can indeed result in marked increases in the activity of this enzyme. In the investigation described here, both corticosterone and dexamethasone strongly depressed AChE activity in the presence of ETOH (41 and 65% of control; $p < 0.005$ and 0.05, respectively).

These studies thus demonstrate that corticosteroid hormones can abolish the ETOH-induced stimulation of ChAT (a rate-limiting enzyme of acetylcholine synthesis) in cultured fetal brain cells of the rat and can further reduce enzyme activity to below unstimulated values. In the presence of ETOH, corticosteroids were also shown to decrease the measured activity of AChE, a key enzyme involved in acetylcholine degradation. More extensive studies will be required to determine the mechanism of action for these steroid hormones in this system and also to determine whether such compounds may have a protective influence on neurologic and behavioral development during fetal alcohol exposure.

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Testosterone changes the electric organ discharge and external morphology of the mormyrid fish, *Gnathonemus petersii* (Mormyriformes)[1]

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Summary. Effects of silastic and pellet methyltestosterone implants on the waveform of the electric organ discharge of the weakly electric African mormyrid, *Gnathonemus petersii*, were investigated. Within seven days of implantation, the duration of the discharge increased dramatically while the associated peak power frequency of the Fourier spectrum decreased in all treated fish. By day 35, hormone-treated fish exhibited up to five-fold increases in EOD duration, as well as multiple discharges and variations in the shape of the positive phase of the discharge. Testosterone treatment also changed body morphology, making immature and adult female fish resemble adult males.
Key words. Gnathonemus petersii; Mormyridae; electric organ discharge; testosterone; sex differences.

Several species of mormyrid fish exhibit sex differences in the characteristics of their electric organ discharge. Such differences appear important for sexual identification[3]. However, the majority of mormyrids investigated failed to exhibit any sexual dimorphism in waveform or discharge pattern under laboratory conditions[4]. Male hormones administered to juvenile or adult females typically result in fish exhibiting male-like discharges[5]. However, only when a natural sex difference is evident under laboratory or field conditions, is it affected by exogenous gonadal hormone manipulation[5]. Thus, it is believed that treatment with gonadal steroids may predict naturally occurring sexually dimorphic electric-organ-discharge (EOD) waveforms[5].

Kramer and Westby[6] did not find a sex difference in the waveform of the EOD of *Gnathonemus petersii*. Landsman, Jou and Moller[7], however, reported a sex difference in the average peak power spectral frequency (PPSF) for this species. Only when the fish were recorded at rest, unrestrained in their shelters, did males exhibit peak power spectra of higher frequencies than females, with some overlap between the sexes. This sex difference was inversely related to the duration of the EOD, with male EODs shorter than those of females.

The present study investigated whether the EOD waveform of *G. petersii* can be influenced by testosterone, to substantiate a natural EOD sex difference in this species.

Materials and methods. Animals. Fourteen juvenile *G. petersii* (standard length: 10.2–12.0 cm; weight: 10.0–19.0 g) and one adult female (16.1 cm, 42 g) were randomly selected from large stock tanks (Lombardos African Fish Imports, Newark, N. J.). Fish were maintained individually in 20-l aquaria on a 12:12 L:D cycle, with lights on at 09.00 h. Water conductivity was kept at 200 ± 40 µS/cm and temperature at 23 ± 0.5 °C. *EOD recordings.* A pair of Ag/AgCl electrodes, extending from plexiglas tubes were fitted to the far ends of the aquarium, approximately 8 cm from head and tail of the fish when at rest in its porous ceramic shelter. EODs were fed directly into an oscilloscope (Tektronix, model 455) which triggered a spectrum analyzer (Hewlett-Packard, model 3582A, range: 0–25 KHz, resolution: 100 Hz). Both EODs and Fourier transformations were plotted with an X-Y plotter (Hewlett-Packard) and photographed from the screens of the oscilloscope and spectrum analyzer, respectively. All recordings were made between 15.00 h and 17.00 h.

Gonadal manipulation and hormone implants. The duration of the EOD was tested in each fish. Then, three of the juveniles were anesthetized (tricaine methane sulfonate, 1:20 000) and either 1) implanted with a silastic capsule (Dow Corning 0.065 inch o.d. and 0.03 inch i.d.) containing 1 mm/2.5 g b.wt packed 17α-methyltestosterone (17α-T) (Sigma), 2) gonadectomized and implanted with a similarly sized 17α-T

silastic capsule, or 3) gonadectomized and implanted with a 1 mg 17α-T pellet. The adult female fish was anesthetized, gonadectomized, and implanted with a 17α-T silastic capsule. The gonad was removed and/or implant inserted into the gut cavity through a 1.5-cm incision on the left ventral surface, approximately 5 mm posterolateral of the ventral fins. Five other juvenile fish were anesthetized, gonadectomized, and implanted with a silastic tube containing cholesterol to control for the effects of non-gonadal steroid hormones. The animals were returned to their aquaria, and all fish, experimental and cholesterol control, were treated with chloramphenicol (Sigma), an antimicrobial agent. Six of the juvenile fish were not subjected to any anesthetization or surgery and served as nonhandled controls. All operated fish, testosterone-treated and cholesterol-implanted controls, showed no visible deficiency in their motor behavior and fed normally.

Results and discussion. Figure 1 a shows a typical EOD of a juvenile *G. petersii* along with its Fourier transformation. Before treatment, we had verified that all fish exhibited EODs of 0.6–0.8 ms in duration with peak power spectrum frequencies (PPSFs) ranging between 2900 Hz and 4400 Hz (fig. 1 a). The EOD duration of the adult female was 0.8 ms with PPSF at 2900 Hz. The EOD duration for all control fish, nonhandled and cholesterol, remained between 0.6 and 0.8 ms throughout the study.

Methyltestosterone exerts profound effects on the EOD of *G. petersii* as evidenced by changes in duration, the associated PPSF, and pulseform. Within the first 7 days post-implant, the 17α-T implanted fish exhibited increased EOD durations ranging from 1.2 to 2.0 ms. These 'day 7' EOD durations (1.26 ± 0.32 ms) were significantly longer than the 'day 0' pre-implant durations (0.67 ± 0.06 ms), $t(3) = -3.67$, $p < 0.05$.

Figure 1 b illustrates changes in duration and Fourier transformations over the length of the study for a gonadectomized, 17α-T pellet-treated fish. By day 4 post-implant, the EOD length had increased from 0.6 ms to approximately 1.0 ms, while the associated PPSFs decreased from 4100 Hz to 2100 Hz. By day 35, the EOD lasted 3.4 ms, a more than 5-fold increase in duration, while the PPSF had decreased to 400 Hz. This increase in the duration of the pulse was clearly reflected in the first three phases of the discharge; the duration of the EOD was inversely related to its PPSF, $r(N = 8) = -.861$, $p < 0.01$, $r^2 = 0.74$.

All control fish exhibited PPSFs ranging from 2900 to 4400 Hz throughout the study. By post-implant day 7, fish #1 (silastic 17α-T implant, intact), fish #4 (silastic 17α-T implant, gonadectomized), fish #5 (17α-T pellet implant, gonadectomized), and fish #23 (adult female, silastic 17α-T implant, gonadectomized) exhibited decreases in the PPSF of the Fourier transformations of their EODs (see insert, fig. 2).

Figure 2 shows the mean PPSFs for all control and experimental fish. An analysis of variance revealed a significant treatment effect by post-implant day 7, $F(2, 12) = 49.50$, $p < 0.0001$, with the Tukey HSD indicating that the 17α-T mean PPSF (1700 ± 440 Hz) was significantly lower than the mean PPSFs for both the nonhandled (3725 ± 264 Hz, $p < 0.01$) and cholesterol (3560 ± 329 Hz, $p < 0.01$) control groups. There was no statistical difference between the two control mean PPSFs.

By day 15, the EOD duration for fish #4 reached its maximum length (2.2 ms) with PPSF of 800 Hz, after which it began to decrease in duration resulting in higher PPSFs (see insert, fig. 2). EOD durations increased with sharply decreasing PPSFs for all implanted fish until day 12, and then began to level off. Fish #5 exhibited the EOD of longest duration (3.4 ms, PPSF = 400 Hz), followed by #1 (2.8 ms, PPSF = 600 Hz), #4 (2.5 ms, PPSF = 850 Hz), and fish #23 (1.9 ms, 1300 Hz). The time-course of changes in PPSFs was identical in all fish with the exception of #4 (see insert, fig. 2).

Changes in EOD duration have been reported in other mormyrids treated with testosterone, but only in species that showed a sex difference under natural conditions[4]. Testosterone-treated immature fish and treated adult females typically exhibit a male-like EOD of longer duration[4], as was found for all of the treated *G. petersii* in the present study. This was unexpected in that results from previous studies[7,8] found that female pulses were longer than those of males. It has been suggested that hormone treatment may reliably induce naturally occurring EOD sex differences in mormyrids[4]. Our results appear to be incongruent with the previously reported sex difference in *G. petersii*, suggesting that at least for this species, testosterone treatment may not reliably indicate a naturally occurring EOD sex difference. It is also possible, however, that the physiological stress of laboratory housing may alter male EODs in the female direction. EOD duration and waveform obtained from mormyrids in captivity were reported to have undergone a natural sex reversal from maleness to femaleness, and such changes may be a function of rapidly fluctuating steroid

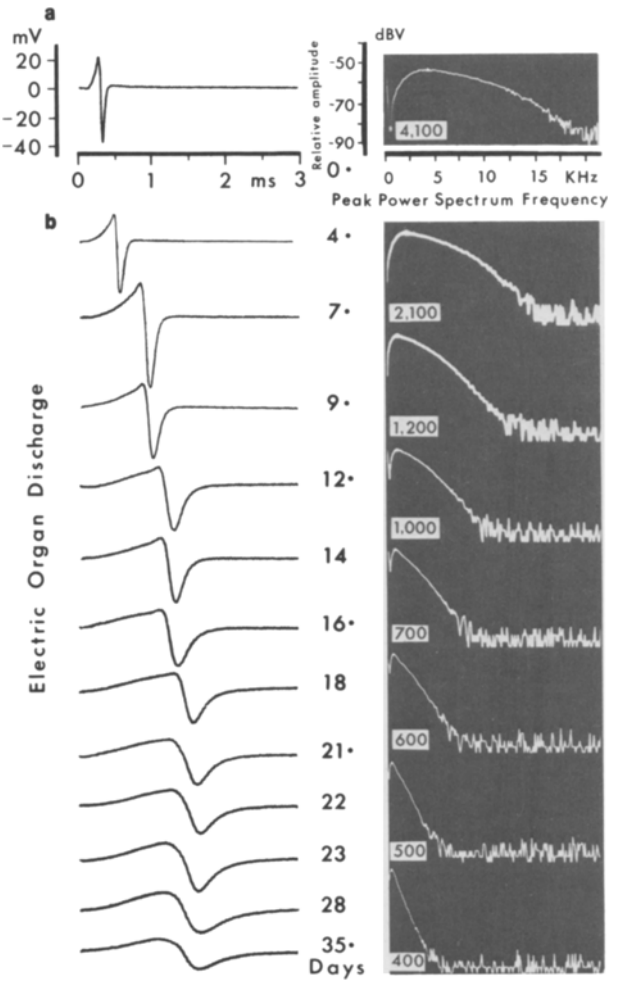


Figure 1. Electric organ discharge waveforms and associated Fourier transformations recorded from a juvenile *G. petersii*, fish #5. *a* Before and *b* following gonadectomy and 17α-methyltestosterone implantation for a period of 35 days. Note increase in EOD duration and corresponding decrease in peak power spectrum frequency. Fourier spectra (right panel) correspond in successive order to EODs (left panel) recorded on days marked with black dot.

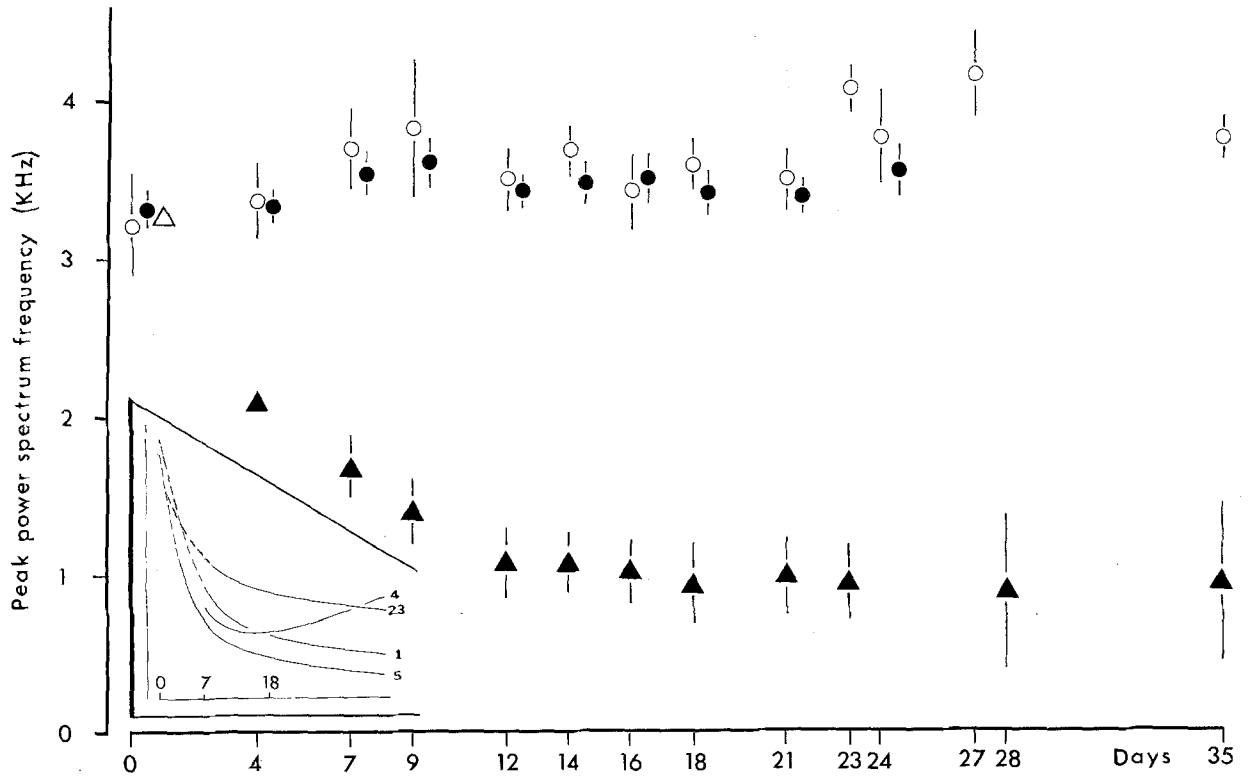
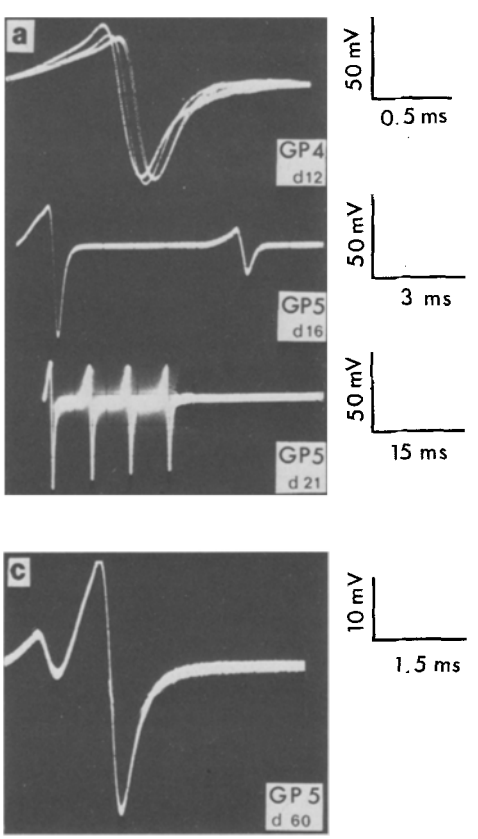


Figure 2. Time-course of hormone treatment on electric organ discharge waveform in *Gnathonemus petersii*. Mean peak power spectrum frequencies (PPSFs) and SEM for controls (open circles-nonhandled fish, n = 6; solid circles-gonadectomized, cholesterol-implanted fish, n = 5) and 17α-methyltestosterone-implanted fish, n = 4 (solid triangles). Mean pre-testosterone implant PPSF ('Day 0', open triangle) was estimated on the basis of pulse duration data. Day 4 solid triangle represents the PPSF from fish #5 only. Insert (left bottom corner) shows time-course over days of 17α-T treatment on PPSF for the individual fish.



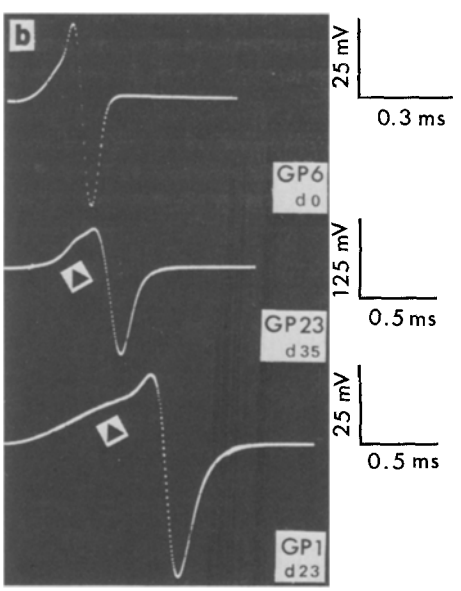


Figure 3. Effects of 17α-methyltestosterone treatment on EOD activity. *a* Multiple discharges, *b* EOD of an untreated fish and EODs of 17α-T implanted fish; note variations in ascending positive phase (arrowheads), and *c* EOD pulseform in fish #5 on day 60 post-implant.

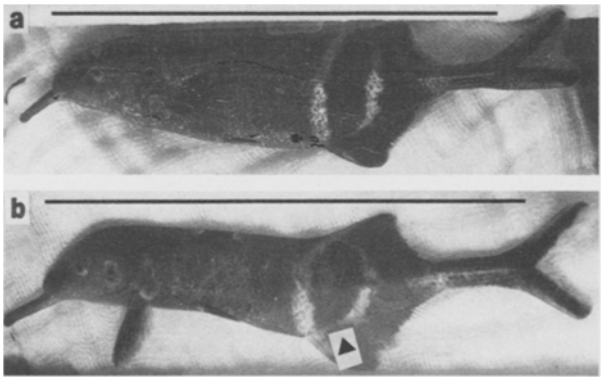


Figure 4. Change in external sexual characteristic in *G. petersii* #5. *a* Day 1 post-implant and *b* day 60 post-implant. Note indentation in base of anal fin by day 60 (arrowhead). Length of black bar: 10 cm. Emaciated appearance of fish is probably due to prolonged hormone treatment [13].

levels [4]. Fish exposed to acute or chronic stress from confinement, capture, and handling exhibited stimulation of the hypothalamo-pituitary-interrenal axis, marked by increased ACTH and cortisol levels, and suppression of the hypothalamo-pituitary-gonadal axis, marked by suppression of plasma androgens [9, 10]. Such environmentally induced hormone responses could explain the previously reported EOD sex difference in *G. petersii*. These differences were: a) accentuated by atypically high aquatic conductivity levels (above 700 µS/cm) [8], b) immediately abolished following physical restraint [7], and c) eliminated within 48 h following sudden, large conductivity changes [8]. Comparable sex-related alterations in EOD as a function of water conductivity were reported in *Pollimyrus isidori* [11]. Testosterone treatment effected other changes in the fish's EOD activity (fig. 3). 1) Frequently, the fish emitted trains of 2–6 multiple discharges that were identical in form and duration and were separated by 6.1-ms intervals (fig. 3a), suggesting autostimulation of the electric organ. Control fish never exhibited such EOD activity. 2) Treated fish exhibited characteristic variations in the ascending portion of the initial positive phase (fig. 3b). 3) On day 60, fish #5 switched between two pulseforms, the elongated EOD (as shown in fig. 1b on day 35) and a discharge of the same duration, best described as a succession of two biphasic pulses with the second lasting twice as long as, and exhibiting about 4 times the peak-to-peak amplitude of the first (fig. 3c). These changes in the generation of EODs could be related to a testosterone-induced increase in electrocyte membrane surface [4].

Testosterone treatment also resulted in external morphological changes. Figure 4 shows the appearance of an indentation in the dorsal margin of the anal fin in fish #5 [12]. The other three 17α-T implanted fish also exhibited this change, whereas none of the control subjects did. In several species of mormyrid fish, such indentation is characteristic of large, mature males. Although the extent of the hormone effects could, in part, be due to pharmacological doses, it is evident that gonadal hormones do influence the electro-communication system in *G. petersii*. Further studies will be required to resolve the issue of a hormone-dependent natural sex difference in the EOD of this species.

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Clonogenic growth of acute non-lymphocytic leukemia cells in serum-free medium

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Summary. We devised a serum-free medium for growth of leukemic colony-forming units (CFU-L), enriched with albumin, transferrin, lipids, insulin, hydrocortisone and oligoelements. Blast cells from 15 patients affected by acute non-lymphocytic leukemia were grown in this medium in the presence of human placental conditioned medium obtained under serum-free conditions (sfHPCM). Their clonogenic growth was comparable with that obtained in a serum-containing system. Furthermore, when serum-free cultures were carried out in absence of sfHPCM, either CFU-L growth was prevented or, if clones were obtained, the cultures showed a marked decrease in clonogenicity, indicating their strict dependence on growth factors.
Key words. Acute non-lymphocytic leukemia; serum-free medium; tumor cell cloning.

The in vitro culture systems employed in the study of hemopoiesis are generally supplemented with animal sera. Since serum may contain growth factors and substances capable of modulating their activity as well as molecules with inhibiting activity, the use of serum-free culture systems is required. Such methods, which were originally described for the study