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# Hormonal control of sex differences in the electric organ discharge (EOD) of mormyrid fishes

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**Summary.** Field studies have demonstrated that several species of mormyrid fish from Gabon, West Africa have a sex difference in the pulse-like waveform of their Electric Organ Discharge (EOD). Administration of androgen hormones (testosterone or dihydrotestosterone) to a female or juvenile can induce the EOD typical of a sexually mature male. Data for two such species – *Brienomyrus brachyistius* (triphasic) and *Stomatorhinus corneti* – are presented, showing that transformation of a female's or juvenile's EOD to a male-like EOD involves a 2–3 fold increase in EOD duration and a downward shift in the peak frequency of the EOD's power spectrum (as determined by Fourier analysis). For *Brienomyrus brachyistius* (triphasic), estradiol can also induce changes in the EOD waveform, although not as dramatic as that for androgens. Changes in EOD duration and power spectra are often accompanied by an alteration of the wave-shape or 'morphology' of the EOD pulse, i.e., the relative amplitude of its peaks and the presence of inflection points in its negative and positive phases.

A third species, *Hippopotamyrus batesii* (triphasic), not previously known to have an EOD sex difference, also responds to testosterone treatment with an increase in EOD duration. Preliminary field data indicate this species may have a sexual dimorphism in its EOD, suggesting that the response to a steroid hormone may be an indicator of a sex difference in a species' EOD waveform. Such findings are discussed in relation to the affects of steroids on vertebrate neurons and muscle, and the evolution of electric communication systems.

*Abbreviations:* *DHT* 5 α-dihydrotestosterone; *EOD* electric organ discharge; *F* mature female; *HTI* interval between peaks (H and T) in EOD's first derivative; *IF* juvenile female; *IM* juvenile male; *M* mature male; *PPW* peak frequency of power spectrum

## Introduction

Several species of weakly electric fish have evolved sex differences in their Electric Organ Discharge or EOD (Hopkins 1972, 1980, 1981; Moller 1980; Hopkins and Bass 1981; Westby and Kirschbaum 1981; Bass and Hopkins, 1982b, 1983; Hagedorn and Carr 1985; Meyer 1983). In some cases, the sex difference is in the repetition rate (rhythm) of the EOD, while in others it is in the EOD pulse waveform itself. The mormyrid electric fish from Africa are all 'pulse' species. Our studies concern the mormyrids from Gabon, in West Africa, where the following three species have a sex difference in the pulse waveform of their EOD: *Brienomyrus brachyistius* (long biphasic), *Brienomyrus brachyistius* (triphasic) and *Stomatorhinus corneti* (Hopkins 1980, 1981; Moller 1980; Hopkins and Bass 1981; Bass and Hopkins 1983). In all three cases, male and female EODs differ both in the overall appearance of the pulse waveform, and in the EOD duration. The male's pulse is typically 2–3 fold longer in duration than the female's (see Fig. 2).

Since the EOD of electric fish is at once a stereotyped behavior important for mechanisms of species recognition and object detection (cf. Heiligenberg 1977; Hopkins and Bass 1981), and a discrete electrophysiological event under the control of a specialized electromotor pathway (Bennett 1971; Bell et al. 1983), it presents a somewhat unique interface between behavior and physiology. As such, the development of sex differences in EODs offers a special opportunity to study the ontogeny of a sexually dimorphic vertebrate be-

havior. Within this context, we have initiated studies of the neuroendocrine mechanisms underlying the development of sex differences in the electromotor system of mormyrid fish. We have discovered for several mormyrids that gonadal androgens can induce the transformation of a female's or juvenile's EOD to a waveform pulse resembling a mature male. In earlier reports, we describe the effects of steroid hormones on the EOD of one species, *B. brachyistius* (long biphasic) (Bass and Hopkins 1982b, 1983). We found the following: (1) *Testosterone* induces a *male-like EOD* in females and juveniles. (2) *Dihydrotestosterone*, another androgenic hormone, has the same effect as testosterone, while *estradiol* has only a weak effect. This suggests the phenomenon is androgen-specific. (3) *Gonadectomized* individuals respond to testosterone as intact ones. This suggests the effect can be induced by the administration of steroid hormones, rather than stimulation of endogenous gonadal steroid activity. (4) The testosterone effect is *reversible*. The EOD of androgen-treated females or juveniles returns to the female-like waveform after treatment ceases. (5) Control specimens maintained in *captivity* or treated with *cholesterol*, a non-specific steroid, show no changes in the EOD waveform (see also Bass and Hopkins 1984).

We also found in another species, *B. brachyistius* (biphasic), in which males and females have similar EODs, that testosterone has no significant effect upon the EOD pulse. This finding suggested to us that only species with known EOD sex differences would be sensitive to the influences of gonadal steroids. To test this hypothesis, we posed a series of questions: How widespread is the phenomenon of steroid effects on an EOD waveform? Do all species with an EOD sex difference respond as *B. brachyistius* (long biphasic), where testosterone induces a 2–3 fold elongation of the EOD pulse? Can estradiol have an effect? Is the response always reversible? And finally, are other species without a known EOD sex difference, unresponsive to steroids as is *B. brachyistius* (biphasic)? By conducting a comparative study, we hoped to understand the range of variation of neuroendocrine control mechanisms underlying the evolution and development of EOD sex differences. In this paper, we describe the effects of steroid hormones on the EOD of two additional species – *B. brachyistius* (triphasic) and *S. corneti* – with a known EOD sex 'dimorphism', plus a third species – *Hippopotamyrus batesii* (triphasic) – for which sex differences in the EOD have not been previously described. Portions of the data for *B. brachyistius* (triphasic) appeared earlier in reports that describe changes in the frequency tuning of electroreceptors in androgen-treated mormyrids (Bass 1983; Bass and Hopkins 1984).

## Methods

*Animals.* All mormyrids were tentatively identified in a previous field season and representative specimens are in the Musée Royal de l'Afrique Centrale in Tervuren, Belgium (Hopkins 1980). Specimens of *B. brachyistius* (triphasic) (29–90 mm, total length), *S. corneti* (32–55 mm), and *H. batesii* (triphasic) (73–102 mm) were caught in small streams flowing into the Ivindo River in the region around Makokou, Gabon (0° 30′ N, 12° 50′ E) during the October-November, 1981 rainy season. Individuals were maintained separately in bowls of 1.2 l of aerated, stream water (conductivity, 20–60 kOhm cm; temperature, 22–23 °C). At the end of each experiment, specimens were sacrificed with an overdose of tricaine methanesulfonate (MS222) and identified as immature (juvenile) male (IM) or female (IF), or mature male (M) or female (F). Some immature (juvenile) specimens (I) could not be identified as to their sex. In mormyrids, only one gonad is developed (see Okedi 1969), being found on the left side. Immature females had a small ovary with translucent, glass-bead-like eggs that were lying dorsal and rostral within the peritoneal cavity. Mature females had an ovary filling the peritoneal cavity with yellow-pigmented eggs. Immature males had a small testis which, unlike mature males, did not extrude any milt when cut open with a scalpel blade and pressed. In Gabon, we never saw male testes expanded to fill the body cavity as seen in specimens from other areas in Africa (e.g. Senegal; Hopkins, unpublished observations). Mature males can be recognized unambiguously from external morphological features by an indentation along the dorsal margin of the anal fin which is absent in females (Figs. 2a, 10a; see also Iles 1960; Nawar 1960; Lucker and Kramer 1981).

*Surgery.* Some specimens were gonadectomized. Fish were first anaesthetized with MS222 and then gently restrained in a plexiglass mount inside a plastic dish filled with stream water. Following an incision in the ventrolateral body wall, the left gonad was carefully excised with forceps and scissors and the area then flushed several times with 0.6% NaCl. In some cases, a pellet of either 17$\beta$-estradiol or 5$\alpha$-dihydrotestosterone was implanted in the gut (see below). In cases where an ovary was not removed, the pellet was placed between it and the body wall. The wound was sutured shut with ethicon thread and the fish revived by passing aerated water over the gills via a glass mouth tube. Each fish was then returned to its own bowl containing a 1:1 mixture of stream water and 0.6% NaCl which seemed to accelerate wound healing. Each individual was gradually returned to 100% stream water after a period of 4–5 days.

### *Hormone treatments*

The following hormone treatments were followed:
*Testosterone:* For 7 individuals (3 I, 1 IF, 1 IM, 2 F) of *B. brachyistius* (triphasic), 2.0–4.0 mg of 17$\alpha$-methyl-testosterone (Sigma Chemical Co.) were directly added to the water at intervals of 24 or 48 h. Two additional specimens (1I, 1 IF) were treated every 24 h. One immature female was treated at 24 or 48 h intervals with testosterone propionate (Sigma Chemical Co.). Testosterone treatment would cease by returning a specimen to 'non-testosterone' stream water.

Seven specimens of *S. corneti* were similarly treated with

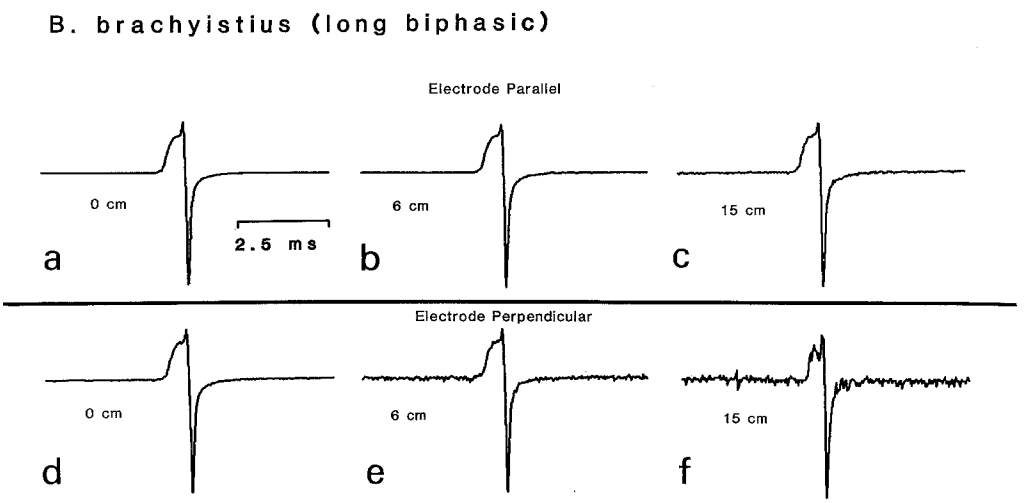


**Fig. 1.** Recordings of EOD waveforms of a female *B. brachyistius* (long biphasic). As electrode orientation varies from parallel (top) to perpendicular (bottom) (relative to anterior-posterior body axis), the basic waveform shape remains unchanged. The amplitude of the signal varies depending on the electrode's distance (0, 6, and 15 cm) from the female. All EODs have been scaled to the same peak-to-peak amplitude to show the similarity in waveform shape

17$\alpha$-methyl-testosterone (3 I, 1 F) or testosterone propionate (2 I, 1 F). We also treated one immature *H. batseii* (triphasic) with 17$\alpha$-methyl-testosterone and a second with testosterone propionate.

*Dihydrotestosterone (DHT) and Estradiol:* Since testosterone can be metabolized to 17$\beta$-estradiol or another androgen, 5$\alpha$-dihydrotestosterone (DHT) (see McEwen 1980), we had to test for androgen specificity of a possible testosterone effect. For *B. brachyistius* (triphasic), one of several DHT (Sigma Chemical Co.) treatments was followed to test for specificity: (1) three specimens (1 I, 1 F, 1 IM), two of which were gonadectomized (1 IM, 1 F), received a DHT pellet (2.0–3.0 mg) implant; (b) one immature specimen received a single 0.02 $cm^3$ DHT injection (concentration of 400 mg/$cm^3$ of peanut oil); (c) two specimens (1 I, 1 F) had 2.0–4.0 mg of DHT added to the water at intervals of 24 or 48 h.

Several specimens of *B. brachyistius* (triphasic) were treated with 17-$\beta$ estradiol (Sigma Chemical Co.). These included: (1) three specimens (1 IM, 1 IF, 1 F) with estradiol pellet (2.0–3.0 mg) implants; (b) one immature specimen with a single 0.02 $cm^3$ estradiol injection (concentration of 400 mg/$cm^3$ of peanut oil); and (c) one mature female with an estradiol injection followed eight days later by an estradiol pellet implant.

*EOD recordings:* All EODs were recorded daily in fresh stream water with differential Ag/AgCl electrodes aligned parallel to a PVC tube holding the fish in an orientation parallel to the electrodes. As shown for *B. brachyistius* (long biphasic) in Fig. 1, the waveform shape of the EOD does not vary with electrode position; only the amplitude of the signal changes. Following amplification, the EOD was photographed, recorded on tape (Nagra IV-SJ, at 38.1 cm/s), or hand traced into a notebook. After returning from Gabon, representative tape-recorded EODs were digitized at a sampling rate of 40 kHz using the 10-bit lab 8e A/D converter with a PDP 8e computer. EOD records were then scaled to the same peak to peak amplitude before digital storage. All EODs are represented with head positive upward.

*EOD quantification:* Although the electric waveform of mormyrids appear relatively simple, we found it difficult to adopt a single standard measure that would measure quantitative properties of the EODs of all species. We were interested in the EOD duration because it appeared to be the most dramatic variable affected by testosterone. The EOD waveform begins and ends with a voltage which approaches a baseline asymptotically. We measure the duration of the EOD by determining the time between the first digitized sample and the last to differ from the baseline voltage by more than some threshold amplitude. Threshold was set to 5% of the peak-to peak-amplitude. This method works well if the EOD waveform returns to the baseline quickly, as in *H. batesii* (triphasic) (Fig. 12) and *B. brachyistius* (long biphasic) (Bass and Hopkins 1983, and Fig. 1). But in species where the waveform tends to have a slow return to the baseline, as in *B. brachyistius* (triphasic) (Fig. 2), the method gives variable measures, even for the same individual. Changing the threshold to 10% still produces variable results. We therefore decided to measure the time between extremes in the slope of the waveform by searching for peaks in the calculated first derivative of the waveform (5-point smoothing). We used the computer algorithm to determine the position of local peaks on the derivative waveform and measured intervals between the first major positive peak (called the 'H' peak) and the major negative peak (called the 'T' peak). We call this the H-T interval or HTI. For example, in Fig. 3 is shown the EOD of a female before (Fig. 3a), and 16 days after (Fig. 3b) testosterone treatment. To the right of each EOD is its first derivative (Fig. 3c, d). Following testosterone treatment, the HTI of the EOD increases from 0.39 ms to 0.96 ms.

For all duration measures, our sample accuracy was 24.8 $\mu$s per point. All EOD durations measured in these ways were either recorded at 22°C or were adjusted to this temperature (recording temperatures ranged from 22–25°C) by scaling the time measurements using a $Q_{10}$ factor of 1.75 (comparable to other electric fish, see Hopkins 1976), as determined for two specimens of *B. brachyistius* (triphasic). The formula for scaling duration ($D$) is: $D\ (22°) = D(T) \times 1.7^{(T-22)/10}$, where $T$ (°C) is the temperature at which the EOD is recorded.

Finally, for species like *S. corneti* (Fig. 10) with brief duration EODs (250 $\mu$s), our digitizing rate was too slow to resolve the subtle changes in duration which we observed after hormone treatment. For these species, as others, we determined the peak frequency of the power spectrum (PPW), as calculated

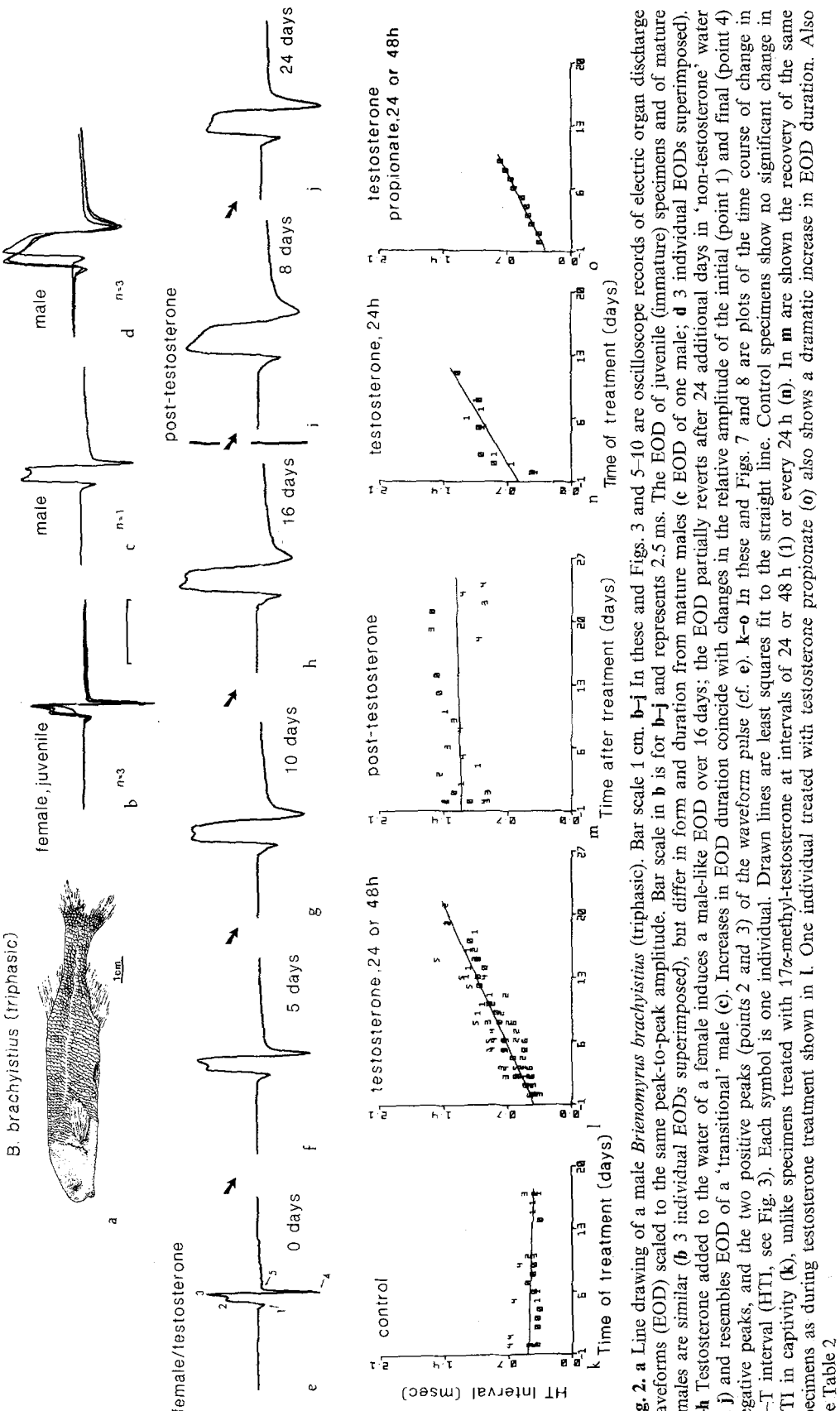


**Fig. 2. a** Line drawing of a male *Brienomyrus brachyistius* (triphasic). Bar scale 1 cm. **b–j** In these and Figs. 3 and 5–10 are oscilloscope records of electric organ discharge waveforms (EOD) scaled to the same peak-to-peak amplitude. Bar scale in **b** is for **b–j** and represents 2.5 ms. The EOD of juvenile (immature) specimens and of mature females are similar (**b** 3 individual EODs superimposed), but differ in form and duration from mature males (**c** EOD of one male; **d** 3 individual EODs superimposed). **e–h** Testosterone added to the water of a female induces a male-like EOD over 16 days; the EOD partially reverts after 24 additional days in 'non-testosterone' water (**i, j**) and resembles EOD of a 'transitional' male (**e**). Increases in EOD duration coincide with changes in the relative amplitude of the initial (point 1) and final (point 4) negative peaks, and the two positive peaks (points 2 and 3) of the waveform pulse (cf. **e**). **k–o** In these and Figs. 7 and 8 are plots of the time course of change in H–T interval (HTI, see Fig. 3). Each symbol is one individual. Drawn lines are least squares fit to the straight line. Control specimens show no significant change in HTI in captivity (**k**), unlike specimens treated with 17α-methyl-testosterone at intervals of 24 or 48 h (1) or every 24 h (**n**). In **m** are shown the recovery of the same specimens as during testosterone treatment shown in **l**. One individual treated with testosterone *propionate* (**o**) also shows a dramatic increase in EOD duration. Also see Table 2

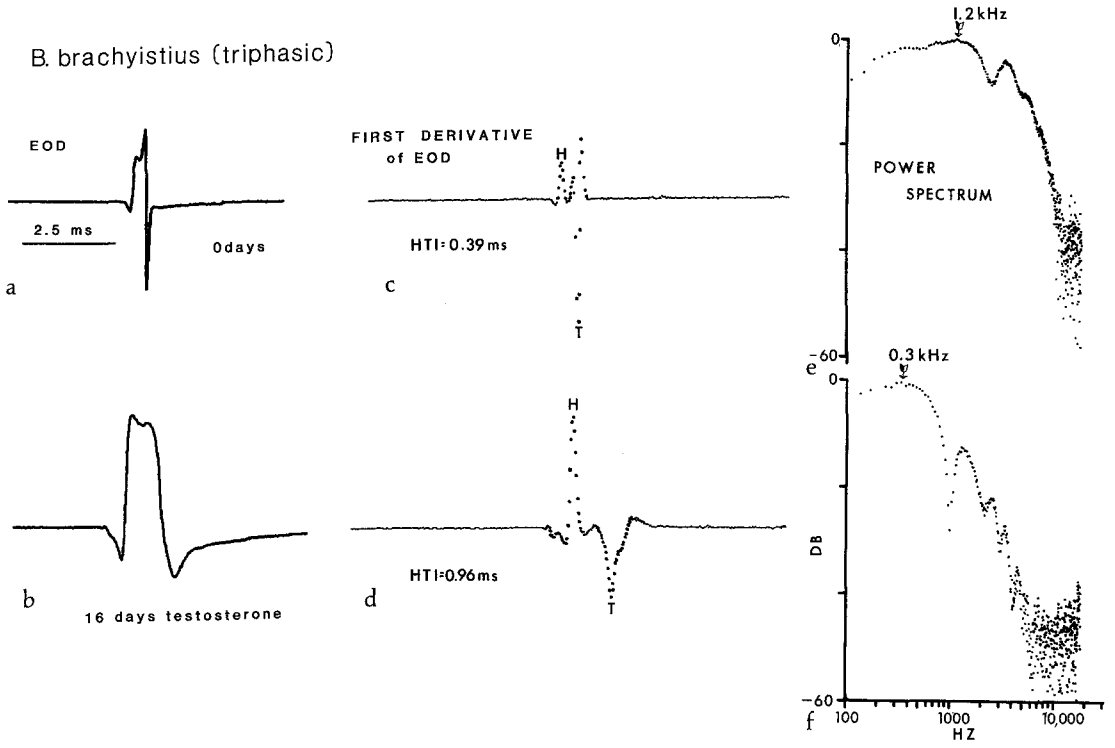


**Fig. 3. a, b** EOD of female *B. brachyistius* (triphasic) before (**a**) and after (**b**) 16 days of testosterone treatment. **c, d** Corresponding digitized plots of EOD's first derivative showing peaks (H and T) in the major negative-positive transitions in the derivative. The time interval between H and T was used as a measure of EOD duration. **e, f** Plots of corresponding EOD power spectra. Frequency is plotted on the x-axis and amplitude (dBs of attenuation) on the y-axis. As EOD duration increases (**a** to **b**), the H–T interval increases (**c** to **d**), and the peak frequency (arrow) in the power spectrum decreases (from 1.2 kHz in **e** to 0.3 kHz in **f**)

from the Fast-Fourier transform. Power spectra are plotted as log frequency versus log amplitude. In general, changes in the PPW are approximately inversely proportional to EOD duration changes (i.e. as EOD duration increases, the PPW decreases and vice versa, cf. Fig. 3).

*Statistics:* When plotting EOD duration over several days treatment, we fit the data to a linear function using the least squares method. The slope of each regression line is then calculated and a two-tailed *t*-test (Sokol and Rohlf 1969) is used to test if the slope is significantly different from zero. Statistical values are presented in Table 2.

## Results

The results are framed within the series of questions we posed during our field studies.

*Question 1: Can testosterone induce a male-like EOD among females and juveniles of species with a natural sex difference in the EOD?*

A. *B. brachyistius* (triphasic) (Fig. 2a): This species has a sex difference in both the duration and appearance of the EOD waveform (Fig. 2b–d). The average HTI (see Table 1 for summary) for imma-

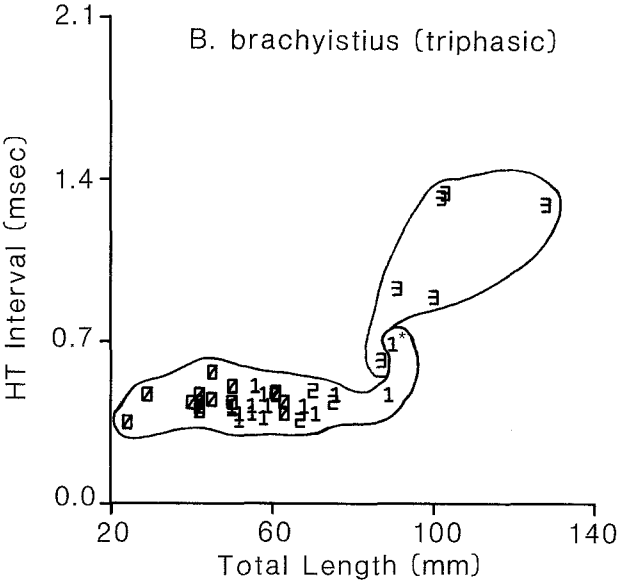


**Fig. 4.** Plot of H-T interval versus total body length for 1981 population of *B. brachyistius* (triphasic). Among males (symbol 3), unlike juveniles with no identifiable sex (0), females (1), and immature males (2), there is a trend toward increasing H-T interval (i.e. EOD duration) with increasing size

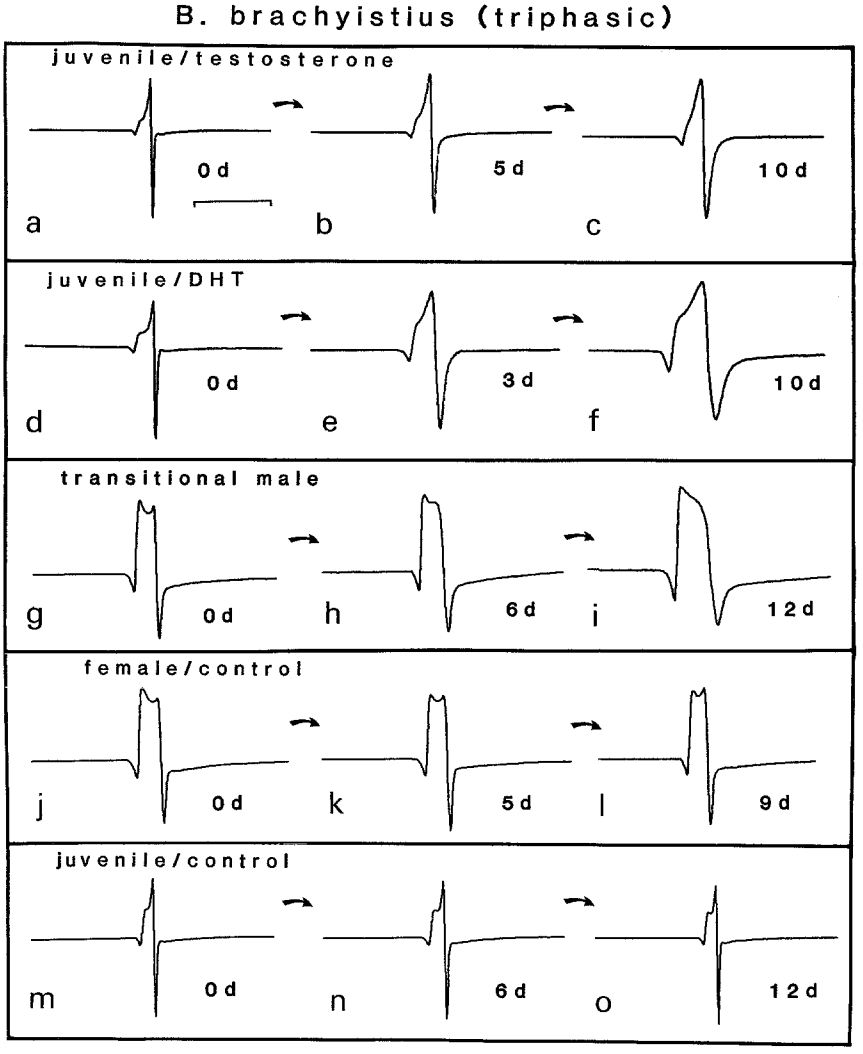


**Fig. 5a–o.** EODs of several individuals of *B. brachyistius* (triphasic). Bar scale in **a** is for **a–o** and represents 2.5 ms. Juveniles treated with 17α-methyl-testosterone (**a–c**) or 5α-dihydrotestosterone (DHT) (**d–f**) show a change in EOD duration over 10 days (10 d), but not waveform appearance as seen for larger females or juvenile males (Figs. 2e–h, 6a–l, 7a–f). The EOD of a captive male with a 'transitional' waveform (**g**) becomes more male-like (**h, i**) in captivity. In contrast to all the above, the EOD of captive females (**j–l**) or juveniles (**m–o**) remains unchanged when in captivity for comparable times

ture specimens (sex unidentified) is 0.44 ms, which is close to that of immature and mature females whose average HTI are respectively 0.42 ms and 0.44 ms.

For immature males the HTI is 0.43 ms, which is similar to that of females and immature specimens. The average HTI of mature males is 1.07 ms, which is more than two-fold greater than all other specimens. Sex differences in HTI correlate with those in the average peak frequency in the power spectrum (PPW, see Table 1 for summary). The PPW is 1.3 kHz for immature, unidentified specimens; 1.4 kHz and 1.2 kHz for respectively immature and mature females; and 1.2 kHz for immature males. The PPW of mature males, as the HTI value, differs dramatically from other individuals and is 0.3 kHz.

For wild-caught, mature males, the HTI value increases with size, ranging from 0.62 to 1.24 ms (Fig. 4). One mature female (not included in above data) has an HTI of 0.69 ms (asterisk, Fig. 4; also see Fig. 5j–l), which is within the low range for mature males (see Fig. 4). This specimen is also the largest female (90 mm) that we caught during the 1981 field season and suggests that females of this size class may show an increase in the HTI, but additional data are needed.

### *Hormone effects on EOD duration*

Six specimens treated with 17α-methyl-testosterone (Fig. 21) at 24 or 48 h intervals show a highly significant increase in the HTI over the treatment period (see Table 1 for all final HTI values and Table 2 for all statistics). The average HTI increases by 202% (0.42 to 1.3 ms). The smallest (29 mm), immature specimen treated with methyl-testosterone shows a less dramatic 35% increase in HTI (0.47 to 0.65 ms) over an 8 day observation period (point 6 in Fig. 21 and Fig. 5a–c). Additional data

**Table 1.** Average EOD duration (H–T Intervals) and peak frequency in the Fourier-derived power spectrum for *B. brachyistius* (triphasic): All values are those at end of observation period. Standard deviations (SD) are also indicated

| | H–T interval (ms) | Peak frequency (kHz) |
|---|---|---|
| Natural sex differences | | |
| Immature, unidentified sex ($n=15$ specimens) | 0.44 (SD=0.4) | 1.3 (SD=0.2) |
| Immature females ($n=9$) | 0.42 (SD=0.06) | 1.4 (SD=0.1) |
| Mature females ($n=6$) | 0.44 (SD=0.08) | 1.2 (SD=0.1) |
| Immature males ($n=3$) | 0.43 (SD=0.07) | 1.2 (SD=0.3) |
| Mature males ($n=6$) | 1.07 (SD=0.3) | 0.3 (SD=0.08) |
| Hormone-treatment groups | | |
| Controls ($n=4$) | 0.43 (SD=0.07) | 1.4 (SD=0.2) |
| 17α-methyl-testosterone (24–48 h) ($n=6$) | 1.30 (SD=0.2) | 0.3 (SD=0.06) |
| 17α-methyl-testosterone (24 h) ($n=2$) | 1.30 (SD=0.2) | 0.4 (SD=0.01) |
| POST-methyl-testosterone (24–48 h) ($n=5$) | 1.30 (SD=0.3) | 0.2 (SD=0.09) |
| Testosterone propionate ($n=1$) | 0.78 | 0.3 |
| 5α-dihydrotestosterone (DHT) ($n=6$) | 1.05 (SD=0.19) | 0.4 (SD=0.1) |
| 17β-estradiol ($n=5$) | 0.78 (SD=0.09) | 0.6 (SD=0.1) |

**Table 2.** Rate of change in EOD duration (or H–T Interval) and power spectra for hormone-treatment groups. Slopes are calculated from a least squares fit to the straight line. The *t*-test (two-tailed) is used to test if the slope is significantly different from zero. T-values (*t*) and the number of degrees of freedom (*df*) are shown. *P* values report the probability that the slope = 0

| Treatment | Linear slope | *t* | *df* | *P* |
|---|---|---|---|---|
| *Brienomyrus brachyistius* (triphasic): Changes in EOD duration (H–T interval) | | | | |
| Control (captive, untreated) | −0.001 ms/day | 1.07 | 23 | >0.2 |
| Testosterone/24–48 h in water | 0.05 | 16.0 | 70 | <0.001 |
| Post-testosterone | −0.01 | 1.52 | 17 | 0.1<0.2 |
| Testosterone/24h in water | 0.08 | 5.15 | 11 | <0.001 |
| Testosterone propionate | 0.05 | 19.7 | 8 | <0.001 |
| Dihydrotestosterone (DHT) pellet | 0.03 | 6.89 | 18 | <0.001 |
| DHT injection | 0.02 | 4.19 | 8 | <0.01 |
| DHT in water | 0.04 | 6.37 | 12 | <0.001 |
| Estradiol pellet | 0.03 | 4.90 | 9 | <0.001 |
| Estradiol injection/pellet | 0.02 | 4.59 | 3 | <0.02 |
| Male/castrated | −0.009 | 1.47 | 5 | 0.1<0.2 |
| Transitional male in captivity | 0.05 | 4.11 | 6 | <0.01 |
| *Stomatorhinus corneti:* Changes in peak frequency of the power spectrum | | | | |
| Testosterone in water | −826 Hz/day | 5.7 | 14 | <0.001 |
| Testosterone propionate in water | −876 | 7.97 | 10 | <0.001 |
| Male in captivity | 16 | 0.24 | 6 | >0.5 |
| *Hippopotamyrus batesii* (triphasic): Changes in EOD duration | | | | |
| Testosterone or testosterone propionate | 0.15 ms/day | 5.85 | 9 | <0.001 |

on the influence of size and age on the effectiveness of steroid treatments is given in a section below. For the five specimens from this group that are tracked after testosterone treatment ceases, there is sometimes a further increase in HTI (Fig. 2i, m) that is followed by a slight return to a transitional male-type waveform (Fig. 2j). However, there is no significant change in the HTI over the entire post-treatment observation period which ranges from 3–25 days (Table 2); the final average HTI value at the end of the post-observation treatment period is still 1.3 ms.

The two specimens treated every 24 h with methyl-testosterone also show a 201% increase in the average HTI (0.43 to 1.3 ms). The EOD duration changes at a faster rate for these individuals (Fig. 2n, 0.08 ms/day) than those treated at varying intervals of 24 or 48 h (0.05 ms/day, Table 2). For the one female treated with testosterone propionate every 24 or 48 h (Fig. 2o), the HTI increases by 117% (0.36 to 0.78 ms).

The HTI value decreases slightly (0.43 to 0.42 ms) for captive females and juveniles (2 I, 1 IF, 1 F) maintained in stream water for similar time periods as the testosterone-treatment groups (Figs. 2k; 5 m–o; Tables 1, 2).

### *Hormone effects on EOD power spectrum*

Power spectra of mormyrids are broad-band and often have multiple peaks or humps. As the duration and HTI of the EOD of *B. brachyistius* (triphasic) increase (Fig. 3a–d), the peak in the power spectrum (PPW) shifts downward (arrow, Fig. 3e, f). The average PPW decreases by over 60% (1.3 to 0.4 kHz) for specimens treated with 17α-methyl-testosterone (excluding the 29 mm juvenile). The female treated with testosterone propionate shows a similar decrease. The PPW of captive specimens does not change significantly (1.5 to 1.4 kHz; see Table 1 for summary).

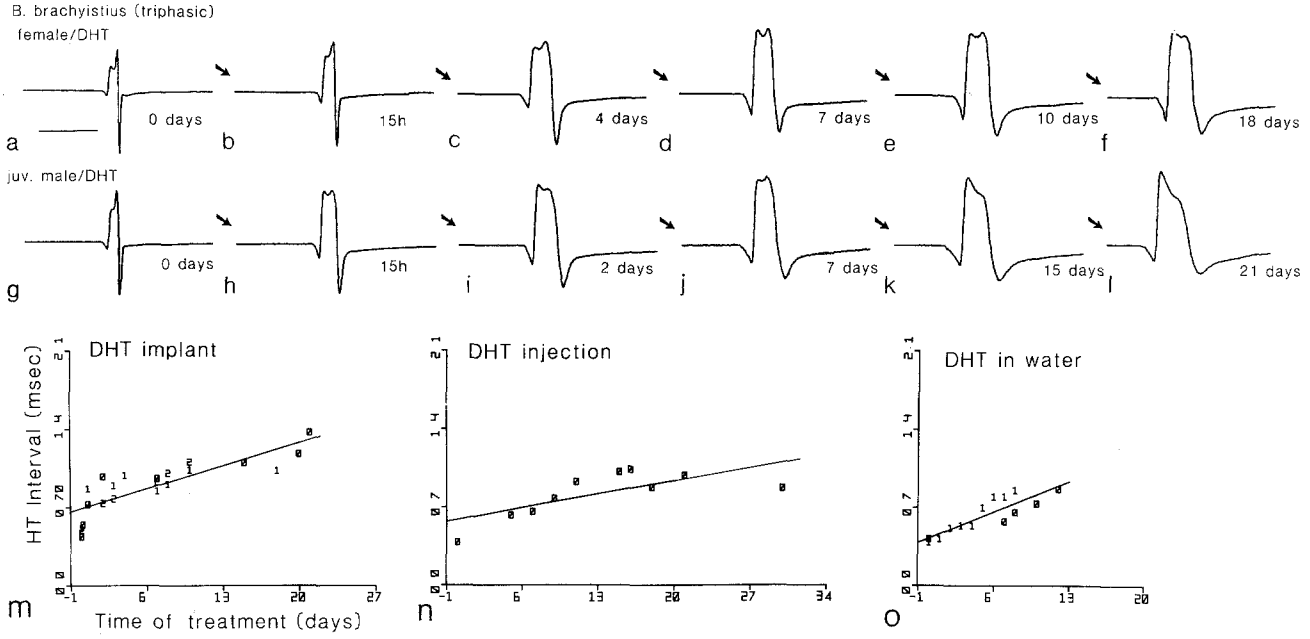


**Fig. 6. a–l** Changes in EOD waveform for female (**a–f**) and juvenile male (**g–l**) *B. brachyistius* (triphasic) with pellet implants of DHT. Bar scale in **a** is for **a–l** and represents 2.5 ms. As for methyl-testosterone (Fig. 2), DHT induces significant changes in EOD waveform shape and duration, with EODs becoming more male-like during the treatment period. Similarly, there are significant changes in the H–T interval for specimens after DHT pellet implants (**m**), DHT injections (**n**) or DHT added to the water at intervals of 24 or 48 h (**o**). See Table 2

*Hormone effects on EOD waveform-shape*

The EOD waveform of *B. brachyistius* (triphasic) has three phases as shown in Fig. 2e. It begins with a small head negativity that has a smooth peak (point 1). This initial negativity is then followed by a major head-positive phase. The positive phase is distinct in having an inflection point or plateau (beginning at point 2) that precedes its major peak (point 3). A final head-negative phase also has a smooth peak (point 4). Aside from changes in EOD duration and power spectrum, testosterone appears to induce a shift in the relative amplitudes of points 2 and 3 of the positive phase: point 2 gradually increases in relative amplitude (Fig. 2f, g) as the entire EOD resembles that of a 'transitional male' (Fig. 2c). Eventually, point 2 becomes the major peak (Fig. 2h, i) and the waveform is similar to the longer duration male EOD (Fig. 2d). The negative phase also changes: in females and juveniles it returns abruptly to the baseline (Fig. 2b, e), while in testosterone-treated individuals, as natural males, it returns more gradually (Fig. 2f–j).

B: *Stomatorhinus corneti* (Fig. 10a): The EOD of *S. corneti* has four phases (Fig. 10b). The waveform of females and juveniles varies with size. Among specimens 32–40 mm in length (Fig. 10b), the EOD has an initial negativity with a smooth peak (point 1), followed by a positive phase with a smooth peak (point 2, Fig. 10b). A second major negative phase (point 3, Fig. 10b) is followed by a final positive phase whose peak amplitude (point 4, Fig. 10b) is greater than the first (point 2). For individuals over 40 mm there is often no initial negativity, the first positive peak is dominant (point 2, Fig. 10c), and there is a final negative phase (point 5, Fig. 10c). The EOD of males resembles that of the smaller female size class excepting that among males the first positive peak (point 2, Fig. 10d) exceeds the second (point 4, Fig. 10d) in amplitude, and the entire EOD is of longer duration. Moller (1980) reports a second type of EOD for juvenile males which closely resembles a transitional stage of hormone-treated specimens (Fig. 10f): the relative amplitudes of the initial negativity and final positivity are not as great as in mature males. The average PPW of males is 3.5 kHz ($n=4$), ranging from 3.3 to 3.6 kHz. For females, the average PPW is 9.0 kHz for the small size class ($n=4$; standard deviation = 2.3 kHz) and 9.8 kHz for the larger size class ($n=5$; s.d. = 2.1 kHz). All females considered, the PPW has a broad range from 6.2 to 11.3 kHz.

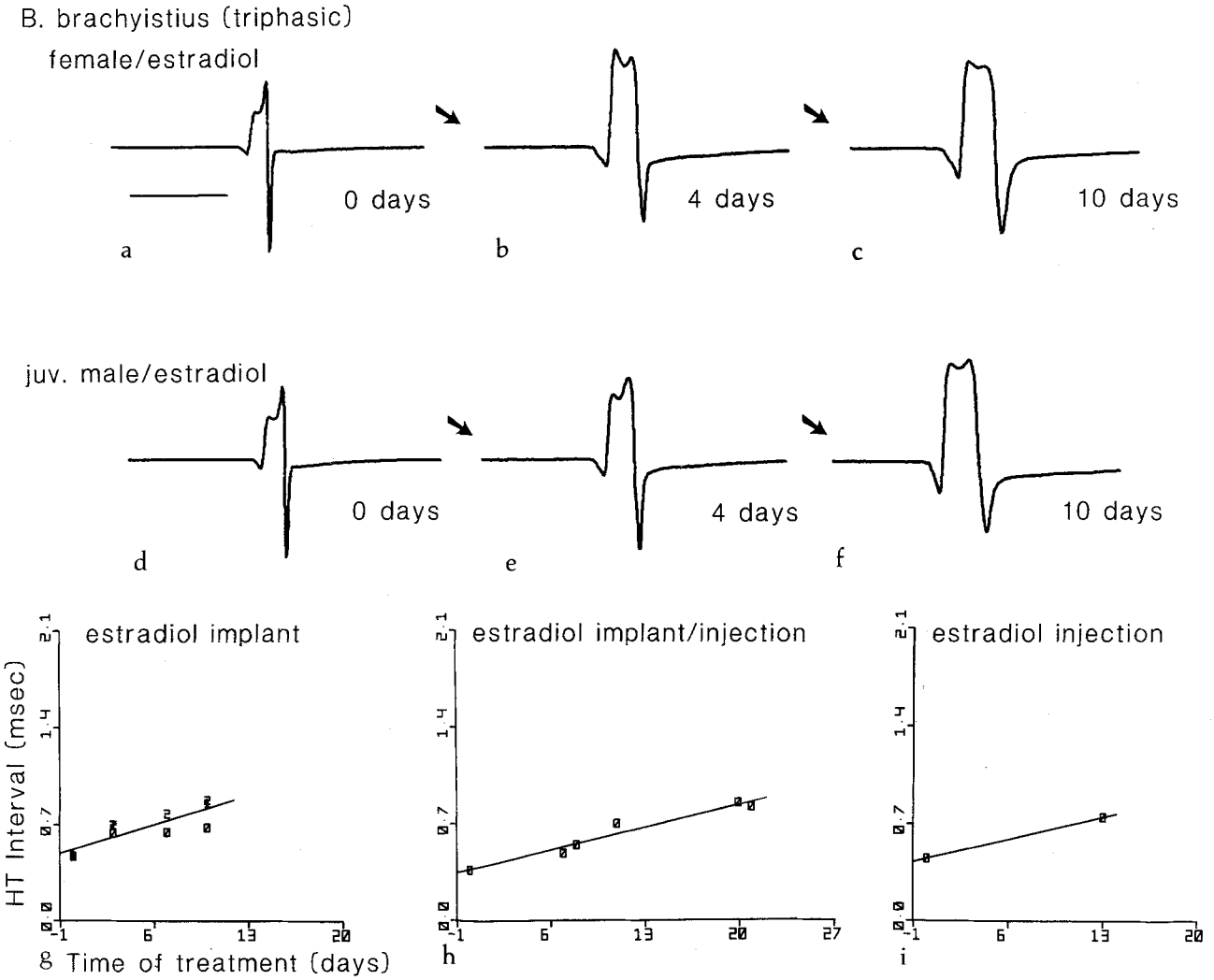


**Fig. 7. a–f** Changes in EOD waveform for female (**a–c**) and juvenile male (**d–f**) treated with 17$\beta$-estradiol pellet implants. Bar scale in **a** is for **a–f** and represents 2.5 ms. As with testosterone and DHT, estradiol induces a significant change in the H–T interval for specimens with pellet implants (**g**), implant combined with an injection (**h**), or an injection alone (**i**). See Table 2

*Hormone effects on EOD*

For *S. corneti* treated with 17$\alpha$-methyl-tetosterone (3 I, 1 F) or testosterone propionate (2 I, 1 F), there is a significant shift in PPW (Fig. 11a, c, e f; Table 2). The final average PPW is 1.6 kHz for the testosterone group (excluding point 3 of Fig. 11a, which was followed for only 2 days) and 3.3 kHz for the testosterone propionate group. For two control females held captive for 6–14 days, the average PPW decreases from 10.6 to 8.2 kHz. The final values of 9.8 and 6.7 kHz are within the female or juvenile range of variation and still 2–4 fold greater than androgen-treated specimens. The EOD did not revert to a female-form during a post-treatment observation period ranging from 6–9 days (Fig. 11b, d). The shift from a female to a male-type EOD is characterized by an increased amplitude of the first positive peak relative to the second (Fig. 10e–g).

*Question 2: Is the response androgen specific?*

This question was addressed only for *B. brachyistius* (triphasic) (for *B. brachyistius* (long biphasic), see Bass and Hopkins 1983)). Both 5$\alpha$-dihydrotestosterone (DHT) and 17-$\beta$-estradiol have a significant effect, comparable to that of testosterone, on the HTI (Tables 1, 2; Figs. 6, 7). The rate of change of HTI for DHT-treated animals depends on the treatment method: 0.02 ms/day for the immature specimen with a single injection; 0.03 ms/day for the three specimens with pellet implants; and 0.04 ms/day for the two individuals

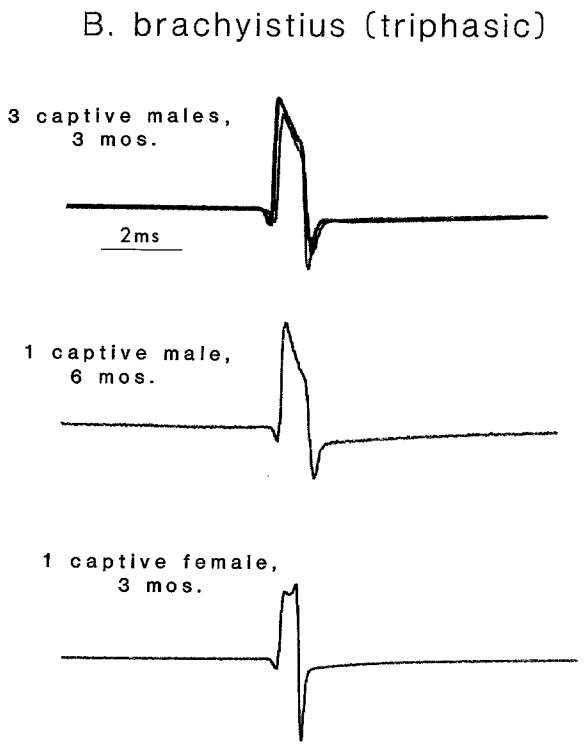


**Fig. 8.** EODs of male and female *B. brachyistius* (triphasic) in captivity for 3 to 6 months

where DHT was directly added to the water (see Table 2). These values compare to those for estradiol-treated individuals: 0.02 ms/day for the specimen with an injection followed by an implant; 0.03 ms/day for those with implants alone. (The slope of the regression line for the specimen with a single estradiol injection is similar to other estradiol-treated individuals, but EODs were recorded only on the first and final days of treatment.) The EOD changes at a faster rate (0.05–0.08 ms/day; Table 2) for individuals treated with testosterone propionate for 17α-methyl-testosterone.

For both the DHT and estradiol-treatment groups, increases in EOD duration are accompanied by a decrease in the average PPW (Table 1) and changes in waveform shape (Figs. 6a–l, 7a–f). For specimens treated with DHT (pooled data for all groups), the average HTI increase is 139% (0.44 to 1.05 ms), while the average PPW decrease is 71% (1.4 to 0.4 kHz). For estradiol-treated specimens (pooled data) the average HTI increase is only 73% (0.45 to 0.78 ms), while the average PPW decreases by 50% (1.2 to 0.6 kHz).

In general, DHT appears to have a greater effect on the EOD than does estradiol. This point is brought out if we compare the largest treatment group in each case, i.e. specimens with a steroid implant. For the three specimens with a DHT implant, the average HTI increase is 129% (0.48 to 1.08 ms) after 10 days of treatment. For the three individuals with an estradiol implant, the average HTI value increase is only 72% (0.47 to 0.81 ms) after 10 days. For all specimens (excepting 29 mm juvenile) treated with methyl-testosterone (which was added to the water directly), the average HTI increases by 201% (0.43 to 1.3 ms) after 10 days. The average HTI increase for DHT and methyl-testosterone treated specimens is, respectively, 2- and 3-fold that of the estradiol-treated individuals. Thus, while both androgens and estrogens have a significant effect upon the EOD of *B. brachyistius* (triphasic), androgens appear to be more effective. The results also show that addition of methyl-testosterone directly to the water has the most dramatic effect of all treatments upon an individual's EOD.

*Question 3: Are there permanent changes in the EOD waveform that may be dependent on gonadal steroids?*

For *B. brachyistius* (triphasic), three observations suggest there are permanent changes in the EOD waveform of sexually mature males (and perhaps females) that are under the control of gonadal ster-

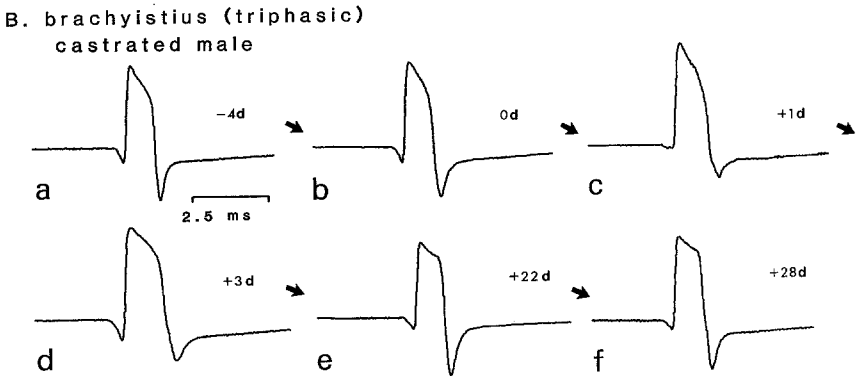


**Fig. 9.** EOD of mature male *B. brachyistius* (triphasic) before (**a, b**) and after (**c–f**) castration. The EOD, as that of captive males (Fig. 8), remains male-like

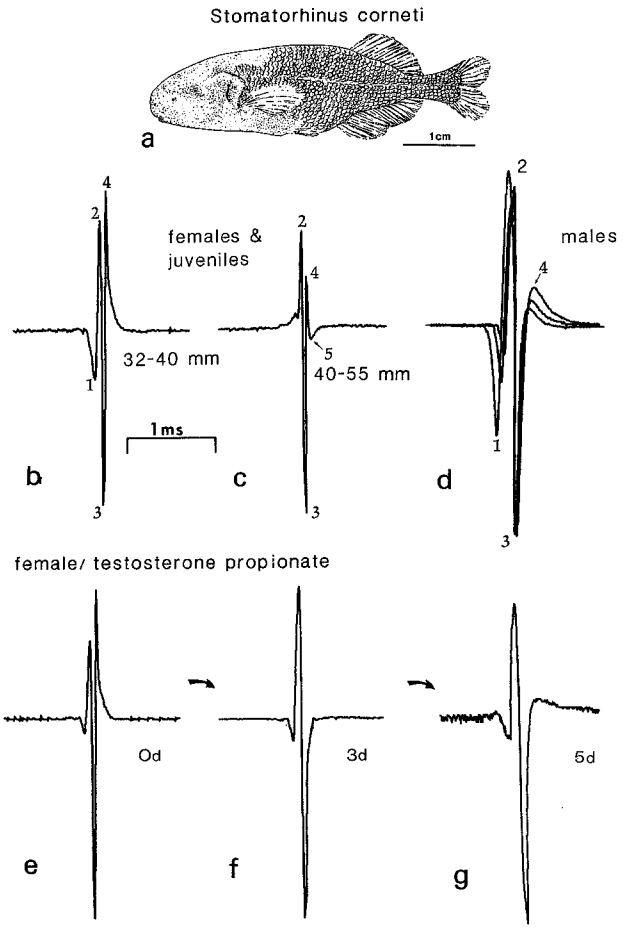


**Fig. 10.** **a** Line drawing of a male *Stomatorhinus corneti*. **b–g** Oscilloscope records of EOD waveforms. Juveniles and females show two EOD forms depending on total body length (**b, c, e**). The EOD of mature males ($n=3$ individual EODs superimposed; **d**) typically has a reduced second positive peak (point 4) and is longer in duration. **e–g** Testosterone propionate added to the water of a female induces a mature male-like EOD over 5 day period

oids. First, the induced male-like EOD of androgen-treated females does *not* revert completely to the female form, even 25 days after hormone treatments end (Fig. 2j, m). There may be some additional reversion with longer post-treatment observation periods. In contrast, in *B. brachyistius* (long biphasic) (Bass and Hopkins 1983), the male-like EOD of hormone-treated females reverts to the female-like waveform within 24 days.

Second, the EODs of mature males maintained in captivity for periods of 3 to 6 months (Fig. 8), do not revert to the female type waveform. Just the opposite occurred in one case where the EOD of a male with a 'transitional' waveform (Fig. 5g–i) became more 'male-like' after 12 days in captivity, the HTI increasing from an initial value of 0.62 ms to a final value of 1.17 ms. The EOD of the one female (Fig. 5j–l) having the longest duration EOD of any female, did not become more male-like after 9 days in captivity, but rather underwent a decrease in HTI from 0.69 to 0.58 ms. For comparison, the EOD of a captive, immature specimen remains constant (Fig. 5m–o). Its HTI decreases (as other controls, see above) from 0.39 to 0.36 ms over 12 days.

Finally, the long duration EOD of one wild-caught male remains male-like during 28 days after castration (Fig. 9). At first, its EOD increases in duration (Fig. 9c), but then later returns to its pre-castration form (Fig. 9d–f; initial and final HTI values are 0.93 and 0.89 ms, respectively). These results suggest the EOD of mature males is permanent, although its appearance may be somewhat modifiable depending on an individual's overall physiological state.

As mentioned earlier, the degree to which hormones affect the EOD duration may be a function of fish size. Figure 5 shows two juvenile specimens where the HTI increases by only 38% (0.47 to 0.65 ms) in the case of the 29 mm juvenile treated with methyl-testosterone (Fig. 5a–c), compared to 131% (0.47 to 1.11 ms) for a 45 mm juvenile treated with DHT (Fig. 5d–f). Changes in waveform shape may also depend on size. For both juveniles, there is not a dramatic change in the appearance of the positive phase of the EOD. Unlike larger individuals treated for similar time periods (e.g. Fig. 2e–j, 71 mm female; 6a–f, 78 mm female; 7a–c, 58 mm female), the appearance of the plateau or inflection point remains nearly the same and the relative amplitude of the first peak does not approach that of the second (see earlier Section).

For one male *S. corneti* with a long duration EOD, we also observed no significant change in the EOD during a 15 day observation period (see Table 2).

*Question 4: Is the hormonal effect specific to species with known sex differences in the EOD?*

So far, we have shown that testosterone increases the EOD duration of three species – *B. brachyistius* (long biphasic), *B. brachyistius* (triphasic) and *S. corneti*; but has no effect on a fourth – *B. brachyistius* (biphasic) (see also Bass and Hopkins 1983). Specimens of *Hippopotamyrus batesii* (triphasic) (Fig. 12a), with no previously described EOD sex differences, also respond to testosterone (Fig. 12c, Table 2) with a 350% increase in average EOD duration (0.6 to 2.7 ms; Table 2), and an 87% decrease in average PPW (3.7 to 0.5 kHz). Prelimi-

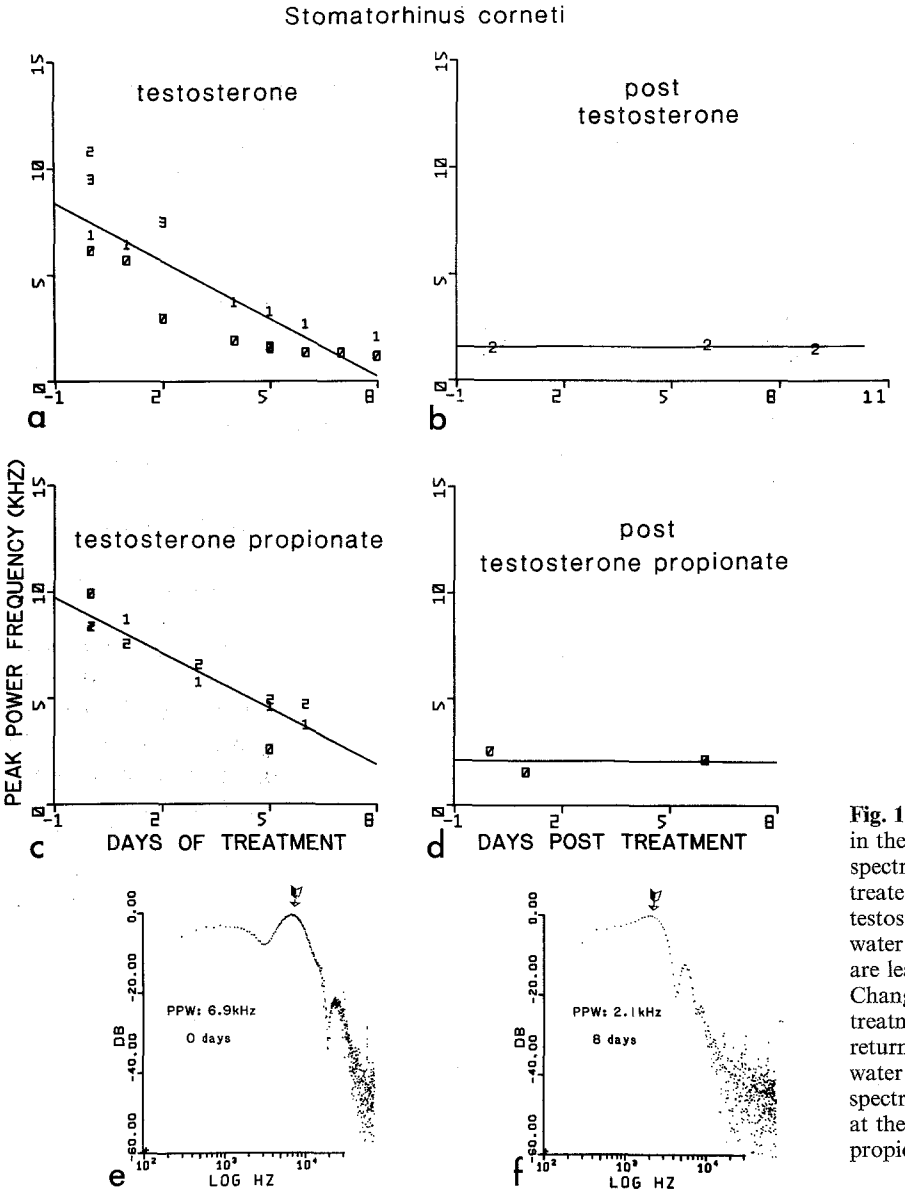


**Fig. 11. a–d** Plots of time course of change in the peak frequency in the power spectrum (PPW) of the EOD of *S. corneti* treated with 17α-methyl-testosterone (**a**) or testosterone propionate (**c**) added to the water at 24 or 48 h intervals. Drawn lines are least squares fit to the straight line. Changes in PPW are significant during treatment but not after specimens are returned to 'non-testosterone' stream water (**b, d**). **e, f** Plots of the power spectrum for a female just prior to (**e**) and at the end of treatment with testosterone propionate (**f**). See Table 2

nary data suggest a sex difference in the EOD of *H. batesii* (triphasic). Just prior to leaving Gabon, we caught several specimens whose EODs were later recorded in France. The EOD duration of a mature male was two-fold that of two mature females. We later reviewed data from a previous field season (collected by Hopkins in 1976) which also suggests a possible sex difference in EOD duration and power spectra for this species (Fig. 12b).

*Question 5: Is the hormone effect at a central (motor pathway) or peripheral (electric organ) level?*

We considered two sites of action where gonadal hormones or their metabolites might induce EOD transformations: the peripheral electric organ or the central motor pathway controlling its excitation (Bass and Hopkins 1982b, 1983). The former seemed more likely since the current generated by a single excitable cell (electrocyte) of the electric organ resembles that of the entire EOD waveform (Bennett and Grundfest 1961; Bennett 1971). Centrally mediated events appear to control only the synchrony and the rate of electrocyte discharges (Bennett 1971). The spinal electromotor nucleus is located at the level of the electric organ and each of its cells fires three spikes prior to the occurrence of each EOD (cf. Bennett 1971). All cells fire synchronously, and the electrocytes generate an EOD pulse after the third spike in the volley. We recorded the highly stereotyped three-spike

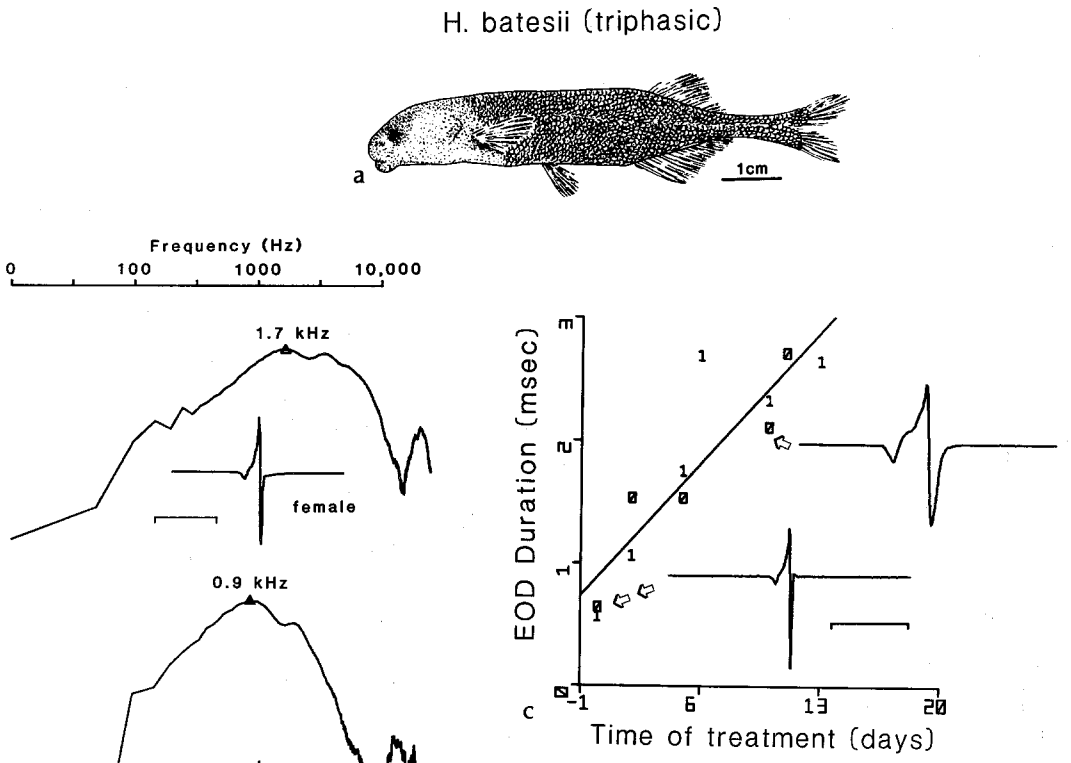


**Fig. 12. a** Line drawing of a female *Hippopotamyrus batesii* (triphasic). **b** EOD and power spectrum for a female (top) and male (bottom). There is a sex difference in EOD duration (bar scale: 2 ms for male and female EOD waveforms) and the peak frequency (triangle) in the Fourier-derived power spectrum. **c** There is a significant change in EOD duration for two specimens treated with 17α-methyl testosterone as the EOD assumes the longer duration male-like (top right) form. Bar scale 2.5 ms

'command signal' in specimens of *B. brachyistius* (triphasic), whose EOD was blocked by intraperitoneal injections of Alloferin, a curare-like drug. The command was monitored by either (a) placing an external, fire-polished, glass capillary electrode next to the caudal peduncle at the level of the electric organ; or (b) inserting two 00-insect pins subdermally at either end of the organ. For females, mature males, and androgen (methyl-testosterone, T; or testosterone propionate, TP; Fig. 13) treated females, the command signal has three major spikes (large arrows, Fig. 13) each separated by about 1 ms. The time interval between the spikes appears the same for all specimens – treated or untreated – with EODs of widely differing form and duration. The appearance of the waveform of the command varies with electrode placement. As in other mormyrids (Bennett 1971; Russell and Bell 1978; Bass and Hopkins 1983), we presume the three spikes represent the synchronous firing of the electromotoneurons. We conclude that the *temporal* properties of the spinal command signal are unchanged in testosterone-treated females. The effects of gonadal steroids on the EOD waveform thus appear to be at the level of the electric organ, distal to the command signal generator in the spinal cord.

## Discussion

### *Hormone-dependent sex differences in EOD waveforms*

The results of this and our earlier (Bass and Hopkins 1983) investigations suggest that the evolution of sex differences in the EOD waveform of mormyrid fish depends upon the development of a hormonal control mechanism that can influence the electrical properties of the excitable cells comprising the electric organ. At first, we expected only a few species, namely those with an observable sex difference, to respond to steroid treatments. Indeed, we found that all mormyrids from Gabon with a known EOD sex difference – *B. brachyistius* (triphasic), *S. corneti* and *B. brachyistius* (long biphasic) (Bass and Hopkins 1983) – respond to ster-

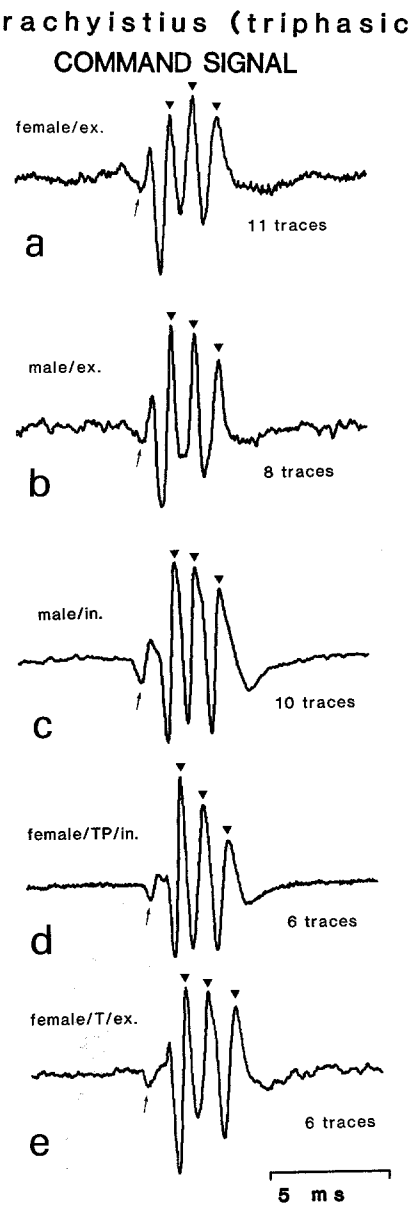


**Fig. 13a–e.** Oscilloscope records of the command signal of electromotoneurons that excite the electric organ. Recordings were either monopolar with an external (*ex.*) electrode or differential with internal (*in.*) electrodes. The command has three major spikes with nearly identical temporal pattern for a *B. brachyistius* (triphasic) female (**a** 11 averaged traces), sexually mature male (**b** or **c**), or a female treated with testosterone propionate (TP, **d**) or 17α-methyl-testosterone (T, **e**). The initial negativity (arrow) probably corresponds to the descending medullary signal that excites the electromotor nucleus (see Bennett 1971)

oid treatments, as does *Pollimyrus isidori* (Bass, unpublished observations), another mormyrid with an EOD sex difference (Westby and Kirschbaum 1981). However, the EOD of *Hippopotamyrus batesii* (triphasic), a species not known to have an EOD sex difference, could also be modified by androgen treatment. Other data now suggest a natural EOD sex difference for this species and so we believe that steroids can be used to predict species that will have a sexually dimorphic EOD waveform. Other mormyrids with a 'hormone-sensitive' EOD waveform include (*Brienomyrus* sp. 2 from Nigeria (Bass 1983; Bass and Hopkins 1984), *Brienomyrus niger, Gnathonemus tamandua* and *Hyperosius bebe* (A.H. Bass, unpublished observations). Hagedorn and Carr (1983) also report a hormone dependent sex difference in the EOD pulse of a gymnotoid electric fish with a pulse-like EOD as mormyrids. Together, the above data suggest that the entire phenomenon of hormone-dependent EOD sex differences may be more widespread among both mormyrid and gymnotoid electric fish.

Steroid hormones are also known to induce changes in the EOD pulse repetition rate (or rhythm) of gymnotoids by shifting the firing rate of central neurons that determine the EOD rhythm (Meyer 1983, 1984; Meyer and Zakon 1982; Meyer et al. 1984; also see Bennett 1971; Hopkins 1972). Some mormyrids may also have a sex difference in the EOD rhythm (Lucker and Kramer 1981) that we may expect to be under the control of gonadal steroids (see Bass et al. 1984). Together with the above data for hormone effects upon EOD waveforms (of both mormyrids and gymnotoids), we may add yet another feature to the convergent evolution of the African mormyrids and South American gymnotoids (review: Bass and Hopkins 1982a; Bullock 1982) – the development of hormone-dependent sex differences in the EOD waveform pulse or rhythm.

### *Species-specificity of hormone effects on EOD*

In all cases where a hormone-sensitive EOD waveform pulse has been discovered, there is an increase in EOD duration following steroid treatment, which may relate to some common effect of steroids on the electric organ's spike-generating properties. But the EOD of hormone-treated individuals does not simply stretch isomorphically in time. The transformation in waveform shape is entirely species-specific with each phase of the EOD changing in its own characteristic fashion: compare *B. brachyistius* (triphasic) to *S. corneti*, to *H. batesii* (triphasic). Moreover, in some cases the hormone effect may be reversible as in *B. brachyistius* (long biphasic) (Bass and Hopkins 1983) and *Brienomyrus* sp. 2 from Nigeria (Bass and Hopkins 1984) and associated with seasonal fluctuations of the EOD waveform (Bass and Hopkins 1983). In other species, such as *B. brachyistius* (triphasic), there

may be more permanent developmental changes in EOD waveforms that are controlled by steroids. And finally, some species as *B. brachyistius* (biphasic), with no observable EOD sex difference, may not be influenced by gonadal steroids (Bass and Hopkins 1983). Species-specific differences in the nature of the hormone-dependent EOD sex dimorphism, or even its absence, probably relate to species differences in the anatomy and physiology of the excitable membranes that comprise the electric organ (see below).

*Androgen-specificity of the EOD hormone effect*

For *Brienomyrus brachyistius* (triphasic), both androgens and estrogens have an effect on EOD duration. Estradiol, as 17$\alpha$-methyl-testosterone, also affects the EOD waveform of *Brienomyrus* sp. 2 from Nigeria and *Brienomyrus niger* (A.H. Bass, unpublished observations). In contrast, estradiol has a small, but insignificant effect on the EOD of *B. brachyistius* (long biphasic) (Bass and Hopkins 1983). Interestingly, one female *B. brachyistius* (triphasic) has an EOD duration nearly 60% greater than that of other immature and mature females, though still nearly half the average of mature males. If estrogens are biologically active steroids among mormyrids, their effect on the EOD waveform may underlie an increase in duration among larger, more mature females. Both testosterone and 17$\beta$-estradiol have been isolated from the blood plasma and gonads of several teleosts (Ozon 1972a, b; Wingfield and Grimm 1977; Fostier et al. 1982; Hannes and Franck 1983; Scott et al. 1983), and are known to stimulate reproductive behavior and the development of secondary sex characteristics in both intact and gonadectomized males and females (review: Jones et al. 1972; Also Fernald 1976; Johansen and Cross 1980; Stacey 1981; Meyer 1983).

Alternatively, the effects of estradiol on the EOD waveform may also depend on its ability to mimic some of the behavioral effects of testosterone, as among mammals (cf. Pfaff 1970). The determination of the differential effects of androgens and estrogens on EOD waveforms requires further studies that define threshold dosages for eliciting a response.

Control experiments in *B. brachyistius* (long biphasic) (Bass and Hopkins 1983) and *Brienomyrus* sp. 2 from Nigeria (Bass and Hopkins 1984) show that cholesterol treatment has no significant effect on the EOD waveform. These results suggest the effects of steroids on the EOD waveform are specific to gonadal steroids.

*Possible mechanisms underlying hormonal influences on EOD waveforms*

*The electric organ.* The discovery of steroid hormones affecting the waveform of the EOD pulse of mormyrids suggests that steroids can influence the most fundamental of electrical activities of neurons and muscle, namely the action potentials of excitable membranes. In an earlier paper, we suggested three possible mechanisms by which steroid hormones might alter the spike-generating properties of the electrocyte (Bass and Hopkins 1983): they might change (1) the physiology of the electromotoneuron-electrocyte junction, (2) the cable properties of electrocytes, or (3) the ionic conductances of the electrocyte's excitable membranes. Before discussing additional findings that now support the hypothesis that steroid hormones directly affect the activity of individual electrocytes, we will briefly review the mechanism of EOD generation by electrocytes in mormyrids, which resembles that of other electric fish (review: Bennett 1971).

The electric organ of mormyrids is derived from muscle and consists of four columns of serially-stacked, disk-shaped cells or electrocytes. Each cell has anterior and posterior faces that are oriented perpendicular to the longitudinal body axis. In addition, the posterior face has a series of finger-like evaginations that fuse into a stalk-like trunk which is innervated by spinal electromotoneurons (Szabo 1958; Bennett and Grundfest 1961). All cells fire synchronously; the number of cells only determines the amplitude of the EOD (Bennett 1971; Bell et al. 1976). Bennett and Grundfest (1961) discovered from microelectrode recordings that each electrocyte face and the stalk produce distinct action potentials. Electromotor axons generate a spike in the stalk that propagates to the posterior face, which then fires a second spike. Current flows across the electrocyte and then depolarizes the anterior face which fires a third and final spike. Bennett and Grundfest conclude for mormyrids that the sequential activity of the posterior and anterior faces accounts respectively for the major positive and negative phases of an EOD waveform, while the stalk's activity can determine the presence of an initial negative phase (e.g. as in *Brienomyrus brachyistius* (triphasic), Fig. 2).

We expected that sex differences in EODs, as species differences (Bennett and Grundfest 1961), would depend upon variation in the appearance of the spikes generated by each face and stalk, or the internal delay between their firing. Such differences may be determined by passive electrical

properties such as total capacitance of the electrocyte's membranes, or active properties related to the number or distribution of different classes of ion channels in those membranes. As regards possible changes in membrane capacitance, preliminary data indicated the electrocyte's anterior face in males or androgen-treated females has a greater surface area compared to natural females (Bass and Hopkins 1983; Bass et al. 1983). We have now extended these findings for *Brienomyrus* sp. 2 specimens from Nigeria (Bass et al. 1984; Bass, in press). Anterior face 'thickness', which probably relates to the degree of invagination of the membrane surface (see Bass et al. 1983), and total electrocyte thickness are increased in testosterone-treated females compared to control females. Increased surface area should change total membrane capacitance of either the anterior or posterior face. While such changes may not significantly change the shape of the spike generated by each face, it would certainly change the time course of spike onset in response to stimulation. This could, for example, affect the delay between firing of the anterior and posterior faces (or even the stalk) and so the compound action potential (i.e. the EOD waveform) produced by the electrocyte. Interestingly, Kendrick and colleagues (Kendrick and Drewett 1979, 1980; Kendrick et al. 1981; Kendrick 1982, 1983) report that the mean absolute refractory period of neurons in the corticomedial amygdala increases in castrated male rats, but decreases in castrated males or intact females treated with testosterone propionate. The authors suggest that hormones may directly affect the cell membrane (also see Dufy et al. 1979), but a specific mechanism is not suggested.

Steroids can also affect the amplitude and perhaps the duration of electrocyte action potentials (Hagedorn and Carr 1985). DHT appears to induce a change in the ratio of spike amplitudes of electrocyte's two faces in a gymnotoid (*Hypopomus occidentalis*) with a pulse-like EOD as mormyrids. There are also increases in electrocyte size. Changes in spike wave-form may depend upon alterations of ionic conductances, although this has not yet been shown.

For comparison, there are also increases in the duration of the spike-like receptor potentials generated by electroreceptors in androgen-treated mormyrids (Bass and Hopkins 1984). Similarly, among wave gymnotoids, the electroreceptor produces an oscillatory receptor potential where the periodicity of oscillation increases in androgen-treated specimens (Meyer and Zakon 1982; Zakon and Meyer 1983). Initial reports showed small, but insignificant affects of steroids on electroreceptor tuning (see above references). Recent data for gymnotoids suggest that steroids can directly affect tuning (K. Keller and H. Zakon, personal communication). Comparable biophysical mechanisms may underlie changes in the electrogenic properties of the membranes comprising both electrocytes and electroreceptors. Whether these changes relate to the active or passive properties of excitable membranes is the focus of future experiments.

*The central electromotor pathway.* Bennett and his colleagues (review: Bennett 1971) show for mormyrids, that the electromotoneurons which innervate the electric organs fire synchronously in response to a descending signal from a 'relay' nucleus in the medulla. Each electromotoneuron characteristically produces a three spike 'command' signal which excites the electrocytes. The first two spikes elicit post-synaptic potentials in the electrocytes; only the third elicits a spike. The electromotoneurons are electrotonically coupled and discharge synchronously, as do the electrocytes. Using gross electrodes, we recorded a three-spike command signal, which we presume represents the synchronous firing of the electromotor neurons. The temporal properties of this signal match those of the electromotoneurons as recorded in *Gnathonemus* (Bennett et al. 1967). Similarly, the three-spike signal appears nearly identical between different sexes, hormone-treated and untreated individuals, as well as different species (Fig. 13; Bass and Hopkins 1983; Bennett 1967; Russell and Bell 1978). The data so far collected do not indicate a significant effect of steroid hormones on the command signal. Further recordings from electromotoneurons may indicate more discrete changes in their individual or coupled activity.

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