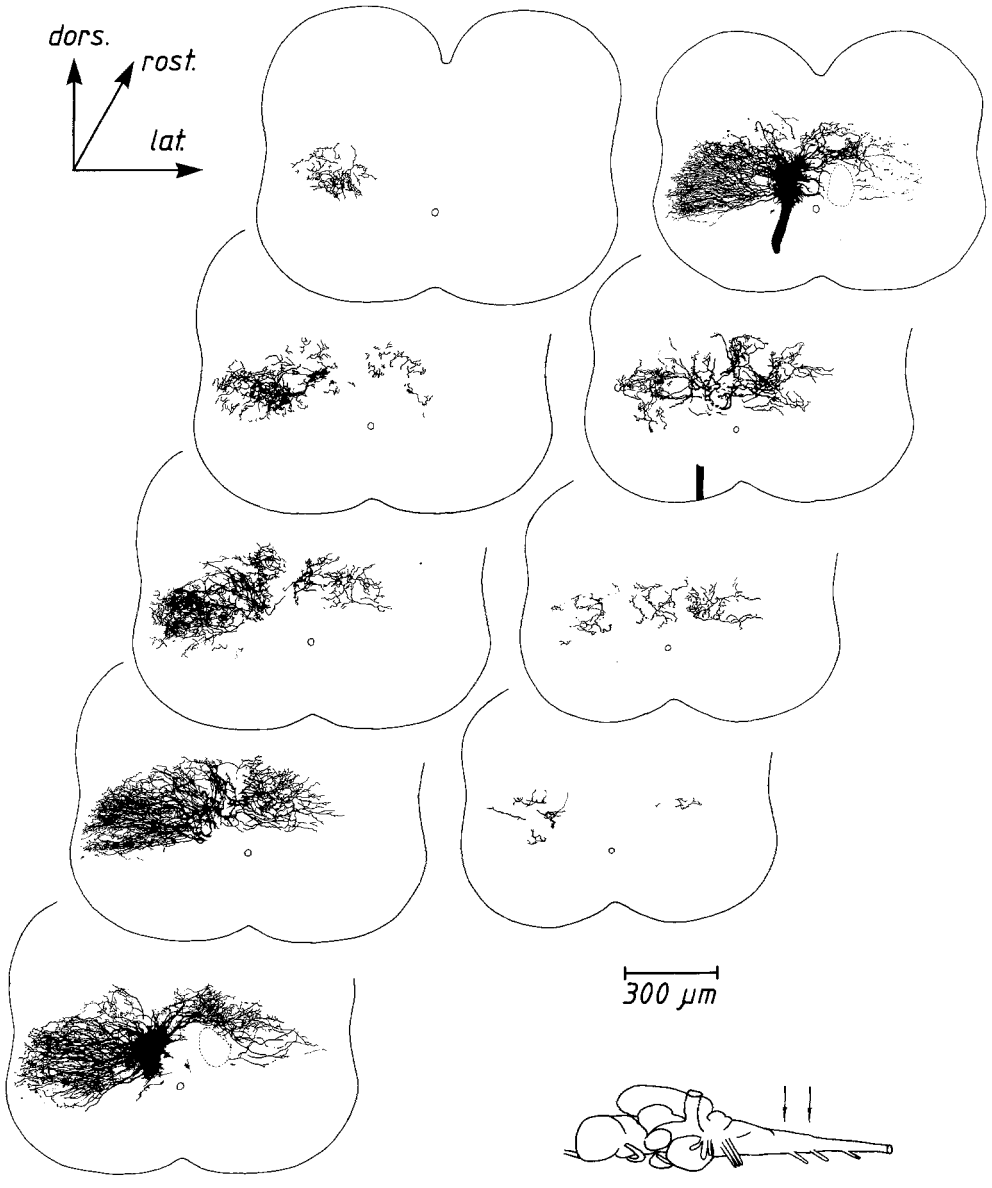


Fig. 4. Reconstruction of 100-µm-thick transverse sections through an HRP-filled electromotoneuron. The range of the rostral spinal cord that has been sectioned is indicated by the *two arrows* at the lateral aspect of the brain of the electric catfish (*bottom right*). Within the lateral funicle, lateral dendrites are observed over a length of at least 1.0 mm, whereas within the intermediate grey matter, the rostro-caudal length of the dendritic tree reaches 0.8 mm. The final few dendrites in the subsequent sections are not shown

commissure. Similar arcuate fibres are observed at the level of the electromotoneurons (Figs. 1B, C, 2C). Some of these originate in the ventral fascicle, whereas others are commissural fibres. Spinal arcuate fibres are loosely associated rather than forming a compact tract as in the brain stem. Some of these fibres proceed in a dorsal direction directly adjacent to the fibre net; here, they branch (Fig. 1A, C). Other arcuate fibres first run laterally and only then turn in a dorsal direction to penetrate the lateral funicle where they branch (Fig. 1C). Horizontal sections clearly reveal the close apposition of arcuate fibres and dendrites of the electromotoneurons at different distances from the soma (Fig. 2C).

Spinal neurons at the level of the electromotoneurons. Numerous neurons are observed within the range of the electromotoneurons. Neurons of the ventral horn including large dorsal motoneurons (about 1000 µm^2) are also situated near the axon hillock of the electromotoneurons (Figs. 1B, 2A). Dorsal to the motoneurons, there is a horizontal column of interneurons of varying sizes (diameters between 7 µm and 33 µm; Figs. 1B, 2C). Their dendrites project into the lateral funicle; the arcuate fibres of the ventral funicle are often adjacent to their somata. Occasionally, dendrites of these interneurons proceed dorsally and run parallel to the arcuate fibres. These interneurons, like electromotoneurons, also display close appositions with fibres of the lateral funicle.

The central area of the intermediate grey matter contains interneurons of a large range of sizes. The largest have a diameter of up to 54 µm (Fig. 1C). These interneurons are also closely adjacent to fibres of the fibre net and to fibres originating from the lateral funicle. Similar interneurons are found rostrally and caudally to the electromotoneuron somata and in other spinal segments, but there they take a more ventral position. It appears that, because of the enormous size of the electromotoneuron somata, the intermediate grey matter

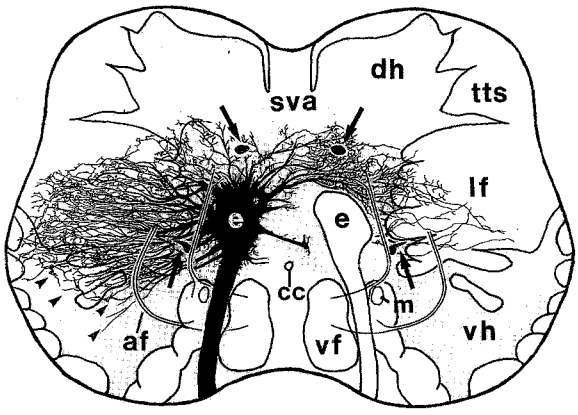


Fig. 5. Schematic overview of the cytoarchitecture of the electromotoneuron within the cervical spinal cord. *Arrows* mark positions of interneurons; *arrowheads* indicate dendrites in the dorsal part of the ventral horn. *White* White matter; *grey* grey matter; *af* arcuate fibres; *cc* central canal; *dh* dorsal horn; *e* electromotoneuron; *lf* lateral funicle; *m* Mauthner axon; *sva* sensory visceral area; *tts* tractus spinalis trigemini; *vf* ventral funicule; *vh* ventral horn

in the electric catfish has become dorsally, rostrally and caudally displaced. This would also explain the extreme ventral location of the central canal in the cervical cord of *Malapterurus* compared with other fish (Fig. 1A).

Morphology of the electromotoneuron as revealed by dye-injection

Extension of the dendritic tree. Only intracellular dye-injections (HRP or cobaltic lysine) reveal the enormous extension of the electromotoneurons (Figs. 3A, 4). In addition to numerous fine dendrites that do not leave the fibre net, the neuron contains at least 50 large primary dendrites. Fine primary dendrites originate even within the perforations of the cell soma.

The large primary dendrites give rise to numerous fine branches resulting in an extremely dense dendritic projection. All ipsilateral dendrites traverse the lateral funicle and almost reach the lateral margin of the spinal cord. In a rostrocaudal direction, the lateral funicule is occupied by electromotoneuron dendrites over a range of 1.2 mm (Fig. 4). The rostral extension thereby exeeds the caudal extension by about 200 µm. Although the dendritic arborizations run through the entire lateral funicle, they do not reach the spinal trigeminal tract (Tuge et al. 1968; Figs. 1A, 3A). Some ventral dendrites branch within the dorsal part of the ventral horn (Fig. 3A).

Rostrally and caudally directed dendrites arborize within the intermediate grey matter, turn laterally and proceed within the lateral funicle. Within the grey matter, the dendritic tree has a rostrocaudal extension of 700–800 µm (Fig. 4).

Median and dorsal dendrites arborize within the intermediate grey matter. They cross the midline and possess an extensive contralateral arborization zone (Figs. 3A, 4). Some of these dendrites enter the contralateral lateral funicle but only a few reach the contralateral margin of the spinal cord (Fig. 4). The dendritic projections have the same extension and are of similar density within the ipsi- and contralateral neuropile. The dorsal dendrites reach the ventral margin of the sensory visceral area but the dorsal horn is free of branches (Figs. 1A, 3A). Furthermore, no electromotoneuron dendrites occur within the ventral funicule. No recurrent axon collaterals of the electromotoneuron have been observed.

Dendritic shape. The diameters of primary dendritic trunks range from 0.7 µm to 22 µm. Diameters are reduced by half, halfway along the dendritic length. Many fine processes originate from these primary dendrites but the formation of second- and third-order dendrites is rarely observed. This results in a radiating structure of the dendritic tree (Fig. 3A). All dendrites carry spines (Fig. 3C) but these occur more often on dendrites within the grey matter. The number of spines varies between individuals. They can be less frequent and small, or numerous and exhibiting long spine stems. The reason for this variability is not clear. The number and shape of the spines are possibly correlated with the variable usage of the electromotor system by animals kept in captivity for different lengths of time. Dendrites are often perforated. Clusters of such perforations give rise to a net-like appearance of the dendrites (Fig. 3B). Fine dendritic processes frequently form a plexus (Fig. 3D) that surrounds cell bodies and processes of other spinal neurons. Dendritic terminals reveal numerous tiny branches (Fig. 3E).

The principal cytoarchitectural features of the rostral spinal cord within the range of the electromotoneurons are summarized in Fig. 5.

Connectivities within the dendritic tree

Innervation of dendrites. Dendrites are not homogeneously innervated. Regions with densely packed synapses alternate with regions lacking axon terminals. Nerve terminals originate from both myelinated and unmyelinated fibres (Fig. 6B, C). Both types of fibres give rise to the two types of nerve terminals that have previously been described (Janetzko et al. 1987): nerve terminals with flat electron-lucent synaptic vesicles (type I) and nerve terminals with round electron-lucent vesicles as well as electron-dense vesicles displaying both chemical and several electrical contacts (gap junctions) (type II). On electromotoneuron dendrites, type-II synapses are the predominating type. Myelinated and unmyelinated fibres often form en passant synapses (Fig. 6B) that are of the second type.

Within the intermediate grey matter, dendrites of the electromotoneurons form a dense neuropile together with dendrites of spinal neurons and axon collaterals of differing origin (Fig. 6A). Electromotoneuron dendrites often reveal a ruffled surface with numerous indentations forming a bowl-like structure and containing axon terminals or dendrites (Fig. 6C). Presynapses

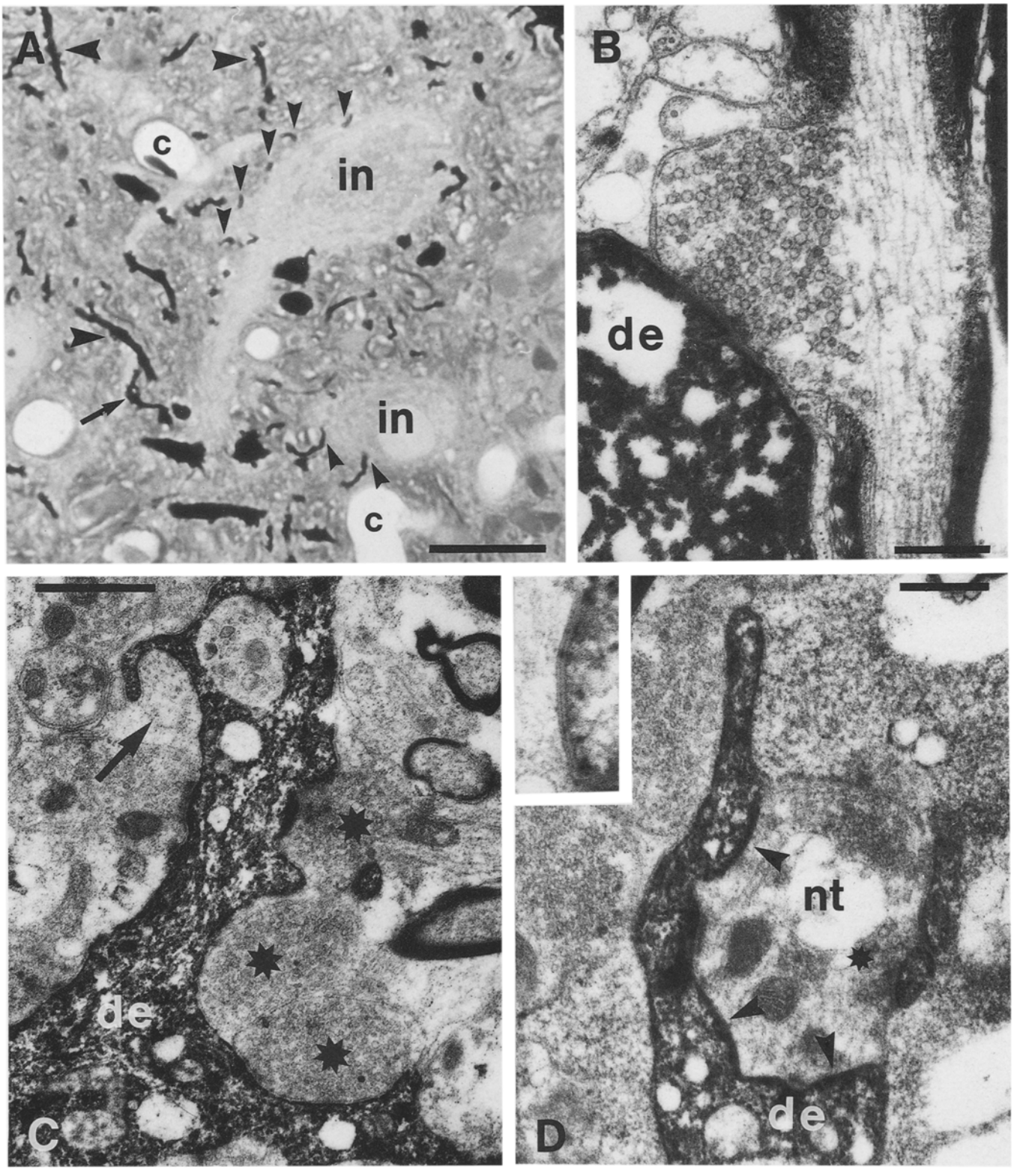


Fig. 6A–D. Contacts of cobaltic-lysine- (**A**) and HRP-labelled dendrites (**B–D**) of electromotoneurons with various cellular elements. **A** Semithin section of the intermediate grey matter after cobaltic-lysine filling. Small dendritic processes (*arrowheads*) are found in close apposition to interneurons (*in*). Dendrites often reveal a ruffled surface (*large arrowheads*). The *arrow* marks the perforation of a dendrite. *c* Capillary. ×1000. **B** Innervation of a labelled dendrite (*de*) of the electromotoneuron by an en passant synapse that originates from a node of Ranvier. ×33500. **C** Innervation of a dendrite with ruffled surface as in **A**. Synaptic terminals are located within indentations of the dendritic surface (*stars*). The dendrite (*de*) also ensheaths a fine unlabelled dendritic process (*arrow*). ×25000. **D** Ultrastructure of a dendritic perforation as in **A**. Such perforations often reveal gap junctions (*arrowheads* and *inset*; *inset*: ×82000) but only rarely chemical synapses (*star*; *nt* nerve terminal). ×31000. *Bars*: 20 μm in **A**; 0.5 μm in **B**; 1 μm in **C**; 0.5 μm in **D**

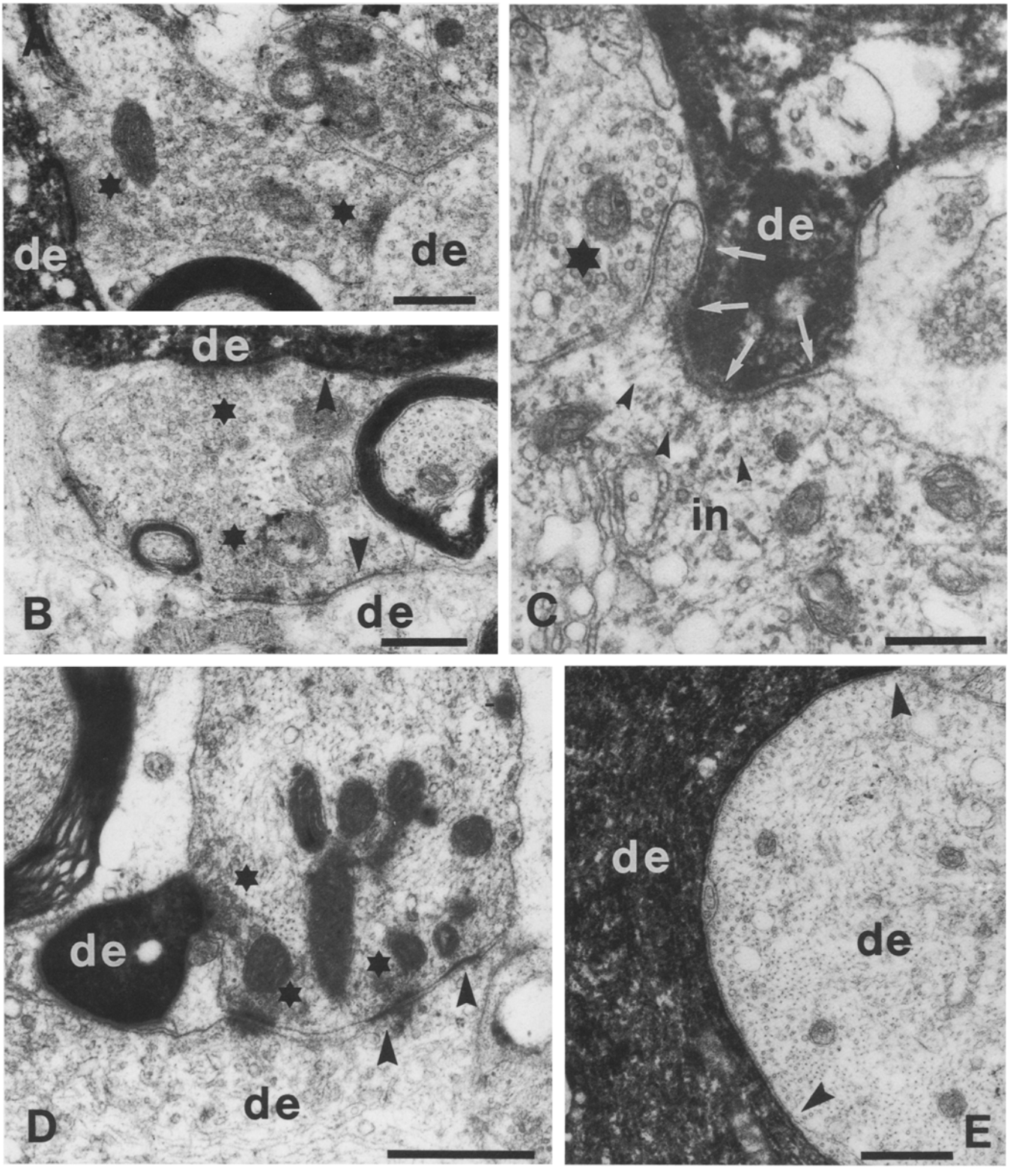


Fig. 7A–E. Innervation of two postsynaptic structures by a common presynaptic element. One electromotoneuron was labelled with HRP. **A** Symmetrical synaptic contact (*stars*; type I) with dendrites (*de*) of both labelled and unlabelled electromotoneuron within the fibre net. ×29000. **B** Mixed chemical (*stars*) and electrical (*arrowheads* gap junction) contacts with a labelled dendrite of the electromotoneuron and a dendritic process of unknown origin. ×30000. **C** Small process of a labelled dendrite in close apposition with the soma of an interneuron (*in*) corresponding to the fine processes marked by *arrowheads* in Fig. 6A. The gap between the two membranes is 20 nm and the total contact as revealed by an analysis of serial sections is up to 3 µm long. At the site of the interneuron, electron-dense structures radiate from the gap. In the adjacent section (not shown), the nerve terminal marked by a *star* forms a mixed electrical-chemical synaptic contact with the interneuron. ×35000. **D** Synaptic terminal contacting a primary dendrite (*de*, black letters) of the unlabelled electromotoneuron and a fine dendritic process (*de*, white letters) of the labelled electromotoneuron. The section reveals gap junctions (*arrowheads*) only at the unlabelled dendrite, whereas the labelled dendrite carries exclusively a chemical contact (*stars*). ×26000. **E** Juxtaposition (within the fibre net) between dendrites of labelled and unlabelled electromotoneurons. The gap is reduced to about 17 nm and is 4 µm long (between *arrowheads*). ×32000. *Bars*: 0.5 µm in **A–C**; 1 µm in **D**; 0.5 µm in **E**

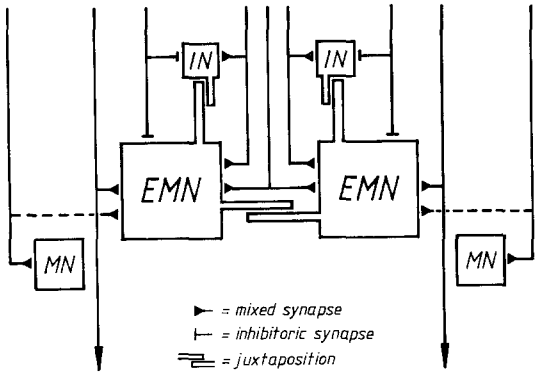


Fig. 8. Schematic summary of connectivities of the electromotoneuron (*EMN*) within the spinal cord, including contacts with interneurons (*IN*) and motoneurons (*MN*). It reveals the high level of common innervation of interneurons and electromotoneurons. *Dashed lines* based only on light-microscopical evidence

aligned in series are separated by dendritic folds that form the rim of the "bowls". The lateral folds of the dendrites do not carry sites of contact. These are confined to the bottom of the indentations and reveal synapses of type I and II. Axons are sometimes completely enclosed, resulting in a perforation of dendrites (Fig. 6D). Electrotonic contacts via gap junctions predominate at these perforations.

Connections with spinal interneurons. Labelled dendrites of electromotoneurons are often observed in close apparent contact with dendrites and somata of interneurons (Fig. 6A). Thick sections reveal that these dendrites sometimes wind around interneurons and their processes (Fig. 3D). Dendrites may approach a cell body producing fine processes to contact the surface (Fig. 6A). Electron-microscopical analyses have rarely revealed direct contacts between cells. However, the dendrites of the electromotoneuron and dendrites or soma of an interneuron are often found to be innervated by a common presynaptic terminal (this type of contact is depicted in Fig. 7A, B). Such synapses can be of either type. They include gap junctions between the presynaptic terminal, and both the electromotoneuron dendrite and the interneuron.

Occasionally, fine processes of the labelled electromotoneuron dendrite have been found to form a synapse-like contact with an interneuron (Fig. 7C). The membrane apposition at such contacts is extended, containing an electron-dense matrix and a postsynaptic-density-like structure. The apposition can be up to 3 μm long, but often has a length of only 0.5 μm–1 μm.

Connections between the two electromotoneurons. Close dendritic appositions can also be found between electromotoneurons. One principal type of contact is depicted in Fig. 7D. A thin dendrite of the contralateral and labelled cell approaches a primary dendrite of the unlabelled electromotoneuron. This primary dendrite carries a mixed electrical-chemical contact that in addition makes a chemical synaptic contact with the labelled dendrite. In addition, within the fibre net, which contains only electromotoneuron dendrites, there are numerous dendritic juxtapositions (Fig. 7E). Here, the dendritic membranes of the two electromotoneurons form ~17-nm-wide contacts over a length of several μm.

A schematic summary of the connectivities of the electromotoneurons within the spinal cord is presented in Fig. 8.

Discussion

Electromotoneuron and motoneurons

The cytoarchitecture of spinal motoneurons bears many similarities with that of the electromotoneurons. Generally, primary (or median) motoneurons of fish have a dorsomedian location and their axons run medial to the Mauthner axon (Meyers 1985; Fetcho 1986, 1987). They have long rostrocaudal dendrites and a contralateral dendritic projection that interacts with contralateral motoneurons. These features are shared by the electromotoneurons of the electric catfish. Moreover, the fine-structural characteristics of electromotoneurons are similar to those of somatic spinal motoneurons. Somatic motoneurons of lower vertebrates are innervated by chemical synapses of both types, including mixed electrical-chemical synapses. They have dendritic juxtapositions and a common presynaptic innervation (Schnitzlein and Brown 1975; Erulkar and Soller 1980) as described for the electromotoneurons. The contralateral dendritic projection results in the electrotonic coupling of motoneurons from opposite sides (Erulkar and Soller 1980). These data agree with the conclusion of Johnels (1957) that the electric organ of the electric catfish is phylogenetically derived from muscle. Thus, the giant electromotoneurons represent modified primary motoneurons.

The two electromotoneurons might be coupled electrotonically via gap junctions of common pre-fibres as previously suggested by Bennett et al. (1967). In addition, juxtapositions occur between dendrites of the electromotoneurons. Dendritic juxtapositions where the distance between cells is reduced to ~17 nm are sites of electrotonic coupling in the motoneurons of frogs (Erulkar and Soller 1980). Two different structural features might therefore effect electrotonic coupling between the two electromotoneurons, viz., direct contacts via juxtapositions (ephapses) and indirect contacts via gap junctions of common prefibres. Electrotonic coupling is the prerequisite for synchronous activity of both electromotoneurons followed by synchronous electric organ discharge.

Electromotoneuron and interneurons

There is a considerable number of interneurons within the dendritic tree of the electromotoneurons. Histologically, these interneurons are not different from other spinal interneurons along the spinal cord. Membrane

appositions similar to those between electromotoneurons also occur between the electromotoneuron dendrites and spinal interneurons. Nevertheless, the apposition is about 20 nm wide and the contacts are shorter. These contacts show some features of chemical synapses, such as a radiation of electron-dense material on one side of the contact. However, in spite of the considerable width of the gap, these membrane appositions may be a site of electrotonic coupling if, for example, the membrane resistivity was sufficently low (Bennett and Auerbach 1969).

The common innervation of electromotoneurons and interneurons is frequent. A shared presynaptic terminal would result in synchronous activition of electromotoneurons and spinal interneurons. We thus predict a participation of the interneurons in the electromotor system of the electric catfish. Our light-microscopical investigations also suggest a similar pattern of common innervation of electromotoneurons and somatic motoneurons.

The structure of the dendritic tree

Whereas somatic motoneurons have only a few primary dendrites, electromotoneurons possess about 50 dendrites spanning the entire lateral funicle and the intermediate grey matter. Furthermore, electromotoneurons might obtain inputs from arcuate fibres that originate partly within the ventral funicle. Thus, the giant dendritic tree of the electromotoneurons is capable of contacting all ascending and descending pathways within the spinal cord. In contrast, somatic motoneurons have smaller and distinct dendritic projections areas within the grey matter (Székely 1976, frogs). In fish, the two main descending pathways are the reticulospinal and the vestibulospinal system (Kimmel 1982; Kimmel et al. 1982; Metcalfe et al. 1986; Rao et al. 1987). It is known that these descending systems participate in the initiation and maintenance of various spinal mechanisms, including locomotion (McClellan 1986; Grillner et al. 1989, lampreys; Livingston and Leonard 1990, stingrays; Kashin et al. 1981, carp; Cruce and Newman 1984, reptilia) and the escape response in fish (Eaton et al. 1991).

Individual dendrites of the electromotoneurons can be up to 600 μm long. Synapses on dendrites form clusters leaving surface areas uninnervated. It can be speculated that this clustering of synapses is related to a specific spatial distribution of synaptic inputs. Possibly, these long dendrites, like those of retinal ganglion cells, are electrically non-uniform and can form subunits that are electrically isolated from each other (Koch et al. 1982). The specific spatial distribution of synapses and the precise timing of synaptic inputs at subunits would form the basis for complex information processing. These include logic operations and directional selectivity to motion in some retinal ganglion cells. Interestingly, compartmentalization of a dendritic tree was originally described for a median motoneuron in the goldfish (Yasargil and Diamond 1968; Diamond and Yasargil 1969). Here, the ventral dendrite forms a functional decoupled compartment that contacts a collateral of the Mauthner axon.

The formation of the extensive fibre net with individual fibres first surrounding the soma before forming synaptic contacts can be viewed in the context of a precise timing of the synaptic input: variations in axonal length would cause a differential delay of signals. This could result in an increased time discrimination at the level of the electromotoneuron.

Comparison with other electric fish

Electrotonic coupling is a common feature of electromotor systems in electric fish. In weakly electric fish, electromotoneurons are directly coupled via gap junctions and are activated mainly by electrical contacts derived from the pacemaker nucleus. In this case, the function of the electromotoneurons is reduced to the simple transmission of signals from the medullary pacemaker neurons directly to the electric organ (Dye and Meyer 1986). Thus, the pattern of electric organ discharge is generated by the pacemaker nucleus only. The cytoarchitectonic organization of spinal electromotoneurons of the electric eel (*Electrophorus electricus*) bears similarities to that of the electric catfish. In the electric eel, electrotonic coupling between electromotoneurons is effected by common prefibres forming mixed synapses (Meszler et al. 1974). There is, however, morphological evidence for the existence of a medullary command nucleus (De Oliveira Castro 1961). Only in electric rays (Torpedinidae) the electromotoneurons ($\sim$60000, situated in the electric lobe) carry a purely chemical innervation. Their function is restricted to a one-to-one transmission of impulses received from the reticular command nuclei (Roberts and Ryan 1975; Fox 1977; Krenz 1985).

The electromotor system of the electric catfish: functional considerations

For effective electric organ discharge, the electromotoneurons of the electric catfish require access to information carried by the various descending or ascending pathways; this information would include, for example, the distance of the prey, its size or smell, or the internal emotional state of the fish. Such information is also required by other spinal motor systems to initiate, for example, locomotion in prey-catching behaviour. Nevertheless, in principle, the activity of the electromotoneurons must remain independent of the activation of, for example, locomotive activity. The electromotoneurons have to extract only the essential information from the bulk passing through the spinal cord. This demands a high capability of information processing that is normally achieved by a mass of neurons. Our results suggest, however, that information processing is performed by a giant soma and about 50 huge primary dendrites in the case of electromotoneurons.

Our data thus support the earlier view (Bennett et al. 1967) that the two electromotoneurons are the main structures for the production of electromotor discharges

in the electric catfish. If this is the case, the electromotor system of the electric catfish would have been established with a minimum of functional reorganization of the nervous system. A pre-existing motor innervation involving only two motor neurons might therefore have evolved to become the control centre of a sophisticated behavioural response by a local increase in size and in the complexity of information processing.

Acknowledgement. The financial support of the Deutsche Forschungsgemeinschaft (SFB 45, C8) is gratefully acknowledged.

References

Bauer R (1968) Untersuchungen zur Entladungstätigkeit und zum Beutefangverhalten des Zitterwelses *Malapterurus electricus* Gmelin 1789 (Siluroidea, Malapteruridae, Lacep. 1803). Z Vergl Physiol 59:371–402

Belbenoit P, Moller P, Serrier J, Push S (1979) Ethological observations on the electric organ discharge behaviour of the electric catfish, *Malapterurus electricus* (Pisces). Behav Ecol Sociobiol 4:321–330

Bennett MVL, Auerbach AA (1969) Calculation of electrical coupling of cells separated by a gap. Anat Rec 163:152

Bennett MVL, Nakajima Y, Pappas GD (1967) Physiology and ultrastructure of electrotonic junctions. III. Giant electromotor neurons of *Malapterurus electricus*. J Neurophysiol 30:209–235

Bilharz T (1857) Das elektrische Organ des Zitterwelses. Engelmann, Leipzig

Braun N, Schikorski T, Zimmermann H (1990) Structural and immunocytochemical characterization of the giant electromotoneuron of the electric catfish *Malapterurus electricus*. In: Pfannenstiel H-D (ed) 83. Jahresversammlung der Deutschen Zoologischen Gesellschaft. Fischer, Stuttgart New York, p 631

Cruce WLR, Newman DB (1984) Evolution of motor systems: the reticulospinal pathways. Am Zool 24:733–753

De Oliveira Castro G (1961) Morphological data on the brain of *Electrophorus electricus*. In: Chagas C, Paes de Carvalho A (eds) Bioelectrogenesis. Elsevier, Amsterdam, pp 171–184

Diamond J, Yasargil GM (1969) Synaptic function in the fish spinal cord: dendritic integration. Prog Brain Res 31:201–209

Dye JC, Meyer JH (1986) Central control of the electric organ discharge in weakly electric fish. In: Bullock TH, Heiligenberg W (eds) Electroreception. Wiley, New York Chichester Brisbane, pp 71–102

Eaton RC, DiDomenico R, Nissanov J (1991) Role of the Mauthner cell in sensorimotor integration by the brain stem escape network. Brain Behav Evol 37:272–285

Erulkar SD, Soller RW (1980) Interactions among lumbar motoneurons on opposite sides of the frog spinal cord: morphological and electrophysiological studies. J Comp Neurol 192:473–488

Fetcho J (1986) The organization of the motoneurons innervating the axial musculature of vertebrates. I. Goldfish (*Carassius auratus*) and mudpuppies (*Necturus maculosus*). J Comp Neurol 249:521–550

Fetcho J (1987) A review of the organization and evolution of motoneurons innervating the axial musculature of vertebrates. Brain Res Rev 12:243–280

Fox GQ (1977) The morphology of the oval nuclei of neonatal *Torpedo marmorata*. Cell Tissue Res 178:155–167

Fritsch G (1883) Die elektrischen Fische im Lichte der Deszendenzlehre. In: Virchow R, Holtzendorff FR von (eds) Sammlung gemeinverständlicher wissenschaftlicher Vorträge, Serie 18. Habel, Berlin, pp 835–898

Fritsch G (1887) Die elektrischen Fische. Erste Abteilung *Malapterurus electricus*. Veit, Leipzig

Gallyas F (1979) Light insensitive physical developers. Stain Technol 54:173–176

Görcs T, Antal M, Oláh É, Székely G (1979) An improved cobalt labelling technique with complex compounds. Acta Biol Acad Sci Hung 30:79–86

Grillner S, Buchanan JT, Wallén P, Brodin L (1989) Neural control of locomotion in lower vertebrates: from behavior to ionic mechanisms. In: Cohen AH, Rossignol S, Grillner S (eds) Neural control of rhythmic movements in vertebrates. Wiley, New York, Chichester Brisbane, pp 1–40

Janetzko A, Zimmermann H, Volknandt W (1987) The electromotor system of the electric catfish (*Malapterurus electricus*): a fine-structural analysis. Cell Tissue Res 247:613–624

Johnels AG (1957) On the origin of the electric organ in *Malapterurus electricus*. Q J Microsc Sci 97:455–464

Kashin S, Brill R, Ikehara W, Dizon A (1981) Induced locomotion by midbrain stimulation in restrained skip jack tuna, *Katsuwonus pelamis*. J Exp Zool 216:327–329

Kimmel CB (1982) Reticulospinal and vestibulospinal neurons in the young larva of a teleost fish, *Brachydanio rerio*. Prog Brain Res 57:1–24

Kimmel CB, Powell SL, Metcalfe WK (1982) Brain neurons which project to the spinal cord in young larvae of the zebrafish. J Comp Neurol 205:112–127

Koch C, Poggio T, Torre V (1982) Retinal ganglion cells: a functional interpretation of dendritic morphology. Philos Trans R Soc Lond [Biol] 298:227–264

Krenz WD (1985) Morphological and electrophysiological properties of the electromotoneurones of the electric ray *Torpedo marmorata* in vivo and in vitro brain slices. Comp Biochem Physiol 82A:59–65

Livingston CA, Leonard RB (1990) Locomotion evoked by stimulation of the brain stem in the Atlantic stingray, *Dasyatis sabina*. J Neurosci 10:194–204

Mahy G (1970) Les deux types morphologique de la famille des Malapteruridae (Pisces – Ostariophysi). Naturaliste Can 97:387–399

Maurer F (1913) Die ventrale Rumpfmuskulatur der Fische (Selachier, Ganoiden, Teleostier, Crossopterygier, Dipnoer). Jen Z 49:1

McClellan AD (1986) Command systems for initiating locomotion in fish and amphibians: parallels to initiation systems in mammals. In: Grillner S, Stein PSG, Stuart DG, Frossberg H, Herman RM (eds) Neurobiology of vertebrate locomotion. Macmillan, London, pp 3–20

Meszler RM, Pappas GD, Bennett MVL (1974) Morphology of electromotor system in the spinal cord of the electric eel, *Electrophorus electricus*. J Neurocytol 3:251–261

Metcalfe WK, Mendelson B, Kimmel CB (1986) Segmental homologies among reticulospinal neurons in the hindbrain of the zebrafish larva. J Comp Neurol 251:147–159

Meyers PZ (1985) Spinal motoneurons of the larval zebrafish. J Comp Neurol 236:555–561

Poll M, Gosse JP (1969) Revison des Malapteruridae (Pisces, Siluriformes) et description d'une deuxieme espece de silure electrique: *Malapterurus microstoma SP. N.*. Bull Inst R Sci Nat Belg 45:1–12

Rao PPD, Jadhao AG, Sharma SC (1987) Descending projection neurons to the spinal cord of the goldfish, *Carassius auratus*. J Comp Neurol 265:96–108

Roberts BL, Ryan KP (1975) Cytological features of the giant neurons controlling electric discharge in the ray, *Torpedo*. J Mar Biol Assoc UK 55:123–131

Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17:208–212

Schikorski T, Zimmermann H (1989) Innervation of the giant electromotor neuron of the electric catfish (*Malapterurus electricus*). In: Elsner N, Singer W (eds) Dynamics and plasticity in neuronal systems. Proceedings of the 17th Göttingen Neurobiology Conference. Thieme, Stuttgart New York, p 236

Schikorski T, Braun N, Zimmermann H (1990) Neuronal control of the electric catfish electric response: functional and structural relations. In: Elsner N, Roth G (eds) Brain, perception, cognition. Proceedings of the 18th Göttingen Neurobiology Conference. Thieme, Stuttgart New York, p 93

Schnitzlein HN, Brown HK (1975) Spinal motoneurons of the goldfish (*Carassius auratus*). Brain Behav Evol 12:207–228

Székely G (1976) The morphology of motoneurons and dorsal root fibres in the frog's spinal cord. Brain Res 103:275–290

Tuge H, Uchihaski K, Shimamura H (1968) An atlas of the brains of fishes in Japan. Tsukiji Shokan, Tokyo

Uchizono K (1974) Excitation and inhibition; synaptic morphology. Igaten Shoin, Tokyo Elsevier, Amsterdam

Yasargil GM, Diamond J (1968) Startle-response in teleost fish: an elementary circuit for neural discrimination. Nature 220:241–243