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ORIGINAL PAPER

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Ontogeny of the electric organ discharge and the electric organ in the weakly electric pulse fish *Brachyhypopomus pinnicaudatus* (Hypopomidae, Gymnotiformes)

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Abstract I recorded the electric organ discharges (EODs) of 331 immature *Brachyhypopomus pinnicaudatus* 6–88 mm long. Larvae produced head-positive pulses 1.3 ms long at 7 mm (6 days) and added a second, small head-negative phase at 12 mm. Both phases shortened duration and increased amplitude during growth. Relative to the whole EOD, the negative phase increased duration until 22 mm and amplitude until 37 mm. Fish above 37 mm produced a "symmetric" EOD like that of adult females. I stained cleared fish with Sudan black, or fluorescently labeled serial sections with anti-desmin (electric organ) or anti-myosin (muscle). From day 6 onward, a single electric organ was found at the ventral margin of the hypaxial muscle. Electrocytes were initially cylindrical, overlapping, and stalk-less, but later shortened along the rostrocaudal axis, separated into rows, and formed caudal stalks. This differentiation started in the posterior electric organ in 12-mm fish and was complete in the anterior region of fish with "symmetric" EODs. The lack of a distinct "larval" electric organ in this pulse-type species weakens the hypothesis that all gymnotiforms develop both a temporary (larval) and a permanent (adult) electric organ.

Key words Weakly electric fish · Gymnotiformes · Development · Electric organ · Electric organ discharge

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Abbreviations *EOD* electric organ discharge · *A1* amplitude of phase one relative to total amplitude · *A2* amplitude of phase two relative to total amplitude · *D1* duration of first phase · *D2* duration of second phase

Introduction

The gymnotiform fish of South America produce weak electric discharges used for electrolocation and communication. The electric organ consists of muscle-derived cells (electrocytes) arranged in series and in parallel, like batteries. Each electrocyte produces one or more action potentials, which sum to produce the electric organ discharge (EOD) (Bennett 1961). Four gymnotiform families contain pulse-type species, which produce EODs separated by relatively long silent intervals. The other three families contain wave-type species, in which the intervals between EODs are less than or equal to the EOD duration (Bullock 1974). Each species has a characteristic EOD waveform consisting of one to four positive and negative phases in characteristic proportions to each other (Bass 1986). In addition, the EOD waveform may differ between males and females of the same species (Bass 1986) and among individuals (McGregor and Westby 1992). The cladogram in Fig. 1 summarizes the relationships of the families and some of their electrogenic characters.

The variety of EOD waveforms and electric organ anatomy has encouraged studies of the functional morphology of electrocytes (Bennett 1971; Schwartz et al. 1975; Comfort 1990; Hopkins et al. 1990; Mills et al. 1992). Unlike studies of mormyrid electric fish, in which electrocyte stalk morphology predicts the number and polarity of EOD phases (Bass 1986), comparative studies of gymnotiforms have not indicated functional reasons for electrocyte shape, arrangement, or the presence or absence of innervated stalks. Because the EOD changes during development (Kirschbaum 1995), ontogeny provides another possible means of correlating anatomy with signal waveform.

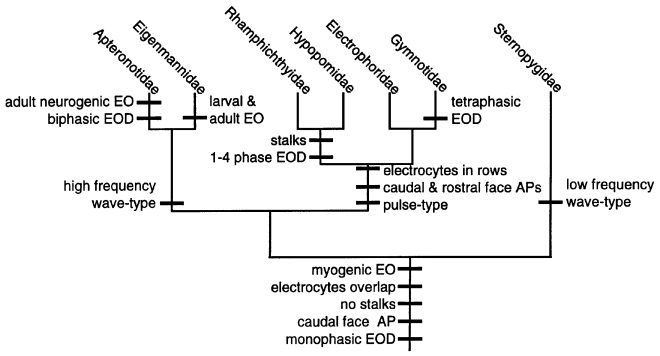

Fig. 1 The current distribution of electrogenic characters and the hypothetical ancestral traits were mapped on the cladogram of the Gymnotiformes (adapted from Alves-Gomes et al. 1995). Descendants share the ancestral trait unless a new character state is shown. The family Sternopygidae has the most numerous primitive characters. Alves-Gomes et al. (1995) are ambiguous on the relationship of Gymnotidae and Electrophoridae to the other families, but this position is supported by Gayet et al. (1994), Triques (1993), and Mago-Leccia (1994). *AP* = action potential; *EO* = electric organ; *EOD* = electric organ discharge

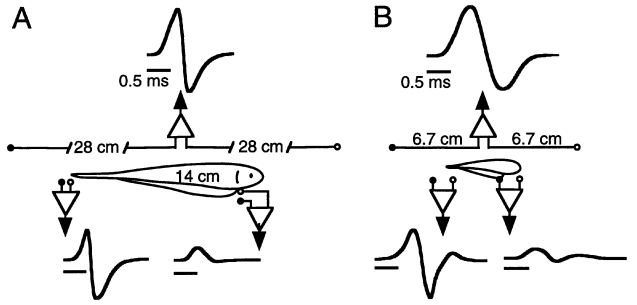

Fig. 2A, B At a distance the head-to-tail EOD is biphasic (*top*), but the local EOD (*below*) varies along the length of a mature female (**A**) and a 5.3-cm juvenile (**B**). The more complex waveforms in **B** result from the different electrode configuration. *Triangles* represent differential amplifiers measuring the signal between the positive (*white circles*) and negative (*black circles*) electrode. Recording electrode placement is shown in scale relative to the fish

Only three previous studies address the development of both electric organs and EOD waveform in gymnotiforms (Kirschbaum and Westby 1975; Kirschbaum 1977, 1983). The wave-type species *Eigenmannia lineata* [previously reported as *virescens*; see Kirschbaum (1995)] and *Apteronotus leptorhynchus* both develop two electric organs during their lifetimes. A *larval electric organ* appears first in development, produces an EOD for a time period that varies by species, and then degenerates while being replaced by and adult electric organ in a separate location (Kirschbaum 1977). The development of the electric organ has not yet been described in any pulse-type species.

Brachyhypopomus (formerly *Hypopomus*) *pinnicaudatus* (Hopkins 1991) is a sexually dimorphic pulse-type species. The standard head-to-tail EOD waveform, which is recorded at a distance from the fish, is a nearly symmetric, biphasic EOD averaging 1.1 ms in duration in females (Fig. 2A). In males, the total EOD duration is longer, and the negative phase of the EOD is smaller in amplitude but longer in duration than the positive phase (Hopkins et al. 1990). Measurements of electric potentials within 1 mm of the skin (local EODs) indicate that electrocytes neither fire simultaneously nor produce the same waveform at all points along the fish's length (Stoddard 1994; Stoddard et al., unpublished observation). The local EOD of *B. pinnicaudatus* is monophasic head-positive in the head region of the fish but biphasic near the caudal filament (Fig. 2A); differences in local timing and waveform average at a distance into the head-to-tail waveform. This heterogeneity contradicts a simple dipole model of the electric organ, in which the waveform is identical at all points along the fish's body.

Pilot studies of *B. pinnicaudatus* showed that the head-to-tail EOD changes from a monophasic head-positive pulse in larvae to the biphasic adult waveform (Franchina 1994). I hypothesized that the "larval" (first) electric organ continues to produce monophasic pulses in the anterior region of adults instead of degenerating, while the "adult" (second) electric organ produces biphasic pulses in the posterior region. Alternatively, a single adult electric organ might contain anterior electrocytes that produce monophasic discharges and posterior electrocytes that produce biphasic pulses, which differences might be reflected in anatomy. This paper describes the development of the electric organ discharge and electric organ anatomy in the pulse-type species, *Brachyhypopomus pinnicaudatus*.

Materials and methods

Breeding

Artificial rainy season conditions stimulated breeding in wild caught and lab-bred *B. pinnicaudatus* (Kirschbaum 1979). The photoperiod was 12 L:12 D or 13 L:11 D. A pump sprinkled recirculated water (rain) on the tank surface three times a day for 2–3 h. The water conductivity, periodically lowered by adding deionized water, ranged from 70 to 300 μS cm^{-1} (14–3.3 kΩ cm^{-1}). Water temperature cycled from 26 to 28 °C daily. One to four males and one to four females were present at a time. I fed adults generously with oligochaetes (blackworms).

Care during development

I placed eggs in 175-ml containers in a 27 °C water bath. A wide spectrum antibiotic was necessary to prevent 100% mortality. I adjusted the conductivity to a standard 250 μS cm^{-1} before hatching and changed the water every 1 or 2 days after feeding began. Starting on day 6, I provided freshly hatched brine shrimp once or twice daily. Fish ≥ 10 mm received chopped blackworms and gradually fewer brine shrimp. I measured the total length of 722 live fish of known age. To calculate the average growth rate, I fitted a regression line to a plot of length versus age. Because I did not identify individuals nor sacrifice all fish after measurement, 512 data points potentially came from the same individuals on different days.

Electric organ discharge measurement

I attempted to record head-to-tail EODs from 329 immature fish 6–88 mm total length (4–135 days old). However, 38 fish (6–7 mm;

3–9 days) had no detectable EOD. Seventy-eight EODs came from the same eight fish at different ages (without sacrifice or individual identification). I placed fish < 60 mm in a 140–mm diameter Petri dish containing 100 ml of 10% gelatin in tank water of conductivity 250 $\mu S \cdot cm^{-1}$ (Fig. 3A). A well in the center of the gelatin constrained the fish without changing the electric field. I placed two 1-mm silver ball electrodes approximately equidistant from the head and tail; the inter-electrode distance was 10–135 mm, depending on EOD amplitude. The ground electrode was perpendicular to the midpoint of the fish and 70 mm away. I restrained fish > 60 mm in a plastic net in a tank 60 cm × 45 cm × 25 cm deep. Carbon rod electrodes were 60 cm apart and the ground was 22 cm perpendicular to the midpoint. The positive electrode was always located nearest to the head. The electrodes connected to an AC-coupled differential amplifier (Charles Ward Electronics; model BMA-831/XR or −200) with lower and upper filters set to 1 Hz and 10 kHz, respectively. A 12-bit (Data Translation DT2821F) or 16-bit (Tucker-Davis Technology AD1) analog-to-digital converter board transformed the signal at 125 kHZ (8-μs intervals).

I wrote a computer program that measured the relative amplitude of each phase from the baseline (A1, A2) and the duration of each phase at 5% of the total amplitude (D1, D2) (Fig. 3B). Capacitive coupling in the amplifier added a long duration, small amplitude, negative component to EODs recorded at gains ≥ 5000. Because the artifact was usually small in amplitude compared to the EOD, a threshold for duration at 5% of the total wave height excluded the artifact from most EODs (Fig. 3B, lower right EOD). However, EODs from 28 fish 7–8 mm long were recorded at gains of 20 000, which increased the artifact to more than 5% of the EOD amplitude (Fig. 3B, lower left EOD, arrow); these 28 EODs were excluded from further analysis. A 1.0-ms square wave recorded in the dish showed the distorting effect of the amplifier (Fig. 3C). An EOD was considered to be biphasic if the amplitude of the head-negative phase was at least 10% of the amplitude of the head-positive phase.

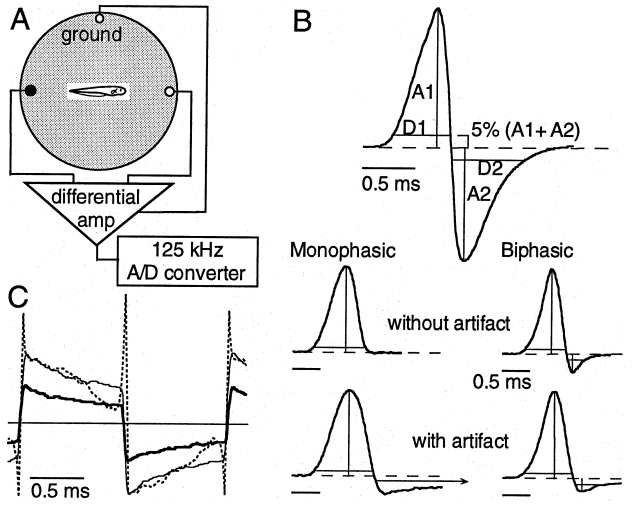

Fig. 3 A I recorded the EODs of fish < 60 mm in a 140-mm Petri dish filled with 10% gelatin in tank water of conductivity 250 $\mu S \cdot cm^{-1}$; a well in the gelatin restrained the fish without interfering with the electric field. The electrodes (not to scale) moved along the *x*-axis to accommodate different size fish. I recorded the EODs of larger fish in a tank (60 × 45 × 25 cm deep) with an identical electrode configuration. **B** The EOD measurement paradigm was derived from that used for adult biphasic EODs (*top*). The computer measured the relative amplitude of each phase (*A1*, *A2*), and the duration of each phase (*D1*, *D2*) at 5% of the total peak-to-peak height (*A1* + *2*). If the second phase was more than twice the duration of the first, it was discounted as amplifier artifact. The larval EODs without artifact (*middle*) were recorded at 2000 gain, while the ones with artifact were recorded at 20 000 gain (*bottom left*) and 5000 gain (*bottom right*). **C** A 1.0-ms square wave, recorded in the dish at gains of 5000 (*heavy line*), 10 000 (*solid line*), and 20 000 (*dotted line*), shows the effect of capacitive coupling within the amplifier

Histology

I anesthetized fish larger than 50 mm with MS 222 before immersing them in fixative or isopentane at −80 °C; smaller fish died in seconds without anesthetic. To reveal the position and size of the electric organ, as well as gross electrocyte morphology, I followed the protocol of Filipski and Wilson (1984) to clear and stain whole fish. Sudan black, normally used to stain the fatty myelin sheath of nerve, also increased contrast in the electric organ. Briefly, I fixed fish in Bouin's fixative or 4–10% buffered formalin, rinsed the fish to remove fixative, then transferred it to a trypsin solution, which denatures proteins and renders most tissues transparent.

To distinguish electric organ from muscle, I used monoclonal antibodies against cytoskeletal proteins. Both desmin and myosin are found in normal muscle cells; myosin is mostly absent in electric organs (Schwartz et al. 1975). In *B. pinnicaudatus*, anti-desmin binds strongly to the electric organ, while anti-myosin labels muscle (H. Zakon, personal communication). I cut frozen fish into 10–15 μm serial sections, placing alternating sections on two sets of slides, one for each antibody, which were then processed concurrently. I labeled sections with monoclonal mouse primary antibodies diluted to 5–15 $\mu g \cdot ml^{-1}$ [D3 or D76 = anti-desmin (Danto and Fischman 1984); MF20 = anti-myosin (Bader et al. 1982)] followed by fluorescein-conjugated secondary antibody (goat anti-rat; Organon Teknika #55756). Specimens stored in isopentane at −80 °C for a period of 24 h up to 2 years showed tissue-specific antibody labeling, but suffered from dehydration. Specimens fixed in 4% formalin and stored in 30% sucrose in phosphate buffer at −80 °C had excellent tissue quality but non-specific labeling. A step-by-step protocol is available from the author.

Results

Developmental staging

The individual growth rate was highly variable; mortality was lower and increases in length faster and more uniform among individuals when there were fewer larvae per volume of water. The average growth rate of all fish (measured one or more times) was 4.0 mm per week (n = 722; r^2 = 0.84), but two individuals kept in separate dishes grew 7.8 mm per week. Because development is better correlated with size than age in most teleosts, developmental events were described by size, unless the fish was less than 1 week old.

The night of spawning was designated day zero of development. The slightly sticky eggs were 2 mm in diameter. Fish did not exhibit parental care and ate eggs that fell to the floor during spawning. Embryos hatched on day three and remained 6–7 mm long during the *early larval stage*, which is defined by endogenous feeding on the yolk (Blaxter 1988).

On day 7, the appearance of digestive organs in the body cavity, followed by exogenous feeding, marked the transition to the *late larval stage* (7–37 mm). The dorsal finfold began to resorb in 9-mm fish and was nearly invisible by 16 mm. Anal fin rays were first visible in 9-mm fish at the anterior of the ventral finfold, just behind the anus at the posterior end of the body cavity; larger larvae added rays caudally. By 14 mm, larvae used

the undulating anal fin for propulsion (like adults), rather than sinusoid motions of the whole body. The anus and the anterior margin of the anal fin moved rostrally during development, until the anal fin reached the midpoint of the body cavity and the anus exited between the opercula after 30 mm, as they do in adults. A gray opaque knob, appearing in a terminal notch between the dorsal and ventral finfold in 9-mm fish, became the caudal filament (that portion of the tail that extends beyond the anal fin). The caudal filament comprised 22 ± 5% of fish 40–60 mm long (n = 10); in adult females the caudal filament is 20–30% of the total length (Hopkins et al. 1990).

The *juvenile stage* is the time period when the fish has most of the characteristics of an adult except mature gonads (Blaxter 1988), but in practice is difficult to define in a species without a metamorphosis. After analyzing the data, I defined a juvenile as a fish with an EOD resembling that of a mature female (see below); fish with female-like EODs also had the body proportions and pigmentation pattern characteristic of adult females. In the field, the smallest fish with mature gonads were 120 mm (Hopkins et al. 1990), so probably none of the fish in this study (≤ 88 mm) were reproductively mature.

Electric organ discharge

The EOD waveform changed gradually from a single head-positive phase (monophasic), to a head-positive phase followed by a smaller head-negative phase (asymmetric), to a nearly symmetric biphasic waveform (Fig. 4). I was able to record the very first discharges at 6.5 days (early stage larvae) only by touching the electrodes to the fish. By day 7 (7 mm), late stage larvae produced monophasic head-positive EODs, which were 1.3 ± 0.2 ms in duration (n = 13) and recordable at a short distance from the fish.

The duration of the head-positive phase decreased as length increased ($y = kx^{-0.30}$; n = 263; r^2 = 0.82) (Fig. 5). Starting at about 12 mm, a small head-negative phase followed the head-positive phase, creating an asymmetric biphasic EOD. Although the duration of the head-negative phase increased at first, in fish above 22 mm, the duration of the second phase decreased at the same rate as the head-positive phase ($y = kx^{-0.29}$; n = 117; r^2 = 0.26) (Fig. 5). The relative amplitude of the head-negative phase also ceased to increase after about 37 mm, but the absolute amplitude of the total EOD continued to increase with length.

The similarity of an immature EOD to that of an adult female was judged by measuring the relative proportions of the phases. In fish 22–88 mm, the ratio of phase one duration to the total was nearly constant (0.56 ± 0.03; n = 117), and similar to that of an adult female EOD (0.55; n = 24; Hopkins et al. 1990) (Fig. 6A). Among fish 38–88 mm, the ratio of phase one amplitude to the total (0.49 ± 0.05; n = 85) was slightly greater than that of adult females (0.44; n = 24; Hopkins et al. 1990) (Fig. 6B). In two fish (56 and 53 mm) the local EOD waveform differed from anterior to posterior, as it does in adults (Fig. 2B). Because juvenile and female EODs are expected to be similar due to a lack of masculinizing androgens, I arbitrarily defined 37 mm as the larva-juvenile transition.

Electric organ anatomy

The electric organ was always located at the ventral boundary of the ventral muscle mass (hypaxial muscle).

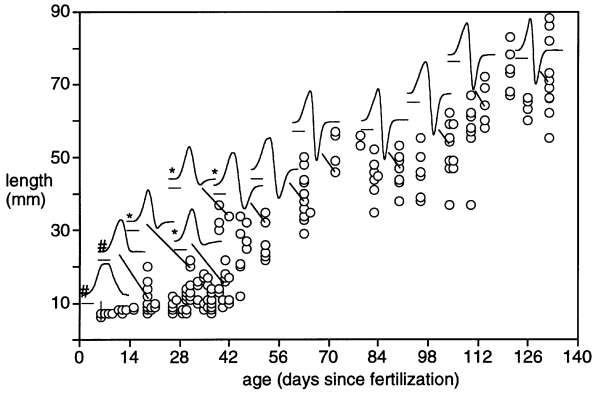

Fig. 4 Early larval stage fish (6–7 mm; days 3–6) had no detectable EODs (n = 38). Late larval stage fish (7–37 mm) had monophasic EODs (n = 144; #EODs) or asymmetric biphasic EODs (n = 68; *EODs). In fish above 37 mm, the positive and negative phases had approximately equal duration and amplitude (unmarked EODs); fish with these "symmetric" EODs were defined as juveniles (n = 79). 142 EODs were potentially recorded from the same fish on different days. All EODs were scaled so that the first phases were the same size; therefore the amplitudes cannot be compared. EOD scale bars = 0.5 ms

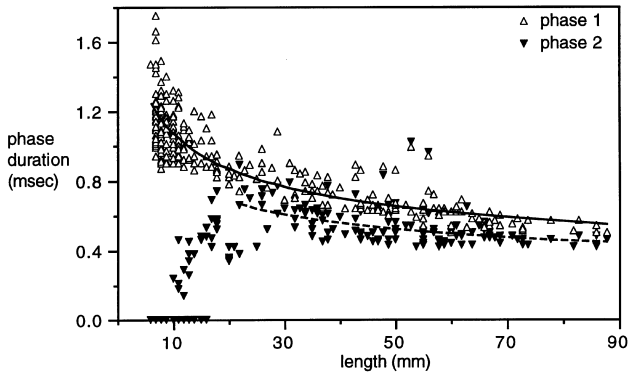

Fig. 5 The total duration of the EOD decreased from an average of 1.3 ms at 7 mm to 1.1 ms at 70–90 mm. The duration of the head-positive phase decreased as length increased (r^2 = 0.82). After a period of rapid increase, the duration of the head-negative phase also decreased (r^2 = 0.26). (The highest r-squared value was obtained when only fish 22–88 mm were included in this regression). Note the similarity in the decline of both phases' duration

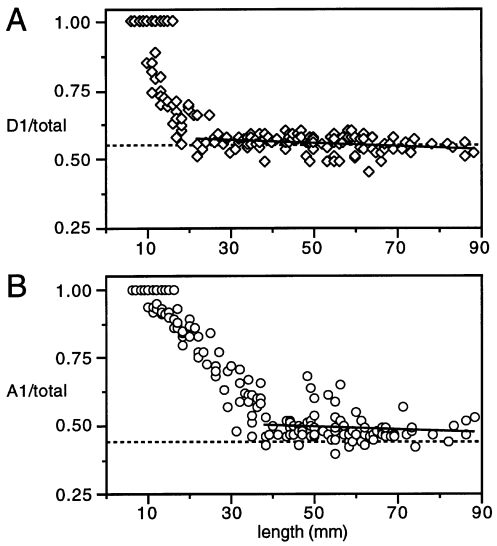

Fig. 6 **A** Until about 22 mm, the head-negative phase duration increased, while the head-positive phase duration decreased, so the relative duration of the head-positive phase (*D1*) decreased with respect to the total EOD. After 22 mm, the duration ratio (*solid line*) was similar to the phase duration ratio of adult females (*dotted line*). **B** The relative amplitude of the head-positive phase (*A1*) decreased with respect to the total EOD until about 37 mm, after which the amplitude ratio (*solid line*) remained slightly above that of female EODs (*dotted line*). Fish > 37 mm were defined as juveniles

At no point did electrocytes disappear from a location occupied in an earlier stage. In gymnotiforms, the electric organ consists of bilateral pairs of columns. Each column is a series of electrocytes inside a connective tissue sheath that runs parallel to the body axis of the fish (Bennett 1961). A row (Hopkins et al. 1990) consists of vertically aligned electrocytes from different columns, as seen in sagittal section.

While anti-myosin labeled muscle in 5-day-old larvae (indicating that the procedure worked), no tissue was specifically labeled by anti-desmin. I first saw electrocytes in cleared specimens and in desmin-labeled sections of 6-day-old larvae. A single pair of columns extended from behind the abdominal cavity for roughly half the length of the "tail" (Fig. 7A). Each electrocyte was a cylinder $\sim$ 250 μm long with tapered ends that overlapped with electrocytes in the same column. They were located between the ventral margin of the hypaxial muscle and the dorsal margin of the ventral finfold (Fig. 7B).

Larvae with monophasic EODs (7–12 mm) added columns and extended columns caudally. By day 8 (7 mm), the electric organ tapered from three pair of columns just behind the abdominal cavity to one pair that did not reach the tail tip. Larvae at 10 mm had four or five longer columns (Fig. 8A). Electrocytes were cylindrical, close together, and had no stalks, like those of 6-day-old fish (Fig. 8B). The anal fin rays passed medially between the bilateral columns of the electric organ (Fig. 8C). The muscle and skin of the anal fin labeled for both desmin and myosin.

In larvae with asymmetric biphasic EODs (12–37 mm), the electric organ advanced rostrally below the abdominal cavity and extended further caudally. The electric organ consisted of three to five bilateral columns along most of its length, but did not extend into the caudal filament. The dorsal column contained the widest electrocytes (in transverse section) and extended furthest below the body cavity (Fig. 9A). Anterior electrocytes had the original cylindrical shape, while posterior electrocytes began to shorten along the rostrocaudal axis, separate into vertical rows, and develop stalks on the caudal face (Fig. 9B, C). The anal fin muscle (*pinnalis analis externalis*) formed a thin layer on both sides of the electric organ and between the ventral-most electrocyte columns.

Juveniles with "symmetric" EODs (> 37 mm) showed the greatest differentiation of the electric organ. The electric organ columns extended from below the abdominal cavity into the caudal filament (Fig. 10A). Electrocytes in the anterior electric organ also developed stalks and separated into rows during this stage (Fig. 10B). Posterior electrocytes resembled those of sexually ma-

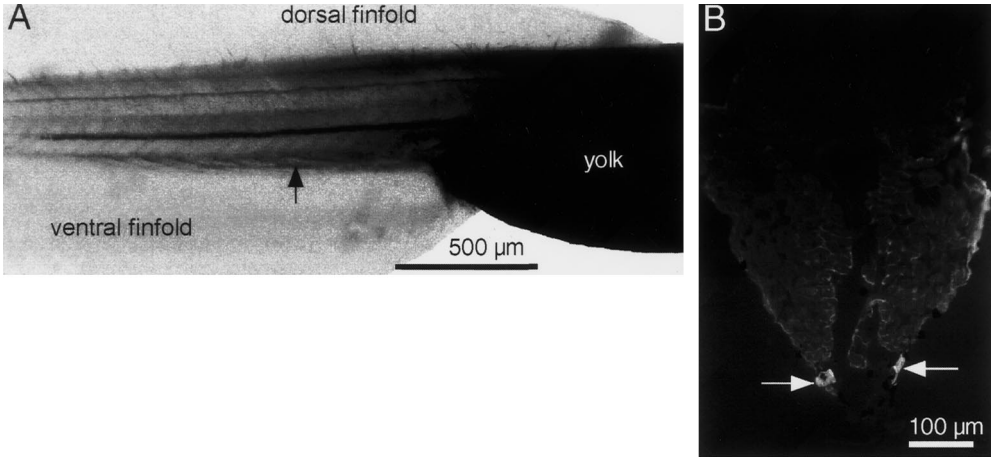

Fig. 7A, B Early larval stage fish (7 mm; day 6) had barely detectable EODs (not shown): **A** cleared and stained fish shows a single faint column of cylindrical, overlapping electrocytes (*arrow*) at the ventral margin of the hypaxial muscle. The *dark horizontal line* is a blood vessel. Rostral = right; **B** a transverse section behind the yolk showed a single bilateral pair of electrocytes (*arrows*). The rest of the cross-sectional area is mostly muscle tissue

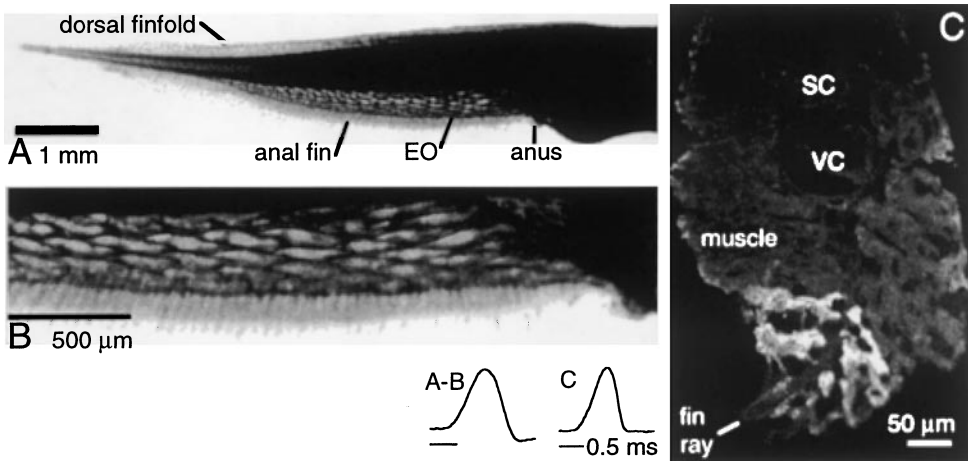

Fig. 8A–C Late larval stage fish at 10 mm with monophasic EODs: **A** cleared and stained fish shows the extent of the electric organ; **B** electrocytes were cylindrical and close together, and had no stalks; **C** in transverse section, an anal fin ray passed between at least two bilateral columns of electrocytes (*arrows*), labeled by anti-desmin; *SC* = spinal cord; *VC* = vertebral column

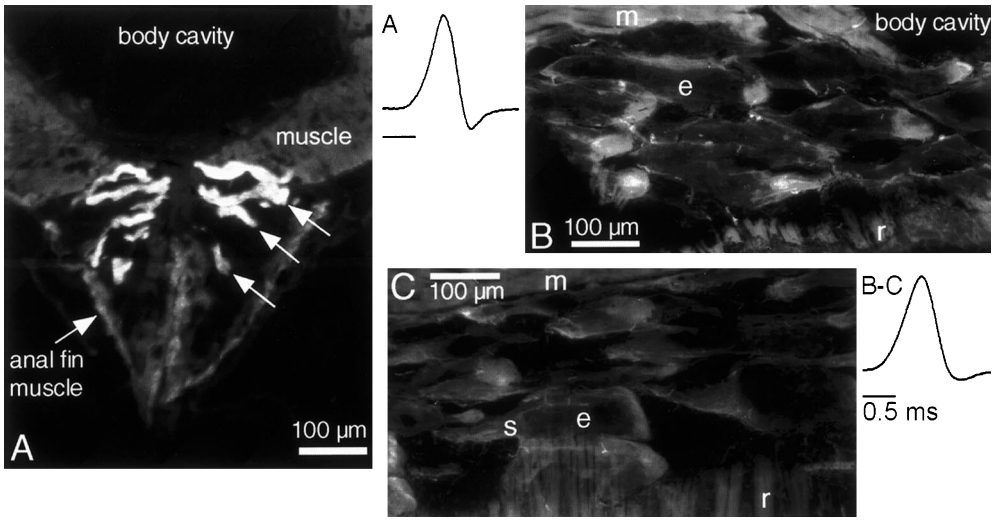

Fig. 9A–C Late larval stage fish with asymmetric biphasic EODs: **A** transverse section from a 20-mm fish shows at least four columns of electrocytes (*arrows*) labeled by anti-desmin, with the anal fin muscle also faintly labeled; **B** sagittal section with non-specific labeling shows the cylindrical anterior electrocytes of a 17-mm fish; *e* = electrocyte; *m* = muscle; *r* = anal fin ray; **C** posterior electrocytes in the same fish have stalks (*s*) on the caudal face; rostral = right; dorsal = up

ture females (Fig. 10C) [see Hopkins et al. (1990) for comparison].

Discussion

No new evidence for a larval electric organ

By Kirschbaum's (1995) definition, a larval electric organ produces the first EODs, exists in a separate location than the adult electric organ, and degenerates during formation of the adult electric organ. While it is impossible to prove the absence of something, my results indicate that *B. pinnicaudatus* does not have a larval electric organ according to this definition. First, the desmin-labeled cells, which do not have striations like muscle tissue and do not label for myosin, are highly likely to be the only electrocytes. There is no reason to postulate additional non-desmin-labeled electrocytes, because the desmin-labeled cells can account for the presence of an EOD on day 6. Second, all electrocytes were located in continuous columns at the ventral margin of the hypaxial muscle (Figs. 7–10); thus, there is a single location for the electric organ throughout life. Finally, once electrocytes were present in a given location, they did not disappear from that location. Although two morphological types of electrocytes were seen: cylindrical, stalk-less, and overlapping (Fig. 8A) versus rectangular, stalked, and separated (Fig. 10), electrocytes with intermediate morphology (Fig. 9B) indicate continuing differentiation of cells rather than replacement of one type by another. Therefore, I concluded that *B. pinnicaudatus* possess a single electric organ throughout life.

With the exception of apteronotids, which possess electric organs composed of modified nerve axons, adult gymnotiforms have myogenic electric organs, meaning that electrocytes evolved from mesoderm tissue that originally gave rise to muscle (Bennett 1971). *Apteronotus leptorhynchus* has a larval myogenic electric organ that degenerates after the adult neurogenic electric organ forms (Krischbaum 1983). Because the neurogenic organ is a derived character unique to apteronotids, it is unlikely that the initial myogenic electric organ of

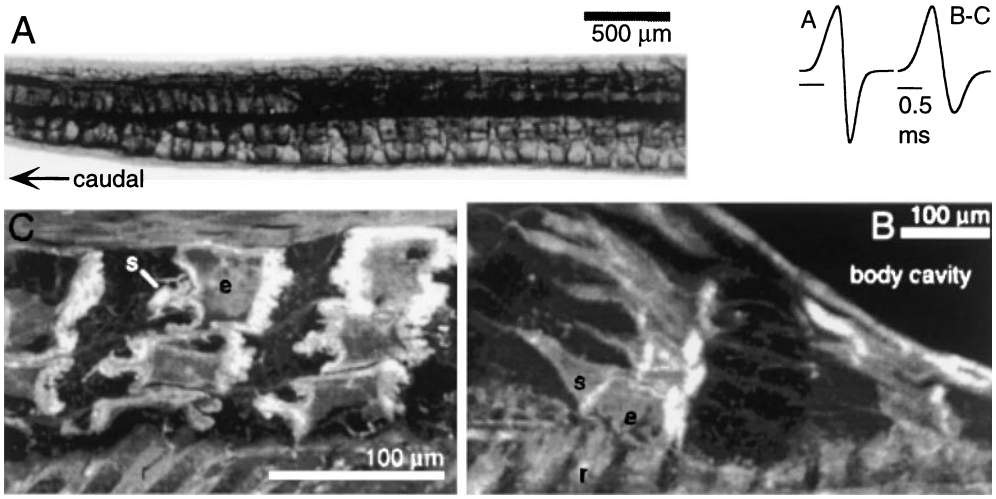

Fig. 10 A The caudal filament of a 46-mm juvenile with a symmetric EOD was filled with stalked electrocytes; their outlines were blurred because electrocyte rows did not align between the right and left sides; rostral = right; dorsal = up. **B** Sagittal section with non-specific labeling of a 34-mm fish with an asymmetric EOD: anterior electrocytes had stalks and spaces between rows; *e* = electrocyte; *r* = anal fin ray; *s* = stalk; rostral = right; dorsal = up. **C** Posterior electrocytes in the same fish have stalks and are separated into distinct rows. Note: magnification is twice that of **B**

apteronotids is homologous to the adult electric organs of other species. Furthermore, the larval electric organ is ventral to the adult organ in *A. leptorhynchus*, whereas the larval organ is dorsal to the adult electric organ in *Eigenmannia lineata*, implying that the larval electric organs in these species are not homologous. Therefore, the replacement of a myogenic electric organ with an adult neurogenic electric organ in the Apteronotidae does not suggest that other families form two myogenic electric organs.

The lack of separate larval and adult electric organs in the pulse-type species *B. pinnicaudatus* weakens the current hypothesis, based on two wave-type species, that all gymnotiforms develop separate larval and adult electric organs. The only evidence of a temporary (larval) myogenic electric organ replaced by an adult myogenic electric organ is the brief report on *E. lineata* (Kirschbaum 1977). My results may reflect fundamental differences between wave-type species and pulse-type species, or differences among families. Examination of species from additional families is essential before drawing conclusions about the ancestral developmental pattern in gymnotiforms. The diversity of electric organs in gymnotiform adults certainly allows for the possibility of equally diverse mechanisms of its formation.

Correlation of electric organ and EOD development

As soon as electrocytes that labeled for desmin were visible, in 6-day-old fish (6–7 mm), EODs were also detectable. Thus, the presence of desmin and the absence of myosin correlate with the functional differentiation of the electric organ in this species. Desmin, an intermediate sized non-contractile filament normally found in muscle, probably has a structural function (Lazarides 1980). Schwartz et al. (1975) found large amounts of intermediate filaments, which could include desmin, keratin, or vimentin, in the electric organs of five species of gymnotiforms. Surprisingly, sternopygid electrocytes label strongly for keratin, normally found in epithelial, or ectodermally derived, cells (Patterson and Zakon 1996). A comparative survey of the types of filaments found in the electric organ may reveal more about its evolution or development.

Bennett (1961, 1971) modeled the electric organ as a dipole in which all electrocytes fire simultaneously (Fig. 11). Connective tissue insulation around the electrocytes channels current along the body axis. In response to an acetylcholine-mediated post-synaptic potential from spinal motoneurons, the innervated caudal faces of electrocytes fire action potentials. Positive ions flow into the cell, carrying current towards the head; current returns to the tail through the environment (Fig. 11A). Thus, an external electrode near the head records a positive voltage relative to the tail during the first phase of the EOD. The headward current may trigger voltage-gated channels in rostral faces, causing a second action potential, during which current flows across rostral membranes towards the tail (Fig. 11B). An external electrode records the second, head-negative phase of the EOD. Species without voltage-gated chan-

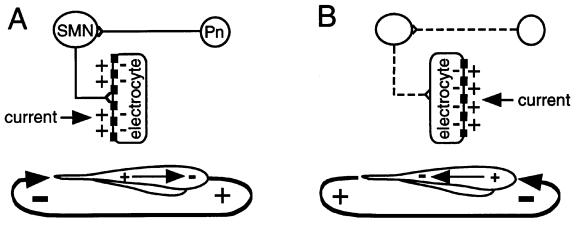

Fig. 11A, B A schematic representation of Bennett's (1961) model: **A** spinal motoneurons (*SMN*) relay the discharge of the pacemaker nucleus (*Pn*) to the electric organ. An excitatory post-synaptic potential triggers an action potential in the caudal, innervated face of the electrocyte. Current flows towards the head inside the fish and returns toward the tail outside, creating the head-positive phase of the EOD; **B** depolarization of the rostral face triggers a second action potential and current flows towards the head outside the fish, creating the head-negative phase of the EOD

nels in the rostral face, such as *Sternopygus macrurus*, have no second action potential and therefore no head-negative phase (Bennett 1971).

The EOD therefore represents the summed action potentials from the caudal and rostral faces of electrocytes. The addition of a second phase at about 12 mm indicates that rostral faces become functional after caudal faces do (Fig. 4). The increase in EOD amplitude during development can be attributed to the addition of electrocytes in series (longer columns) and in parallel (more columns) (Figs. 7–10). If the synchronization of electrocytes improves due to changes in the nervous system, the EOD would not only become larger in amplitude but also shorter in duration. The decrease in phase duration (Fig. 5) could also reflect ontogenetic changes in the voltage-gated ion channels that shape the action potential in each face. In *S. macrurus* for example, the inactivation time-constant of the sodium channel correlates with individual differences in EOD duration; both increase after androgen injections (Ferrari et al. 1995). In *B. occidentalis*, Hagedorn and Carr (1985) found that the action potential duration was longer in the rostral than in the caudal face of electrocytes, accounting for the longer second phase of the EOD; hormone injections selectively increased the duration of the rostral action potential and thereby the second EOD phase.

The addition of the second phase coincided with a change from overlapping, stalk-less electrocytes (cylindrical) to spaced out electrocytes with stalks (rectangular). Individuals with monophasic EODs had only cylindrical electrocytes (Figs. 7, 8), while those with asymmetric biphasic EODs had some rectangular electrocytes in the posterior portion of the electric organ (Fig. 9B). Fish with a larger second phase seemed to have greater numbers of rectangular electrocytes. Fish with "symmetric" EODs had rectangular electrocytes throughout the length of the electric organ (Fig. 10).

However, the concurrent changes in electrocyte morphology and EOD waveform appear to be coincidental. In species whose electrocytes have stalks, the stalk is the site of innervation and initiation of the first action potential (Bennett 1971; Bass 1986). I assumed that when larvae developed stalks, the innervation shifted from the caudal face to the stalk. The pattern of development in *B. pinnicaudatus* suggested that stalks are correlated with active rostral faces and a second phase. However, species in the family Gymnotidae have no stalks but do have active rostral faces (Bennett 1971); therefore, stalks are not necessary for the second action potential. Furthermore, stalks are not sufficient for an active rostral face, because juvenile and adult *B. pinnicaudatus* produce monophasic pulses from the anterior electrocytes with stalks. Furthermore, a hypopomid species with a monophasic head-to-tail EOD has stalks (Schwartz et al. 1975). Because stalks are found only in the sister taxa Rhamphichthyidae and Hypopomidae (Fig. 1), innervated stalks may be a trait shared by common descent and functionally unrelated to EOD waveform. The possible physiological effect of changing from overlapping electrocytes to rows of electrocytes is even more obscure. Relatively large inter-electrocyte spaces along the rostrocaudal axis are present in all families with pulse-type EODs, but again both traits could be shared by common descent rather than functionally related (Fig. 1).

All gymnotiform larvae studied to date produce monophasic head-positive EODs at first, despite the variety of adult waveforms: *S. macrurus* (Hopkins 1974); *Electorphorus electricus* (Assunção and Schwassmann 1995); *Gymnotus carapo* (Crampton and Hopkins personal communication); *B. beebei* (Westby 1988); *B. brevirostris* (M. Kawasaki, personal communication); *B. occidentalis* (Hagedorn and Carr 1985); *A. leptorhynchus* (Kirschbaum 1983); *A. rostratus* (Meyer et al. 1987); *Adontosternarchus* sp. (C.H. Keller, personal communication); *E. lineata* (Kirschbaum and Westby 1975). Therefore, the developmental change from a simple to complex EOD waveform supports cladistic analyses, which show that the monophasic EODs of the sternopygids are probably a primitive character of the gymnotiforms (Alves-Gomes et al. 1995) (Fig. 1). In addition, the change in electrocyte morphology in *B. pinnicaudatus* supports the hypothesis that overlapping, stalk-less electrocytes are a primitive character.

Testing hypotheses about the adult EOD

My hypothesis that a non-degenerating "larval" electric organ produces monophasic pulses in the anterior region of adults cannot account for the differences in local adult EODs, because only one electric organ ever formed. Alternatively, the differences in local adult EODs might have been correlated with a difference in morphology between anterior and posterior electrocytes in the adult electric organ. Because the local EOD in the anterior region remains monophasic (P.K. Stoddard et al., unpublished observation), one might have predicted that electrocytes in the anterior electric organ would remain cylindrical throughout life. But by the time a fish produced an adult-like EOD, all electrocytes had stalks and were separated into rows (Fig. 10). The hypothesis suggested by this study is that the anterior electrocytes do not have voltage-gated channels in the rostral face. In the pulse-type species *Gymnotus carapo*, direct stimulation of the rostral face of anterior electrocytes produces no action potential (Lorenzo et al. 1988). Future attempts to describe the heterogeneity of the electric organ should focus on electrocyte physiology rather than anatomy.

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