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Research Articles

Mauthner cells in the medulla of the weakly electric fish *Gymnotus carapo*

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Summary. The medulla of the gymnotoid fish *Gymnotus carapo* contains two large neurons exhibiting all the characteristics of Mauthner cells (M-cells). Their presence was demonstrated by means of Golgi-like labeling with horseradish peroxidase. This is the first description of M-cells in a fish belonging to the order Gymnotiformes.
Key words. Mauthner neurons; electric fish; HRP-labeling.

One of the most typical characteristics of the central nervous system (CNS) of teleosts and certain amphibians is the presence in the medulla of two large nerve cells whose thick axons extend all the way along the spinal cord. These two peculiar nerve fibers were discovered by Mauthner in 1859[1]. Approximately thirty years later, Goronvitsch[2] found in the medulla of the sturgeon the cell bodies from which the pair of axons discovered by Mauthner originate. Physiological studies have demonstrated that excitation of a single Mauthner cell (M-cell) elicits the so-called Mauthner-reflex. The reflex consists of: a) a forceful contraction of the contralateral muscles of the trunk and tail, b) sudden movements of both eyeballs, c) contraction of both opercular muscles and d) synchronous movement of the lower jaw. As pointed out by Diamond[3], this response resembles the startle reaction of a free-swimming fish, caused by mechanical or photic stimuli.

The morphology of the M-cell has been the subject of numerous investigations covering a variety of species[4–7]. However, investigations on the presence of M-cells in the so-called 'weakly electric fish' seem to be very scarce. According to the lists provided by Zottoli[7] in his review chapter, M-cells do occur in the African Mormyriformes. On the other hand, the important group of the South-American Gymnotiformes has hardly been investigated, with the exception of gymnotoid eels, which were included by Zottoli[7] in the list of fish in which these peculiar neurons have not been found.

This report is concerned with the location and morphology of the M-cells in the weakly electric gymnotoid *Gymnotus carapo*. Interest in the identification of M-cells in weakly electric fish goes beyond the goal of adding new members to the list of species in which these peculiar neurons have been found. In all fish investigated, the dorso-lateral dendrites receive afferents from vestibular

and lateral line mechanoreceptors while the ventral dendrites mainly integrate optic stimuli[11]. In the particular case of gymnotoid fish, in which electroreception is the dominant sensory modality, it could be important to investigate whether the ventral dendrite also receives inputs from the electroreceptive centers. The present investigation is the first essential step in any attempt to initiate this more ambitious research goal.

We have succeeded in staining the M-cells by applying horseradish peroxidase (HRP) crystals (Sigma, Grade VI) to small lesions made in the dorsal portion of the spinal cord. For this purpose the animals were maintained deeply anesthetized (3% ether emulsified in water). Oxygenation was secured by passing aerated water through the gills. Gymnotids which had recovered from anesthesia were maintained in normal conditions in the aquaria. After five days' survival (pilot experiments had indicated that the best histological images can be obtained after this survival period), anesthetized fish were perfused with a phosphate-buffered fixative mixture consisting of 1% glutaraldehyde and 1% paraformaldehyde (pH, 7.4). Serial transverse sections of both the medulla and the first segments of the spinal cord were cut with the aid of a Lancer Vibratome. The presence of HRP was revealed using 3'-3' diaminobenzidine tetrahydrochloride as chromogen agent. Staining was intensified with cobaltous chloride[8].

Sections passing through the level of entrance of the VIIIth nerves have revealed the occurrence of two large cell bodies fulfilling all the criteria proposed by Zottoli[7] for identification of the M-cells (fig. 1). Their somata are large, reaching in this species a diameter of 45–50 μm.

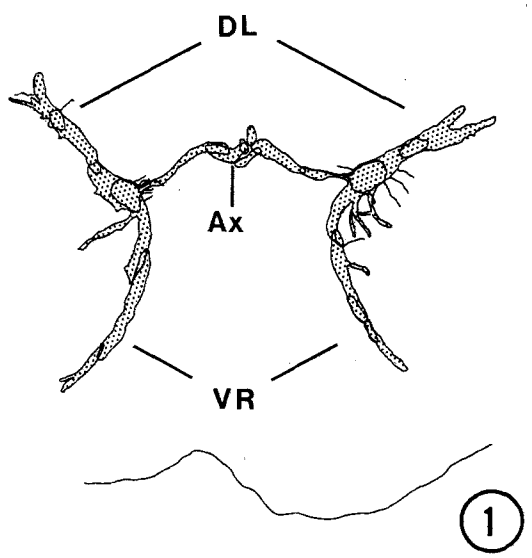

Figure 1. This is a computer-assisted reconstruction of the M-cells of *G. carapo*. It is based on six thick serial sections passing through the medulla at the level of entrance of the VIII nerves. The neurons were HRP-labeled. Note the presence of the dorso-lateral (DL) and ventro-rostral (VR) dendritic trunks. Thinner dendritic branches can be seen arising from the somata and the main dendrites. The axons (Ax) cross the midline and project to the contralateral side. ×90.

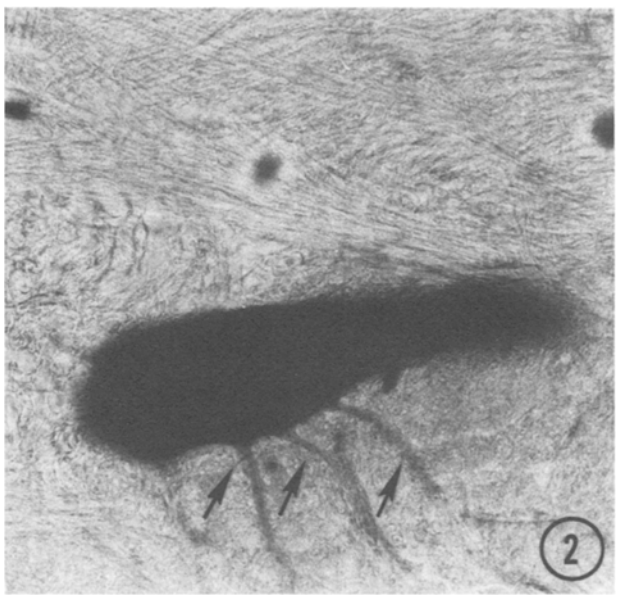

Figure 2. This microphotograph shows the thinner dendritic branches (arrows) usually arising from the M-cell somata. ×450.

Two thick dendritic trunks arise from each soma and project towards the dorso-lateral and ventro-rostral regions of the medulla, respectively. Thinner dendritic branches were observed stemming from the cell body or the main dendritic trunks (fig. 2). The dorso-lateral dendrite extends approximately 250 μm from its site of origin (defined by convention, since there are no well-defined boundaries between the cell body and the main dendritic trunks) to the region of entrance of the fibers of the VIIIth nerve. This dendritic trunk terminates in two or three thinner branches which usually appear lightly stained. Consequently, their more distal segments were difficult to visualize. The ventro-rostral dendrite is thinner but longer (350 μm) than the dorso-lateral one and its terminal branches also appeared faintly stained. Both the axon-hillock and the first unmyelinated portion of the axon were found to be covered by a conspicuous, typical 'axon cap' (fig. 3). As was first reported by Bartelmez[4] in *Ameiurus*, the cap consists of a complex array of neural and non-neural elements (see review by Nakajima and Kohno[9]).

At the medullary level, the M-cell axons had diameters ranging between 25 and 30 μm. Along their pathways within the medulla and the spinal cord they send out short collateral processes; it is commonly accepted that these subserve synaptic functions. It is important to mention that when HRP crystals are applied on severed regions of the dorsal funiculi not only the M-cells were labeled but also the relay cells of the medullary electromotor nucleus and a variety of reticular neurons. It is probable that collaterals of some of the descending reticulo-spinal axons may give rise to the terminal processes and synaptic knobs that were observed contacting both the cell bodies of the M-cells and their main dendritic trunks (fig. 4).

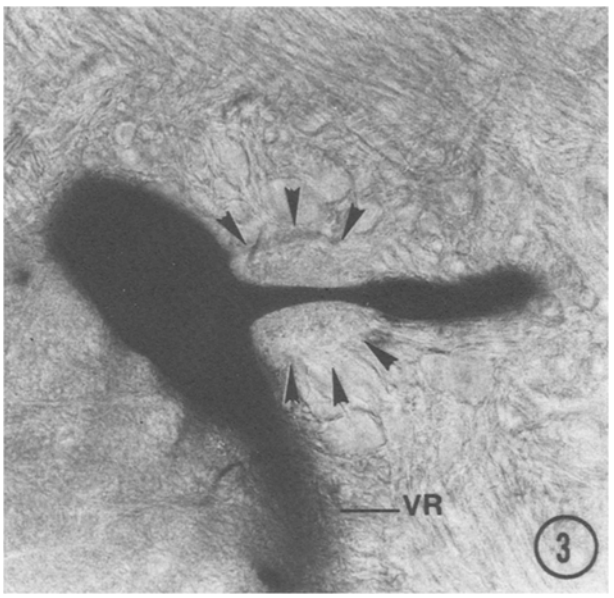

Figure 3. Microphotograph showing the cell body and the first axon segment of an HRP-labeled M-cell. The limits of the axon-cap are indicated by the arrow-heads. The ventro-rostral dendritic trunk (VR) appears ill-defined in the lower half of the picture. ×450.

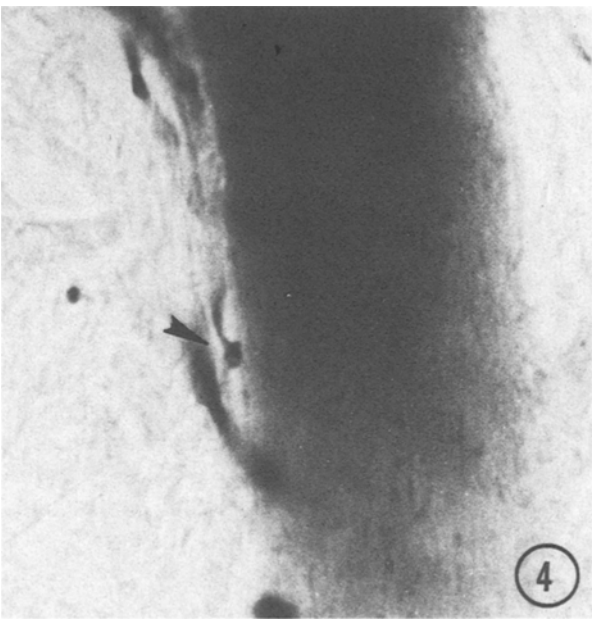

Figure 4. When the HRP-labeled M-cells are examined at a higher magnification, typical synaptic knobs can be seen contacting either the cell bodies or the main dendritic trunks. The arrow-head indicates a synaptic knob impinging on the ventro-rostral dendrite. ×2000.

A few lines have to be devoted to speculation about the mechanisms allowing M-cells to become labeled when HRP crystals are applied on severed dorsal regions of the spinal cord. Since the M-cell axons run in the undamaged ventral region of the cord, the possibility of retrograde transport of the substance should be discarded. Therefore, it seems reasonable to postulate the trans-synaptic transfer of the enzyme via neuronal systems projecting descending fibers within the dorsal funiculi. On the other hand, the Golgi-like density of the marked cells indicates an unusual permeability of the synapses involved in this particular case of transcellular transport of non-conjugated HRP.

In addition to the anatomical features which allowed the identification of M-cells in *G. carapo*, Pereda and Borde (pers. comm.) have recorded, in the medulla of this species, all-or-none, low-threshold, short latency, large negative fields evoked by spinal cord stimulation. It is unanimously accepted that this kind of field characterizes M-cell extracellular spikes [10].

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