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Ultrastructural Features and Hormone-Dependent Sex Differences of Mormyrid Electric Organs

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ABSTRACT

The electric organ of mormyrid fishes is composed of action potential-generating cells called electrocytes that together produce a species-typical electric organ discharge (EOD). The electrocytes of mormyrids are disc-shaped cells with distinct anterior and posterior faces, and a series of evaginations of one face that form a stalklike structure that is the site of innervation by spinal electromotoneurons (Bass: *J. Comp. Neurol. 244:*313–330, '86a). Here, we describe the major ultrastructural features of mormyrid electrocytes, which include surface invaginations along each face, myonuclei, myofilaments, and neuromuscularlike junctions formed by the axons of spinal electromotoneurons. The degree of surface invaginations along the anterior face is the most dramatic interspecific variable and is usually greater for species with the longer duration EODs. Among species with sexually dimorphic EODs, natural males, or females treated with gonadal steroid hormones, have longer-duration EODs and thicker electrocytes with more surface invaginations along the anterior face. The results are discussed in relation to the action potential-generating properties of the electrocyte's membranes.

Key words: electrocytes, EOD, steroid hormones, spike-generation

Electric organs generate a readily observable signal, the electric organ discharge (EOD) (Fig. 1), which is both a discrete electrophysiological event and a behavior important for mechanisms of orientation (electrolocation), social communication (electrocommunication), or prey capture and defense against predators (reviews: Bennett, '71; Heiligenberg, '77; Hopkins, '83; Bass, '86b). This paper is the second in a series (see Bass, '86a) focusing on the evolution of electromotor systems in one family of electric fish from Africa, the Mormyridae (see Taverne, '72), which use their EOD for electrolocation and social communication.

We are interested in the anatomical variation of both species- and sex-specific characters of the electric organ's action potential-generating cells, the electrocytes (after Bennett, '71), in relation to the development and evolution of species- and sex-specific differences in EODs. As detailed in a previous light microscopic paper (Bass, '86a), each electrocyte is a disc-shaped cell that is characterized by three elements. Two are its anterior and posterior faces; a third is a stalklike structure that is usually an evagination of the posterior face and is innervated by spinal electromotoneurons. In some cases, the stalk actually penetrates through the electrocyte proper to emerge on the anterior side, where it is then innervated.

An *EOD waveform* describes the appearance of a single electrical pulse generated by the entire electric organ (Fig. 1). A second element, the *EOD rhythm*, is the rate at which the electric organ generates an EOD waveform and is under the control of a central electromotor pathway (see Bass, '86a,b). Each face and stalk of a single electrocyte produces a distinct action potential; their summed activity determines the appearance of the EOD waveform (Bennett and Grundfest, '61). The penetrating or nonpenetrating rela-

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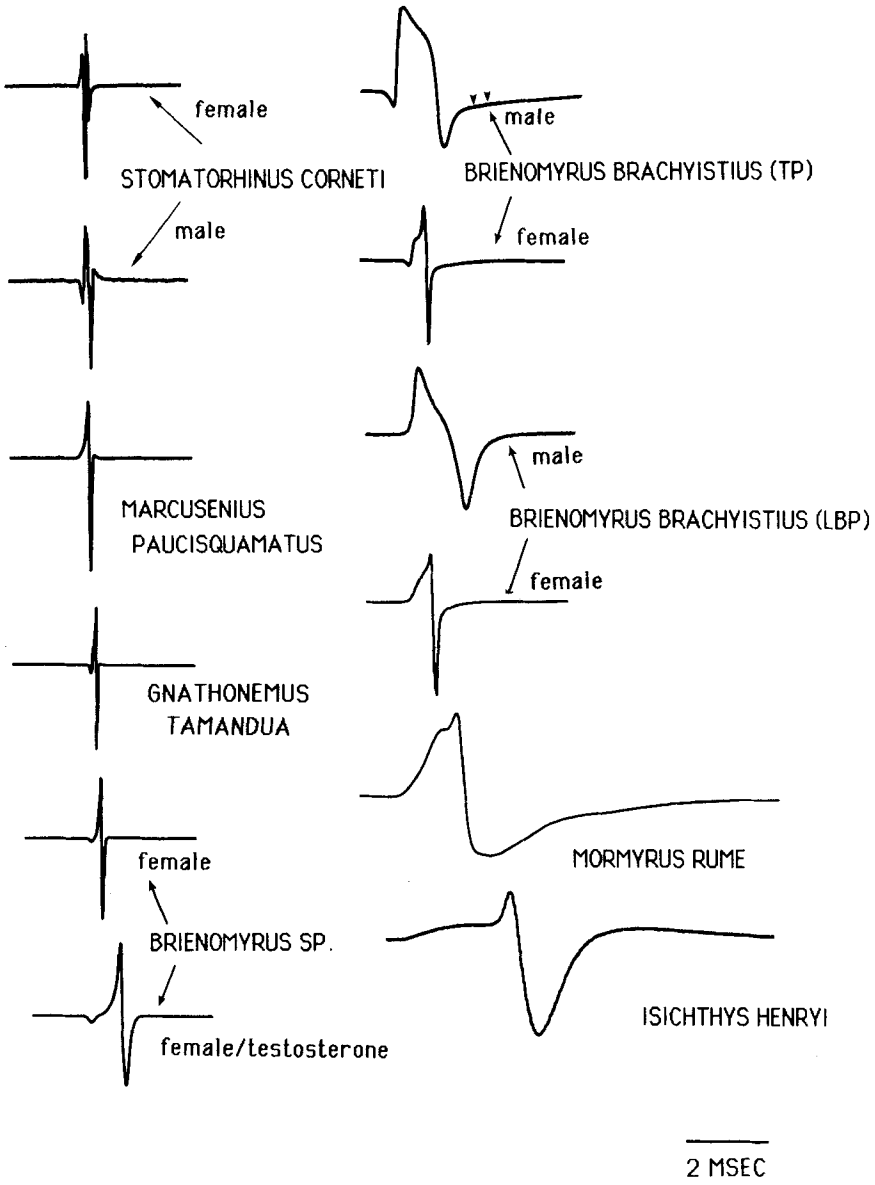


Fig. 1. Oscilloscope records of representative electric organ discharges (EOD) for the mormyrid fish used in this study. Included here are two subgroups of the species complex referred to as *Brienomyrus brachyistius*: "triphasic" (TP) and "long biphasic" (LBP). EODs were recorded with differential Ag/AgCl electrodes aligned parallel to a polyvinyl-chloride tube holding the fish in an orientation parallel to the electrodes. The positive electrode was near the head. All records were scaled to the same peak-to-peak amplitude; positive is up in all cases.

tionship between the stalk and the electrocyte proper is referred to as the geometry of the cell. Geometry can predict the polarity and number of phases in the EOD waveform (Bass, '86a) in accordance with a model proposed by Bennett and Grundfest ('61). The main principle of the model is that the direction of current flow along the electric organ is longitudinal. For example, a head-positive, biphasic EOD waveform (e.g., *Marcusenius paucisquamatus* [Fig. 1]) is found for those species in which the stalk extends away from the posterior face and is innervated on the posterior side of the electrocyte. In this case, an initial spike is generated in the stalk and propagates along its membrane until it invades the posterior face. Current flow along the stalk is radial. The posterior face generates a second spike, but current now flows across the electrocyte in a posterior to anterior direction. A pair of head-positive electrodes lying external to the electric organ would record an initial positivity for the EOD waveform. Current flow across the electrocyte then excites the anterior face, which also generates a spike, but the direction of current flow is now reversed and goes from anterior to posterior. The external electrodes record a final negative phase for the biphasic waveform. In species with a penetrating-stalk system (e.g., *Gnathonemus tamandua* [Fig. 1]), the stalk is usually innervated on the anterior side of the electrocyte and then penetrates through the main body of the electrocyte to the posterior side, where it is continuous with the posterior face. Such species have an EOD waveform with an initial negativity that arises

TABLE 1. Androgen-Dependent Sex Differences in Electrocyte Size[1]

	Total electrocyte and anterior face thickness (μm, SEM)	
	Average total thickness	Average anterior face thickness
BBTP females		
(n = 2 animals)	15.5	4.5
26 electrocytes	SEM = 0.5	SEM = 0.1
BBTP males		
(n = 2)	27.0	10.3
40 electrocytes	SEM = 0.6	SEM = 0.1
BBLBP female		
(n = 1)	28.4	3.9
14 electrocytes	SEM = 0.5	SEM = 0.2
BBLBP testosterone-treated female		
(n = 1)	37.6	6.7
41 electrocytes	SEM = 0.6	SEM = 0.2
B. sp. females		
(n = 5)	14.4	3.7
121 electrocytes	SEM = 0.2	SEM = 0.09
B. sp. males		
(n = 2)	14.5	3.9
43 electrocytes	SEM = 0.2	SEM = 0.1
B. sp. cholesterol-treated females		
(n = 3)	20.0	5.7
89 electrocytes	SEM = 0.5	SEM = 0.2
B. sp. testosterone-treated females		
(n = 7)	29.9	11.8
168 electrocytes	SEM = 0.6	SEM = 0.2
B. sp. testosterone-treated male		
(n = 1)	30.6	12.4
18 electrocytes	SEM = 0.4	SEM = 0.1

[1]Abbreviations: *BBTP* = *Brienomyrus brachyistius* (triphasic); *BBLBP* = *Brienomyrus brachyistius* (long biphasic); *B.* sp. = *Brienomyrus* sp.; SEM = standard error of the mean.

from anterior to posterior current flow generated at the site where the stalk penetrates the electrocyte (see Bennett and Grundfest, '61; Bass, '86a for detailed figures).

Despite correlations between electrocyte geometry and the shape of an EOD waveform, geometry does not predict other species-typical characters of EOD waveforms such as their duration, the presence of inflection points in each phase, or sex differences. EOD sex differences provide a focal point for questions regarding the functional morphology of electrocytes. Different sexes have the same electrocyte geometry (Bass, '86a) so that sex differences, as other species-typical characters, must also depend upon other electrocyte features. A series of field studies show, for species with a natural sex difference in their EOD waveform, that gonadal steroid hormones can induce females and juveniles to produce an EOD waveform that is typical of sexually mature males (review: Bass, '86c). As shown here and elsewhere (Bass and Volman, '85), steroid hormones provide a tool with which to manipulate the anatomical and physiological properties of mormyrid electrocytes. An understanding of hormonal influences on electrocytes and EODs may further help us understand the structural and functional bases for species-typical electrocytes and EODs.

We now describe several ultrastructural characters of electrocytes that may influence the production of EODs. In particular, emphasis is placed on the electrocyte's anterior face because (a) it is the most dramatic interspecific variable, and (b) gonadal steroids induce changes in its morphology. Some of these results were reported earlier (Bass et al., '84).

MATERIALS AND METHODS

Interspecific data

A previous light microscopic study focused on the mormyrid fishes from Gabon, West Africa (Bass, '86a). The following mormyrids from Gabon were also included in this study: *Stomatorhinus corneti, Marcusenius paucisquamatus, Brienomyrus brachyistius* (triphasic), *Brienomyrus brachyistius* (long biphasic), and *Isichthys henryi*. For *B. brachyistius*, the terms "long biphasic" and "triphasic" denote differences in EOD waveforms for species that appear similar on the basis of external morphology (Hopkins, '80). There are also species differences in electrocyte morphology for each member of the *B. brachyistius* complex (Bass, '86a). Additional material was prepared for *Gnathonemus tamandua, Brienomyrus sp.* (an unidentified species within the *Brienomyrus* complex; also referred to as *Brienomyrus sp.* [2] in Bass and Hopkins, '84), and *Mormyrus rume*, which are commercially available from American fish dealers. This sampling is representative of the diversity found for the EOD waveforms and electrocytes of mormyrids (see Bass, '86a). Representative EODs of the study species are shown in Figure 1.

All tissue samples were obtained from specimens that were first anesthetized with an overdose of MS222 (tricaine methane sulfonate) and then perfused transcardially with 0.1 M phosphate buffer (pH 7.2) followed by 2.5% glutaraldehyde in 0.1 M phosphate buffer. Tissue was postfixed in 2.5% glutaraldehyde for an additional 1–2 hours and then stored in 0.1 M phosphate buffer. Secondary fixation was in

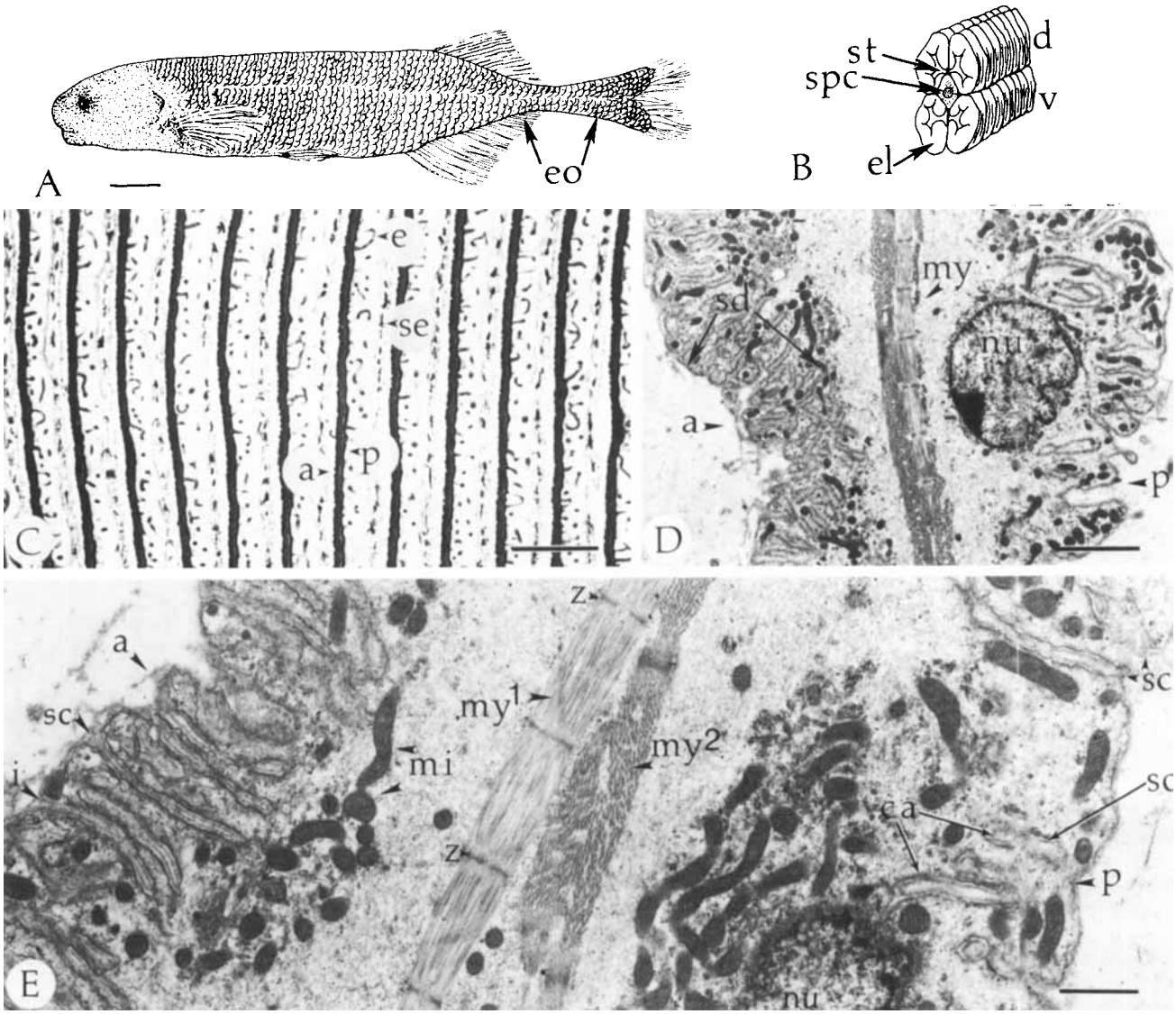


Fig. 2. Overview of the organization of the electric organ in mormyrids. A. Line drawing of *Brienomyrus* showing that the electric organ (eo) is located in the tail. Bar = 1 cm. B. As shown in this schematic line drawing of an oblique view, the electric organ is found on either side of the spinal cord (spc) and consists of dorsal (d) and ventral (v) columns of serially stacked, disc-shaped cells—the electrocytes (el). Spinal electromotoneurons innervate the electrocytes along their stalk (st). C. This sagittal section of Mallory-stained electrocytes from *Brienomyrus brachyistius* (long biphasic) also shows that each electrocyte is aligned along the longitudinal body axis. Each cell has distinct anterior and posterior faces (a and p, respectively). Connective tissue septa (se) separate adjacent electrocytes into compartments. One face, usually the posterior (as shown here), gives rise to a series of fingerlike evaginations (e) that form the stalk. Bar = 200 μm. D. An electron micrograph of a low-power, sagittal view of an electrocyte from *Brienomyrus* sp. showing some of the principal features of electrocytes: anterior and posterior faces with a characteristic surface density (sd), a central zone of myofilaments (my), and nuclei (nu) along the face. Bar = 2.5 μm. E. Montage of electron micrographs from *Brienomyrus* sp. showing that the surface density along each face is characterized by invaginations (i) and mitochondria (mi). Some invaginations, called calveoli (ca), do not open onto the surface. The surface invaginations are covered by a coating material (sc). Z = Z band. Bundles of myofilaments (my^1, my^2) are not oriented in parallel. Bar = 1 μm.

2% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2). Following this, the tissue was taken through a graded series of alcohols and embedded in Araldite or Polybed. The electrocytes were first studied in 1 μm-thick sections stained with 1% toluidine blue. Thin sections were picked up on copper grids, stained with a 1% uranyl acetate and Reynold's lead citrate stain (Reynolds, '63), and examined under a Siemens 102 or Phillips 201 electron microscope. Measurements of electrocyte thickness and anterior face surface density (Table 1) were based on 1-μm, toludine-blue-stained sections viewed at a magnification of ×1,000.

We also prepared electrocytes of *Brienomyrus* sp. for scanning electron microscopy. Perfusion, fixation, and tissue storage were as described above. Tissue was postfixed in 2% unbuffered osmium tetroxide and then incubated in 8.0 N HCl for 1 hour at 60°C. After a distilled water wash, the tissue was dehydrated through a graded series of alcohols, air dried, and then mounted on stubs. Each sample was

then gold-coated with a sputter coater (Balzers) and viewed under a Phillips Scanning Electron Microscope. This procedure was originally adapted for mammalian muscle by Miriam Salpeter and Margaret Marchaterre from the procedure of Desaki and Uehara ('81).

Sex differences and hormone treatments

Several species of mormyrids have a sex difference in their EOD waveform (Hopkins, '80; Moller, '80; Westby and Kirschbaum, '82; Bass and Hopkins, '83, '84, '85). The sex difference may be a permanent or reversible phenomenon as suggested respectively for *Brienomyrus brachyistius* (triphasic) and *B.b.* (long biphasic) (Bass, '86c). We collected material for electron microscopy (as above) from two females (56 and 60 mm, total body length) and two sexually mature males (90 and 97 mm) of *B.b.* (triphasic). The small sample size relates to the scarcity of *B.b.* (triphasic) in the field and their lack of availability from commercial dealers. We have included this data because we consider it as crucial for the documentation of natural sex differences in the electrocytes of mormyrids.

Owing to the reversible nature of a sex dimorphism and a scarcity of specimens, we could not study the electrocytes of male *B.b.* (long biphasic) with a long duration EOD. However, testosterone can induce females to produce an EOD that is typical of sexually mature males (Fig. 1) (Bass and Hopkins, '83; Bass, '86c). Fortunately, we could properly fix the electric organs from one normal female (95 mm), and a second female (68 mm) that had an EOD resembling a sexually mature male after being treated with 17a-methyl testosterone (Sigma, St. Louis, MO). Each female was housed in a bowl of 1.2 l of water (22–23°C). For the treated female, 2–4 mg of testosterone were added directly to its water every 24 hours for 7 days. Considering the extensive documentation of the effects of steroids on the EOD of this species, we again considered it important to report these limited results.

Brienomyrus sp. is the species we are using in our extensive laboratory studies. When maintained in captivity in nonreproductive condition, males and females of this species have similar EODs, as has been found in field studies of *B.b.* (long biphasic) (Bass, '86c). As detailed elsewhere (Bass and Hopkins, '84; Bass, '86c), testosterone induces a 2–3-fold elongation of the EOD waveform (Fig. 1). We often express changes in EOD duration in terms of the signal's power spectrum. An increase in the duration of the EOD corresponds to a lowering of the peak frequency in the power spectrum (PPW) of the EOD (see Bass and Hopkins, '84). All experimental animals were housed individually in water maintained at 22–23°C. The majority of animals used in this preliminary study were females (76–116 mm) that were divided into three experimental groups. One group included controls of untreated specimens (n = 5), whose average PPW was 4.1 kHz. Members of the two remaining groups received a 2–3-mg pellet implant of either 17a-methyl testosterone (n = 7) or cholesterol [5(6)-cholestene-3-ol, Sigma] (n = 3). Following anesthetization with MS222, an incision was made in the ventrolateral body wall and a pellet was inserted along the swim bladder. Wounds were sutured shut, and the animals were then revived and placed in a 1:1 mixture of aquarium water and 0.4% NaCl to help prevent infection. After 3–4 days, animals were maintained in aquarium water alone. Survival times ranged from 6 to 26 days for the testosterone group, and 6 to 18 days for the cholesterol group. The final average PPW was 1.1 kHz for testosterone-treated females and 3.6 kHz for cholesterol-treated females. One male (122 mm) was treated with testosterone for 20 days and had a final PPW of 1.4 kHz. Two additional untreated males (111 and 112 mm; average PPW = 4.2 kHz) were also included in the study. All animals in the steroid treatment studies were sacrificed as above, and the electric organs were prepared for transmission electron microscopy.

RESULTS

Overview of electrocyte morphology

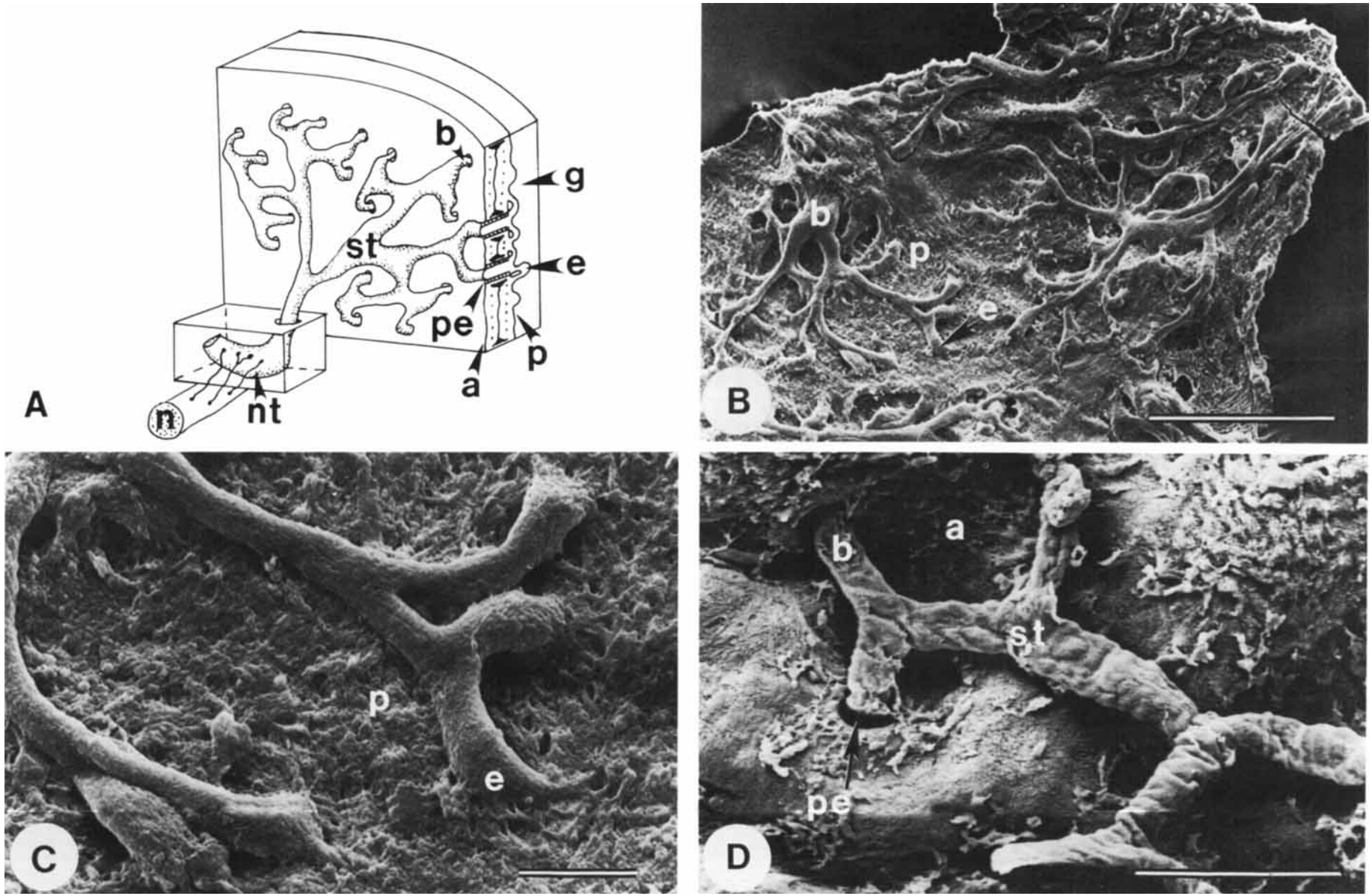
Figure 2 presents an overview of the gross and ultrastructural features of electrocytes. The electric organ of mormyrids is located in the tail (eo, Fig. 2A) and consists of four columns (two on either side of the midline) of serially stacked cells called electrocytes (el, Fig. 2B). The electrocyte columns surround the spinal cord (spc, Fig. 2B),which contains the electromotor nucleus. Connective tissue septa (se, Fig. 2C) separate adjacent cells into compartments. For the species described here, electrocyte thickness ranged from 6 μm (*Stomatorhinus*) to 50 μm (*Isichthys*). Each electrocyte has anterior (a, Fig. 2C–E) and posterior (p, Fig. 2C–E) faces and a series of fingerlike evaginations arising from the posterior face (e, Fig. 2C), which fuse into a stalklike structure (st, Fig. 2B and below) that is innervated by electromotor axons.

Each face is characterized by a surface density (sd, Fig. 2D) that mostly consists of invaginations (i, Fig. 2E) and mitochondria (mi, Fig. 2E). Surface invaginations have also been called tubules or canaliculi (see Schwartz et al., '75). Invaginations usually appear as tubulelike structures that open onto the outer surface of the electrocyte. However, they may also appear as enclosed tubules (called calveoli by Luft, '58) if they are oriented oblique to the sagittal plane (ca, Fig. 2E). As in other electric fish (Schwartz et al., '75), a coating material (sc, Fig. 2E) overlies the outer surface of the electrocyte's invaginations and the "inner" surface of the calveoli. The surface coating probably consists of mucopolysaccharides, glycoproteins, and acid mucopolysaccharides, as in the electric eel, *Electrophorus* (Benchimol et al., '77). Mitochondria are scattered throughout the electrocyte. They often form a dense population near the base of the invaginations but are more scattered within the folds created by the invaginations themselves.

Mormyrid electrocytes are derived from myotomes (Szabo, '60; Denizot et al., '78, '82), as denoted by a central zone of myofilaments that lies parallel to the electrocyte's faces (my, Fig. 2D,E). Bundles of myofilaments form recognizable Z bands (Z, Fig. 2E), but adjacent bundles are not usually arranged in parallel (my^1, my^2, Fig. 2E). Myofilaments are not continuous with surface invaginations, although they are often found near the base of the stalks that extend from the posterior face.

Electrocytes are multinucleated cells, since they form by the fusion of myoblasts (see Srvistava and Szabo, '72). The nuclei (nu, Fig. 2D,E) are arranged in a single row along either face but are usually scattered within the stalk (also see Bass, '86a).

As noted above, the evaginations of the posterior face fuse into a major stalklike structure, whose gross morphology imparts a special geometry upon the overall shape of the electrocyte (see Bass, '86a, for details). The three-dimensional structure of the stalk is best visualized with scanning electron micrographs, as shown for the electrocyte of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Brienomyrus sp., which has a penetrating-stalk system (schematized in Fig. 3A). Along the posterior face, there are many small fingerlike evaginations (e, Fig. 3A–C). The evaginations fuse into larger branches (b, Fig. 3A,B) that penetrate through the main body of the electrocyte to emerge on the anterior side of the cell (Fig. 3A,D). The major branches of the stalk fuse into a single trunk, which is innervated in a restricted zone (nt, Fig. 3A) by electromotoneurons.

The synaptic contact between the axons of spinal electromotoneurons and the electrocyte's stalk resembles other vertebrate neuromuscular junctions. Electromotor axons envelop the base of the stalk (large arrows, Fig. 4A,B) and are myelinated up to their terminations (small arrows, Fig. 4C). Synaptic vesicles (small arrows, Fig. 4D) and active zones (large arrows, Fig. 4D) are found presynaptically. The synaptic cleft is on the order of 80 nm (see Denizot et al., '82, for more details).

As shown for *Brienomyrus* sp., the outer edge of each electrocyte forms a footlike end plate (Fig. 5A), that closely apposes an overlying sheath of connective tissue and collagen (ct, Fig. 5A). The major surface of the foot plate is formed by the anterior face of the electrocyte. The number of invaginations along the anterior face decreases dramatically near its contact zone with the connective tissue sheath. As the anterior face "turns" to form the edge, there is a gradual decrease in the gap between its surface and that of the connective tissue. At the most distal end of the foot plate, the gap (ga, Fig. 5B) appears to be "filled" by a fibrillarlike material. A similar substance also lies between the stalk and the electrocyte proper at the site of a stalk penetration (pe, Fig. 5C,D). The fibrillarlike substance present at the edges of electrocytes may form high-resistance barriers to prevent local current flow and so help direct current across the electrocyte faces (see Bell et al., '76).

Surface invaginations — comparative aspects

In general, surface proliferation along the anterior face exceeds that of the posterior face (see Fig. 2E). However, there is a wide range of variation in the extent of anterior face proliferation that is least in species with short EODs (0.5 msec or less, Fig. 1) such as *Brienomyrus* sp. (Fig. 2D,E), *Marcusenius paucisquamatus* (Fig. 6A,B), *Stomatorhinus corneti* (Fig. 6C,D), and *Gnathonemus tamandua* (Fig. 6E,F). Proliferation of the anterior face is far more extensive in those species with the longest EODs (3 msec or more, Fig. 1), such as *Mormyrus rume* (Fig. 7A,B) and *Isichthys henryi* (Fig. 7C,D). Invaginations are dense along both faces in *I. henryi*. The two species with sexually dimorphic EODs—*B.b* (triphasic) and *B.b.* (long biphasic)— are intermediate in the extent of anterior face invaginations (Figs. 8–10; see below) and in EOD durations (0.8 msec for females and 2.5–3.0 msec for males, Fig. 1). In *B.b.* (long biphasic), the anterior face's surface area is further amplified by large folds or papillae (pa, Fig. 10A,B), as in *Isichthys henryi* (Fig. 7C).

Sex differences in electrocytes

The electrocytes of *Brienomyrus brachyistius* (triphasic) males and females have a single penetrating-stalk pattern of organization (cf. Fig. 3A). One striking feature of its electrocyte is the stalk, which is quite large in comparison to those of other species (Fig. 8A,B) (also see Bass, '86a). Although the stalk appears larger in males than females at the penetration site (Fig. 8A,B), we have not devised an accurate way to assess these size differences.

Overall electrocyte thickness is also greater in males than females (Figs. 8A,B, 9A,B, Table 1). The thickness or density of the anterior face is more extensive in male electrocytes compared to the posterior face in male, or either face in female, electrocytes (Figs. 8A,B, 9A,B, Table 1). In comparison to females (Fig. 9D), surface invaginations appear wider and more extensive along the anterior face in the electrocytes of males (Fig. 9C). An increase in surface density may also relate to differences in the number of mitochondria, in this and other species, but this feature has not yet been assessed.

Hormone-sensitive electrocytes

In *Brienomyrus brachyistius* (long biphasic), we compared electrocyte morphology between a normal female and a testosterone-treated female with a fully transformed male-like EOD. Total electrocyte thickness is greater in the testosterone-treated female, as is the depth of surface folds and the density of the anterior face (Fig. 10A,B, Table 1). As with the natural sex difference in *B.b.* (triphasic), the difference in face density relates in part to surface invaginations, which appear more extensive in the testosterone-treated female (Fig. 10C,D). There is no obvious difference between the posterior faces of untreated and testosterone-treated females, which have few surface invaginations.

Just as captive male and female *Brienomyrus* sp. have similar EODs, the thickness and surface morphology of their electrocytes are nearly the same (Figs. 11A,B, 12A,B, Table 1). Testosterone has a dramatic effect upon electrocyte morphology. In comparison to untreated females and males, total electrocyte thickness and anterior face surface density is two to three times greater in testosterone-treated females and males (Figs. 11C, 12C, Table 1). Again, changes in anterior face surface density are related to surface invaginations, which are especially apparent in *Brienomyrus* sp. because the majority of invaginations appear to be aligned perpendicular to the electrocyte surface. The depth of surface invaginations is two to three times greater in the electrocytes from the testosterone-treated specimens. Overall electrocyte thickness and anterior face surface density is also greater in cholesterol-treated females (Figs. 11D, 12D, Table 1) in comparison to untreated females. However, the effects of treatments with cholesterol are not as dramatic as those with testosterone. Neither cholesterol or testosterone had any obvious effects on the surface morphology of the posterior face. Future reports will address steroid-induced changes in surface area in a more quantitative fashion.

Fig. 3. A. Line drawing of an electrocyte with a penetrating-stalk system. Shown is a cutaway view of one-half of an electrocyte. The three fundamental elements of an electrocyte include anterior (a) and posterior (p) faces and a stalk (st). The entire cell is enclosed in a compartment containing a gelatinouslike matrix (g). In the penetrating-stalk cell, the fingerlike evaginations of the posterior face join into branches (b) that penetrate the main electrocyte body to the anterior side at penetration sites (pe). The "stalk" 's branches fuse into a major trunk that is innervated in a restricted zone (nt) by the electromotor nerve (n). B–D. Scanning electron micrographs of the electrocyte of *Brienomyrus* sp. which has a penetrating-stalk system. B. The stalk arises from the posterior face (p) as a series of evaginations (e) that fuse into branches (b). C. Higher magnification of the stalk shown to the far left in B. D. Penetration sites (pe) where the stalk (st) branches (b) emerge along the anterior face (a). Bar = 50 μm in B; 7.5 μm in C; 50 μm in D.

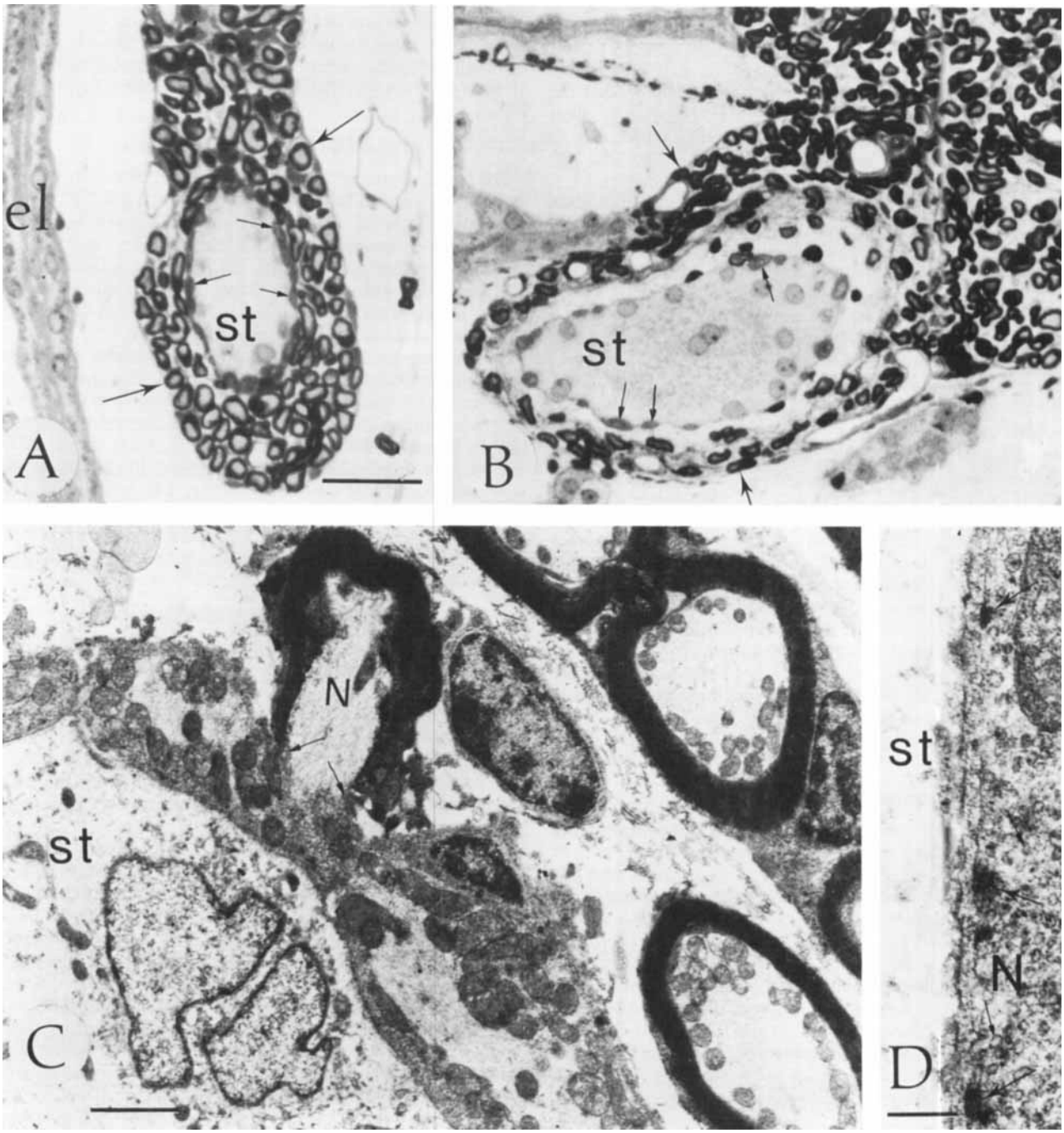


Fig. 4. A,B. Light micrographs of 1-μm, toluidine-blue-stained sections showing innervation of the electrocyte's stalk by spinal electromotor axons. A. *Brienomyrus brachyistius*—the efferent myelinated fibers (large arrows) of the electromotor nerve are arranged in a concentric fashion around the stalk (st). Note the presence of terminal zones on the stalk (small arrows). Bar = 50 μm for A and B. B. *Stomatorhinus corneti*— this species has a space between the myelinated fibers (large arrows) and the surface of the stalk. Terminal zones (small arrows) are also shown. C,D. Electron micrographs of the stalk—(st) electromotor axon (N) interface of *B.b.* C. Note the interruption of the myelin sheath (arrows) near the stalk membrane. Bar = 1 μm. D. Electromotoneuron-stalk synapse showing active zones (large arrows) along the presynaptic membrane and synaptic vesicles (small arrows). Bar = 0.2 μm.

DISCUSSION

Morphological and electrical properties of electrocytes

In general, the results of this study suggest that increasing duration of an EOD waveform is associated with increasing surface proliferation, especially along the anterior face of an electrocyte. The comparative studies of M.V.L. Bennett and colleagues (review: Bennett, '71) suggest that more extensive surface proliferation is associated with greater duration of the action potential or spike generated by an electrocyte face. The duration of each face's spike will

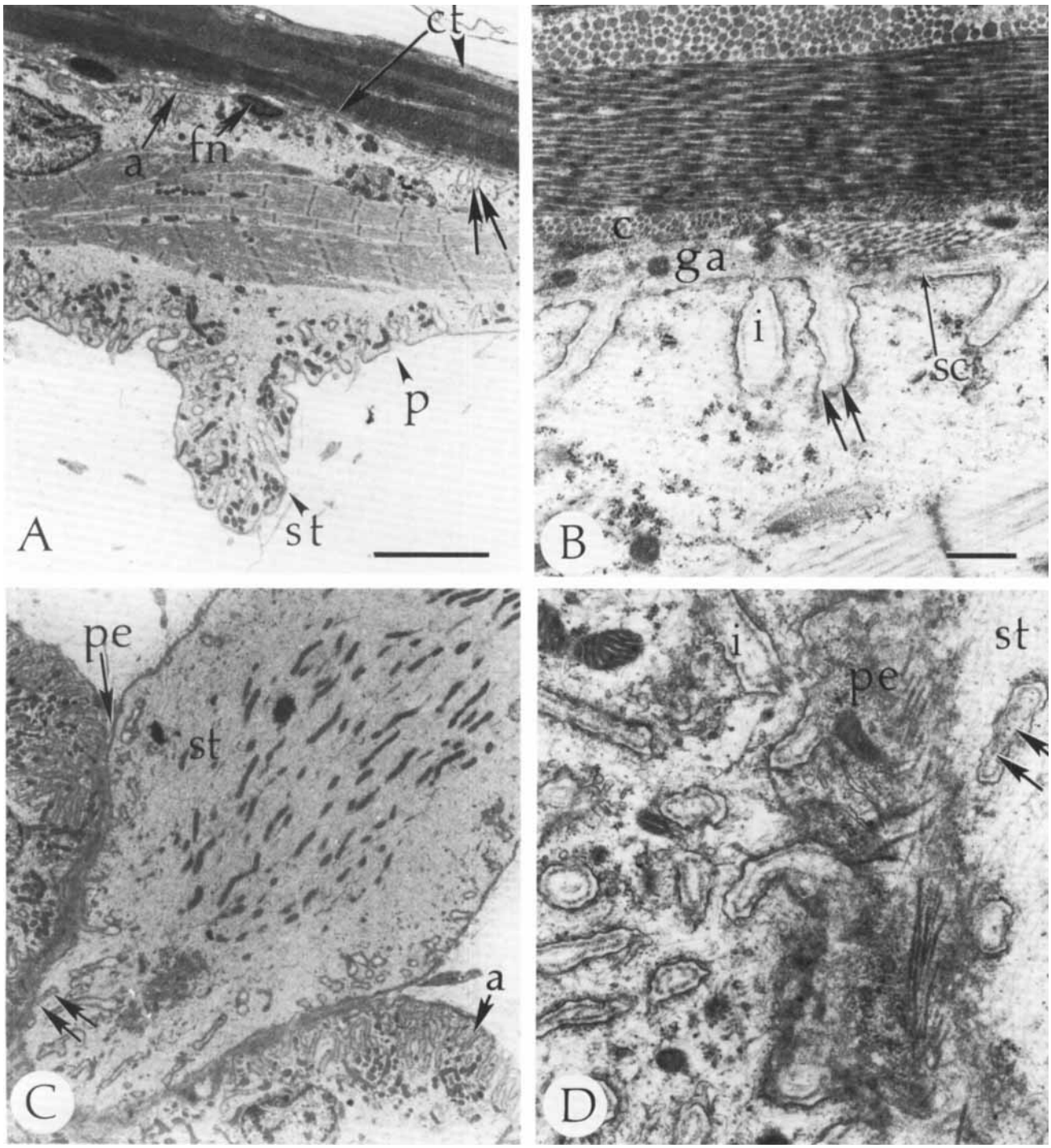


Fig. 5. A–D. Electron micrographs of thin sections showing the electrocyte's edges. A. The outer edge of an electrocyte forms a "footplate" that apposes the connective tissue sheath (ct) surrounding the electric organ. The anterior face (a) of the electrocyte forms the major surface of the plate lying against the connective tissue surface. Fibroblast nuclei (fn) are also indicated. Bar = 4 μm for A and C. B. Higher magnification of A; double arrows are corresponding positions. The gap (ga) between the surface coating (sc) over the invaginations (i) and collagen bundles (c) is filled with a fibrillarlike material. Bar = 0.5 μm for B and D. C. View of a stalk as it begins to penetrate the electrocyte body. D. Higher magnification of C. Double arrows are corresponding points. Note fibrillar substance in penetration (pe) zone.

in turn affect the duration of a particular phase of the EOD waveform, as stated in Bennett and Grundfest's ('61) model (see above). In *Gnathonemus petersii*, a mormyrid with a penetrating-stalk electrocyte (Bennett and Grundfest, '61), surface invaginations along the anterior face are not extensive and are similar to those of the posterior face (see Bruns, '71; Schwartz et al., '75). Both faces produce brief-duration spikes and the major positive and negative phases of the EOD are also brief (Bennett and Grundfest, '61). In contrast, the anterior face of *Mormyrus rume*'s electrocyte has

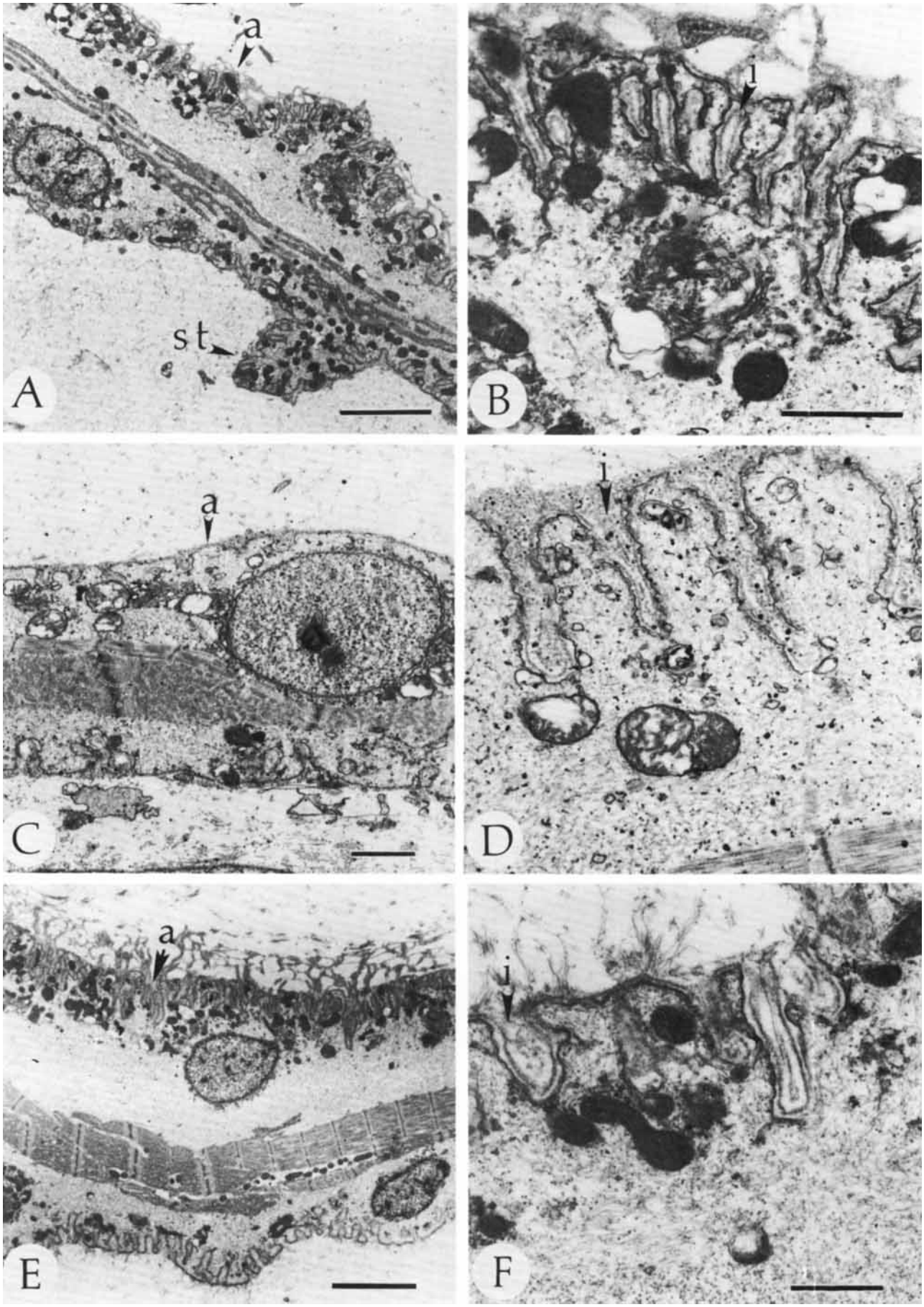


Figure 6

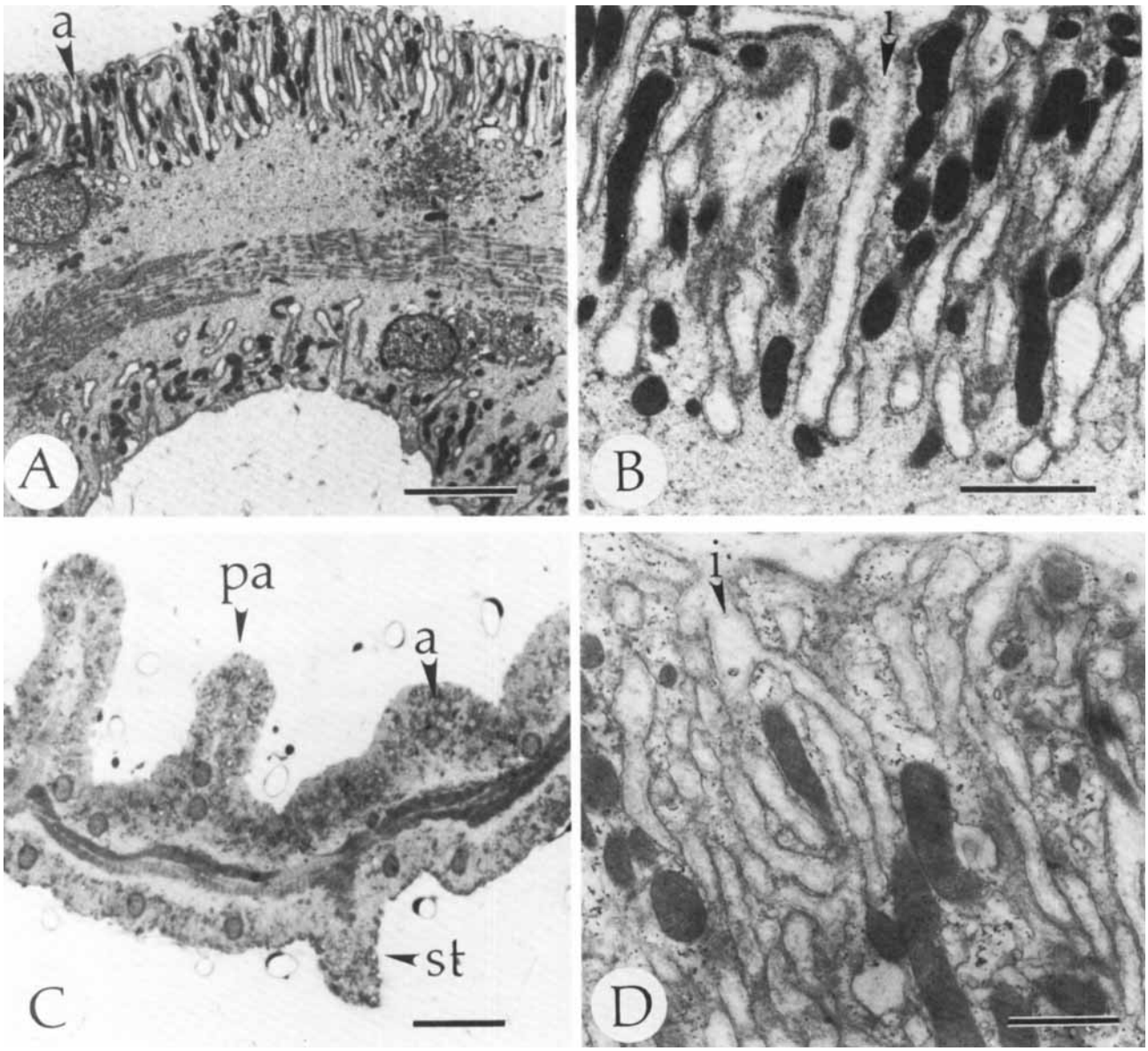


Fig. 7. Photomicrographs of the electrocytes of two species with long-duration EOD waveforms (see Fig. 1) and nonpenetrating stalk systems. A,C = sagittal views of electrocytes; a indicates anterior face; st indicates stalk. B,D = anterior face; i indicates invaginations. A,B. *Mormyrus rume* male. C,D. *Isichthys henryi* juvenile. Low-power view of *I. henryi* electrocyte (C) is a light micrograph. The anterior face has large foldings or papillae (pa), whose size varies between cells (see Bass, '86a). Bar = 6 μm in A; 2 μm in B; 50 μm in C, and 1 μm in D.

Fig. 6. The electrocytes of three mormyrids with short-duration EOD waveforms (see Fig. 1). Figures 6,7, 9–12 are electron micrographs of sagittal views of an entire electrocyte or of the anterior face. A,C,E = sagittal views of electrocyte; a indicates anterior face, st indicates stalk. B,D,F = high-magnification view of anterior face; invaginations (i) are indicated. A,B. *Marcusenius paucisquamatus* (male), which has a nonpenetrating stalk system. C,D. *Stomatorhinus corneti* (female) has a stalk system that includes both penetrating and nonpenetrating elements (see Bass, '86a). E,F. *Gnathonemus tamandua* (female) has a penetrating stalk system. Bar = 4 μm in A; 1 μm in B and D; 2 μm in C; 5 μm in E; and 0.5 μm in F.

a far greater surface area (Fig. 7A,B; also see Luft, '58) and produces a spike of longer duration than the posterior one (Bennett and Grundfest, '61). The negative phase of the EOD (associated with anterior face current flow) is also of longer duration than the positive phase (see Fig. 1 and Bennett and Grundfest, '61).

While we have not recorded the action potentials generated by the electrocytes of our study species, there are comparable correlations between surface area and EOD phase duration. For example, in *Marcusenius paucisquamatus*, *Stomatorhinus corneti*, and *Gnathonemus tamandua*, the major negative and positive phases of the EOD are brief; the surface area of the anterior face, as in *G. petersii*,

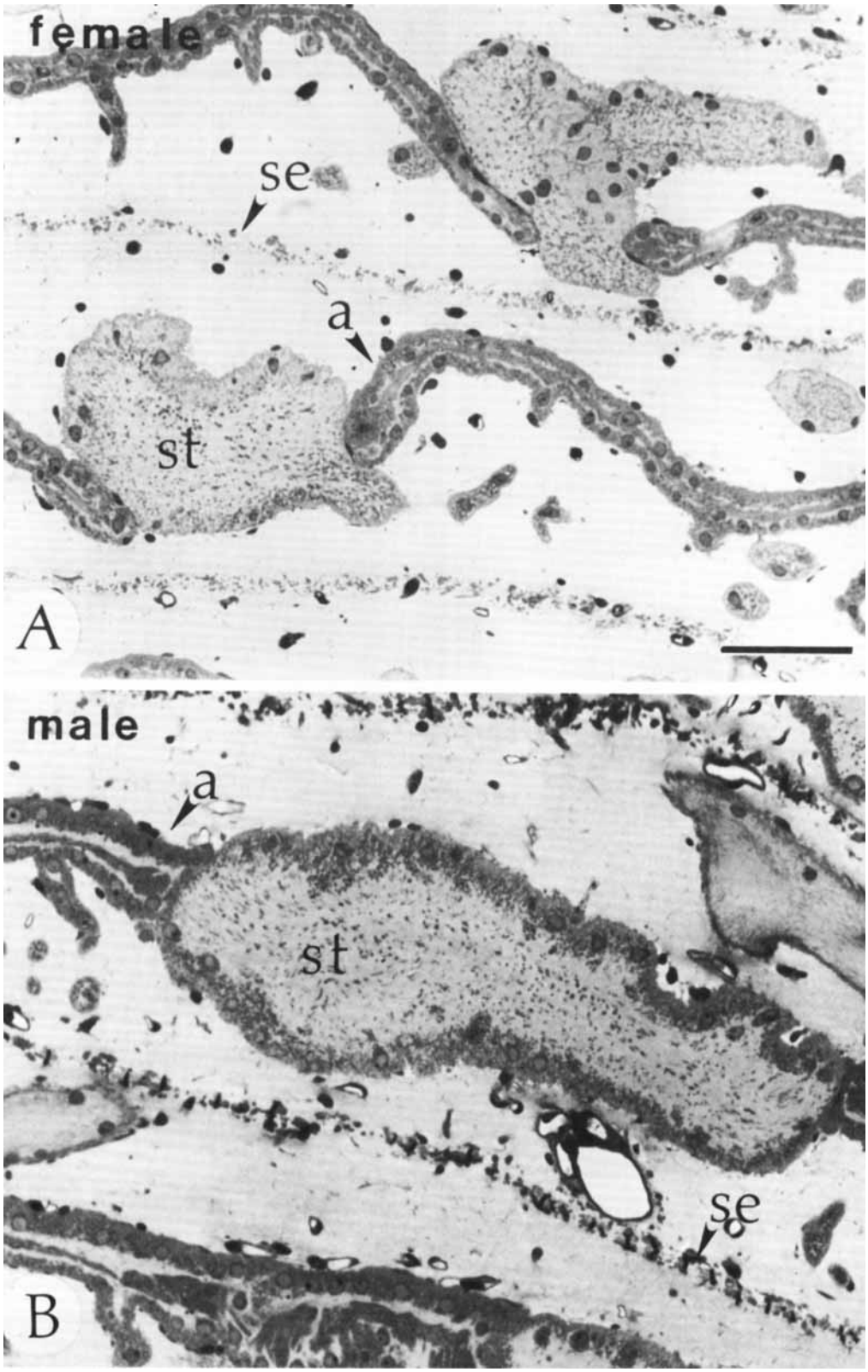
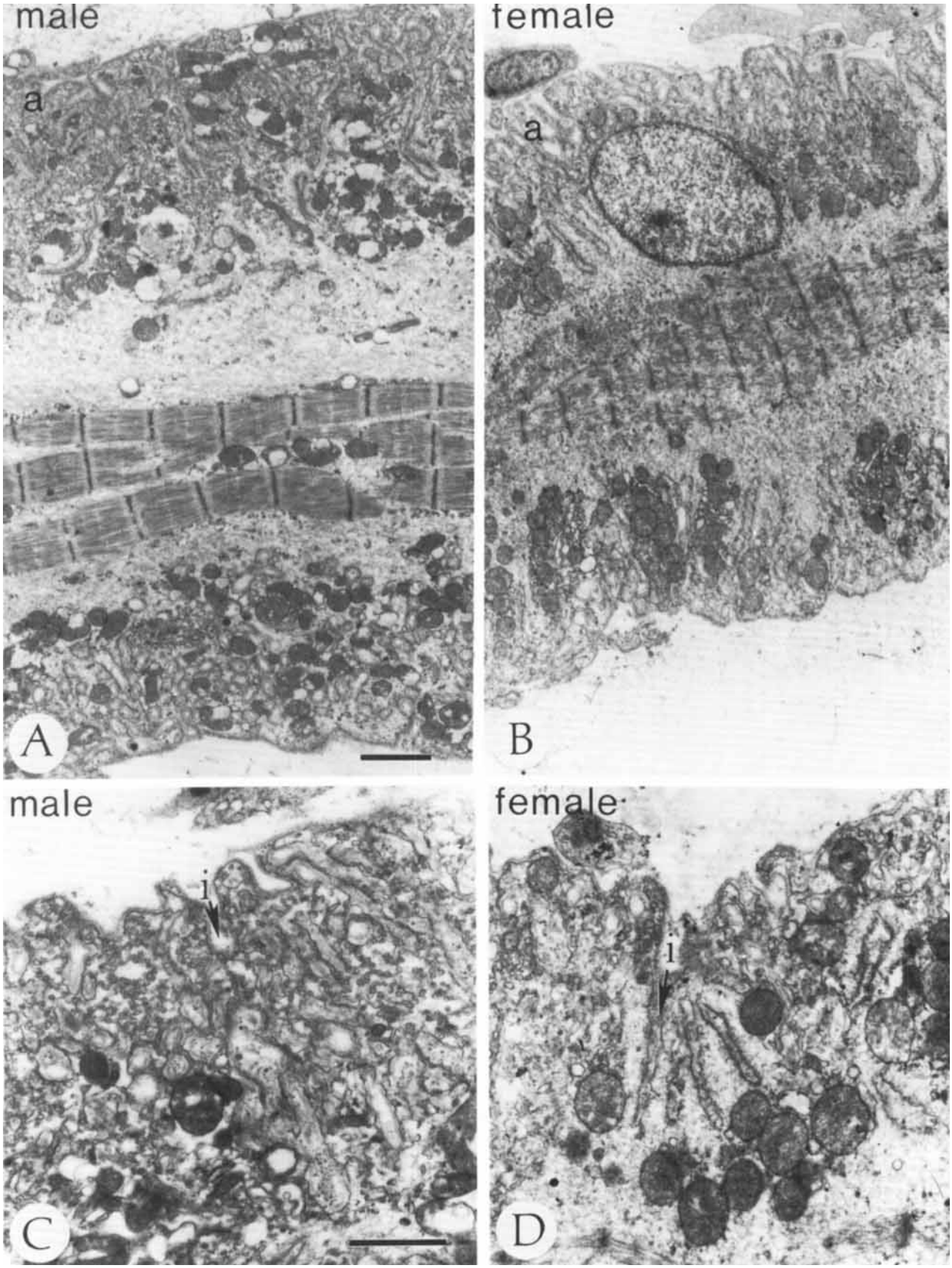


Fig. 8. Photomicrographs of the penetrating-stalk electrocyte of *Brienomyrus brachyistius* (triphasic), which has a sex difference in the EOD waveform (see Fig. 1). Sagittal views of female (A) and male (B) electrocytes. Abbreviations: a, anterior face; st, stalk; se, connective tissue septa. Bar = A is 50 μm for A,B.


male
a
A
female
a
B
male
i
C
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i
D

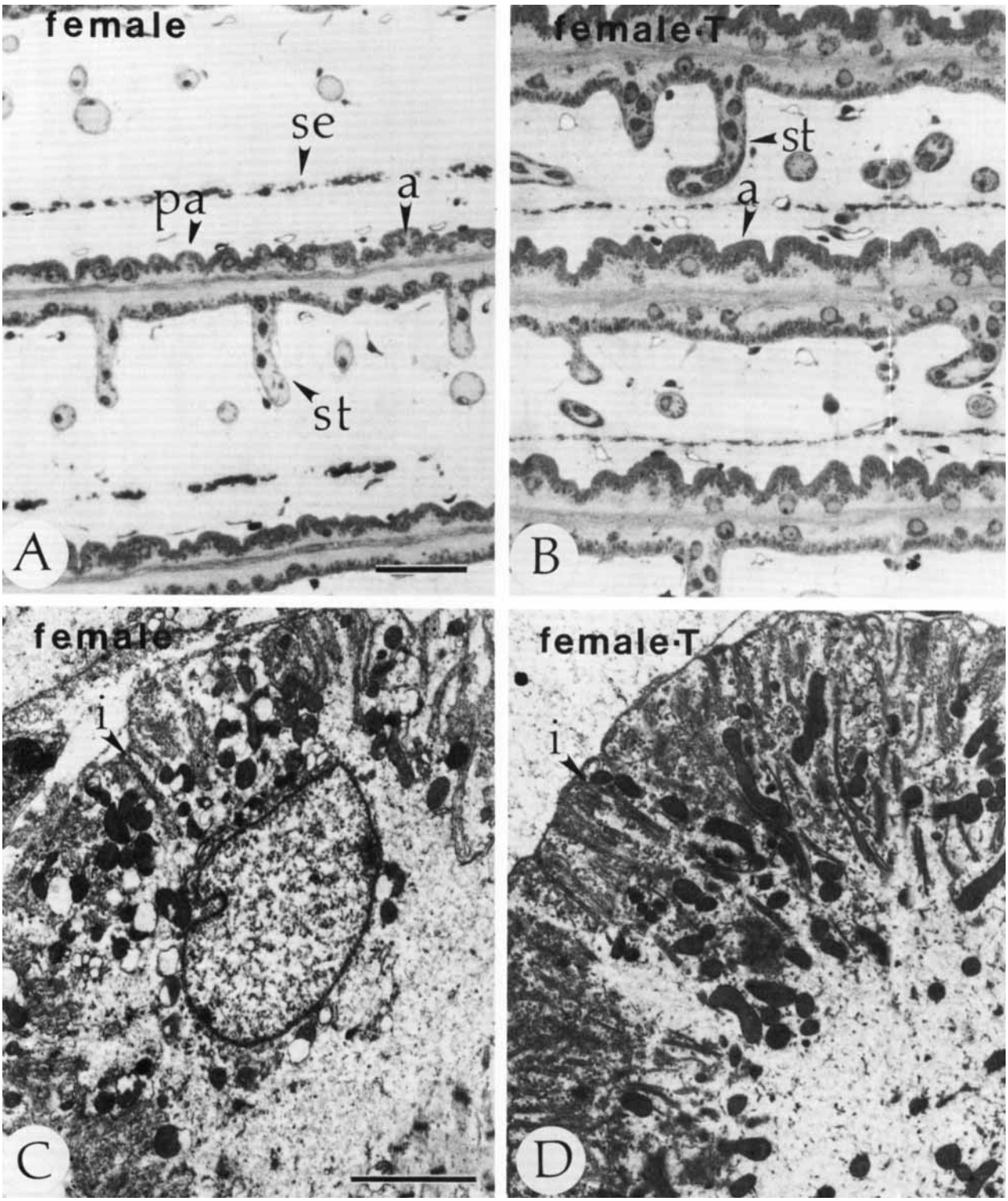

Fig. 10. Photomicrographs of the electrocytes from untreated (A,C) and testosterone (T)-treated females (B,D) of *Brienomyrus brachyistius* (long biphasic). The EOD of the testosterone-treated female is nearly three times the duration of the untreated female (see Fig. 1). A,B. Sagittal views of electrocytes; anterior face (a) is indicated. The electrocyte has foldings or papillae (pa) along the anterior face that effectively increase its surface area. Connective tissue septa (se) and stalks (st) are also indicated. C,D. View of one fold along the anterior face. Bar = is 40 μm in A for A, B; 2.5 μm in C for C,D.

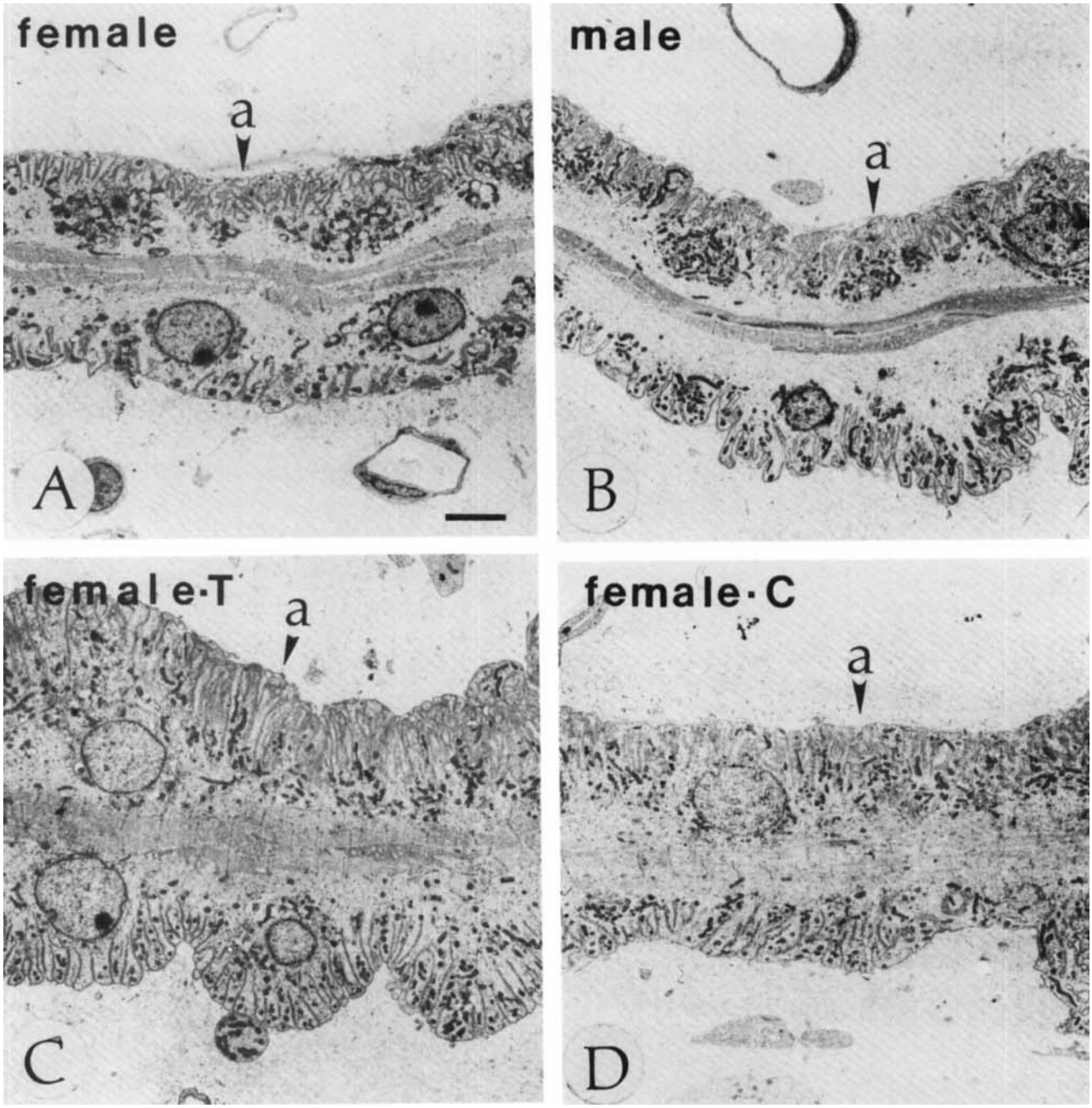

Fig. 11. Photomicrographs of sagittal views of the electrocytes of *Brienomyrus sp.* Anterior face (a) is indicated A. Female. B. Male. C. Testosterone (T)-treated (6 days) female. D. Cholesterol (C)-treated (6 days) female. Testosterone induces a twofold increase in the thickness of electrocytes. Bar = 4 μm in A for A–D.

is not extensive and similar to that of the posterior face. The negative phase of the EOD waveform of male *Brienomyrus brachyistius* (triphasic) has a slow return to the baseline (double arrows, Fig. 1) making it much longer in duration than the positive phase; the anterior face, as in *Mormyrus rume*, is more proliferated than the posterior one. In *I. henryi*, both electrocyte faces have extensive invaginations and both phases of the EOD are long.

Yet there are cases in which the correlation between surface area and EOD phase duration is not straightforward. In female *Brienomyrus brachyistius* (long biphasic) and *B.b.* (triphasic), the duration of the major positive phase of the EOD waveform exceeds or equals that of the negative phase, but the anterior face is more proliferated. While such findings do not detract from the general conclusion that increasing surface area (especially of the anterior face) correlates with increasing *total* EOD duration, they do suggest that in some cases, the determination of the duration of a particular phase of the waveform is not a simple function of the activity generated by one face (or the stalk).

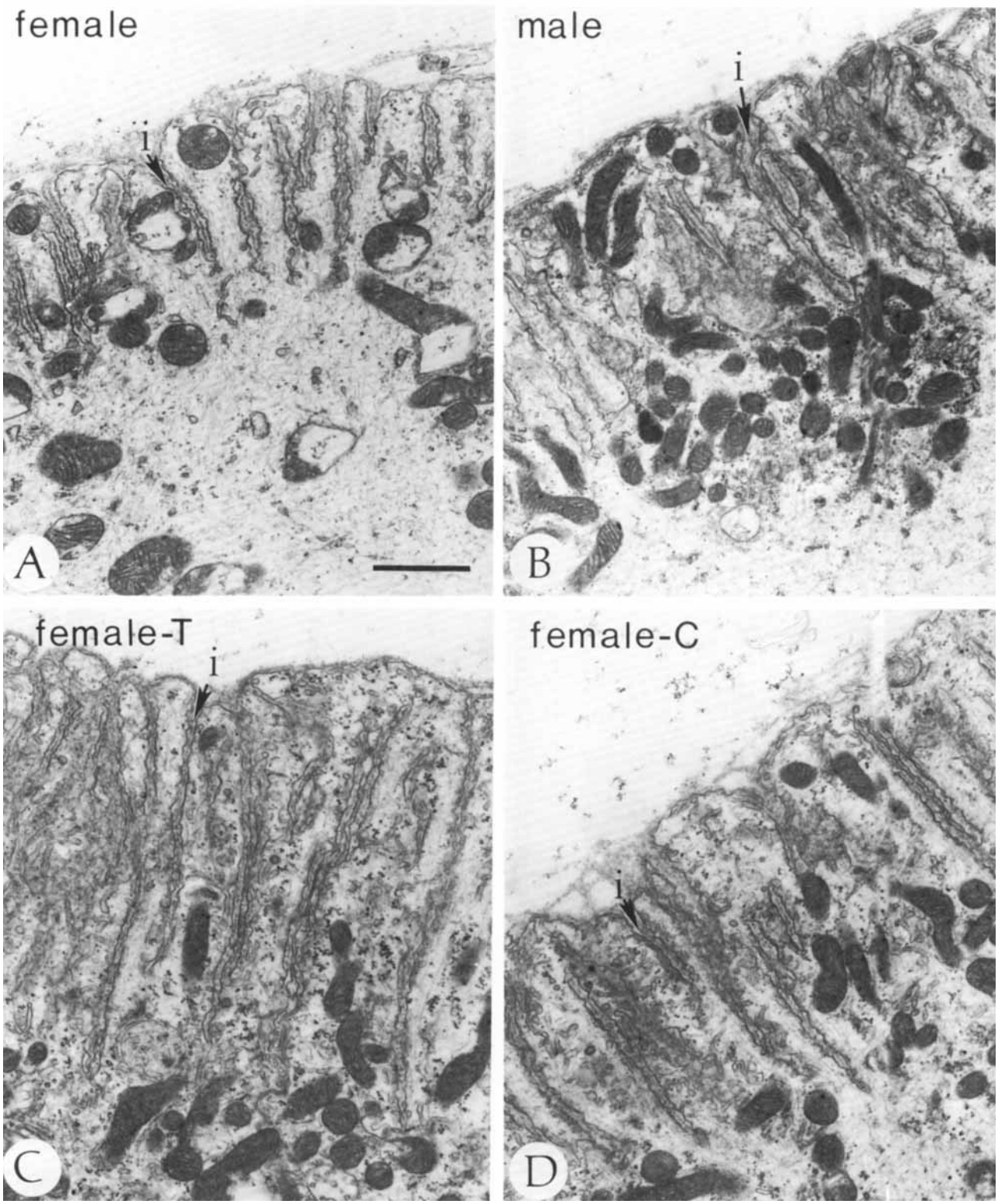

Fig. 12. Photomicrographs of the anterior face of electrocytes of *Brienomyrus* sp. shown in Figure 11. Invaginations (i) are indicated. A. Female. B. Male. C. Testosterone (T)-treated female. D. Cholesterol (C)-treated female. Testosterone induces a dramatic increase in the depth of surface invaginations along the anterior face. Bar = 1 μm in A for A–D.

We know that testosterone induces an increase in the duration of electrocyte action potentials in *Brienomyrus* sp. (Bass and Volman, '85). Since testosterone also induces an increase in electrocyte surface area (this report), then changes in membrane capacitance, which should increase with increasing surface area (Hobbie, '78), can potentially account for the observed changes in spike duration (A.H. Bass and B. Land, unpublished observation). However, testosterone appears to induce an increase in the duration of spikes generated by both the anterior and posterior faces (Bass and Volman, '85), while there are dramatic morphological changes in only the anterior face. The data suggest that steroids may also be affecting the active, ionic properties of the electrocyte's faces.

The action potential-generating properties of the electrocyte's membranes are probably determined by both passive (area-related) and active (conductance-related) factors. Consider the "main" organ of the electric eel, *Electrophorus*. The electrocyte's anterior face is highly proliferated and electrically inexcitable; the posterior face is of far less surface area and alone fires a spike (Keynes and Martins-Ferriera, '53; Luft, '57; Machado et al., '76). Ellisman and Levinson ('82) have shown, using immunocytochemistry, that the excitable posterior face has a much greater density of sodium channels. How might the distribution or density of voltage-gated channels in the electrocyte's membranes change as their surface area increases in hormone-treated individuals? How do such features differ between species? These questions are the focus of future studies.

Electrocytes: androgen-target tissue

Using biochemical methods, we can now characterize the electric organ of mormyrids as an androgen-target tissue, much as mammalian levator ani muscles (see Dube et al., '76), the avian syrinx (Lieberburg and Nottebohm, '79), and the anuran larynx (Segil et al., '83). Androgen-binding activity in the electric organ of *Brienomyrus* sp. is severalfold greater than in other myogenic structures such as trunk musculature (Bass et al., '84, in press).

Steroid hormones are known to increase muscle fiber diameter in levator ani (Venable, '66a,b; Galavazzi and Szirmai, '71) and anuran larynx (Sassoon et al., '83). The increase in overall thickness of myogenic electrocytes of testosterone-treated mormyrids is consistent with the above data showing steroid-induced hypertrophy of vertebrate muscle fibers. Steroid hormones also induce an overall increase in the diameter of electrocytes in a gymnotiform electric fish that has a sex difference in its EOD waveform (Hagedorn and Carr, '85). The effects of cholesterol on electrocyte morphology may be due to their further metabolism to a gonadal steroid such as testosterone (see Wilson et al., '80). Cholesterol also has some effect on the duration (or power spectrum) of the EOD (see Materials and Methods), as it does on the duration of action potentials generated by electrocytes (Bass and Volman, '85).

The surface invaginations of electrocytes have been compared to the junctional folds (Wachtel, '64) and the transverse tubules (Schwartz et al., '75) of vertebrate muscle. A comparison with junctional folds is reinforced by recent documentation of acetylcholinesterase (AchE) activity along the anterior face (although it is absent on the posterior face) of mormyrid electrocytes (Tsuji and Verma, '77; Denizot et al., '78, '82). The functional significance of AchE activity along one face remains an enigma.

For mormyrids, the observed increase in the extent of the electrocyte's surface invaginations, a possible homolog of the more typical vertebrate transverse tubules, is especially intriguing. Considering the role of the transverse tubule system in the coupling of excitation and muscle contraction (e.g., see Bianchi and Narayan, '82), mormyrid electrocytes may provide some insight into the possible effects of steroid hormones on the ionic milieu so integral to vertebrate skeletal muscle excitability. On the other hand, a possible homology with junctional folds may provide insight into the possible effects of steroid hormones on neuromuscular transmission. Erulkar and Wetzel ('85) have already shown that androgenic steroids can influence the conductances of acetylcholine-activated channels in cultured myotubes of another androgen-target muscle, the anuran larynx.

SUMMARY

The electrocytes of mormyrid fish are multinucleated cells that have three morphological/functional components—two faces and a stalk. This study, and a previous one (Bass, '86a), show that interspecific differences in EOD waveforms relate to differences in the morphology of the stalk and each face. Three features characterize the stalk. The first regards the presence or absence of a penetrating-stalk system, which is simply designated as cell geometry. Two other stalk features are their branching pattern, which determines the number of penetration sites, and their size. Surface proliferation, especially of the anterior or nonstalk face, is the most salient feature characterizing the electrocyte's main body.

Taken together, the above features—cell geometry, stalk morphology, surface proliferation—can characterize species-typical differences in the anatomy of mormyrid electrocytes. The highly differentiated stalk system is not found in other families of electric fish and so probably relates specifically to the evolution of mormyrid EODs. In contrast, surface proliferation is one feature common to the electrocytes of most groups of electric fish and most likely underlies the determination of functional characters common to the electrocytes of many different unrelated species (see Bass, '86b).

As noted for mormyrids, it is usually the anterior face that varies most from species to species. Anterior face morphology also best distinguishes male and female electrocytes in species with a steroid-dependent sex difference in the EOD. More detailed analyses of the effects of gonadal steroid hormones on electrocyte morphology will probably lend insight into the role of anatomical features in determining both intra- (sex) and interspecific differences in EODs. But indeed, a next step in understanding the coadaptation of EODs and mormyrid electric organs is further characterization of the electrocyte's active electrical properties.

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LITERATURE CITED

Bass, A.H. (1986a) Species differences in electric organs of mormyrids: Substrates for species-typical Electric Organ Discharge waveforms. J. Comp. Neurol. *244:*313–330.

Bass, A.H. (1986b) Electric organs revisited: Evolution of a vertebrate communication and orientation organ. In T.H. Bullock and W. Heiligenberg (eds): Electroreception. New York: John Wiley and Sons.

Bass, A.H. (1986c) A hormone-sensitive communication system in an electric fish. J. Neurobiol. *17:*131–156.

Bass, A.H., and C.D. Hopkins (1983) Hormonal control of sexual differentiation: Changes in electric organ discharge waveform. Science *220:*-971–974.

Bass, A.H., and C.D. Hopkins (1984) Shifts in frequency tuning in androgen-treated mormyrid fishes. J. Comp. Physiol. *155:*713–724.

Bass, A.H., and C.D. Hopkins (1985) Hormonal control of sex differences in the electric organ discharge (EOD) of mormyrid fishes. J. Comp. Physiol. *156:*587–604.

Bass, A.H., N. Segil, and D. Kelley (1984) A steroid sensitive electromotor pathway in mormyrid fish: Electric organ morphology, androgen receptor biochemistry and steroid autoradiography. Neurosci. Abstr. *10:*972.

Bass, A.H., N. Segil, and D. Kelley (1987) Androgen binding in the brain and electric organ of mormyrid fish. J. Comp. Physiol. (in press).

Bass, A.H., and S.F. Volman (1985) Androgen-induced changes in action potential waveforms of an electric organ. Neurosci. Abstr. *11:*159.

Bell, C.C., J. Bradbury, and C.J. Russell (1976) The electric organ of a mormyrid as a current and voltage source. J. Comp. Physiol. *110:*65–88.

Benchimol, M., W. De Souza, and R.D. Machado (1977) An electron microscopic investigation of the surface coat of the electrocyte of *Electrophorus electricus*. Cell Tissue Res. *183:*239–253.

Bennett, M.V.L. (1971) Electric organs. In W.S. Hoar and D.J. Randall (eds): Fish Physiology, Vol. V. New York: Academic Press pp. 347–491.

Bennett, M.V.L., and H. Grundfest (1961) Bioelectrogenesis. In C. Chagas and A. Carvalho (eds): Studies on Morphology and Electrophysiology of Electric Organs. III. Electrophysiology of Electric Organs in Mormyrids. London, New York, Princeton: Elsevier, pp. 113–135.

Bianchi, C.P., and S. Narayan (1982) Muscle fatigue and the role of transverse tubules. Science *215:*295–296.

Bruns, V. (1971) Elektrisches Organ von *Gnathonemus* (Mormyridae). Z. Zellforsch *122:*538–563.

Denizot, J.-P., F. Kirschbaum, G.W.M. Westby, and S. Tsuji (1978) The larval electric organ of the weakly electric fish *Pollimyrus (Marcusenius) isidori* (Mormyridae, Teleostei). J. Neurocytol *7:*165–181.

Denizot, J.-P., F. Kirschbaum, G.W.M. Westby, and S. Tsuji (1982) On the development of the adult electric organ in the mormyrid fish *Pollimyrus isidori* (with special focus on the innervation). J. Neurocytol. *11:*913–934.

Desaki, J., and Y. Uehara (1981) The overall morphology of neuromuscular junctions as revealed by scanning electron microscopy. J. Neurocytol. *10:*101–110.

Dube, J.Y., R. Lesage, and R.R. Tremblay (1976) Androgen and estrogen binding in rat skeletal and perineal muscles. Can. J. Biochem. *54:* 50–55.

Ellisman, M., and S. Levinson (1982) Immunocytochemical localization of sodium channel distributions in the excitable membranes of *Electrophorus electricus*. PNAS *79:*6707–6711.

Erulkar, S.D., and D.M. Wetzel (1985) The effects of 5a-Dihydrotestosterone on the acetylcholine receptor in myotubes cultured from adult male *Xenopus laevis* larynx. J. Physiol. (Lond.) *363:*35P.

Galavazi, G., and J.A. Szivmai (1971) Cytomorphometry of skeletal muscle: The influence of age and testosterone on the rat M. levator ani. Z. Zellforsch *121:*507–530.

Hagedorn, M., and C. Carr (1985) Single electrocytes produce a sexually dimorphic signal in South American electric fish, *Hypopomus occidentalis* (Gymnotiformes, Hypopomidae). J. Comp. Physiol. *156:*511–523.

Heiligenberg, W. (1977) Principles of Electrolocation and Jamming Avoidance in Electric Fish. A Neuroethological Approach. In V. Braitenberg (ed): Studies of Brain Function. New York: Springer-Verlag, pp. 1–85.

Hobbie, R.K. (1978) Intermediate Physics for Medicine and Biology. New York: John Wiley and Sons.

Hopkins, C.D. (1980) Evolution of electric communication channels of mormyrids. Behav. Ecol. Sociobiol. *7:*1–13.

Hopkins, C.D. (1983) Functions and mechanisms in electroreception. In R.G. Northcutt and R.E. Davis (eds): Fish Neurobiology, Vol. I. Univ. Michigan Press, Ann Arbor pp. 215–259.

Keynes, R.D., and H. Martins-Ferriera (1953) Membrane potentials in the electroplates of the electric eel. J. Physiol. (Lond.) *119:*315–351.

Lieberburg, I., and F. Nottebohm (1979) High-affinity androgen binding proteins in syringeal tissues of songbirds. Gen. Comp. Endocrinol. *37:*286–329.

Luft, J.H. (1957) The histology and cytology of the electric organ of the electric eel (*Electrophorus electricus*, L.). J. Morphol. *100:*113–139.

Luft, J.H. (1958) The fine structure of electric tissue. Exp. Cell Res. *5:*168–182.

Machado, R.D, W. DeSouza, G. Cotta-Pereira, and G. Oliveira Castro (1976) On the fine structure of the electrocyte of *Electrophorus electricus* L. Cell Tissue Res. *174:*355–366.

Moller, P. (1980) Electroreception. Oceanus *23:*44–54.

Reynolds, E.S. (1963) The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. *17:*208–213.

Sassoon, D., N. Segil, and D. Kelley (1983) Androgen induced proliferation in the larynx of juvenile *Xenopus laevis*. Neurosci. Abstr. *9:*1093.

Schwartz, I.R., G. Pappas, and M.V.L. Bennett (1975) The fine structure of electrocytes in weakly electric teleosts. J. Neurocytol. *4:*87–114.

Segil, N., L. Silverman, D. Kelley, and T. Rainbow (1983) Androgen binding in the laryngeal muscle of *Xenopus laevis*: Sex differences and hormonal regulation. Neurosci. Abstr. *9:*1093.

Srivastava, C.B.L., and T. Szabo (1972) Development of electric organs of *Gymnarchus niloticus* (Fam. Gymnarchidae): 1. Origin and histogenesis of electroplates. J. Morphol. *138:*375–386.

Szabo, T.H. (1960) Development of the electric organ of mormyridae. Nature *188:*760–762.

Taverne, L. (1972) Osteologie des genres *Mormyrus* (Linne), *Mormyrops* (Muller), *Hyperopisus* (Gill), *Isichthys* (Gill), *Mormyrus* (Boulenger), *Stomatorhinus* (Boulenger) et *Gymnarchus* (Cuvier). Considerations générales sur la systématique des poissons de l'ordre des mormyriformes. Musée Royale de l'Afrique Centrale, Tervuren, Belgique. Annales. Serie In-8°, Sciences Zoologiques, n° 200.

Tsuji, S., and V. Verma (1977) Synaptic and non-synaptic acetylcholinesterase activity in the electroplaque of Mormyrid fishes. J. Neurocytol. *6:*119–123.

Venable, J.H. (1966a) Morphology of the cells of normal, testosterone-deprived and testosterone-stimulated levator ani muscles. Am. J. Anat. *119:*271–302.

Venable, J.H. (1966b) Constant cell populations in normal, testosterone-deprived and testosterone-stimulated levator ani muscles. Am. J. Anat. *119:*263–270.

Wachtel, A. (1964) The ultrastructural relationships of electric organs and muscle. I. Filamentous systems. J. Morphol. *114:*325–359.

Westby, G.W., and F. Kirschbaum (1982) Sex differences in the waveform of the pulse-type electric fish, *Pollimyrus isidori* (Mormyridae). J. Comp. Physiol. *145:*399–403.

Wilson, J.D., F.W. George, and J.E. Griffin (1980) The hormonal control of sexual development. Science *211:*1278–1284.