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Labelling of Electroreceptive Afferents in a Gymnotoid Fish by Intracellular Injection of HRP: The Mystery of Multiple Maps

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Summary. Physiologically identified primary electroreceptive afferents in the gymnotiform fish, *Eigenmannia,* were labelled by intracellular injection of horseradish peroxidase (HRP) in order to determine their termination sites in the posterior lateral line lobe (PLLL) (Fig. 1). For each terminal field we mapped the location of the associated receptor pore on the body surface and found:
1) Ampullary units project in a somatotopically ordered manner to the medial PLLL.
2) Tuberous units, both P- and T-types, project in a somatotopically ordered manner to three separate regions of the PLLL, called central-medial, central-lateral and lateral (Figs. 2–4). Each tuberous unit projects to all three maps and the projections of P- and T-units are in somatotopic register.

In addition to electroreceptive units, mechanoreceptive units were also encountered in the anterior lateral line nerve ganglion, but their central projections were found outside of the PLLL, in the anterior lateral line lobe (ALLL) and in the eminentia granularis. This finding is in accordance with the notion of modality-specific separation of central projections, forwarded by Maler et al. (1974).

Tuberous electroreceptive afferents have larger somata and faster nerve conduction the further their receptor pores are located towards the tail end of the body (Fig. 5, 6). The faster nerve conduction of afferents from more distant regions of the body surface minimizes temporal disparity in the arrival of spikes linked to synchronous electrical events in widely separated regions of the body surface.

Abbreviations: *ALLL* anterior lateral line lobe; *EOD* electric organ discharge; *HRP* horseradish peroxidase; *PLLL* posterior lateral line lobe

Introduction

The anterior lateral-line nerve ganglion of gymnotiform electric fish contains the somata of primary afferents from electroreceptors and mechanoreceptors which are distributed over the animal's body surface. According to neuroanatomical studies by Maler et al. (1974), these afferents project to modality-specific targets in the central nervous system: Electroreceptor afferents terminate in the posterior lateral line lobe (PLLL), while mechanoreceptor afferents terminate in the anterior lateral line lobe (ALLL), eminentia granularis and a region of the lobus caudalis.

Electroreceptors are classified into ampullary and tuberous types, which respond to low frequency and high frequency signals respectively. The latter type, which is most sensitive at the animal's own electric organ discharge (EOD) frequency (Hopkins 1976), again is classified into T- and P-units (see review by Bullock 1974). T-units, which fire one spike on each EOD cycle, are phase locked to the zerocrossing of the stimulus and encode modulations in instantaneous phase of the local EOD signal. P-units, which fire intermittently at a rate positively correlated to stimulus amplitude, encode modulations in instantaneous amplitude of the local EOD signal (Scheich et al. 1973; Heiligenberg and Partridge 1981).

Detailed anatomical studies of the PLLL by Maler (1979) and Maler et al. (1981) strongly suggested that P- and T-units project to different targets in the PLLL. Yet the targets of the ampullary afferents remained unknown. Subsequent labelling with horseradish peroxidase (HRP) of various branches of the anterior lateral line nerve by Carr et al. (1982) gave evidence of a somatotopic organization of electroreceptive projections but could

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not distinguish tuberous from ampullary terminations.

In order to resolve these issues, we have labelled single primary afferent cells by intracellular injection of HRP, after testing their physiological response characteristics and locating their receptor pore on the animal's body surface. Neuroanatomical analysis of this material has revealed one ampullary and three tuberous somatotopically organized maps in distinct regions of the PLLL. Most surprisingly, each tuberous afferent cell has been found to project to all three tuberous maps. The functional significance of this triple representation of identical inputs is as yet unresolved.

Methods

Animals of the species *Eigenmannia virescens*, 10 to 12 cm in total length, were paralyzed by a minimal intramuscular injection of Flaxedil (less than 0.05 cm^3 of a 1% solution in Hickman's Ringer solution) in order to silence their electric organ discharges (EODs) and to arrest the movements of their opercula. These injections were of sufficiently low dose that opercular movements recovered within 2 to 3 h, by which time the experiment was normally completed. After an additional 2 to 3 h, the animal swam in an almost natural manner and could survive indefinitely.

The animal was respirated through a mouth tube which supplied, at a rate of approximately 1 drop/s, aerated water of the same temperature as the bath, 26 to 27 °C. While the animal was gently held in a foam rubber collar, a small hole of less than 1 mm diameter was drilled through the skull, approximately 2 mm dorsally and caudally from the eye, to expose the anterior lateral line nerve ganglion. A small area of skin was removed from the parietal and frontal bones, and, with a drop of Eastman 910 adhesive, a Plexiglas rod was glued to the exposed bone, holding the animal with only the upper right half of its head above the water surface. At this point, the foam rubber collar was removed so that the animal's body was freely suspended in the water. The pacemaker activity was monitored through a small suction electrode placed over the tip of the tail (as described in Heiligenberg et al. 1978).

A sine wave stimulus, similar in frequency and intensity to the animal's EOD prior to curarization, was applied either between an electrode in the mouth and an electrode near the tip of the tail or between a pair of external electrodes straddling the animal longitudinally. The first stimulus regime mimics the geometry of the natural EOD field and thus approximates the local transepidermal stimulus intensity more closely than any other placement of the electrodes (Heiligenberg et al. 1978). This is an important condition for the classification of receptors into P- or T-types since most P-units will respond like T-units if they are driven with supernormal stimulus intensities (Scheich et al. 1973). However, this stimulus regime also yields large stimulus artefacts when units in the ganglion are recorded monopolarly, and units can only be recognized clearly after successful penetration. The longitudinal stimulus regime, on the other hand, with proper placement of the ground electrode near the outside of the animal, yields no stimulus artefacts, and the faint activity of a unit is readily detected as the electrode approaches the cell membrane. Therefore we chose the longitudinal regime while searching for a unit and then, after successful penetration, switched to the mouth-tail electrode regime in order to classify the unit. In addition to tuberous units, ampullary afferents were encountered, which are characterized by spontaneous activity that is modulated by stimulus frequencies as low as 1/100 of the frequency of the EOD substitute. The location of the pore of an electroreceptor on the body surface was determined with a small stimulus dipole, held by hand at the end of a thin glass rod while the normally applied stimulus was turned off. Passing the dipole close to a single small region of the body surface caused a vigorous response of the primary afferent and allowed for the localization of the pore with an accuracy of a few millimeters.

We recorded and iontophoretically filled cells with glass capillaries (AM and WPI microdot glass) of resistances between 40 and 200 MOhm. Following the method described by Katz and Gurney (1981) we filled capillaries with a 10% HRP (Sigma, type VI) solution in 0.5 mol/l KCl and 0.1 mol/l Tris buffer (pH 7.4). An Ag-AgCl wire was placed inside the capillary for monopolar recording with a WPI amplifier, and a similar wire, cast into a small block of Agar, prepared and stored in a 0.5 mol/l KCl solution, served as a ground electrode. The recording electrode was advanced in steps of 2 to 3 µm by means of a hydraulic drive (D. Kopf Instruments) while the experimental table was gently tapped to facilitate the penetration of cells. In some instances we used electrodes bevelled in a spinning bath of Buehler Micropolish (0.05 micron gamma alumina), which lowered their resistance by as much as 50%. These electrodes seemed to penetrate cells more easily. After the response characteristics of the cell and the location of its receptor pore had been determined, a current, oscillating sinusoidally at 4 Hz between 0 and 5 nA, was passed through the HRP containing electrode over a period of 10 to 15 min. Excessive clogging of the electrode was overcome by temporarily adding a negative bias current. Quite commonly, the resting potential of the cell fell considerably during the injection, and often was indiscernible after 10 to 15 min of injection. However, even in many of these cases, cells were sufficiently filled with HRP so that their processes could be accurately mapped.

After filling of a cell, the electrode was retracted, the ganglion was covered with gelfoam and the skull sealed with tissue glue (Histoacryl). If the animal became too lively, it was released from the holder, and the exposed bone was sealed with tissue glue before the animal was allowed to swim freely. After survival times of 3 to 5 h, the animal was anaesthetized with MS222. Via a cannula, inserted through the opened ventricle into the conus, the blood was driven out with Hickman's Ringer solution to which heparin (1 mg/15 ml) and $NaNO_2$ (1 mg/ml) were added to avoid clotting. This procedure was followed by 10 min of perfusion with 4% glutaraldehyde in phosphate buffer and another 20 min of perfusion with the same fixative in 30% sucrose. The rate of flow was adjusted so that the conus of the heart stayed inflated to approximately twice its normal diameter.

The brain was removed from the skull and embedded in a small block of gelatine which was placed for 40 to 60 min in the fixative with 30% sucrose. The block was stored at 4 °C in a 30% sucrose solution in phosphate buffer until it was cut into 40 µm sections on a freeze microtome. The sections were reacted according to the Hanker-Yates method as modified by T. Finger (Bell et al. 1981).

Results

1. The Distinction of Cell Types

1.1. Tuberous Electroreceptive Afferents. This kind of cell was impaled most frequently, presumably

because it has the largest soma, 20 to 30 μm in diameter. Units which, under near natural stimulus intensities, fired one spike per stimulus cycle, phase-locked to the zero-crossing of the stimulus, were identified as T-units. Units which, under this same condition, fired intermittently and raised their rate of firing with increasing stimulus amplitude were identified as P-units. Approximately 15 units of each type, after being filled with HRP, showed intensive labelling up to and including their termination sites which are as far as 2 mm away from the location of the soma. Many more T-units could have been filled since they were most frequently encountered. However, we usually abandoned such units in our search for less common cell types.

Each tuberous unit is found to terminate in three separate locations of the PLLL (see description of somatotopic organization in Sect. 2). Terminal fields are located in the most ventral layer of cells and have a diameter of 20 to 40 μm. T-unit terminals often form clusters around spherical cells (see Fig. 1), which are their assumed targets (Maler et al. 1981), and the presence of club like endings suggests synaptic contacts. In three instances HRP reaction product is seen in spherical cells contacted by T-unit terminals. Presumably, in these cases HRP crossed membranes via micropinocytosis (Triller and Korn 1981), which strongly suggests the presence of synaptic contacts. Less evidence has been obtained about the synaptic targets of P-unit afferents insofar as their terminals do not form particular clusters in the vicinity of any identified cell type, although they also appear to contact spherical cells in several cases. In one instance, HRP has reached a polymorphic cell (see Fig. 1), and this cell type is indeed believed to be one of the targets of P-unit afferents (Maler et al. 1981).

In an effort to demonstrate synaptic connections directly, we filled approximately a dozen additional tuberous afferents with Lucifer Yellow. Since various afferent synapses in the PLLL are electrotonic (Maler et al. 1981), we had hoped to demonstrate these connections by dye-coupling (Stewart 1978). However, for still unknown reasons, we failed to observe any dye-coupling despite excellent staining of the terminal fields. Therefore, we plan to study synaptic connections by electron-microscopic analysis of HRP-filled terminals.

1.2. Ampullary Electroreceptive Afferents. It is extremely difficult to impale and to hold ampullary afferents since their somata are only approximately 10 μm in diameter, and, as are the other cells in the ganglion, they are heavily myelinated (Carr et al. 1982). In several instances the amount of HRP we were able to inject was insufficient to label the terminals of the cell which are located in the medial, and hence most distant part of the PLLL (see description of somatotopic organization in Sect. 2). Only 5 cells showed sufficient labelling of their terminals to allow accurate mapping. Only one terminal site was found in each case, in the most ventral layer of cells in the medial PLLL. Ampullary afferents are readily recognized on the basis of their rather regular firing rate which is modulated by low-frequency electric stimuli (0 to 10 Hz).

1.3. Mechanoreceptive Afferents. These afferents also have small somata, approximately 10 μm in diameter, and therefore are encountered rarely. Only 4 cells were filled and all projected to the ALLL, with a collateral termination in the eminentia granularis. Both sites have been identified as mechanoreceptive areas by Maler et al. (1974). We found no labelling in an additional mechanoreceptive area, a region of the lobus caudalis (Carr and Matsubara 1981), presumably because this site is too remote from the soma of the cell. Mechanoreceptive afferents are spontaneously active, cannot be driven by electrical stimuli of natural intensity but respond vigorously to slight vibrations of the bath in which the fish is held.

1.4. Cells of Uncertain Function. We encountered a fourth type of cell which was known to us from previous extracellular recordings (unpublished data). These cells are characterized by a very regular, spontaneous discharge rate between 60 and 180 Hz, which cannot be modulated by mechanical or low frequency electrical stimuli. However, these cells may modulate their rate of firing in synchrony with an amplitude modulation of an electrical sine wave stimulus of a carrier frequency near that of the animal's EOD. In some instances, a pore could be located on the body surface through which the unit could be driven most efficiently. In one instance, when a sufficiently strong stimulus was applied, a unit followed the stimulus in a one-to-one manner, much as a T-unit. After such stimulation, the unit was silent for a few seconds before it resumed its regular spontaneous activity. These cells are also characterized by the largest spike potentials found in the ganglion. However, we never obtained large resting potentials and failed consistently to label such cells with HRP. In spite of the fact that they fire at a very stable rate, we believe these cells are unhealthy tuberous afferents that do not live long enough after penetration to show good labelling in the anatomical preparation.

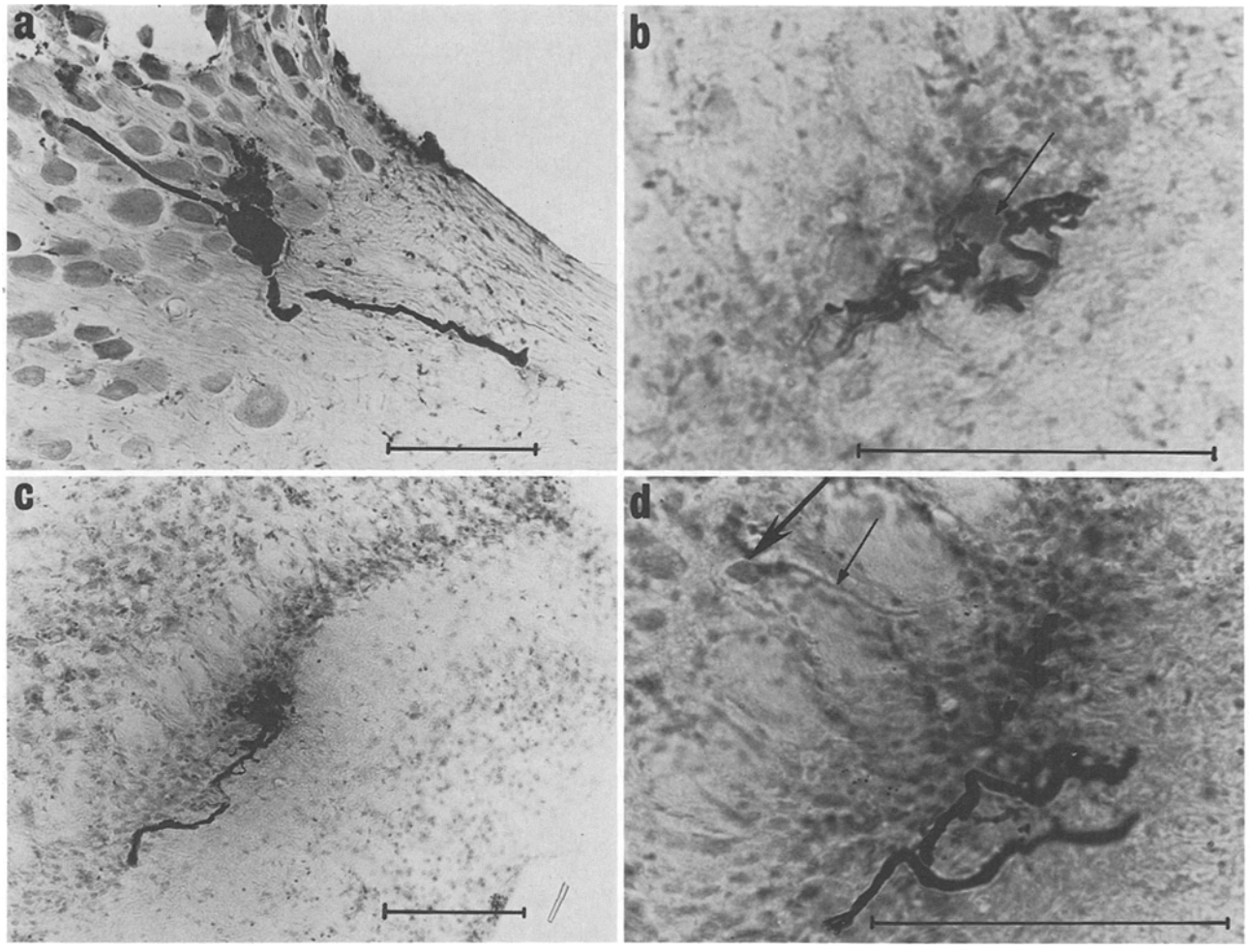

Fig. 1 a–d. HRP-labelled primary afferent electrosensory cells. **a** Soma of P-unit with distal (right) and proximal axon in anterior lateral line nerve ganglion. A small amount of HRP leaked out into the space above the soma. **b** Terminal field of T-unit in lateral part of the PLLL. Terminals cluster around a spherical cell (arrow) which contains a small amount of HRP. Note thick, club-like terminals on the soma of the spherical cell. **c** Terminal field of a P-unit in central-lateral part of PLLL. The field wraps around a spherical cell which contains a small amount of HRP that is not visible in this micrograph. **d** Same field as in **c**, but 40 µm more caudal. The large arrow points to the soma, the small arrow points to a dendrite of a polymorphic cell which contains a small amount of HRP. The transport of HRP by one and the same primary afferent to a spherical and to a polymorphic cell suggests synaptic contacts with both types of cells. All scaling bars are 100 µm, and all micrographs are from the right side of the brain. Dorsal is up and lateral is to the right

2. The Somatotopic Organization of Tuberous and Ampullary Afferent Projections

On each side of the hindbrain the PLLL forms a horizontal sheet that curls up dorsally in its rostral-lateral portion. We have reconstructed the horizontal layout of the PLLL from transverse sections and plotted its contours as they would appear if it were rolled out flat (see Fig. 2). A close inspection of the laminar organization of the PLLL revealed to us a previously unrecognized subdivision in one of the three regions of the PLLL, described earlier by Maler (1979) as medial, central and lateral. A barely visible discontinuity in the horizontal alignment of cells, comparable to a small geological fault line, divides Maler's central region of the PLLL into a central-medial and a central-lateral segment (see Figs. 2, 3). In only a few instances can this separation easily be seen, and one of the clearest cases has been chosen for Fig. 2. In other genera of gymnotiform fish, such as *Hypopomus*, we have found this subdivision to be much more prominent than in *Eigenmannia*. Ampullary units project exclusively to the medial PLLL whereas each tuberous unit projects to each of the three remaining areas of the PLLL: central-medial, central-lateral and lateral.

In a fish with a total length of 10 to 12 cm, the PLLL extends approximately 1.3 mm in both the caudal-rostral and medial-lateral directions. The location of terminal fields has been measured along two coordinates: longitudinally as the frac-

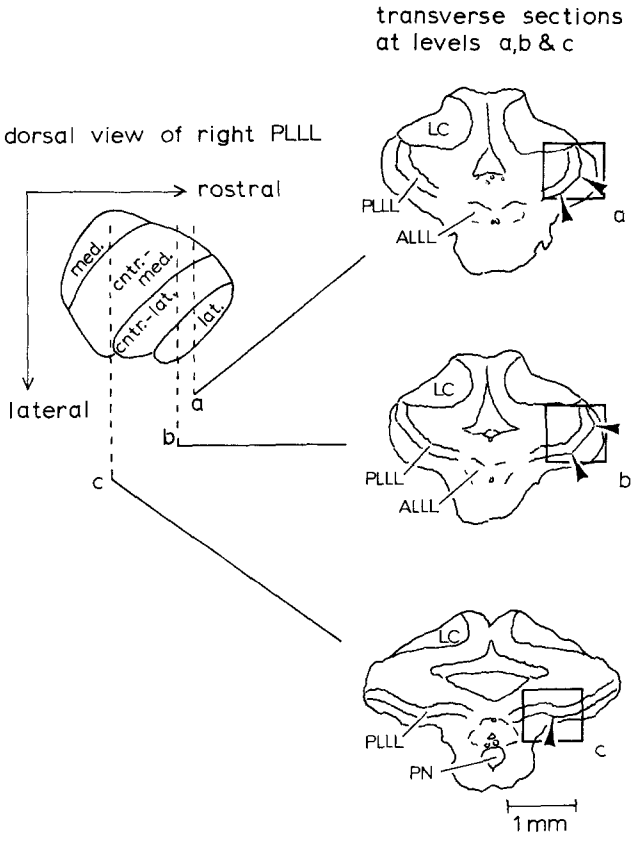

Fig. 2. A horizontal layout of the right *PLLL* and transverse sections at the levels indicated by dashed lines. The *PLLL* is drawn by a double contour with the lower line indicating the most ventral layers of cells (spherical cells) and the upper line indicating the most dorsal layer (polymorphic cells). The frames represent the boundaries of the micrographs a, b and c shown in Fig. 3. *LC* is the lobus caudalis, *PN* is the medullary pacemaker and *ALLL* is the anterior lateral line lobe. Pointers indicate the boundaries between adjacent parts of the *PLLL*, which is subdivided into four parts: lateral, central-lateral, central-medial and medial *PLLL* (see layout on left)

tion of the distance from the rostral end of the PLLL to its total length and transversely as the fraction of the distance from the lateral boundary of the particular region of the PLLL to the total width of this region at this level. Distances in the transverse direction have been measured by following the curvature of the ventral layer of the PLLL. These two coordinates were then plotted in a horizontally flattened layout of the PLLL (Fig. 4). Each terminal field is labelled to indicate the position of the cell's receptor pore on the body surface: a letter D, M or V for a dorsal, medial or ventral location respectively and a number between 0 and 1 to indicate the relative location in the longitudinal direction, with 0 being the tip of the snout and 1 being the tip of the tail. With a label of two decimal places, all units can be distinguished from one another in the data.

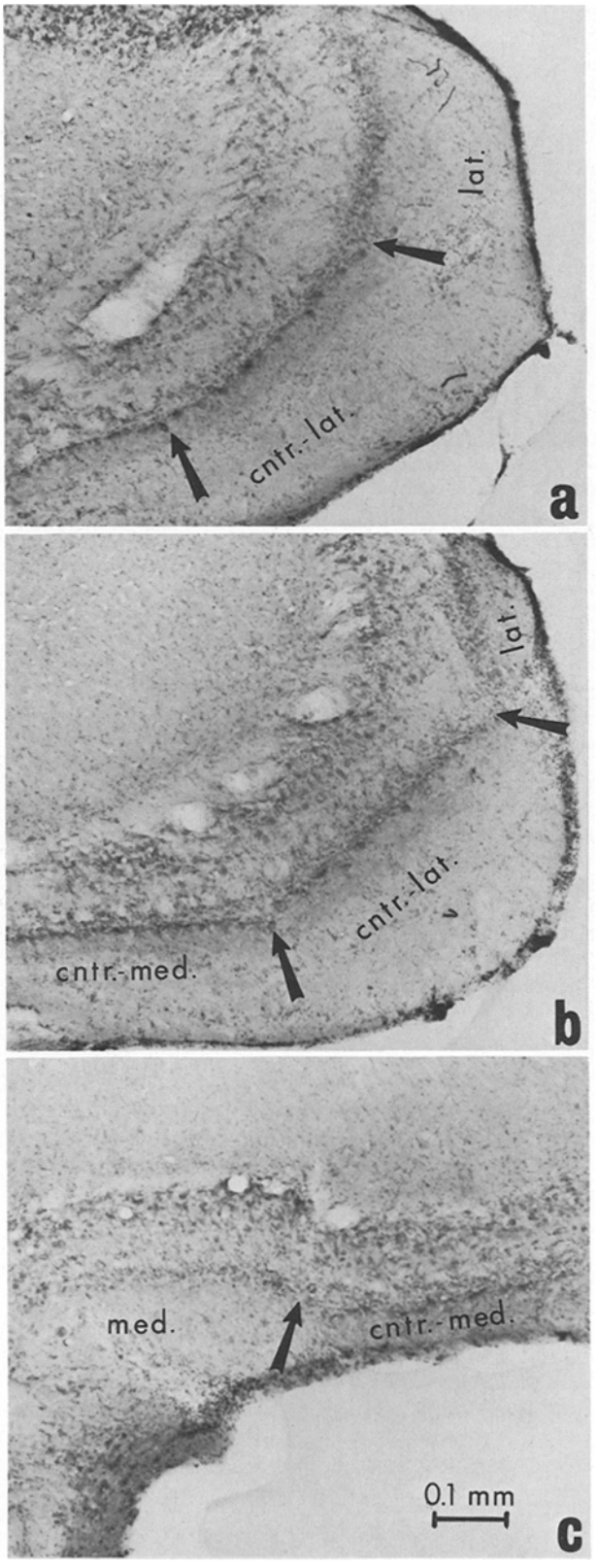

Fig. 3a–c. Micrographs from the areas a, b and c framed in Fig. 2. Pointers mark the spherical-cell layer in the same positions as in Fig. 2. *HRP*-filled fibers can be seen ventral and lateral from the spherical-cell layer in a and b. These fibers are branches of a single primary afferent heading for different parts of the *PLLL*

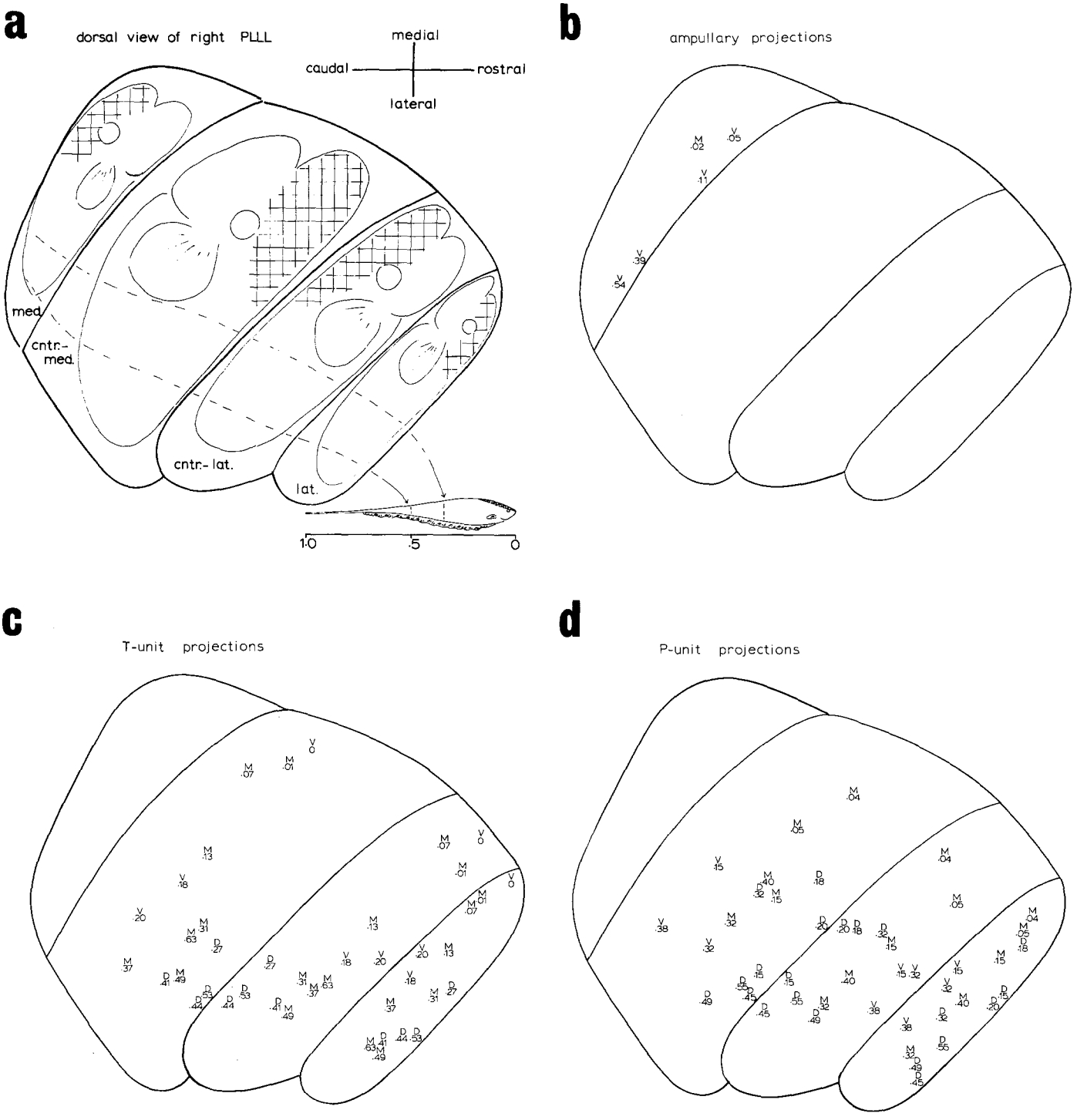

Fig. 4a–d. Somatotopic organization of ampullary and tuberous projections, plotted in horizontal layouts of the PLLL as shown in Fig. 2. **a** Sketches of the representation of animal's right side of body surface. The representation in the medial part of the PLLL is ampullary, the other three are tuberous. Hatched areas indicate dorsal surface of head which was above the water surface while units were recorded and labelled. No receptors could therefore be localized in this part of the body surface. Dashed lines indicate approximate rostral-caudal levels. Although no receptors are located on the pectoral fin, it has been drawn in as a landmark. **b** to **d** indicate locations of terminal fields of ampullary and tuberous units. Each tuberous unit, T-type as well as P-type, projects to all three tuberous maps. Only one cell was labelled in any animal, and individual variation in the local scaling of maps may account for the irregularities in the somatotopic order of these representations. The location of a given cell's receptor pore is indicated by the code of the terminal field. A capital letter, D, M or V stands for dorsal, medial or ventral location, and a two-digit number indicates the rostral-caudal level, with 0 being the tip of the head and 1 being the tip of the tail (see part a)

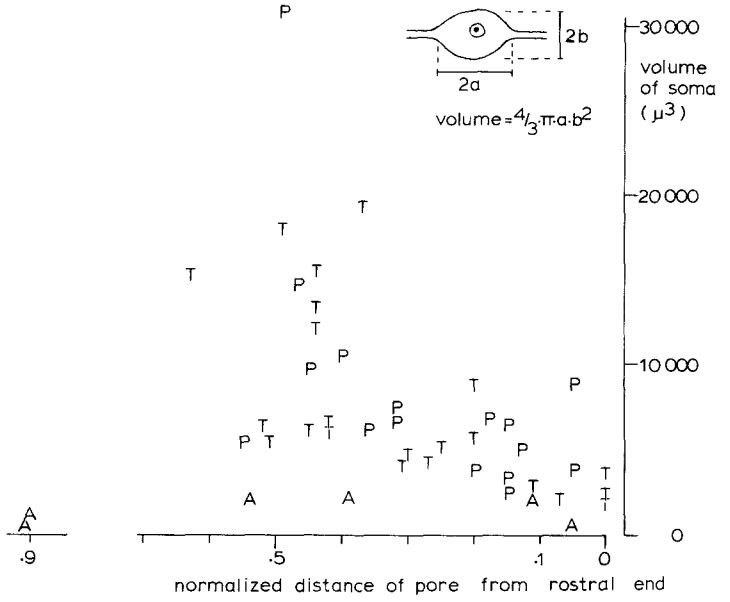

Fig. 5. The volume of ampullary (*A*), tuberous T (*T*) and tuberous P (*P*) units as a function of the normalized distance of their receptor pore from the tip of the head, with a distance of 1 being the tip of the tail. The soma of primary afferents can be approximated by an ellipsoid to compute its volume (see insert)

Based upon these data, the somatotopic organization of ampullary and tuberous projections is sketched in Fig. 4a. The data for the ampullary case may seem insufficient for the construction of an entire body map. However, Carr et al. (1982) have confirmed this map by HRP-labelling of whole afferent nerve branches originating in the dorsal, medial and ventral part of the head and in the posterior section of the trunk. All four "pisciculi", one ampullary and three tuberous, are characterized by an oversized head region which reflects the higher receptor density in this part of the body surface (Szabo 1974). Note, that neighboring pisciculi adjoin at the same area of the body surface, i.e., either back to back or belly to belly, and that all face in the same direction.

3. Soma Size and Nerve Conduction Velocity as Functions of Electroreceptor Location

While searching for units, we impaled tuberous cells with receptor pores at the tail end of the body approximately 10 times more often than cells with receptor pores on the head, regardless of the particular portion of the ganglion that we tested and in spite of the fact that receptors are much denser in the head region (Szabo 1974). The answer to this puzzle is given in Fig. 5, which shows that the volume of the cell soma increases with the distance of the location of the cell's receptor pore from the head of the animal. The somata of primary afferents with more distant receptor pores thus offer larger targets for the impaling electrode.

Since cells with larger somata also appeared to have thicker axons, although this was impossible to measure reliably due to the irregular shrinkage of fibers, we suspected that these cells also had higher nerve conduction velocities. The following experiment has confirmed this suspicion. After the receptor pore of a given tuberous unit had been localized, an electrical stimulus was applied between an electrode placed directly above the pore with a micromanipulator and an electrode inside the animal's gut. The stimulus consisted of a sinusoidal pulse of 0.04 ms duration, repeated at a rate of approximately 40 Hz. The amplitude of the stimulus was increased until the cell responded with at least one spike to each stimulus pulse; not more than a fraction of 1 V was required for this purpose. The latency of the first spike with respect to the onset of the stimulus pulse is plotted for various cells by open symbols in Fig. 6. The amplitude of the stimulus was then raised further, and the latency of the first spike shortened non-linearly to a new steady value which did not change further even if the stimulus amplitude was raised as high as 8 V. These latencies are plotted by filled symbols in Fig. 6, and they are approximately 2 ms shorter than those at the threshold intensity for regular firing. Based upon Bennett's (1967) findings, we interpret these shorter latencies as the result of direct stimulation of the endings of the primary afferent nerve at the site of the receptor pore. This latency therefore reflects the total nerve conduction time, from receptor to cell soma. The additional 2 ms of latency under the lower stimulus intensity would then be due to the regular transduction mechanism in the electroreceptor. As one

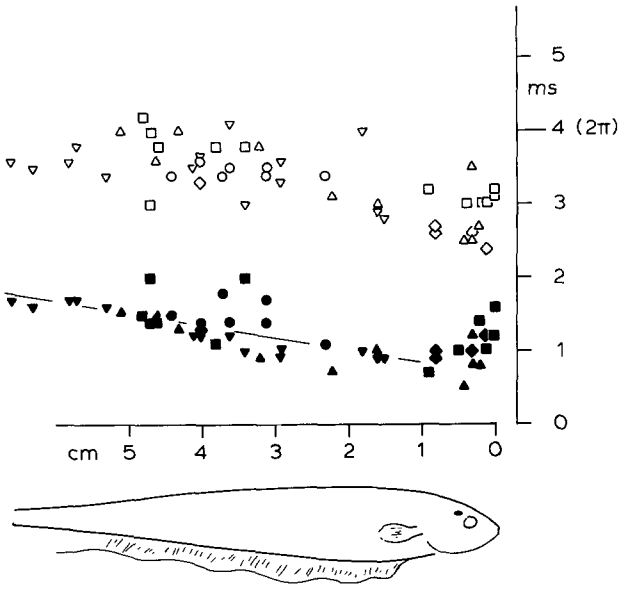

Fig. 6. Latencies of spike arrivals at the soma of tuberous afferents as a function of the distance of the receptor from the tip of the head. The data of four individuals, all between 10 and 11 cm long and with an EOD frequency near 250 Hz, are plotted by differently shaped symbols. Open symbols: latencies for stimulus intensities at the threshold of firing one spike in response to each stimulus cycle. Closed symbols: shortest possible latencies obtained by increasing stimulus amplitude. The regression line was calculated for data points with values on the abscissa above 1 cm. The black dot behind the eye indicates the location of the ganglion which harbors the somata of the primary afferents. Since animals had an EOD frequency of 250 Hz, the EOD period (2π) was 4 ms long. Latencies were measured by using single, sinusoidal pulses of 0.04 ms duration, presented at a rate of 40 Hz

can calculate from the data in Fig. 6, spikes originating in a receptor located 5 cm away from the tip of the head reach the ganglion within approximately 1.5 ms and thus travel at a speed of approximately 30 m/s. The value for a receptor site 2 cm away from the tip of the head is approximately 15 m/s. This compensation mechanism is not perfect; spikes originating simultaneously in the head and in the tail region do not arrive simultaneously in the central nervous system. However, the disparity of their arrival times would be far greater if nerve conduction velocities were identical for all receptor locations. The possible significance of this compensation mechanism will be discussed below.

Discussion

This study has confirmed the postulate of Maler et al. (1974) that mechanoreceptive and electroreceptive afferents, which jointly enter the brain via the anterior lateral line nerve ganglion, nevertheless project to different targets. In addition, we have learned that, even within the electroreceptive modality, two subclasses of afferents, ampullary and tuberous, project to different somatotopically organized areas of the PLLL. What surprises is the existence of 3 tuberous maps with identical electroreceptive inputs (see Fig. 3). A similar situation exists in the nonrelated mormyrid electric fish in which, as Bell and Russell (1978) demonstrated, there are three electroreceptive projections in the hindbrain, one ampullary and 2 tuberous, although we do not know in this case whether the same tuberous afferents project to both areas.

At this point one can only speculate about the significance of 3 separate tuberous representations in the hindbrain of gymnotoid fish. According to Maler and Sass (1982), the PLLL receives descending input from the nucleus praeeminentialis and the lobus caudalis, two higher-order electrosensitive areas. It is possible that the 3 tuberous maps in the PLLL receive different descending inputs and thus may be specialized for different tasks of feature extraction in electroreceptive afferences.

On the basis of a highly detailed, neuroanatomical study of the laminar organization of the PLLL, Maler et al. (1981) postulate that T-unit afferents terminate on the spherical cells whereas P-unit afferents terminate on granule, basilar pyramidal and polymorphic cells. As a consequence of this organization, a spherical cell should fire one spike that is phase-locked to the zero-crossing of the electrical signal at that part of the body surface from which the cell receives T-unit input. Spherical cells would thus encode local phase modulations.

Pyramidal cells, on the other hand, would monitor modulations in stimulus amplitude which are reported to them via P-unit afferents. Whereas the results of the present study do not contradict this notion, they are not greatly supportive. In particular, we have reasons to doubt a strict separation of tuberous receptors into P- and T-types (Viancour 1979). The receptor types now appear to be more the ends of a spectrum than exclusive and distinct classes, since we commonly encounter units which can not be clearly classified as either P- or T-type (see Fig. 2 in Bastian and Heiligenberg 1980). These ambiguous units fire at a rate higher than typical P-units and the phase of their firing within the EOD cycle is far more regular, although it is not as steady as in the case of a typical T-unit. Moreover, we have seen terminations of cells, characterized as P-units, in the proximity of spherical cells although we have no firm evidence yet for

synaptic contacts. This question awaits electron microscopical analysis of single, identified cell fills for its final resolution. However, we feel that a strict separation into T- and P-categories is not necessary and that a statistical rule for connections of cell types in the PLLL would suffice to render the spherical cells phase coders and the pyramidal cells amplitude coders of electroreceptive input.

By this scheme, one and the same tuberous cell could contact both spherical cells as well as granule, basilar pyramidal and polymorphic cells. The closer this cell would be at the T-type end of the spectrum the more predominant would be its contacts with spherical cells. Again, such a rule would have to be tested by electron-microscopic analysis of terminals of physiologically identified afferents. Spherical cells, which apparently integrate input from many tuberous afferents, could then produce one spike on each EOD cycle even in the absence of a regular firing pattern in any one afferent cell, and this spike would fall in the correct phase of the EOD cycle where the spikes of tuberous afferents cluster. Maler indeed suggests, on the basis of the fine structure of spherical cells, that they should only fire in response to near coincident arrival of several spikes and that singly arriving spikes would likely not be transmitted. This mechanism actually must hold in the case of apteronotid fish which, although they have no T-type afferents (Hopkins 1976), have spherical cells that fire one spike at a stable phase on each EOD cycle, as we have learned from preliminary intracellular recordings. In summary then, although phase and amplitude information may not be conveyed by separate classes of primary afferents, such a separation can be brought about at the level of the PLLL by virtue of its connectivity.

With regard to the fact that primary afferents conduct with greater speed the more distant their receptor pore is located from the brain, the following interpretation can be offered. In the case of the Jamming Avoidance Response (JAR), the animal compares phase modulations of its EOD in different areas of the body surface (Heiligenberg and Bastian 1980). A correct evaluation of this input requires that simultaneous zero crossings of the signal in two parts of the body surface are also simultaneously reported in the central nervous system. This in turn requires that the animal compensate for delays which are due to longer distances by increasing conduction velocity. This compensation is not perfect, and behavioral experiments indeed show that phase comparisons between sufficiently distant areas of the body surface give incorrect results (see Fig. 7 in Heiligenberg and Bastian 1980). This compensation mechanism on the afferent side parallels a similar mechanism on the efferent side: in order to fire the entire electric organ simultaneously, the efferent command to more proximal regions of the organ is slowed. This is accomplished by axons of smaller diameter and unnecessary length innervating the proximal electric organ (Bennett 1971).

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