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Coordination of EOD frequency and pulse duration in a weakly electric wave fish: the influence of androgens

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Summary. 1. The electric organ discharge (EOD) of wave-type weakly electric fish is generated as an extremely regular series of electric organ pulses. Measurements of pulse duration and EOD frequency were made in the species *Sternopygus* and *Eigenmannia*. Pulse duration is highly inversely correlated with EOD frequency in a population of fish, so that the EOD waveform remains quasi-sinusoidal over the species range of EOD frequencies. This places most of the energy of the EOD within the fundamental harmonic to which the electroreceptors are most sensitive.

2. Treatment with the anesthetic MS-222 transiently lowers EOD frequency, but does not change pulse duration. This demonstrates that pulse duration is independent of immediate EOD frequency and, therefore, of the medullary pacemaker nucleus (PMN).

3. In *Sternopygus*, small monophasic potentials could be recorded outside the tails of curarized fish. These were of constant duration and shape across all fish regardless of the fish's EOD frequency. The location along the body where this potential reversed polarity was close to, although not identical with, the isopotential line for the EOD pulse. These potentials were partially blocked by the curare. They probably represent the summed psps of the synchronously-firing electrocytes. This result supports the hypothesis that differences in EOD waveform, specifically pulse duration, arise from electrocyte membrane events following the psp (postsynaptic potential).

4. Implantation of *Sternopygus* with 5-α-dihydrotestosterone (DHT) in silastic capsules results in decreased EOD frequencies, as previously reported for this species (Meyer and Zakon 1982; Meyer 1983). In addition, corresponding significant increases in EOD pulse duration occurred (a mean increase of about 1.3 ms or 24%). No changes were measured in controls implanted with empty capsules. The maximum decrease in EOD frequency and increase in pulse duration plateau at different times, suggesting that they are the result of different processes. After removal of the hormone capsules, EOD frequency and pulse duration reverted to baseline levels within a few weeks.

5. These results demonstrate a degree of coordination between the PMN and the electric organ not previously recognized. It is likely that androgens play a role in this process. If, as proposed, the electrocyte membrane properties determine the characteristics of the waveform, then the androgen must act on both the PMN and the electrocytes to keep EOD frequency and pulse duration in register.

Introduction

The EOD of weakly electric fish is used as both a communication signal with conspecifics (Hopkins 1972, 1974a, b, 1980) and for electrolocation (Bastian 1981; Bullock 1982). The EOD is produced either by a myogenic (mormyriforms, all gymnotiforms except *Apteronotus*) or neurogenic (*Apteronotus*) electric organ in the tail. The cells of the electric organ, the electrocytes, are excited simultaneously and their action potentials sum to produce the EOD (Bennett 1971). The discharge frequency is determined by the firing frequency of the medullary pacemaker nucleus ([PMN]; Bennett et al. 1967; Ellis and Szabo 1980), which can be transiently modified for communication (Hopkins 1974a, b; Hagedorn and Heiligenberg 1985) or to

Abbreviations: *DHT* 5-α-dihydrotestosterone; *EOD* electric organ discharge; *FFT* Fast Fourier Transform; *PMN* medullary pacemaker nucleus; *psp* postsynaptic potential

preserve electrolocation abilities (the jamming avoidance response [JAR]; Bullock et al. 1972; Heiligenberg 1977).

Weakly electric fish may be classified into two groups based on the characteristics of the waveform of their discharge: 'wave' and 'pulse' fish. The quasi-sinusoidal waveform of wave fish can be thought of as a highly regular series of pulses. Most wave fish produce a simple, monophasic pulse each time the electric organ fires. The duration of the interval between successive pulses is the same as or only slightly more than the duration of each pulse, producing approximately a 50% duty cycle; hence the designation of the waveform as quasi-sinusoidal. The EOD of 'pulse' fish consists of brief pulses, produced at a highly variable rate. The interval between pulses is long compared to the duration of the pulse itself. The pulse waveform may be mono-, bi-, or triphasic, and so may be complex in the time domain (Bennett 1971; Bastian 1977; Westby and Kirschbaum 1982; Bass and Hopkins 1983, 1984, 1985; Hagedorn and Carr 1985).

Sexual dimorphisms are seen in the EOD in both wave and pulse fish. Male *Eigenmannia* and *Sternopygus* discharge at lower EOD frequencies, whereas male *Apteronotus* discharge at higher EOD frequencies than the females of those species (Hopkins 1972; Westby and Kirschbaum 1981; Meyer 1983; Hagedorn and Heiligenberg 1985; Meyer et al. 1986). In sexually dimorphic pulse fish, the waveforms of the two sexes may differ in the number, amplitude, and duration of various phases and, usually, in the total duration of the pulse (Westby and Kirschbaum 1982; Bass and Hopkins 1983, 1986; Hagedorn and Carr 1985). In all cases observed to date, the EOD pulse of females and juveniles is of shorter duration than that of mature males. These differences are likely due to the effects of steroids on the tissues involved in producing the EOD, as they may be elicited in the laboratory by treatment with sex steroids (Meyer and Zakon 1982; Bass and Hopkins 1983, 1985; Meyer 1983; Hagedorn and Carr 1985; Meyer et al. 1986). It is currently thought that in wave fish, sex steroids affect the PMN, thereby changing EOD frequency, whereas in pulse fish, these hormones affect the electric organ, where they influence pulse waveform properties. We have re-examined this line of thinking, and demonstrate that androgens also affect the EOD pulse duration in the wave fish *Sternopygus*, and that this effect is likely due to direct actions of the hormone on the spiking membrane of the electrocytes. Some of the preliminary results of this study have appeared elsewhere in abstract form (Mills and Zakon 1986).

Materials and methods

Experimental subjects. Twenty-seven *Eigenmannia virescens* and 44 *Sternopygus macrurus* were used. The *Eigenmannia* were supplied by a dealer (and were most likely from Peru), and the *Sternopygus* were caught in the Apure drainage basin, Venezuela. The *Eigenmannia* ranged in length from 12.5–21.0 cm, and in EOD frequency from 270–504 Hz; the *Sternopygus* from 11.5–40.7 cm, and 43–165 Hz. Length was measured from the tip of the snout to the tip of the tail with a ruler. The fish were housed in aerated 20–60 gallon tanks. *Sternopygus* were kept singly or in groups of less than eight. All *Eigenmannia* were in one tank. The aquaria were located in environmentally-controlled rooms with a 12 h light/12 h dark cycle. Tank temperatures were maintained at approximately 25 °C. Most tanks contained snails, gravel, live and/or plastic plants, and short lengths of PVC tubing for fish to hide in. The fish were fed a daily diet of frozen bloodworms (chironomid larvae) or live earthworms (only the *Sternopygus*).

Recording procedure. The fish were individually moved from their home tanks to a $39 \times 36 \times 16$ cm recording tank. The recording tank was maintained at 25.0 ± 0.2 °C, so that it would be similar to the home tank temperature. The larger *Sternopygus* were kept aligned with the electrodes by a flat plastic barrier placed in the recording tank parallel to the bar bearing the electrodes. All *Eigenmannia* and the smaller *Sternopygus* were suspended near one side of the recording tank, in a straight, narrow nylon net to restrict lateral movement. This was to prevent any distortion in EOD waveform which might have resulted from the fish bending its body during recording. There is some distortion in the EOD measurements if there are any obstructions within five electric organ lengths of the fish (Frey and Eichert 1972). Our recording tank was not large enough to prevent such distortions. However, this did not affect the validity of our measurements, since most of the distortion is in the EOD amplitude rather than duration. Also, because the fish were always in the same place when recordings were made, any distortions present appeared in all measurements, and so would not have affected the results.

EODs were recorded with head-to-tail electrodes (head positive) through a Grass P-15 AC-coupled amplifier (3 Hz–10 kHz passband). The electrodes were separated by a distance of 30 cm. After waiting at least 5 min for the fish to accommodate to the tank, two 400 or 800 ms samples of each fish's EOD were stored on a Nicolet 4094 digital oscilloscope, sampling rate of 100 or 200 μs per point, respectively. Unless otherwise specified, all EOD measurements were made with the Nicolet 4094. Upon completion of the experiments, the fish were returned to their home tanks. If the experiment required direct handling of the fish, Shieldex (Aquatronics) slime-coat inducer and/or MarOxy (Mardel Laboratories, Inc.) fungicide were added to the aquaria afterwards, to reduce the likelihood of infection.

Measurement of the location of the reversal in polarity for the EOD pulse was made with a fish in a net in the center of the recording tank. The EOD was recorded differentially between an electrode near the body and an indifferent electrode. The indifferent electrode was placed in a beaker filled with aquarium water and contacting the recording tank through a wick, in order to reduce contamination by the EOD. The elec-

trodes were made from insulated copper wire and the exposed tip was 1 mm in diameter. The active electrode was placed near the middle of the tail and moved along the fish's body in millimeter increments by a micromanipulator. Traces were stored on the Nicolet digital oscilloscope or passed to a storage oscilloscope. EOD pulse duration was measured similarly by placing the active electrode in various locations along the fish's body. Repeated measurements showed the accuracy to be ± 0.1 ms.

MS-222 treatment. Three *Sternopygus* were examined. They were individually placed in a shallow plastic tray containing 2000 ml tank water. The tray was floated in the recording tank, and the water in the tray was kept at an average temperature (for all recording sessions) of 25.3 ± 0.3 °C. The temperature varied during each recording session by no more than ± 0.1 °C. Two head-to-tail samples of the EODs were stored every minute for 10 min as control data. Next, each fish was given 50 mg of the anesthetic MS-222 (tricaine methanesulfonate, Sigma Chemical Co.) every 2 min, until the EOD ceased. The EOD was recorded every minute, as long as the discharge was present. The total dose of the drug was 200 mg for two of the fish and 250 mg for the third. A similar experiment was carried out on six *Eigenmannia*.

Measurement of 'psps'. Two methods were used to record 'psp' (postsynaptic potential) durations. For the first method, fish were injected with curare (0.1–0.2 mg/g) in the dorsal musculature of the back and placed in a net near one side of the recording tank. The EOD was recorded differentially with one electrode placed near the tip of the tail and the other, acting as an indifferent electrode, at the other side of the tank (a distance of about 20 cm). After injection, fish usually lay still in the net, thereby permitting the placement of the electrode nearby to record the changes in the EOD as the curare took effect. Data from fish which moved during this time were discarded. Measurements of pulse duration were made directly from stored traces on the oscilloscope or from photographs of traces. Repeated measurements of the same traces gave a measurement error of ± 0.1 ms.

The second technique allowed us to measure the 'psp' of uncurarized fish. We utilized the same step as in recording reversal site for the EOD. With the electrode carefully placed so as to lie at the site of reversal in potential for the EOD, the 'psp' potential could still be recorded over a short segment of the tail.

Hormone treatment. Nine *Sternopygus* were housed in three 20 gallon tanks, each with two water-permeable plastic dividers, so that each fish had a separate compartment. The aquaria were maintained at approximately 25.0 °C. EOD records from each fish were taken and then animals were implanted with either Silastic capsules containing 5-α-dihydrotestosterone (DHT, Sigma Chemical Co.) or empty Silastic (Dow Corning) capsules (see Keller et al. 1986). Each tube was placed in the peritoneal cavity of an MS-222-anesthetized fish, through a small hole cut on the anterior ventrolateral part of the fish, slightly caudal and ventral to the pectoral fin, on the right side. Each capsule was pushed as far anteriorly as possible to reduce the chance of its falling out later. After insertion, the hole was sealed with histoacryl tissue glue (B. Braun Melsungen AG, West Germany). The fish were returned to their home tanks after recovering from the anesthesia. EODs were recorded and stored every 6 to 8 days following implantation for a total of 8 weeks. The temperature of the recording tank was 25.1 ± 0.2 °C throughout the experiment with a maximum variation of ± 0.1 °C within a single recording session.

On day 57 post-implantation, the capsules were removed from three of the experimental fish, allowing them to return to their original EOD frequencies (Meyer 1983). An incision of 1–2 cm was made at the implantation scar, and the capsule was removed with forceps. The cut was then sutured and sealed with tissue glue. To control for any effects of surgery alone on the EOD, the other three experimental fish and the three control fish were incised, forceps were moved around inside the fish, and the cut was sutured and sealed without capsule removal. All fish were sexed at the end of this experiment. To be certain of accurate identification, a sample of gonadal tissue was removed and inspected under the microscope.

Implantation rather than injection was chosen to administer the DHT for two reasons. The use of implants appears to be less stressful to the fish than daily injections (Keller et al. 1986). This helps to eliminate any effects of stress on the EOD (Mazeaud et al. 1977). Another advantage is that implants ensure a more constant release of the hormone than daily injections. Although blood androgen concentrations were not determined in this experiment, measurement of the plasma DHT concentration in *Sternopygus* implanted in this way in a previous study indicated levels on the order of a few to ten ng/ml (Keller et al. 1986), which is within the normal physiological range of androgen titers in teleosts (Fostier et al. 1983). (DHT is likely an analog to the normally occurring androgens in most teleosts, testosterone and 11-ketotestosterone).

Data analysis. Measurements of pulse duration (the positive-going phase of a single EOD cycle) and interval duration (the time between the end of one pulse and the beginning of another), in ms, were taken from baseline. Baseline was established by lining up the horizontal cursor of the oscilloscope screen with the bottom of the displayed waveform. Five pulse duration and five interval duration measurements were taken from each of the two stored waveforms, and mean values were calculated for each measure. Thus each data point represents ten measurements of each parameter for each fish. In addition, duty cycles were calculated as pulse/(pulse + interval), to determine the relationship of this variable to EOD frequency. EOD frequency was calculated as 1/(pulse + interval), the inverse of the period. EOD frequencies were not adjusted for temperature, as there was minimal temperature drift (see Enger and Szabo 1968).

A Fast Fourier Transform (FFT) program, which was supplied with the Nicolet 4094, was used to analyze the distribution of energy in the EOD waveform into its component series of harmonics. For the fish not involved in the hormone or MS-222 studies, samples from waveforms of one or two high, medium, and low EOD frequency fish were analyzed by FFT. The heights of the first three peaks (little energy was seen in the higher harmonics) of the resulting power spectra were measured, and converted to a decibel (dB) scale for comparison. For the MS-222 experiment, one FFT was performed on a waveform sample from each fish prior to, and another after, administration of the anesthetic and the ratios of amplitudes of the first three harmonics were compared. EOD waveforms of the DHT-implanted and control fish were also analyzed before implantation, again at 35 and 56 days post-implantation, and on days 32 and 63 following capsule removal.

Statistical tests. Two statistical tests were employed to analyze the data from the hormone-treated fish. Comparisons made within a group were analyzed with the Wilcoxon matched pairs signed ranks test. Comparisons between groups were analyzed with the Mann-Whitney U test. Both were one-tailed tests, with a significance level of $P \leq 0.05$.

Results

EOD characteristics

The EOD of both *Eigenmannia* and *Sternopygus* is a nearly sinusoidal wave. The waveform of *Eigenmannia* is smoother and more rounded at the bottom. The EOD of *Sternopygus* has a flatter baseline, which may be horizontal or sloped. Figure 1 shows the EODs of three individual *Sternopygus*, with discharge frequencies ranging from high to low values. The EODs of *Eigenmannia* essentially resemble the top trace of this figure (Fig. 1 A), except on a faster time scale. In *Sternopygus*, but not *Eigenmannia*, a small inflection can sometimes be seen in the rising phase of the EOD, especially in fish with low frequency EODs (arrows in Fig. 1 B and C).

Measurement of EOD pulse duration at various locations along the body in *Sternopygus* ($N=4$) indicate that the pulse is of virtually identical duration for each individual. However, pulse duration varied considerably *between* fish. Note in Fig. 1 that the duration of a single EOD pulse increases as EOD frequency decreases in these three fish. This relationship is true for both *Eigenmannia* and *Sternopygus* (Figs. 2 and 3).

Figures 2A and 3A quantitatively show that fish of higher EOD frequencies have shorter pulse durations than fish of lower EOD frequencies. *Eigenmannia* (Fig. 2A) pulse durations range from about 1.7–2.3 ms (mean $=2.0\pm0.2$ ms), a span of only 0.6 ms (35% increase) over a 234 Hz (87% increase) frequency range. The interval durations (Fig. 2B) are from 0.3–1.4 ms (mean $=0.7\pm$ 0.3 ms). In contrast, *Sternopygus* pulse durations are from 4.0–13.6 ms (mean $=7.3\pm1.9$ ms), a difference of 9.6 ms (240% increase) over a 122 Hz (284% increase) frequency range (Fig. 3A). The interval durations are from 2.0–9.6 ms (mean $=$ 4.8 ± 2.1 ms; Fig. 3B). Like pulse duration, then, the interval durations are shorter in higher frequency fish than in lower frequency fish. Not surprisingly, pulse duration and interval duration are strongly correlated with EOD frequency ($r=-0.90$ and -0.94, respectively, in *Eigenmannia*, $r=-0.89$ and -0.91, respectively, in *Sternopygus*).

In order to determine whether pulse and interval durations co-vary by the same amount in fish of various EOD frequencies, the duty cycle was calculated for both species. Duty cycle, which is shorter in lower frequency fish, ranged from 61–84%, (mean $=74\pm7\%$) in *Eigenmannia*; in *Sternopygus*, duty cycle ranged from 53–74% (mean $=62\pm6\%$). This was also highly correlated

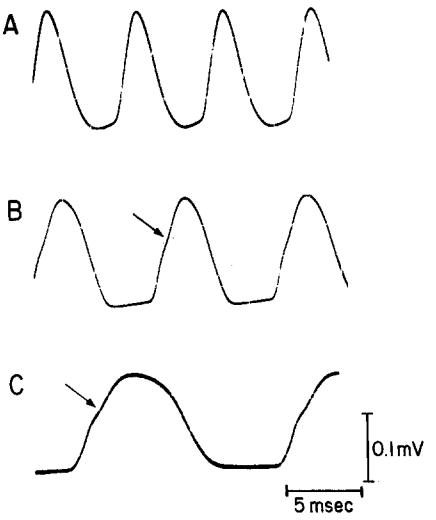

Fig. 1. The EODs of three *Sternopygus*. **A** EOD frequency = 160 Hz. **B** EOD frequency = 125 Hz. **C** EOD frequency = 70 Hz. Arrows denote inflections

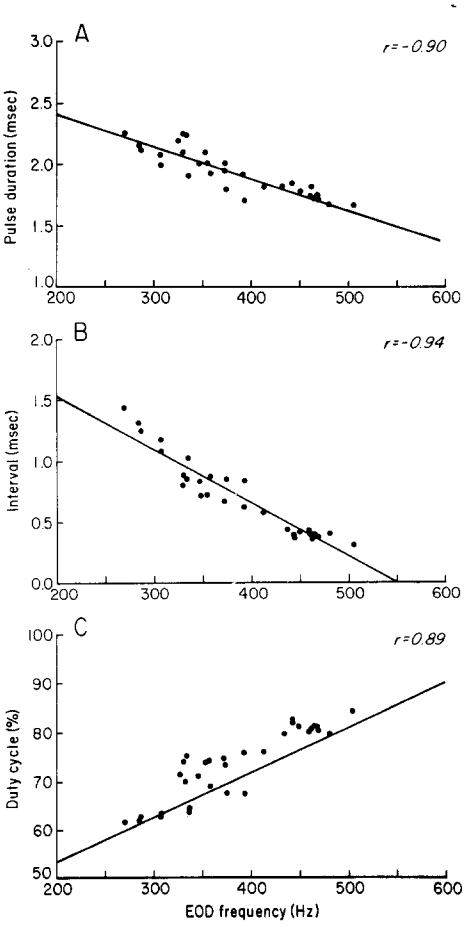

Fig. 2. Pulse duration (**A**), interval duration (**B**) and duty cycle (**C**) as a function of EOD frequency for *Eigenmannia* ($N=29$). The regression line is drawn and the correlation coefficient is in the upper right-hand corner of the figure. Each point represents data from one fish

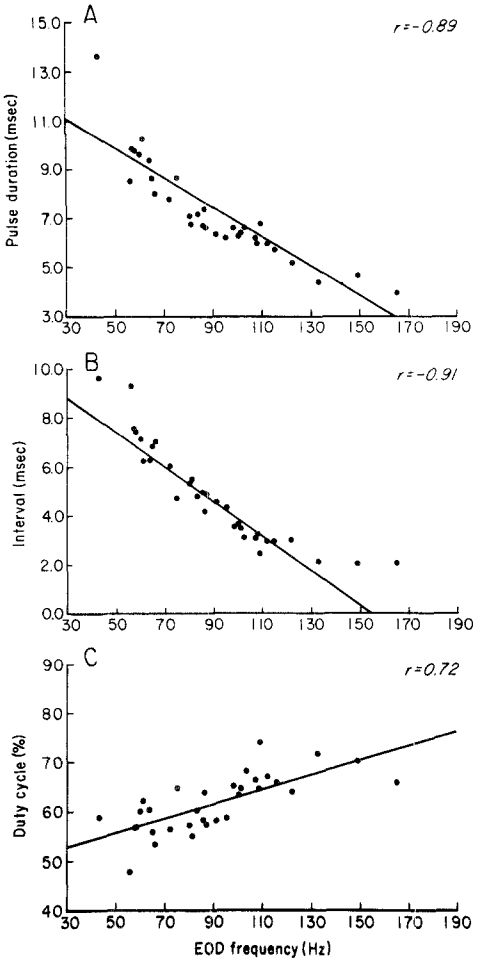

Fig. 3. Pulse duration (**A**), interval duration (**B**), and duty cycle (**C**) as a function of EOD frequency for *Sternopygus* ($N=32$). The regression line is drawn and the correlation coefficient is in the upper right-hand corner of the figure. Each point represents data from one fish

with EOD frequency (Figs. 2C and 3C; *Eigenmannia*: $r=0.89$; *Sternopygus*: $r=0.72$).

There was no detectable correlation between length and EOD frequency in either *Sternopygus* ($r=-0.34$) or *Eigenmannia* ($r=0.08$). There is a demonstrated correlation between these two parameters within each sex in *Sternopygus* such that they are negatively correlated in males and positively correlated in females (Hopkins 1974b; Meyer 1983). However, as we did not note the sex of the fish and therefore pooled the data from both sexes for analysis, the lack of correlation between length and EOD frequency is not surprising.

FFT data showed that most of the energy of the EOD is in the fundamental harmonic. The higher harmonics were low in amplitude compared to the fundamental. In *Eigenmannia*, the first harmonic is 8.0–15.4 dB (mean = 11.7 dB, +3.3 dB, −5.5 dB) greater in amplitude than the second

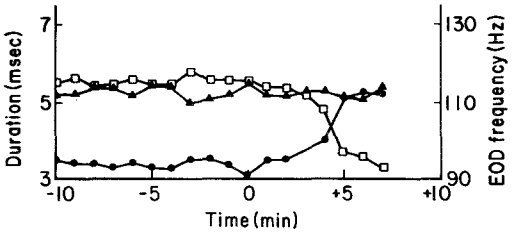

Fig. 4. Pulse duration (filled triangles), interval duration (filled circles) and EOD frequency (open squares) of a representative *Sternopygus* before and during treatment with MS-222. Time 0 represents the first in the series of doses of the drug administered. Note that pulse duration is constant throughout, whereas EOD frequency decreases and interval duration increases

harmonic, and 21.6–24.1 dB (mean = 23.0 dB, +1.2 dB, −1.3 dB) greater than the third. In *Sternopygus*, the first harmonic was 7.0–15.8 dB (mean = 14.1 dB, +2.0 dB, −2.7 dB) greater than the second, and 17.8–27.8 dB (mean = 22.5 dB, +2.9 dB, −4.3 dB) greater than the third. There was no obvious relationship between EOD frequency and relative amount of energy in the first three harmonics. Hence, most of the energy produced by the electric organ is alloted to the fundamental harmonic, and very little to the higher harmonics. This is to be expected since, with shifts in pulse duration, the EOD remains nearly sinusoidal across a range of EOD frequencies.

Determinants of pulse duration

Two experiments were undertaken to determine whether the duration of the pulse is controlled at the level of the electric organ, as is usually the case (Bennett 1971; Bass and Hopkins 1983; Hagedorn and Carr 1985), or whether some aspect of pulse duration is additionally influenced by central mechanisms. First, in order to observe if the EOD pulse duration is affected by the firing frequency of the PMN on a cycle by cycle basis, the PMN was slowed down by MS-222 (Bullock et al. 1972). Second, the EOD pulse was blocked by curare to see if the potentials that remain, which we believe to be summated electrocyte postsynaptic potentials (psps), differ in their duration.

The effects of MS-222. The EOD frequencies of all of the MS-222-treated *Sternopygus* declined an average of 27 ± 9 Hz (range = 21–37 Hz) during the first 7 min; subsequently, the EOD abruptly ceased. Figure 4 shows the data from a typical fish. The control data, from the same fish prior to anesthetic treatment, showed a mean change of 0.3 ± 2.2 Hz (range = +2 to −3 Hz). The pulse durations varied no more than ±0.3 ms during both

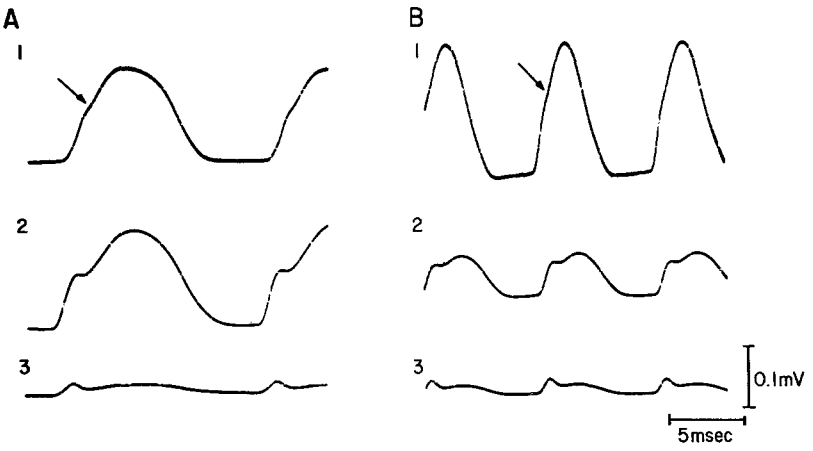

Fig. 5A, B. Effects of curare on the EOD. **A** data from a fish with an EOD frequency of 70 Hz, **B** data from a fish with an EOD frequency of 125 Hz. In both cases the EOD wave is shown: 1) prior to, 2) ~3 min after, and 3) ~5 min after injection of 10–20 mg curare. Note inflections (arrows) in baseline traces

the control and drug experiments. Interval durations from the control data varied at most ±0.1 ms (Fig. 4). The experimental data yielded a mean increase in interval duration of 2.0±0.3 ms. The duty cycle, which during the control period varied no more than 4%, dropped 9–17% (mean=13± 3%) in the three fish during the experimental period. Similar results were obtained with *Eigenmannia*. Thus, MS-222 acutely lowers EOD frequency without affecting pulse duration. This decrease is maintained over several minutes, during which tens of thousands of EOD cycles are produced.

As the effect of MS-222 is to increase the interval between pulses with no effect upon pulse duration, the EOD becomes less sinusoidal. This is shown in the FFT. Baseline recordings showed second harmonics from 4.8 to 15.9 dB (mean= 11.0 dB, +4.4 dB, −9.4 dB) down from the fundamental and the third harmonic down 22.3 to 23.1 dB (mean=22.8 dB±0.4 dB) from the fundamental. After MS-222 treatment, the second harmonic was only 3.2 to 9.1 dB (mean=6.4 dB, +2.6 dB, −3.7 dB) down and the third was 15.3 to 23.0 dB down (mean=19.5 dB, +3.2 dB, −5.1 dB). This indicates that the energy in the EOD was partially redistributed to the higher harmonics as the EOD became less sinusoidal.

Measurement of the 'psps'. The amplitude of the EOD began to decrease 3–5 min after injection of curare. As the amplitude fell, the small inflection became more noticeable (Fig. 5). The amplitude of the pulse waned rapidly within the next few minutes, until the pulse was almost or completely absent. This revealed the initial inflection to be the rising phase of a smaller potential. However, the amplitude of this potential also diminished as the EOD pulse fell. This small potential could either represent the 'spinal command signal' which drives the electric organ, or the summated postsynaptic potentials of the synchronously-driven electrocytes. We feel that the latter interpretation is most likely correct (see Discussion), so we refer to the small potential as the 'psp' component. Figure 6 indicates that the 'psp' duration is virtually identical (~1.9 ms) in all fish recorded, even though the pulse duration of their EODs varied from almost 10.0 ms to less than 4.0 ms.

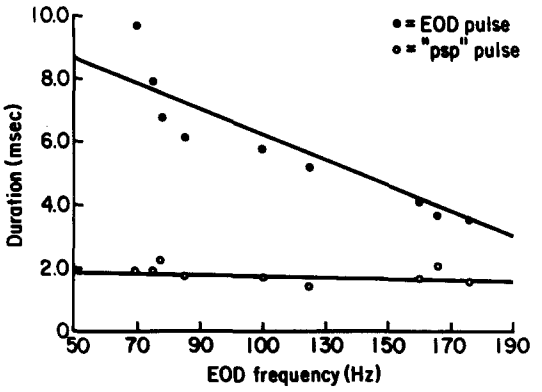

Fig. 6. Duration of the EOD pulse (open circles) and the 'psp' pulse (filled circles) as a function of EOD frequency ($N=9$)

Since the electric organ acts as a dipole, the amplitude of the EOD falls to zero as an electrode reaches the organ's electrical midpoint and the EOD reverses polarity on the other side of it. When the electrode was moved along the fish's body after curarization, the 'psp' was seen to drop to zero and reverse its polarity at about the same place as the EOD before curarization (2 fish). In one additional fish with a regenerated tail, the reversal point for both potentials was again at about the same location, but 3–4 cm farther back along the body than normally observed.

After making these initial measurements, we discovered that the 'psp' could be recorded in uncurarized specimens so that reversal points for both EOD and 'psp' could be determined with

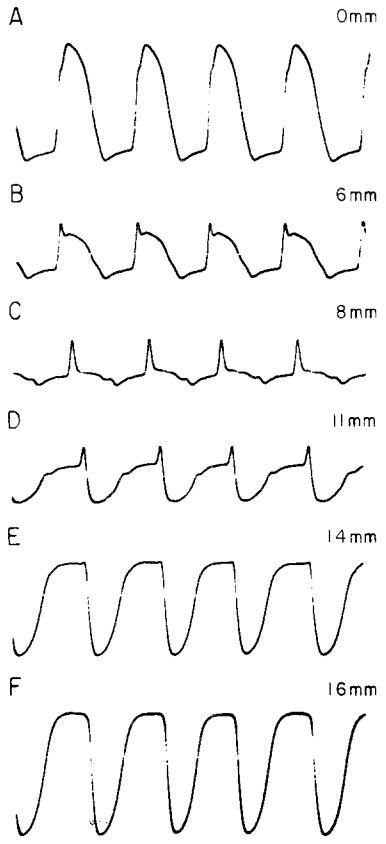

Fig. 7A–F. Difference in reversal point for EOD and 'psp' pulse. Electrode positioned in various locations along the tail flanking both reversal points. It is moved progressively more caudalward from its initial position (**A**), arbitrarily designated as 0 mm, through a series of other positions (**B–F**). The distance of each position with respect to the zero point is given in the left corner above each trace. Calibration bar = 20 ms

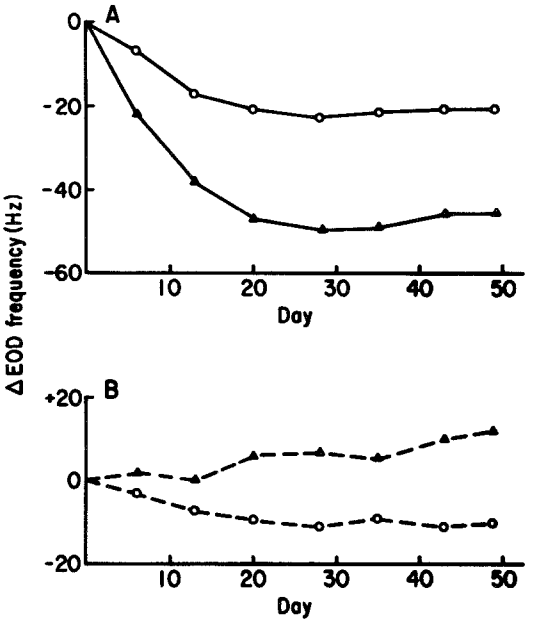

Fig. 8. Change in EOD frequency over time in 2 experimental (**A**) and 2 control (**B**) fish. The four fish represented are those of the largest and smallest change in pulse duration within each group. Notice that both DHT-treated fish show a marked decline in EOD frequency. The control fish do not change significantly

greater accuracy in a fish without changing its position. Observation of the location of reversal sites for the 'psp' (Fig. 7E) and EOD pulse (Fig. 7C) components in noncurarized fish indicated that they are similar, although not identical. Figure 7 illustrates the change in EOD as the active electrode approached and passed the location of the polarity reversal for the EOD. The 'psp' then reversed and blended into the EOD pulse. Note that there was a difference of 6 mm in the location of the zero isopotential lines of these two components (see Discussion). Measurements of 'psp' duration made under these conditions also give its duration at about 1.8–1.9 ms.

The effects of DHT

As reported previously (Meyer and Zakon 1982; Meyer 1983; Zakon and Meyer 1983; Keller et al. 1986), the EOD frequencies of all of the androgen-implanted *Sternopygus* decreased significantly from the control fish as early as the first measurements made (day 6 following implantation). Figures 8 and 10B show the changes in EOD frequency over the course of the experiment. By day 6, the frequencies of the experimental fish were also significantly different from their day 0 values. The maximum decrease for each fish (-20 to -50 Hz or 20.1 to 40%) occurred between the 20th and 28th day following implantation. The control fish achieved a maximum change in EOD frequency between days 20 and 49, but this change, ranging from -12 to $+12$ Hz, was not significantly different from the day 0 values. The maximum percent changes were from -8.6 to $+11.8$%. Most EOD frequencies of experimental fish had increased by up to 5.5 Hz from their lowest values by day 35, and then plateaued. Figure 8 shows the rate of change of EOD frequency in four fish: the experimental (Fig. 8A) and control (Fig. 8B) fish showing the most and least change in pulse duration. The average decrease in EOD frequency in DHT fish was -35.3 ± 12.7 Hz, or -28.9 ± 7.0%. The average change in EOD frequency in control fish was -3.7 ± 13.6 Hz, or -2.9 ± 11.0%. Figure 10B displays the average EOD frequencies of the control and experimental fish over time.

In the experimental fish, interval duration increased 61–122% (mean $= 92 \pm 23$%), and the duty

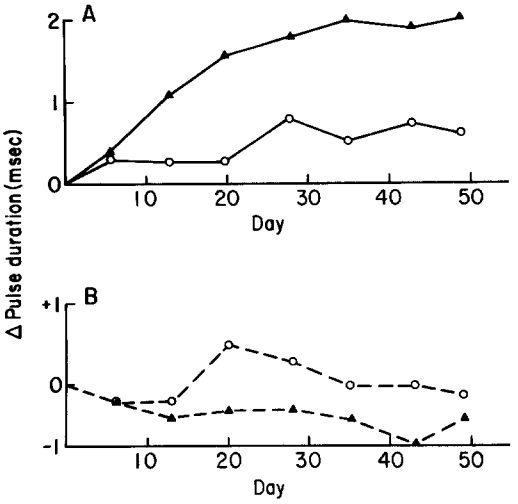

Fig. 9. Change in pulse duration over time in 2 experimental (**A**) and 2 control (**B**) fish. The data are from the fish which showed the most and least change in pulse duration within each group (same fish as in Fig. 8)

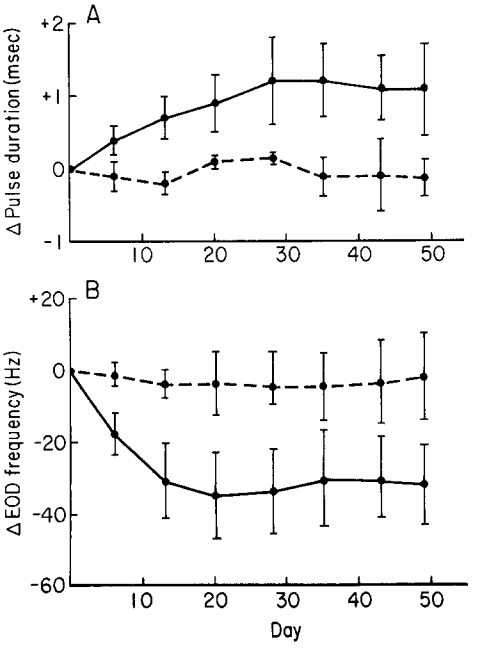

Fig. 10. Each point represents the average change from the pretreatment (day 0) values of the six DHT fish or the three control fish. (**A**) Change in pulse duration. (**B**) Change in EOD frequency

cycle decreased −15–21% (mean = −18 ± 2%). These changes were statistically significant from the day 0 values. In the control fish, the change in interval duration varied from −18 to 31% (mean = +8 ± 18%), and in duty cycle from −11 to +4% (mean = −3.2 ± 5.5%). These values varied without any particular trends, and the differences were not statistically different from the day 0 values.

Pulse durations of the hormone-implanted fish gradually increased following implantation, and were significantly different from pre-implantation values by as early as the first measurements taken (day 6); the changes were also significantly different from those of the control fish. The maximum increase was from 0.8–2.3 ms (13.3–43.4%), mean by day 28. Figure 9A shows the pulse durations of two hormone-treated fish, the ones which underwent the most and least amounts of change in pulse duration. Pulse durations of control fish had changed at most −0.7 to +0.5 ms (−10.9 to +9.6%) at their maximum change (Fig. 8B). The mean maximum change in pulse duration of DHT fish was +1.3 ± 0.6 ms, or +24.4 ± 11.7%. The mean maximum change in control fish was +0.1 ± 0.7 ms (+2.5 ± 11.6%), but was not significantly different from the day 0 values. Figure 10A illustrates the mean changes in pulse duration over time in the control and DHT fish.

The average rate of increase from the day of implant in hormone-treated fish to the day the maximum value of pulse duration occurred was 3.0 ± 0.6%/day. During this time, the rate of change of EOD frequency was 4.5 ± 0.7%/day. These rates of change are not strikingly different. The days at which the average values of EOD frequency and pulse duration plateaued at their new values, however, differed: EOD frequency by day 20, pulse duration by day 28. Therefore, androgen treatment gradually decreases EOD frequency and increases pulse duration, but the changes in these two characteristics follow a different time course and so must be due to different processes.

On day 57, implants were removed from three of the DHT-implanted fish. These fish showed a gradual decrease in pulse duration and a concurrent increase in EOD frequency approaching, to within 3% of, baseline values. Both parameters reached their maximum recovery by day 63 post-removal. The recovery occurred with a slower rate and took almost twice as much time to reach maximum change as the initial DHT-induced changes (recovery rates of 1.7 ± 0.2%/day for EOD frequency, 1.5 ± 0.2%/day for pulse duration). In contrast, the 3 fish whose implants were not removed retained their lowered EOD frequencies and lengthened pulse durations.

Recordings of 'psp' duration were made in hormone-implanted ($N=3$), control ($N=2$) and implant-removed ($N=2$) fish by placing an electrode on the reversal point for the EOD pulse. Accurate measurements could be made from un-

curarized fish which were stationary inside a narrow net, as described previously. The 'psp' duration of all individuals was between 1.8–2.0 ms, showing that it was uninfluenced by androgen treatment.

Examination of gonads at the termination of the experiment indicated that individuals of both sexes were included in both the hormone and control groups. Thus, as has been previously demonstrated for the PMN and electroreceptors in this species (Meyer 1983; Keller et al. 1986), androgen affects the electric organ of both sexes.

Discussion

The relationship between pulse duration and EOD frequency

We have analyzed the EOD of wave fish in terms of the duration of the positive-going phase of the single EOD cycle. Bennett (1971) showed that each EOD pulse arises from a head-negative baseline, so we measured pulse duration from that baseline, which is most consistent with the biophysics of pulse production. Furthermore, Bennett (1971) found that the individual synchronously-fired action potentials which sum to produce the EOD are identical in appearance and duration to the EOD pulse. The waveshape of the EOD pulse is thus a good reflection of the waveshape of the action potentials, and the interval of the EOD represents the time during which the electrocytes are repolarized, before being instructed to fire again.

In *Eigenmannia* and *Sternopygus*, there is a high correlation between EOD frequency and pulse duration. Fish of higher EOD frequencies have shorter pulse durations, while fish of lower EOD frequencies have longer pulse durations. The EODs of both *Eigenmannia* and *Sternopygus* are nearly sinusoidal, based on appearance and power spectra. The high correlation between EOD frequency and pulse duration in both species is therefore not surprising. For a waveform at a given frequency to be sinusoidal, pulse duration and interval duration must be nearly equal (duty cycle = 50%). The interval between successive pulses, then, is greater in low frequency fish, and lesser in high frequency fish. Duty cycles measured here were greater than 50%, so the EOD deviates from being a perfect sinusoid, especially at high frequencies. It is interesting that the pulse durations varied less among *Eigenmannia* than *Sternopygus*, despite the greater frequency variation in *Eigenmannia*.

Gottschalk (1981) analyzed the waveforms of the wave fish *Sternopygus* and *Eigenmannia* in terms of the head-positive (P) and the head-negative (N) half-waves of a single EOD cycle, measured from the zero potential line. He showed that the P/N ratio is directly correlated with EOD frequency. In agreement, we find that the duty cycle, a ratio of pulse duration/(pulse + interval), is directly correlated with EOD frequency. Both measures demonstrate that the EOD pulse and interval durations decrease with EOD frequency but that the interval duration decreases more. He also found that the P/N ratios differ between male and female *Sternopygus* but not *Eigenmannia*. We did not sex our fish during the first part of the study. Nevertheless, since there is a sexual dimorphism in EOD frequency in *Sternopygus* such that EOD frequencies of females are above and males below 100 Hz (Hopkins 1972; Meyer 1983), there is undoubtedly a difference in the duty cycles of the two sexes. In our study, pulse duration was always greater than or equal to the interval duration, while in Gottschalk's, the opposite was true. However, this difference can be attributed to differences between the measurements of pulse duration in these two studies.

The correlation between pulse duration and EOD frequency, whether in the untreated fish or following DHT treatment, results in the maintenance of a nearly sinusoidal EOD waveform. This is evidenced by comparison of the results of Fast Fourier Transforms of waveforms of fish with different EOD frequencies, to those from fish during the course of an MS-222 experiment, in which case the sinusoid is not preserved. Under MS-222, as the EOD frequency decreases but pulse duration remains constant, more energy appears in the higher harmonics. The importance of this lies in the nearly exclusive electroreceptor sensitivity to the fundamental harmonic of the EOD waveform; higher harmonics are useless, because the receptors cannot detect them. Thus, in order to most efficiently expend energy in signal production, the EOD must be kept sinusoidal.

Aside from the necessity for preservation of a sinusoidal waveform, another potential role for the differences in pulse duration may be for temporal encoding of EOD during communication. Physiological and behavioral evidence indicates that the temporal properties of the EOD waveform in this species provide sufficient information to identify the fish as a female conspecific. Gottschalk (1981) demonstrated that the response of a male *Sternopygus* to a conspecific female EOD stimulus was inversely correlated with P/N ratio. Hopkins and Bass (1981) have shown that temporal cues alone can stimulate species recognition.

MS-222-induced changes in EOD

Treatment with MS-222 resulted in a constant pulse duration with decreasing EOD frequency, showing that the two can be manipulated independently. At least in the short term, then, pulse duration is not determined by the medullary pacemaker nucleus, as is EOD frequency.

In some respects, it may be surprising that a general anesthetic like MS-222 affects the PMN but not the electrocytes. Presumably, the effect of MS-222 on the pacemaker is to block voltage-sensitive Na^+ and K^+ channels (see Shanes 1950; Taylor 1959; Blaustein and Goldman 1966; Caldwell 1976 for the effects of cocaine and its derivatives on excitable tissues). The electrocyte membranes also contain voltage-sensitive Na^+ and K^+ channels (Keynes and Martins-Ferreira 1953; Nakamura et al. 1964; Bennett 1971), which are active in spike production. Why then would the drug not affect the excitable membranes of the electric organ? One explanation is that once it enters the gill capillaries, it takes longer to reach the electric organ than the brain. This appears unlikely, because a fish anesthetized with MS-222 for over an hour showed no change in pulse duration. It is possible that the properties of the MS-222-targeted active ion channels in the pacemaker might be different in structure from any corresponding channels in the electric organ, and thus are not affected by the drug. In fact, there is evidence (Nakamura et al. 1964) that at least the K^+ channels in the electrocytes have properties other than those of classical squid axon K^+ channels (Hodgkin and Huxley 1952). On the other hand, the K^+ channels of the PMN are likely to be A-type (P. Stys and L. Maler, personal communication). In any case, it appears that MS-222 does not affect the electrocytes.

Identity and significance of the 'psp'

Classic work on the neuromuscular junction (Fatt and Katz 1951) has relied on the fact that low doses of curare block the muscle action potential, allowing underlying psps to be studied. We suggest a similar phenomenon occurs at the neuroelectrocyte junction. Curare exerts its effects by blocking the psps in the electrocytes along the length of the electric organ. As psp amplitude is lowered below action potential threshold, the EOD amplitude would gradually fall until all, or most of, the electrocytes failed to spike, while psps would be diminished but still present. Thus, a small reduction of psp size could result in a large reduction in EOD amplitude.

The reduction of 'psp' amplitude during curarization is consistent with this interpretation. It would be difficult to explain the reduced amplitude of the potential if it were a spinal command signal, as the descending axons from the PMN are electrotonically coupled to the electromotor neurons (Elekes and Szabo 1981). This means that curare would not likely exert an influence on the synapse between the descending axons and the electromotor neurons. Second, both potentials behave as dipoles with their zero isopotential lines in nearly the same place. This result suggests that they share the same source (the electrocyte), a conclusion emphasized by the abnormally more caudal location of the electrical midlines in a fish with a regenerated tail. The slight misalignment (6 mm) between the reversal sites is possibly due to the fact that the exact source for each potential is not the same (chemosensitive versus electrogenic membrane) within the electrocyte. Further, there is no reason to suspect that the spinal cord would act as a dipole source. Westby and Kirschbaum (1978) have also identified a small curare-sensitive potential with many similar properties in the mormyrid *Pollimyrus isidori*, and have similarly concluded that it is a summated psp.

The most compelling piece of evidence in favor of this interpretation is that intracellular records from *Sternopygus* electrocytes show an early inflection on the spike, identical to that observed in the EOD around the animal (Bennett 1971). When the cell is hyperpolarized by current injection, the spike is eliminated, leaving a true psp similar in appearance to our presumptive psp. The intracellular psp which Bennett recorded is of slightly longer duration than the 'psp' potential recorded in this study.

The 'psp' was of the same duration in all of the nine curarized fish which were examined in this study, even though their EOD pulses varied in duration by a factor of two. The 'psps' of intact fish were of the same duration. This observation suggests that all neural processes before this point have little or no influence on EOD pulse duration. This and other evidence in this study (independence of EOD frequency and pulse duration with MS-222; similar duration pulses when EOD is recorded anywhere around the fish) suggests that the sole determinants of EOD pulse duration are in the electric organ, and probably due to events which follow the generation of the psp.

Steroid effects on EOD pulse

As in both gymnotiform and mormyriform pulse fish, where androgens are known to prolong EOD

pulse duration, DHT caused significant increases in EOD pulse duration in *Sternopygus*, a wave gymnotiform. In this study, the increase in pulse duration ranged from 13–43%. These changes are not as extreme as those produced in pulse fish under similar hormone treatment. In a mormyrid species, Bass and Hopkins (1985) found a 60–87% decrease in peak power frequency; pulse durations typically increased 100–350% (Bass and Hopkins 1983, 1984, 1985). Likewise, Hagedorn and Carr (1985) found that the EOD of female *Hypopomus occidentalis* decreased in peak power by 71% and that the duration of the second phase of the EOD increased significantly.

It is interesting that the rate of return of the EOD and pulse duration to baseline values was slower than the rate of change of these parameters in the opposite direction upon the initial implantation. Based on this, it would be tempting to conclude that pulse duration is capable of a more rapid increase than a decrease. However, since we do not know the time course for clearance of steroids from the bloodstream nor the influence of the implants upon the otherwise intact hypophyseal-gonadal axis of these fish, further work must be done to examine this suggestion.

We presume that the primary site of androgen action is the electrocyte. A less likely alternative, however, is that DHT could exert a direct effect only upon the PMN and not the electrocytes. The changes in pulse duration and the realignment with the new firing rate of the PMN might then be due to long-term effects of the electrocytes being driven at a different frequency. This must be directly tested by determining whether electric organ pulse durations are increased in hormone-treated fish with a PMN lesion or spinal transection (and which is therefore electrically silent). The fact that androgens act directly on the other elements in the electrosensory system, the PMN and the receptor cells, renders the former alternative more likely (Meyer et al. 1984; Keller et al. 1986; Ferrari and Zakon, in prep.). Furthermore, the presence of androgen receptors has been confirmed in the electric organ of a mormyrid (Bass et al. 1986b).

The effects of hormones on the waveshape of the EOD may be understood in terms of the electrical behavior of the electrocytes and their contribution to the EOD pulse. The different waveforms and durations of the EOD pulse in various species of weakly electric fish are determined by the electrical properties of the electrocyte membranes (Bennett 1971; Bass 1986a, b). The waveform produced by *Sternopygus* and *Eigenmannia*, for example, is relatively simple. When the electrocytes are stimulated to fire by the spinal motoneurons, only the caudal, innervated face spikes, producing a monophasic EOD. In the pulse mormyrids, both anterior and posterior faces generate action potentials. In these electrocytes, the posterior membrane forms stalks which may also penetrate the anterior face of the electrocyte, thus conveying the action potential with precise timing into various compartments of the cell (Bass 1986a). In most pulse gymnotiforms, both faces of the electrocyte are excitable, and fire asynchronously (Bennett 1971; Hagedorn and Carr 1985).

In keeping with their physiological diversity, the locus of action of steroids upon the electrocytes from various species differs. The spikes from both faces of the electrocyte of the mormyrid, *Brienomyrus brachyistius*, are changed in duration after DHT (Bass and Volman 1985). In contrast, Hagedorn and Carr (1985) demonstrated that it is only the spike emanating from the anterior face of the electrocyte in *Hypopomus* which is altered after treatment with DHT. Although we have not yet made intracellular recordings from hormone treated fish, we presume that DHT affects only, or mostly, the posterior (spiking) membrane of the electrocytes of *Sternopygus*.

Since spinal cord command signals appear similar in males and females of species with sexually dimorphic EOD waveforms and of hormone-treated and intact fish, it has previously been proposed (Bass and Hopkins 1983; Hagedorn and Carr 1985), that the effect of hormones on the action potentials in these cells may be by alteration of one or more of the following properties: 1. physiology of the electromotor junction (emj). 2. cable (passive) properties of the electrocyte membrane. 3. the voltage-sensitive (active) ion channels.

In *Sternopygus*, the locally recorded EOD pulse duration is the same and the presumptive summed psps do not vary in shape or duration in fish of different EOD frequencies. These findings imply that the mechanism responsible for waveform differences lies beyond the emj. This is a reasonable assumption as, once the psp brings the membrane to spiking threshold, the regenerative voltage-dependent mechanisms will proceed regardless of psp duration.

Passive properties of the electrocyte membrane could limit its charging time and therefore influence spike duration and waveshape. The size and shape of the electrocyte could in turn influence its passive properties. In some species, sexual dimorphism exists in the morphology of the electrocytes. DHT not only masculinizes the EOD of the females of these species, but also results in a more

male-like appearance of the electrocytes. In species with a sexual dimorphism of the electrocytes, the anterior (uninnervated) face of the male electrocyte is thicker, more infolded and, therefore of greater area (Bass and Hopkins 1983; Hagedorn and Carr 1985; Bass 1986b; Bass et al. 1986a). Treatment of females with DHT increases the thickness and infolding or the total size (Bass and Hopkins 1983; Hagedorn and Carr 1985; Bass 1986b; Bass et al. 1986a) of the electrocyte which would likely influence its time constant.

We have not studied the electrocytes of *Sternopygus* histologically, so we are unaware of any sex- or individual-specific variation in the gross morphology of the electrocytes. However, convincing evidence against the membrane time constant determining spike durations is presented in the work of Nakamura et al. (1964) on *Electrophorus*, and Bennett (1961) on *Sternopygus* and *Eigenmannia*, three closely-related gymnotids. The membrane time constant for these species is around 100–200 μs. Since the electrocytes of *Sternopygus* have a much slower rise-time, it is likely that the cable properties of the electrocyte membrane do not determine the characteristics of a significant portion of each EOD pulse. Thus, we would expect that DHT would have little or no influence on the membrane time constants. These results implicate the active properties of the electrocyte membrane in determining pulse duration in *Sternopygus*.

Little is known about the ionic conductances in the electrocytes of *Sternopygus*, but the ionic currents underlying the spike have been studied in the electric eel, *Electrophorus* (Keynes and Martins-Ferreira 1953; Nakamura et al. 1964). The muscle-derived electrocytes of *Electrophorus* possess Na^+ and K^+ channels. The Na^+ channel seems similar to the 'classical' Na^+ channel, but the K^+ channel shows some interesting differences from the classical K^+ channel (Hodgkin and Huxley 1952). At rest, the K^+ channels are conducting. However, during depolarization, the K^+ channels initially inactivate allowing the depolarization to reach a peak value and delaying the repolarization of the cell. During the falling phase of the spike the K^+ channel becomes activated and aids repolarization of the membrane potential. This sequence of conductance changes ensures a large voltage excursion from resting potential and a prolonged discharge (Bennett 1961; Nakamura et al. 1964). It appears, then, that at least the properties of the active K^+ channels play a significant role in determining EOD waveform characteristics, particularly pulse duration. Whether the duration of the pulse is solely a function of the kinetics of the K^+ current, or whether modification of the Na^+ conductance also occurs are questions for future research. Voltage clamp studies of the ionic currents in electrocytes from fish whose EOD pulse durations vary will pinpoint the ionic mechanisms responsible for differences in pulse duration and a potential substrate for androgen effects upon the pulse.

The electrocytes of most pulse fish generate a complex pattern of ionic current flow since each EOD pulse comprises the summated voltages of two or more action potentials out of phase with each other. Unraveling the contribution of the various ionic currents of each face to the waveshape of the electrocyte spike, even under voltage clamp, becomes a difficult task. Because they possess only a single active membrane, the electrocytes of gymnotiform wave fish such as *Sternopygus* offer a potentially more tractable preparation for addressing these questions.

Coordination of PMN and electric organ

It is well known that the tuberous electroreceptor cells are tuned to the power spectrum of the species-specific EOD in both wave and pulse fish (Hopkins 1976; Viancour 1979; Meyer and Zakon 1982; Zakon and Meyer 1983; Bass and Hopkins 1984). In species with sexual dimorphisms or individual differences in EOD frequency composition, the receptors are usually best-tuned to a prominent peak in the individual's power spectrum. The matching of tuning to the EOD is maintained throughout developmental (Meyer et al. 1986; Zakon 1986) and hormonally-induced changes (Meyer and Zakon 1982; Zakon and Meyer 1983) in EOD frequency.

We have shown in this study that the EOD frequency and pulse duration are coordinated in two species of wave fish with myogenic organs. It would also be interesting to find out if such a correlation exists in *Apteronotus* as well, in which the electric organ is neurogenic.

We have also shown that these two parameters shift in a well coordinated manner after systemic treatment with androgens, suggesting that hormones are normally important in their coordination. These results emphasize a level of control and coordination not previously recognized in weakly electric fish. Interestingly, the EOD frequency and pulse duration change with different time courses and peak at different days following DHT treatment, with the changes in pulse duration lagging behind those in frequency. This suggests that the

hormone affects two different, and probably independent, processes. Shifts in tuberous electroreceptor tuning also lag behind the changes in EOD frequency in *Sternopygus* (Zakon and Meyer 1983).

Complementary to androgen-induced decreases in EOD frequency in *Sternopygus*, female sex hormones appear responsible for eliciting increases in EOD frequency: EOD frequency decreases after ovariectomy, and increases after injections of estradiol 17-β and human chorionic gonadotropin (Meyer 1983; Yan and Zakon, unpubl.). Accordingly, the EOD pulse duration decreases significantly when females are injected with gonadotropin, which occurs due to the secretion of the fish's own steroids (Yan and Zakon, unpubl.). This finding strongly underscores the role of sex steroids in the natural coordination of these parameters.

Thus, in *Sternopygus*, the membrane properties of three different tissues, the PMN (neural tissue), the tuberous receptor cells (ectodermal derivative) and the electric organ (myogenic derivative) must be kept in register. This appears to be accomplished by sex steroids. This species offers a unique opportunity to study the actions of steroids on the electrical properties of three distinct tissues and the mechanisms by which those electrical properties may be coordinated.

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References

Bass AH (1986a) Electric organs revisited: Evolution of a vertebrate communication and orientation organ. In: Bullock TH, Heiligenberg W (eds) Electroreception. John Wiley, New York, pp 13–70

Bass AH (1986b) Species differences in electric organs of mormyrids: substrates for species-typical electric organ discharge waveforms. J Comp Neurol 244:313–330

Bass AH, Hopkins CD (1983) Hormonal control of sexual differentiation: changes in electric organ discharge waveform. Science 220:971–974

Bass AH, Hopkins CD (1984) Shifts in frequency tuning of electroreceptors in androgen-treated mormyrid fish. J Comp Physiol A 155:713–724

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

Bass AH, Volman SF (1985) Steroid-induced changes in action potential waveform of an electric organ. Soc Neurosci Abstr 11:159

Bass AH, Segil N, Kelley DB (1986) Androgen binding in the brain and electric organ of a mormyrid fish. J Comp Physiol A 159:535–544

Bass AH, Denizot J-P, Marchaterre MA (1986a) Ultrastructural features and hormone-dependent sex differences of mormyrid electric organs. J Comp Neurol 254:511–528

Bastian J (1977) Variations in the frequency response of electroreceptors dependent on receptor location in weakly electric fish (Gymnotoides) with a pulse discharge. J Comp Physiol 121:53–64

Bastian J (1981) Electroreception II. The effects of moving objects and other electrical stimuli on the activities of two categories of posterior lateral line lobe cells in *Apteronotus*. J Comp Physiol 144:481–494

Bennett MVL (1961) Modes of operation of electric organs. Ann NY Acad Sci 54:458–509

Bennett MVL (1971) Electric organs. In: Hoar WS, Randall DJ (eds) Fish physiology, vol V. Academic Press, New York, pp 347–491

Bennett MVL, Pappas GD, Gimenez M, Nakajima Y (1967) Physiology and ultrastructure of electrotonic junctions IV. Medullary electromotor nuclei in gymnotid fish. J Neurophysiol 30:236–300

Blaustein MP, Goldman DE (1966) Competitive action of calcium and procaine on lobster axon: a study of the mechanism of action of certain local anesthetics. J Gen Physiol 49:1043–1063

Bullock TH (1982) Electroreception. Annu Rev Neurosci 5:121–170

Bullock TH, Hamstra RH, Scheich H (1972) The jamming avoidance response of high frequency electric fish. J Comp Physiol 77:1–22

Caldwell J (1976) Behavior, psychic, neuropharmacologic, and physiological aspects: physiological aspects of cocaine usage. In: Mule SJ (ed) Cocaine – chemical, biological, clinical, social, and treatment aspects. CRC Press, Cleveland, pp 188–199

Elekes K, Szabo T (1981) Comparative synaptology of the pacemaker nucleus in the brain of weakly electric fish (Gymnotoidae) In: Szabo T, Czeh G (eds) Sensory physiology of aquatic lower vertebrates. Pergamon Press, New York, pp 225–278

Ellis DB, Szabo T (1980) Identification of different cell types in the command (pacemaker) nucleus of several gymnotiform species by retrograde transport of horseradish peroxidase. Neurosci 5:1917–1929

Enger PS, Szabo T (1968) Effects of temperature on the discharge rates of the electric organ of some gymnotids. Comp Biochem Physiol 27:625–627

Fatt P, Katz B (1951) An analysis of the end-plate potential recorded with an intra-cellular electrode. J Physiol 115:320–370

Fostier A, Jalabert B, Billiard R, Breton B, Zohar Y (1983) The gonadal steroids In: Hoar WS, Randall DJ, Donaldson EM (eds) Fish physiology, vol 9A. Academic Press, New York, pp 277–372

Frey AH, Eichert ES (1972) The nature of electrosensing in the fish. Biophysics J 12:1326–1358

Gottschalk B (1981) Electrocommunication in gymnotid wave fish: significance of a temporal feature in the electric organ discharge. In: Szabo T, Czeh G (eds) Sensory physiology of aquatic lower vertebrates. Pergamon Press, New York, pp 225–278

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

Hagedorn M, Heiligenberg W (1985) Court and spark: electric

signals in the courtship and mating of gymnotid fish. Anim Behav 33:254–265
Heiligenberg W (1977) Principles of electrolocation and jamming avoidance. In: Braitenberg V (ed) Studies of brain function. Springer, Berlin Heidelberg New York, pp 1–65
Hodgkin AL, Huxley AF (1952) Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. J Physiol 116:449–472
Hopkins CD (1972) Sex differences in electric signalling in an electric fish. Science 176:1035–1037
Hopkins CD (1974a) Electric communication in fish. Am Sci 62:426–437
Hopkins CD (1974b) Electric communication in the reproductive behavior of *Sternopygus macrurus* (Gymnotoidei). Z Tierpsychol 35:518–535
Hopkins CD (1976) Stimulus filtering and electroreception: Tuberous electroreceptors in three species of gymnotoid fish. J Comp Physiol 111:171–207
Hopkins CD (1980) Evolution of electric communication channels of mormyrids. Behav Ecol Sociobiol 7:1–13
Hopkins CD, Bass AH (1981) Temporal coding of species recognition signals in an electric fish. Science 212:85–87
Keller CH, Zakon HH, Sanchez DY (1986) Evidence for a direct effect of androgens upon electroreceptor tuning. J Comp Physiol A 158:301–310
Keynes RD, Martins-Ferreira H (1953) Membrane potentials in the electroplates of the electric eel. J Physiol 119:315–351
Mazeaud MM, Mazeaud I, Donaldson EM (1977) Primary and secondary effects of stress in fish: some new data with a general review. Trans Am Fish Soc 106:201–212
Meyer JH (1983) Steroid influences upon the discharge frequencies of a weakly electric fish. J Comp Physiol 153:29–37
Meyer JH, Zakon HH (1982) Androgens alter the tuning of electroreceptors. Science 217:635–637
Meyer JH, Zakon HH, Heiligenberg W (1984) Steroid influences upon the electrosensory system of weakly electric fish: direct effects upon discharge frequencies with indirect effects upon electroreceptor tuning. J Comp Physiol A 154:625–631
Meyer JH, Leong M, Keller CH (1987) Hormone-induced and ontogenetic changes in electric organ discharge and electroreceptor tuning in the weakly electric fish *Apterotnotus*. J Comp Physiol A 160:385–394
Mills A, Zakon HH (1986) EOD frequency and pulse duration are independently determined but coordinated. Soc Neurosci Abstr 12:313
Nakamura Y, Nakajima S, Grundfest H (1964) Analysis of spike electrogenesis and depolarizing K^+ inactivation in electroplaque of *Electrophorus electricus* L. J Gen Physiol 49:321–349
Shanes AM (1950) Drug and ion effects in frog muscle. J Gen Physiol 33:729–744
Taylor RE (1959) Effects of procaine on electrical properties of squid axon membrane. Am J Physiol 196(5):1071–1078
Viancour TA (1979) Electroreceptors of a weakly electric fish. II. Individually tuned receptor oscillations. J Comp Physiol 133:327–338
Westby GWM, Kirschbaum F (1978) Emergence of development of the electric organ discharge in the mormyrid fish, *Pollimyrus isidori* II. Replacement of the larval by the adult discharge. J Comp Physiol 127:45–59
Westby GWM, Kirschbaum F (1981) Sex differences in the electric organ discharge of *Eigenmannia virescens* and the effects of gonadal maturation. In: Szabo T, Czeh G (eds) Sensory physiology of aquatic lower vertebrates. Pergamon Press, New York, pp 179–194
Westby GWM, Kirschbaum F (1982) Sex differences in the waveform of the pulse-type electric fish *Pollimyrus isidori* (Mormyridae). J Comp Physiol 145:399–403
Zakon HH (1986) The emergence of tuning in newly generated tuberous electroreceptors. J Neurosci 6:3297–3308
Zakon HH, Meyer JH (1983) Plasticity of electroreceptor tuning in the weakly electric fish, *Sternopygus dariensis*. J Comp Physiol 153:477–487