

Shifts in frequency tuning of electroreceptors in androgen-treated mormyrid fish

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Summary. Several species of mormyrid electric fish have a sex difference in the pulse waveform of their electric organ discharge (EOD). Field studies in Gabon, West Africa have shown for one such species, *Brienomyrus brachyistius* (triphasic), that the sexually mature male EOD differs in shape and is nearly twice the duration of the EODs of females and juveniles. Fourier analysis reveals that differences in EOD duration correlate with those in the EOD power spectrum which has a peak at 0.3 kHz in males and 1.3 kHz in females and juveniles. We find a corresponding sex difference in the frequency tuning of at least one class of electroreceptors known as Knollenorgans. The average 'best' or 'characteristic' frequency of Knollenorgans is lower in males compared to females and juveniles. This correlates with a lower peak in the power spectrum of the male's pulse. When females are treated with gonadal androgens, their EODs increase 2–3 fold in duration, and the power spectra of their pulses are correspondingly lowered to match that of mature males. The average best frequency of Knollenorgans decreases by nearly 1 kHz which matches the downward shift of their EOD's power spectrum.

For a second species of *Brienomyrus* (sp. 2) which is commercially imported from Nigeria, we have not detected a sex difference in the power spectrum or duration of the EOD. The power spectrum peaks at about 4.2 kHz in males, females, and juveniles. Androgens, however, do cause a coincident downward shift in the average peak of the EOD power spectrum (from 4.2 to 1.3 kHz) and the average best frequency of Knollenorgans (from 2.3 to 1.4 kHz).

Specimens of *Brienomyrus* (sp. 2) that have been electrically silenced by surgical means are tuned, on the average, only 0.2 kHz higher than control animals. Silenced animals that have been

treated with androgens are tuned, on the average, 0.2 kHz below controls. The results suggest that electroreceptor tuning is only partially modifiable during androgen treatment if the electroreceptors are *not* being stimulated by an external electrical stimulus, i.e. the animal's own EOD. Since androgen treatment has a dramatic effect on receptor tuning *only* in intact fish, it seems likely that retuning is *not* due to a direct action of androgens on receptors, but rather due to the action of the principal electrical stimulus upon the receptors, i.e. the EOD. The implications of such results for the development of species and sex differences in electroreceptor tuning is discussed.

Introduction

For some species of mormyrid and gymnotoid electric fish, gonadal androgens can induce changes in the electric organ discharge (EOD) which mimics 'natural' changes that males undergo during sexual maturation. In some cases, the pulse waveform of the EOD is altered by androgens (Bass and Hopkins 1982, 1983; Hagedorn and Carr 1983), while in others the rate at which the EOD is generated by the electric organ (*EOD frequency*) is affected (Meyer and Zakon 1982; Meyer 1983; Fig. 1). Recently, Meyer and Zakon (1982) demonstrated that androgen-treated gymnotoids (*Sternopygus*), with a natural sex difference in the EOD frequency (Hopkins 1972; Meyer 1983), fired its electric organ at a lower male-like frequency. During the downward shift in the EOD frequency, there was a re-tuning of an individual's electroreceptors to lower best frequencies (see also Zakon and Meyer 1983). The change in receptor tuning may have been the result of the direct action of

steroid hormones on the cells comprising the electroreceptors. Alternatively, as Meyer and Zakon pointed out, androgens may have affected the electroreceptors only indirectly. Androgens may have had a direct, *primary* affect on the discharge frequency of the electric organ which induced a *secondary* change in tuning so that the best frequency of the receptor would be matched to the EOD frequency. The latter hypothesis, supported by recent studies in gymnotoids (Meyer et al. 1984), suggests the EOD can entrain the best frequency of an electroreceptor. Hopkins (1976) already demonstrated a close match between receptor best frequencies and the EOD frequency for individuals of three different species of 'wave' gymnotoids. So, perhaps the mechanism that underlies the tuning of an individual's receptors to its own species-typical discharge can also account for the re-tuning of receptors of androgen-treated specimens. We wanted to test this hypothesis in a fish with a pulse discharge rather than a wave discharge because pulse fish are usually tuned to frequencies which are very different from the EOD pulse repetition rate.

We conducted field studies in Gabon, West Africa during the October–December, 1981 rainy season. We showed for several species of mormyrids that androgens could induce a transformation of the EOD of females such that they resembled the EOD of natural males (Bass and Hopkins 1983). This transformation involves an increase in duration of the EOD waveform, accompanied by a lowering of the dominant frequencies in the EOD power spectrum. In parallel with these changes we found that androgen-treated females had re-tuned their electroreceptors – there was a similar downward shift in EOD power spectrum and electroreceptor best frequencies. We have now been able to reproduce these observations in a different mormyrid species which is commercially available. Furthermore, when an individual's EOD is surgically silenced, there is only a slight shift in tuning even after androgen treatment. The results suggest that the EOD and not the androgens, are the *primary* stimulus that 'drive' the downward shift in frequency tuning of the receptors. As pointed out, however, androgens may still play a role in the development of sex differences in tuning by creating a hormonal milieu for the electroreceptor's cells which permits an ontogenetic shift in tuning to occur.

We emphasize that some electric fish like mormyrids, produce brief pulses separated by long intervals (so-called pulse fish), while some gymnotoids produce wave-like discharges where the pulses are separated by short intervals (Fig. 1). The

physiological mechanism underlying androgen-related shifts in electroreceptor best frequencies may be completely different for 'pulse' mormyrids and 'wave' gymnotoids, which are distant relatives (Greenwood et al. 1966). Among mormyrids, we have been studying species- and hormone-dependent sex differences in the waveform of the EOD pulse (Hopkins 1980; Bass and Hopkins 1983); differences that seem to depend on the anatomy and physiology of the *peripheral* electric organ (Bennett and Grundfest 1961; Bass et al. 1983, 1984, see also Hagedorn and Carr 1983). For wave gymnotoids, there are species and hormone-dependent sex differences in the EOD frequency (Bennett 1971a; Hopkins 1972, 1974; Meyer and Zakon 1982), which are under the control of a *central* pacemaker nucleus in the medulla oblongata (Bennett 1971a).

Preliminary reports of a portion of these findings appeared elsewhere (Bass and Hopkins 1980; Bass 1983).

Materials and methods

This report has two sections. The first concerns field studies conducted in Gabon, West Africa; the second concerns laboratory studies.

Field studies: Field work was conducted during the October–November, rainy seasons of 1979 and 1981 in the Ivindo River District of Gabon (0° 30' N, 12° 50' E). Specimens tentatively identified as *Brienomyrus brachyistius* (triphasic) (Hopkins 1980 and Fig. 3) were caught in local streams. Details of capture techniques and of the field site appeared elsewhere (Hopkins 1980).

Each individual fish was housed separately in 1.2 l plastic bowls containing aerated, home stream water (22–23 °C; conductivity = 20–60 K Ω -cm). Juvenile and female specimens underwent the following treatment with androgens (see also Bass and Hopkins 1983):

a. For one group ($n=9$ individuals), 2–4 mg of 17 α -methyl testosterone (Sigma Chemical) were added directly to the water at 24–48 h intervals. Testosterone treatment was terminated by returning fish to fresh stream water that was changed on a daily basis.

b. A second group ($n=6$) was treated with 5 α -dihydrotestosterone (DHT) (Sigma Chemical). Specimens were lightly anaesthetized with tricaine methanesulfonate (MS222), an incision made in the ventrolateral body wall, and a 2–3 mg pellet of DHT implanted in the body cavity. In some cases, the unilateral gonad (see Okedi 1969) was excised. Wounds were sutured shut and animals were placed in a 1:1 mixture of stream water and 0.6% NaCl to help prevent infection. The water was changed daily and specimens were gradually returned to bowls containing only home stream water after a period of 4–5 days.

All EODs were recorded daily on a Nagra IV-SJ tape recorder at 38.1 cm/s and later digitized on a PDP 8/e with Lab 8/e A/D converter (sampling at 24.83 μ s/point), and then Fourier analyzed to find the peak frequency of the power spectrum.

Knollenorgan electroreceptors are seen easily on the skin surface as large (75–300 μ m), unpigmented spots (Bennett 1965, 1971 b). We recorded the spike-like receptor potentials (Fig. 7c) of Knollenorgans non-invasively by placing a fire-polished glass

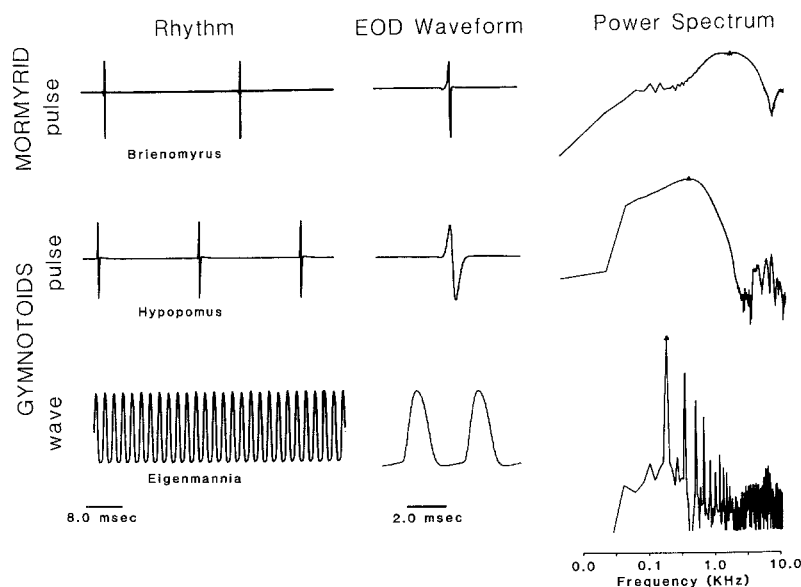


Fig. 1. Comparison of the Electric Organ Discharge (EOD) of 'Pulse' and 'Wave' electric fish. The EOD rhythm describes the rate at which the EOD waveform is generated by the electric organ. For mormyrids and gymnotoids with a pulse-like EOD rhythm, the interval between successive pulses is long compared to the duration of the pulse itself. For wave gymnotoids the EOD rhythm has a quasi-sinusoidal appearance since the inter-pulse interval is similar to the duration of the pulse. To the right of each EOD waveform is shown the power spectrum of the pulse. In pulse fish, the EOD waveform has a broad power spectrum with 'humps'. For wave gymnotoids it is difficult to isolate a single waveform pulse and so two cycles of the EOD are shown on an expanded time scale. Essentially, the power spectrum has a fundamental frequency (usually the EOD repetition rate) and several harmonics. For all traces head positive polarity is up and negative is down. For *Eigenmannia*, the EOD waveform is a head-positive pulse superimposed on a negative baseline (Bennett 1971a)

capillary electrode (filled with stream water) over the receptor pore. The same electrode was used both for recording and stimulating by use of a bridge circuit (WPI 707). Signals were amplified and displayed on a Hewlett-Packard 1707B oscilloscope. Stimuli consisted of 100 ms tone bursts of varying frequencies, but constant amplitude. Stimulus amplitude was changed with a variable attenuator (Kay Electronics).

The 'best frequency' of a receptor organ is determined by at least one of two measures – frequency tuning curves or constant-amplitude response profiles (Fig. 2). Frequency tuning curves measure the threshold for evoking spikes at different sinusoidal stimulus frequencies. Thresholds were determined by adjusting amplitude so that a receptor responded with one to two spikes per trial above the background firing rate. Ten stimuli were presented during each trial. For each trial, a digital up-down counter was used to subtract the spike count accumulated during a 100 ms no-stimulus period from the count accumulated during a 100 ms stimulus period. Ten trials were averaged for each stimulus frequency. Response profiles simply measure the number of spikes above background per trial for sine wave tone bursts of constant amplitude.

The best frequency of a tuning curve is defined as the frequency with the lowest threshold (as measured in dB of attenuation). For response profiles, the best frequency is that frequency which evokes the greatest number of spikes. For individual receptors, there is a close match between the best frequency derived from either method (Fig. 2).

While making electrophysiological recordings, the electric organ, which is derived from muscle, was silenced by an intraperitoneal injection (0.1 μ g) of Alloferin (Hoffman-LaRoche), a curare-like drug. Each fish was then placed in a plexiglass holder and the entire fish was submerged below the surface

of stream water in a plastic recording dish and respired with aerated water flowing over the gills.

Laboratory experiments. Laboratory studies focused on a commercially available mormyrid imported from Nigeria that is identified tentatively as *Brienomyrus* species number 2 (*sp.* 2). It is similar in overall appearance to *Brienomyrus brachyistius* (triphasic) but it has a different waveform EOD (compare Figs. 3 and 5).

Recording techniques were similar to field methods with the following exceptions: EODs were recorded by digitization into a PDP 11/34 computer (sampling rate of 50 kHz). Best frequencies were determined by a computer-automated spike response profile program (Fig. 7a, b).

Some fish had their EOD eliminated by permanently silencing the electric organ by surgical means. Animals were anaesthetized with MS222 and the spinal cord was severed with a sharp scalpel blade inserted just anterior to the electric organ near the origin of the dorsal fin. Spinal transection interferes with a descending signal from a 'command' nucleus in the medulla oblongata which excites spinal electromotoneurons located at the level of the electric organ. The motoneurons in turn excite the electrically excitable cells of the electric organ (cf. Bennett 1971a; Bell et al. 1983). The surgery did not appear to interfere with an animal's ability to locomote since the muscles (and their innervation) controlling movement of the caudal peduncle originate anterior to the electric organ (Moller 1976). Tail movements are controlled via tiny styliform 'Geminger's bones' which run longitudinally above and below the electric organ from a point of attachment to the muscles which is anterior to the surgical cut (cf. Lissmann 1958). Several experimental groups were established for electrophysiological recordings:

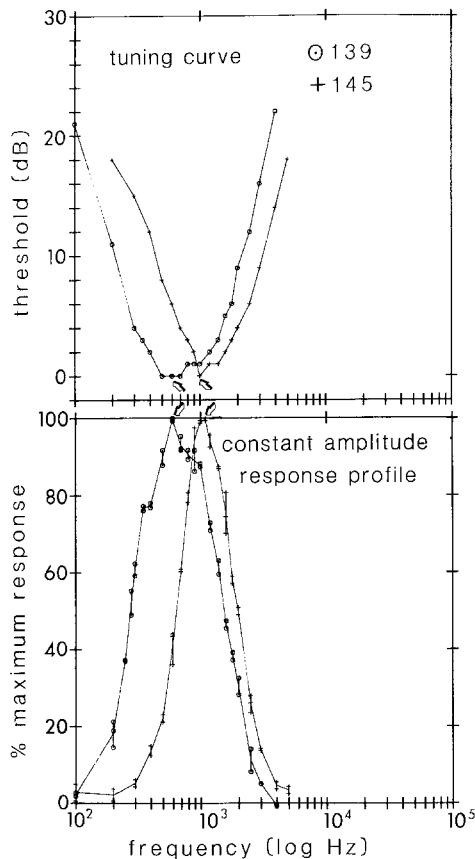


Fig. 2. *Brieniomyros brachyistius* (triphasic). Representative tuning curves (top) and constant amplitude response profiles (bottom) for two Knollenorgan electroreceptors. Attenuation values (top) and spike responses (number/100 ms) (bottom) are normalized since stimulus intensity is variable between receptors because the proximity of the recording and stimulating electrode to a receptor is variable. For response profiles, the actual maximum spike number for receptors 139 and 145 were 16.1 (at 36 dB) and 25.5 (at 48 dB). There is a close match (arrows) for each receptor between the best frequency derived from either method

1. A control group of intact, untreated specimens ($n=7$).
2. A group of gonadectomized specimens with a pellet implant of 2–3 mg of 17α -methyl testosterone or DHT (as described above) ($n=5$).
3. A group of gonadectomized specimens with a 2–3 mg implant of cholesterol (5(6)-cholestene-3-ol; Sigma Chemical) ($n=2$).
4. An intact, but silenced group of animals ($n=3$).
5. A group of gonadectomized and silenced individuals with a methyl testosterone or DHT pellet implant ($n=3$). All surgery for each fish was performed on the same day.

Histogram plots. We present receptor best frequencies as histogram plots (e.g. Fig. 3). The data have been normalized by dividing the observed number of Knollenorgans in a given bin by the number that would have been expected if they were evenly represented on a log axis in the bandwidth of 0.1–10 kHz (the observed/expected value in Figs. 3, 5, 6, 8, 9). Thus, if 100 Knollenorgans are sampled and the histogram contains 20 bins between 0.1 and 10 kHz, we would expect 5 Knollenorgans per bin, given an even distribution. As can be seen from the histogram plots, Knollenorgan best frequencies occur over a broad, though restricted range, and are not evenly distributed.

Results

Field study: Gabon, West Africa

Hormones and EOD sex difference. Two androgens, 17α -methyl testosterone or 5α -dihydrotestosterone (DHT), can induce transformation of the EODs of females and juvenile *B. brachyistius* (triphasic) to an EOD typical of sexually mature males (cf. Fig. 3, and Bass and Hopkins, submitted). The male's EOD has a distinctive waveform and is nearly double the duration of that of either females or juveniles (Fig. 3). If EODs are Fourier analyzed (cf. Fig. 1), it is found that the average peak frequency in the EOD power spectrum of mature males is 0.3 kHz ($n=6$), while for females and juveniles it is 1.3 kHz ($n=31$). Androgen-treated females and juveniles ($n=14$) show a decrease in average peak frequency from 1.3 to 0.3 kHz. Controls ($n=4$) maintained in captivity for similar time periods do not change (1.4 kHz peak).

Electroreceptor tuning. There is also a male-female difference for *B. brachyistius* (triphasic) in the frequency tuning characteristics of Knollenorgans. The electrophysiological properties of Knollenorgans are detailed elsewhere (Bennett 1965, 1971 b; Szabo 1974; Hopkins and Bass 1980, 1981; Bass and Hopkins 1980). As in several other mormyrid species (Bass and Hopkins 1980), we found two modal values of best frequencies (BFs) for Knollenorgans: one population localized just dorsal to the operculum covers an area about the size of the eye and is tuned below 1 kHz. We refer to these as Type K_I receptors (Fig. 4). A second population, K_{II} receptors, includes the majority of Knollenorgans which are scattered over the trunk and head and have BFs above 1 kHz. Best frequencies of K_{II} receptors in males are significantly (two-tailed t -test for difference in means) lower than K_{II} receptors in females or juveniles (Table 1). The average BF of K_{II} receptors is 1.3 kHz for males (90–100 mm total length, $n=4$ individuals) and 1.8 kHz for females and juveniles (49–74 mm, $n=6$). There is no significant sex difference in the average best frequency of K_I receptors which is 0.6 kHz for males and 0.7 kHz for females and juveniles.

In a pilot study, we examined the frequency tuning of Knollenorgans of two *B. brachyistius* (triphasic) treated with androgens. For one juvenile (BBTP62, 67 mm), testosterone treatment lasted for 12 days and was followed by a 24 day recovery period prior to our recordings. Its EOD had a power spectrum that peaked at 0.4 kHz (initially it was 1.4 kHz), which is comparable to that of

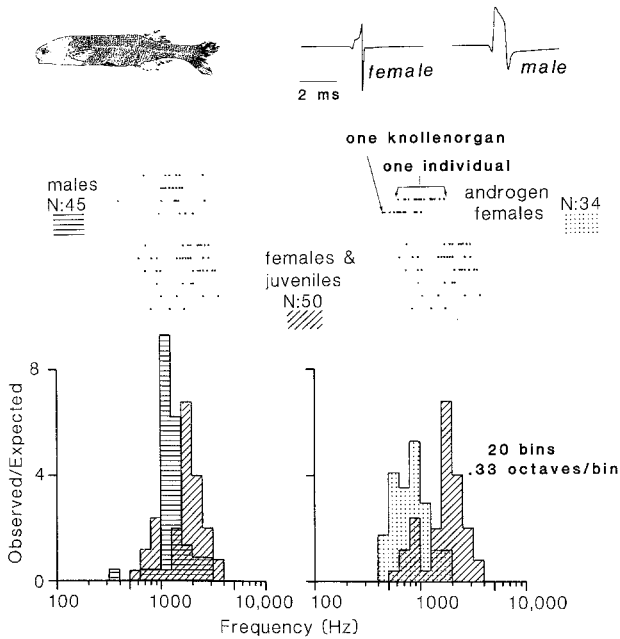
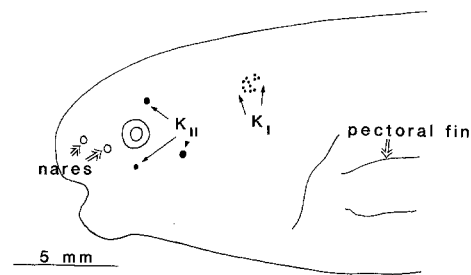


Fig. 3. At top a mormyrid fish, *Brienomyrus brachyistius* (triphasic), the species used in our field studies. Also shown are the EOD waveforms of males and females (juveniles have a similar EOD). All EOD waveforms shown for this figure and Figs. 5–8 have been digitized from tape recordings (this Fig.) or directly from recording electrodes (Figs. 5–8) and scaled to the same peak to peak amplitude. There is a male – female/juvenile difference in the shape and duration of the EOD waveform. The latter correlates with a difference in the average peak frequency of the power spectrum of the EOD which is 0.3 kHz among males and 1.3 kHz among females and juveniles. Bottom histograms of best frequencies (BF) of Knollenorgan electroreceptors. For this Fig. and Figs. 5–8, each histogram is a raster display of BFs, corresponding to the frequency scale of the histogram. Each line represents receptor recordings from one specimen; each dot is one Knollenorgan. Number of receptors/bin is expressed as observed/expected where the expected value is the total number of receptors recorded (*N*) divided by number of bins (in this case 20). So, the value for each bin shows a deviation from an even distribution if there were no clustering of BFs about modal values. For males and females, there is a sex difference in the tuning of one class of Knollenorgan electroreceptors (K_{II} receptors) that are scattered on the head and trunk. A second class of Knollenorgans that are localized near the operculum (K_I receptors; cf. Fig. 4) show no sex difference. Only the BFs of K_{II} receptors are shown in the histograms since this class has a sex difference in the average BF (1.3 kHz among males and 1.8 kHz among juveniles and females; histogram to the left) and shows a shift in average BF (from 1.8 to 0.9 kHz) among androgen-treated females (histogram on the right)

male (BBTP102, 78 mm) had been gonadectomized and retained a DHT pellet for 18 days at the time of the recordings; its EOD had a male-like peak frequency of 0.3 kHz (initially it was 1.1 kHz).

K_{II} receptors of hormone-treated females were tuned significantly lower (average BF = 0.8 kHz) than control females (average BF = 1.8 kHz) or

Brienomyrus brachyistius (triphasic)



Brienomyrus sp.2

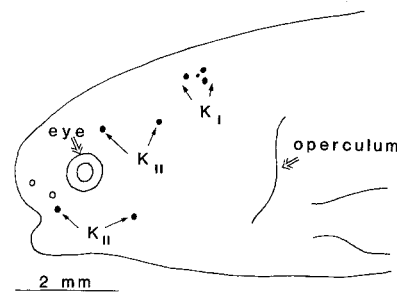


Fig. 4. Camera lucida drawings indicating the position of the low-frequency patch of K_I electroreceptors for a specimen of *Brienomyrus brachyistius* (triphasic) from Gabon and *Brienomyrus* (sp. 2) from Nigeria. Positions of other large ('super') Knollenorgans (K_{II}) are indicated and serve as landmarks (along with the eye, nares, and operculum). The low-frequency patch along with the other super-Knollenorgans are constant in their position from specimen to specimen

even males (average BF = 1.3 kHz) (Fig. 3, Table 1). K_I receptors, sampled for one female (BB102), were tuned close to 0.6 kHz as for control females and males (Table 1). The average BF for K_{II} receptors was significantly lower in the DHT-treated female (0.6 kHz) than the testosterone treated female (1.0 kHz) and may have been due to continued treatment with DHT up to the time of the experiment. Also, the DHT female had an EOD with a slightly lower frequency power spectrum (above). However, it is apparent for both females that lowering of Knollenorgan BFs was coincident with the androgen-induced downward shift in the EOD spectrum.

Laboratory studies

Hormones and EOD sex difference. Although specimens of *Brienomyrus* (sp. 2) imported to our laboratory from Nigeria respond to androgen treatment with a prolonged EOD, untreated males and females have nearly identical EODs under laboratory conditions. The average peak frequency of the power spectrum is 4.2 kHz for females ($n=35$, 76–120 mm), and 4.2 kHz for males ($n=16$, 110–134 mm). If a natural sex difference occurs

Table 1. Average BFs of type K_{II} and K_I Knollenorgan electroreceptors. N =Number of animals recorded for each group; $N(K_I)$, $N(K_{II})$ number of Knollenorgan receptors for each group. S.D. for each group is expressed in kHz. Average peak frequency in the power spectrum (PPW) determined at the time of electrophysiological recordings. PPW values for the silenced groups are based on recordings prior to silencing. Q_h values are a measure of the quality or sharpness of tuning; see text) for the large sample size of the commercially imported *Brienomyrus* (*sp. 2*). There was no significant difference ($P=0.05$) in this value between controls and any experimental group. All statistics based on a two-tailed *t*-test for difference in mean BF. *P* values represent a comparison between controls and each other group shown

Brienomyrus brachyistius (triphasic) (Gabon)

Group	<i>N</i> fish	Average power spectrum peak, PPW (kHz)	<i>N</i> (K_I)	Average BF (kHz)	<i>P</i> (vs control females)	<i>N</i> (K_{II})	Average BF (kHz)	<i>P</i> (vs control females)
Control females and juveniles	6	1.3	16	0.7 (SD=0.1)	–	50	1.8 (SD=0.7)	–
Control males	4	0.3	9	0.6 (SD=0.2)	0.05	45	1.3 (SD=0.4)	0.0001
Androgen-treated females and juveniles	2	0.4	9	0.6 (SD=0.2)	0.05	34	0.8 (SD=0.3)	0.00001

Brienomyrus (*sp. 2*) (Nigeria)

Group	<i>N</i> fish	Average power spectrum peak, PPW (kHz)	<i>N</i> (K_I)	Average BF (kHz)	<i>P</i> (vs control females)	Q_h	<i>N</i> (K_{II})	Average BF (kHz)	<i>P</i> (vs control females)	Q_h
Control females	7	4.2	29	1.1 (SD=0.3)	–	0.88	245	2.3 (SD=1.1)	–	0.76
Androgen-treated females	5	1.3	16	0.9 (SD=0.2)	0.007	0.75	183	1.4 (SD=0.8)	0.00001	0.69
Cholesterol-treated females	2	3.7	9	1.1 (SD=0.2)	0.05	0.81	79	2.3 (SD=1.1)	0.05	0.68
Silenced females	3	4.2	14	1.1 (SD=0.3)	0.05	0.81	127	2.5 (SD=1.2)	0.05	0.75
Silenced, androgen- treated females	3	4.2	12	1.1 (SD=0.3)	0.05	0.76	113	2.1 (SD=0.9)	0.05	0.81

in this species, it may be strictly a seasonal phenomenon (cf. Bass and Hopkins 1983). One specimen, a 142 mm male received soon after importation from Africa, had a peak frequency in its EOD of 1.8 kHz. This is more than an octave lower than the average peak frequency of our general laboratory population. Unfortunately, this male developed a skin infection and did not survive long enough to see if its EOD would revert to a shorter duration form.

Brienomyrus (*sp. 2*) responds to androgen treatment with an increase of its EOD duration and a consequent frequency lowering of its power spectrum (Fig. 5). Among gonadectomized females ($n=13$), 17α -methyl-testosterone or DHT pellet implants can induce a 2–3 fold increase in EOD duration which is matched by an average decrease of 2.9 kHz (from 4.2 to 1.3 kHz) in the peak frequency of the power spectrum (Fig. 5). Although

androgen-treatment periods ranged from 6–52 days, the average decrease was 2.7 kHz after just 10 days of treatment. Two males (not gonadectomized) treated with methyl-testosterone showed an average decrease of 2.4 kHz (3.7 to 1.3 kHz; 20–27 days treatment), while gonadectomized females ($n=3$) treated with DHT showed an average decrease in peak frequency of 2.9 kHz (4.2 to 1.3 kHz; 6–60 days treatment). In contrast, three gonadectomized females treated with cholesterol had an average decrease of only 0.6 kHz (4.3 to 3.7 kHz; 6–60 days treatment). A single male (not gonadectomized) treated with cholesterol for 6 days showed no change in its peak frequency (3.5 kHz). Untreated, gonadectomized control females ($n=3$) showed a slight decrease of 0.5 kHz in the peak frequency of their power spectrum (from 4.6 to 4.1 kHz; 21–37 days observation). So, the response to cholesterol or to androgens may

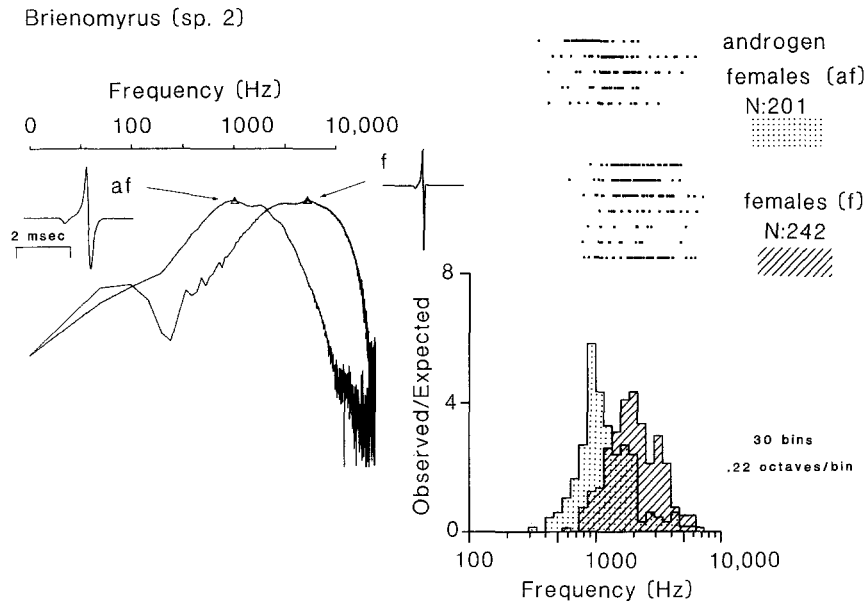


Fig. 5. Androgen-induced shift in EOD waveform and power spectrum shown to the left for the mormyrid fishes from Nigeria. Average peak frequency of the power spectrum is 4.2 kHz among normal females (*f*), 1.3 kHz among androgen-treated females (*af*). To the right are frequency histograms indicating the shift in BFs among androgen-treated females compared to normal females. For this Fig. and Figs. 6–8: (a) each histogram contains 30 bins, 0.22 octaves/bin, (b) both K_I and K_{II} receptors are plotted together as each class shows a significant change in the average BF in androgen-treated females of this species (see Table 1), and (c) each experimental group will be compared to the histogram for normal females

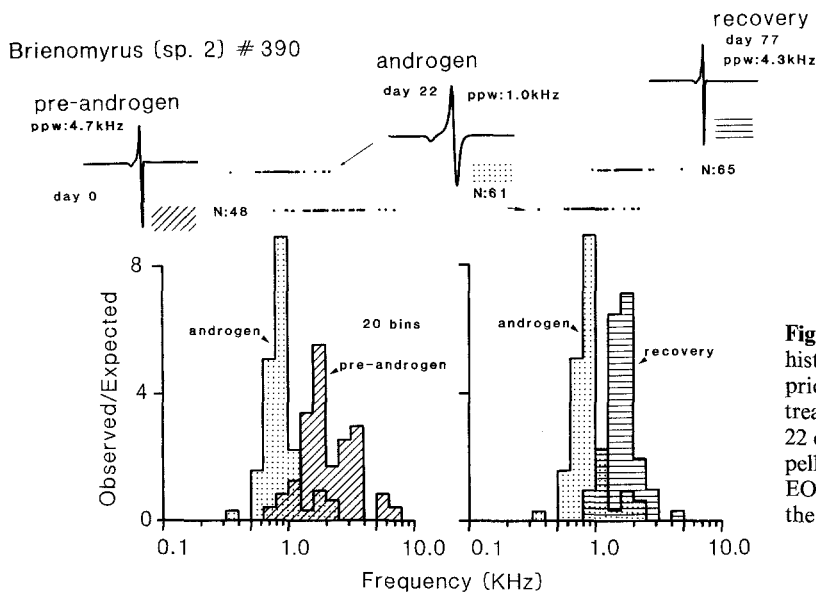


Fig. 6. Changes in EOD waveform and BF histogram for a single specimen recorded from prior to androgen treatment (hatching), after treatment with a 17α -methyl testosterone pellet for 22 days (dots), and 55 days after removal of the pellet (horizontal lines). The BF histogram and the EOD power spectrum (cf. Fig. 5) overlap within the frequency domain

be due in part to surgical trauma. The final peak power frequency for both cholesterol controls and surgical controls however remain nearly three fold greater than those of androgen-treated specimens.

Electroreceptor tuning. The histogram of Knollenorgan BFs spans the range from about 0.3 kHz to 7.0 kHz (Fig. 5). A circumscribed population of K_I receptors near the operculum (Fig. 4) was tuned lower than the K_{II} receptors scattered on the head and trunk (Table 1). After androgen treatment, the average BF of female K_{II} electroreceptors is 1.4 kHz ($n=5$ individuals, 15–22 days treatment), which is significantly lower ($P=0.00001$, two-tailed t -test for difference in means) than the

2.3 kHz for normal females ($n=7$) (Table 1). There is also a significant ($P=0.007$) decrease in the average BF for K_I receptors which is 0.9 kHz for androgen-treated females and 1.1 kHz for normal females. We illustrate the re-tuning of Knollenorgans by plotting BF values in histogram form in Fig. 5. In some cases, we tracked each individual before and after treatment (e.g. Fig. 6). Recovery from the first electrophysiological recording session usually took 4–6 h. About two weeks after removal of the androgen pellet, the EOD of hormone-treated females returns to the short-duration, female form. Coincident with the upward shift in EOD spectrum (as the EOD shortens in duration) there is an upward shift in the BF histo-

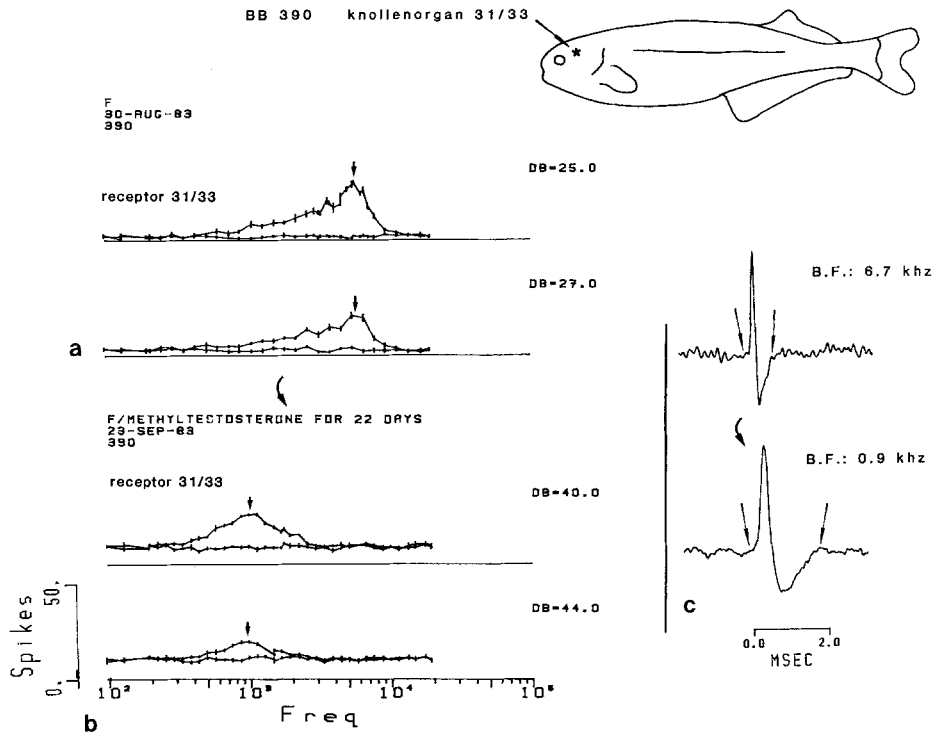


Fig. 7a-c. *Brienomyrus* (*sp. 2*). Changes in tuning of a single Knollenorgan receptor before and after androgen-treatment. **a** and **b** computer-automated constant amplitude spike response profiles: **a** For Knollenorgan 31/33 (labelled as cell number 31 and 33 respectively before and after androgen treatment) the BF (arrow) was 6.7 kHz as shown for two stimulus amplitudes (dB of attenuation). **b** After androgen treatment BF decreases to 0.9 kHz. **c** Changes in BF are correlated with increase in the duration of the spike-like receptor potential generated by Knollenorgans – the duration nearly doubles after androgen treatment (compare top and bottom traces)

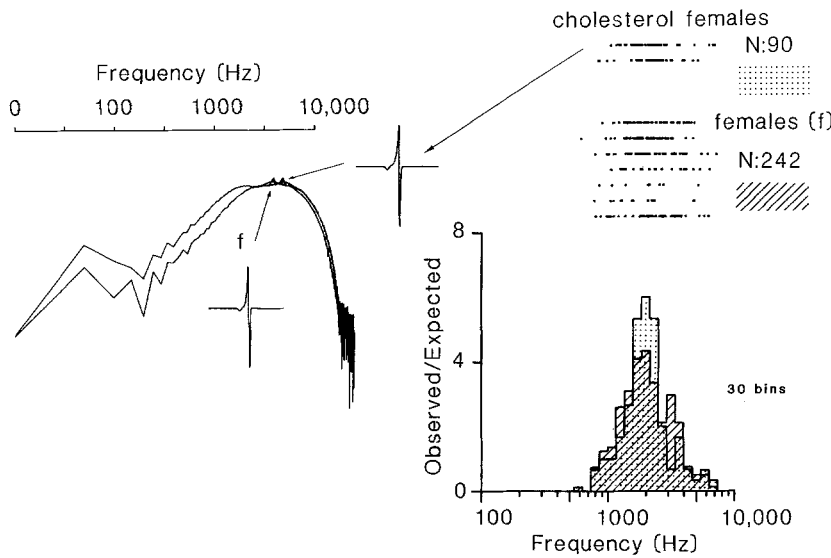


Fig. 8. *Brienomyrus* (*sp. 2*). Data for two gonadectomized females treated with cholesterol implants. Cholesterol, which controls for a non-specific effect of steroids, has no influence upon the EOD waveform duration or its power spectrum, nor upon the BF histogram. So, the downward shift in power spectrum and Knollenorgan tuning is related to the specific effects of gonadal steroid hormones

gram. The EOD power spectrum and Knollenorgan tuning slide together.

It is possible to track changes in the tuning of single receptor organs by recording from recognizable pores before and after hormone treatment. For example, Figure 7 shows that the BF of K_{II} receptor 31/33 for specimen number 390 was 6.7 kHz and 0.9 kHz respectively before and after androgen-treatment. The change in tuning was associated with nearly a two-fold increase in the duration of the externally recorded receptor potential (Fig. 7c).

Knollenorgan tuning was unchanged in specimens treated with cholesterol ($n=2$, 6–60 days treatment) (Fig. 8, Table 1).

Effects of androgens on electrically-silenced individuals (Fig. 9, Table 1). Three *Brienomyrus* (*sp. 2*) were electrically silenced by spinal surgery (19–26 days silencing). The average BF of K_{II} receptors was 2.5 kHz which is slightly higher than in control females (average BF=2.3 kHz). For silenced fish treated with androgens ($n=3$, 18–102 days silencing and treatment) the average

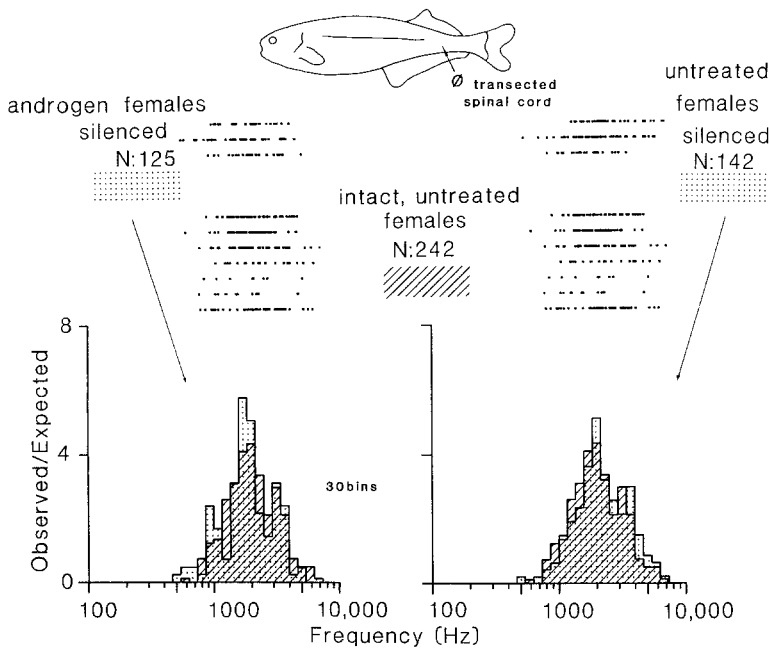


Fig. 9. *Brienomyrus* (*sp. 2*). Effects of 'silencing' upon frequency tuning. The spinal electromotor nucleus that innervates the electric organ is excited by a descending volley from a 'command' nucleus in the medulla oblongata (Bennett 1971a). If the spinal cord is surgically transected (line drawing, top) at the anterior pole of the electric organ, rostral to the electromotor neurons found at the same level as the electric organ, an animal is effectively 'silenced'. Silenced specimens treated (lower left histogram) or untreated (lower right histogram) with androgens do not show a dramatic shift in tuning when compared to normal, intact females. However, as shown in Table 1, there is a slight elevation and depression in the average BF of untreated and treated animals, respectively

BF was 2.1 kHz which is slightly lower than in controls. There was no change in the average BF of K_I receptors for either experimental group.

The average BFs for K_{II} receptors are significantly lower ($P=0.003$) for androgen-treated, silenced fish compared to untreated, silenced fish. The results suggest that Knollenorgan tuning is somewhat labile in the absence of the animals' own EOD, and that androgens can actually have a weak lowering effect upon tuning.

Sharpness of tuning. The quality of sharpness of tuning was determined for each of the Knollenorgans in our large sample from *Brienomyrus* (*sp. 2*). We measured a ' Q_h ' value as the best frequency divided by the bandwidth at 50% of the peak number of spikes as measured by the response profiles. Q_h values are listed in Table 1. There was no significant difference ($P=0.05$) for Q_h values between control females and any experimental group. But, Q_h values are slightly lower among androgen and cholesterol-treated specimens compared to controls and silenced (at least for K_{II} receptors) females. The Q_h for K_{II} receptors of androgen-treated, silenced females is slightly elevated compared to controls and silenced, untreated females.

Discussion

It is apparent from both field and laboratory studies of *Brienomyrus*, that androgen hormones can

induce coincident shifts in the power spectrum of EOD waveforms and the best frequencies of Knollenorgan electroreceptors. Moreover, the absence of dramatic shifts in receptor tuning among specimens deprived of their own EOD waveform supports the hypothesis that the observed changes are 'driven' primarily by the external EOD stimulus. But, as noted below, the silencing experiments suggested some lability in electroreceptor tuning even in the absence of an individual's EOD.

A similar phenomenon is found in *Sternopygus*, a gymnotoid electric fish. It has a hormone-dependent sex difference in the frequency or repetition rate of its wave-like EOD and the best frequencies of its electroreceptors (see Fig. 1 and Meyer and Zakon 1982). In addition, silenced specimens show no significant change in receptor tuning after androgen treatment (Meyer et al. 1984). Together, the data for mormyrids and wave gymnotoids emphasize the dependency of electroreceptor best frequencies upon the power spectrum of the external EOD stimulus.

Previous interspecific studies have also shown a match in power spectra and receptor best frequencies for mormyrids (Bass and Hopkins 1980); and gymnotoids (Hopkins 1976; Bastian 1976, 1977; Hopkins and Heiligenberg 1978; Watson and Bastian 1979). Individual differences in wave EOD frequency are matched by individual differences in tuning (Hopkins 1976; Viancour 1979). Because different species and different individuals vary in their EOD characteristics, each is tuned to itself. This suggests that the re-tuning observed

among androgen-treated specimens may be due to a similar mechanism.

Interspecific and sex differences in frequency tuning would seem related to some property of the receptor cell membrane (Bennett 1965). The best frequency of a Knollenorgan appears to be determined by the time constants controlling the spike-like receptor potential. As the receptor potential duration increases, its best frequency decreases and vice-versa (as shown in Fig. 7). Similarly, among wave (and pulse, Fig. 1) gymnotoids, the electroreceptor produces an oscillatory generator potential where the frequency of the oscillation parallels the receptor's best frequency (Viancour 1979; Watson and Bastian 1979; Meyer and Zakon 1982). The biophysical bases for a mechanism by which an external electrical stimulus could entrain a receptor cell's oscillatory period remain perplexing.

While it is clear that the primary stimulus inducing the change in electroreceptor tuning is the animal's own EOD, the silencing experiments did suggest that tuning was somewhat labile and that androgens might have some weak and independent influence upon tuning (see also Meyer et al. 1984). Our experiments remain inconclusive as to the possible *direct* effect of gonadal steroids on electroreceptors. Such evidence will depend upon *in vitro* studies of receptors or the demonstration that electroreceptors bind steroid hormones. Androgen-related changes in the morphology or physiology of the receptor cells comprising each Knollenorgan (cf. Bennett 1965; Szabo 1974) could underlie shifts in receptor cell potentials and frequency sensitivity. For comparison, androgens appear to induce changes in the surface area of the electric organ's excitable cells (electrocytes) (Bass et al. 1983, 1984) which could affect their passive properties such as total capacitance or active properties like the number and distribution of different ion channels. Such changes could underlie the androgen-induced transformation of EOD waveforms. Comparable morpho-physiological changes in the receptor cells of Knollenorgans may affect its frequency sensitivity.

Alternatively, re-tuning of electroreceptors may not depend upon changes in already existing receptor cells. Perhaps, there is a turnover of receptor cells periodically within a receptor organ, with the frequency sensitivity of new cells determined by the external EOD whose power spectrum may be changing as in androgen-treated fish. Such a turnover mechanism for a peripheral sensory receptor (as the olfactory epithelium, cf. Takagi 1971) would seem adaptive in view of the 'stress' that

a fish might experience during seasonal fluctuations in environmental variables. Furthermore, steroid hormones might stimulate a periodic turnover of receptor cells or change the rate of turnover and in this way have a direct effect upon the receptor organ and 'permit' its frequency sensitivity to be shaped or entrained by a changing EOD power spectrum. While such hypotheses are indeed speculative they remain testable with anatomical techniques.

Meyer and Zakon initially (see also Meyer et al. 1984) suggested that hormones could be exerting their direct effect on the tuning of electroreceptors and that shifts in EOD frequencies occur secondarily to match the best frequency of the receptor. However, we know for several vertebrate groups, including electric fish, that gonadal steroids are binding to muscles, and the medullary or spinal nuclei implicated in their motor control (Arnold et al. 1976; Breedlove and Arnold 1980; Erulkar et al. 1981; Kelley 1980; Fine et al. 1982; Segil et al. 1983; Bass et al. 1984). A primary, direct effect of hormones on the neuromuscular unit, as that controlling the EOD waveform or frequency, seems probable.

It is also intriguing to speculate that the development of frequency tuning of cells of some vertebrate auditory receptors and central auditory nuclei could be affected by external stimuli and possibly by gonadal steroids. 'Electrical' tuning has been compared to the second non-mechanical filter of auditory receptors (Viancour 1979). Crawford and Fettiplace (1981) provide support for the comparison – receptor potentials elicited from hair cells of the cochlea of turtles have oscillations whose frequency approximates the best frequency of the cell.

We are further impressed by the morphological data emphasizing the similarities between the modified hair cells of electroreceptors and those of auditory receptors (Szabo 1974), and the possible origin of electroreceptors (modified lateral line receptors) and auditory receptors from a common anlage, the dorsolateral placodes (cf. Northcutt 1980). Do these phylogenetic similarities extend to a functional level whereby both electroreceptors and auditory receptors have evolved comparable mechanisms for the development of sex (and species) differences in frequency tuning? Narins and Capranica (1976) have already shown a sex difference in the tuning properties of eighth nerve afferents in a tree frog (*Eleutherodactylus coqui*), although the basis for this difference is unknown (see Moffatt and Capranica 1978). Gonadal hormones may alter the morpho-physiological fea-

tures that underly the passive and active electrical properties of receptor cell membranes of both electroreceptors (cf. Bennett 1965, 1976) and auditory receptors. Similarly, the biophysical mechanisms underlying the development of frequency sensitivity in auditory receptors, as electroreceptors, may be susceptible to entrainment by external stimuli of an electrical or mechanical nature.

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