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Hormone-induced and maturational changes in electric organ discharges and electroreceptor tuning in the weakly electric fish *Apteronotus*

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Summary. Plasticity in the frequency of the electric organ discharge (EOD) and electroreceptor tuning of weakly electric fish was studied in the genus *Apteronotus*. Both hormone-induced and maturational changes in EOD frequency and electroreceptor tuning were examined. *Apteronotus* is different from all other steroid-responsive weakly electric fish in that estradiol-17β, rather than androgens, induces discharge frequency decreases. This result can account for the 'reversed' discharge frequency dimorphism found in *Apteronotus* in which, counter to all other known sexually dimorphic electric fish, females have lower discharge frequencies than males. Studies of electroreceptor tuning in *Apteronotus* indicate that electroreceptors are closely tuned to the frequency of the EOD. This finding was noted not only in adult animals, but also in juvenile animals shortly after the onset of their EODs. Tuning plasticity in *Apteronotus*, as in other species studied, is associated with altered EOD frequencies and was noted in both maturational EOD changes and in estrogen-induced changes. Thus, tuning plasticity appears to be a general phenomenon which occurs concurrent with a variety of EOD changes.

Introduction

Active sensory systems, such as those found in echolocating bats or electrolocating fish, are systems in which animals obtain information about the external environment by analysis of the modifications that occur in self-generated signals as a result of external objects. Weakly electric fish, which gather environmental information and communicate by producing electric organ discharges (EODs), provide an example of this process. EODs are detected by electroreceptors in the skin, and small transepidermal voltage fluctuations present in the reafferent EODs are used to provide information about external objects. A frequent finding in active sensory systems is for motor outputs and sensory inputs to be matched in temporal and/or spectral properties. In both wave-type fish (which produce quasi-sinusoidal EODs) and pulse-type fish (which produce short-duration EODs), the electroreceptors of an animal are often tuned to its own discharge frequency (Bennett 1971; Hopkins 1976; Viancour 1979; Meyer and Zakon 1982; Bass and Hopkins 1984). Electroreceptors, thought to be modified hair cells derived from the lateral line, must achieve their frequency selectivity through electrical filters located in the cell membrane (Bennett 1971; Hopkins 1976; Viancour 1979; Zakon and Meyer 1983). Such electrical filters also occur in hair cells which detect mechanical signals (Crawford and Fettiplace 1981; Lewis and Hudspeth 1983; Ashmore 1984; Fuchs 1985). Thus, an understanding of electroreceptor tuning processes may be pertinent to hair cells in general.

One dynamic aspect of electroreceptor tuning is tuning changes associated with changes in EOD frequency which may occur during an animal's life. One such change is the sexual dimorphism in EODs found in adults of many species of both the African order Mormyriformes and the South American order Gymnotiformes. EOD waveforms are altered in pulse-type fish (Bass and Hopkins 1983; Hagedorn and Heiligenberg 1985) and EOD frequencies are altered in wave-type fish (Hopkins 1974; Westby and Kirschbaum 1981; Meyer 1983). These differences may result from elevated gonadal steroid levels, since administered steroids effect EOD changes similar to the naturally-occurring

changes. Androgen implants broaden the EODs and lower peak EOD spectral frequencies in pulse-type discharging fish (Bass and Hopkins 1983, 1985; Hagedorn and Carr 1985), while repeated androgen injections decrease the discharge frequencies of the wave-type discharging gymnotoids *Sternopygus* (Meyer and Zakon 1982; Meyer 1983) and *Eigenmania* (Leong, unpublished data). Such changes in the predominant EOD spectral frequencies would, without any compensation in receptor selectivity, result in a mismatch between an animal's EOD and its electroreceptors. Yet compensation apparently occurs: administered androgens induce concomitant and equal changes in both EOD frequencies and electroreceptor best frequencies (Meyer and Zakon 1982; Zakon and Meyer 1983; Bass and Hopkins 1984).

A limitation to further understanding of the plasticity of electroreceptor tuning has been that studies conducted to date have been restricted to systems in which elevated androgen levels induce decreases in EOD frequency and electroreceptor best frequency. Long-term alterations in EODs, however, also occur in other situations. In this study we therefore have examined the characteristics of two other types of EOD frequency changes seen in the South American wave-type gymnotoid *Apteronotus rostratus*. The first of these is the maturational increase in EOD frequency noted shortly after young animals begin to discharge, as originally reported by Kirschbaum (1983). The second of these changes is the development of a sexual dimorphism in which females have lower EOD frequencies than males (Kirschbaum 1983; Hagedorn and Heiligenberg 1985). This dimorphism is opposite to all other known dimorphic species of electric fish, where males have lower EOD frequencies than females, and suggests the involvement of gonadal steroids with either estrogens causing discharge frequency decreases or androgens causing discharge frequency increases. A previous study found that repeated steroid injections caused short-term pharmacological changes in *Apteronotus* discharge frequencies but no long-term effects that could account for the sexual dimorphism (Meyer 1984). In this study, we have continued to investigate long-term influences of steroids on the discharge frequencies of *Apteronotus*, now using implants for steroid administration. By examining EOD changes and any associated receptor tuning changes in the two situations listed above, we hoped to shed light on the regulation of EOD frequency and to achieve a broader understanding as to the generality of matched EOD-receptor tuning alterations.

Materials and methods

Apteronotus rostratus, commonly called brown ghosts, were collected from rivers in Panama or obtained from local fish importers. Stock animals were maintained in 50–100 l aquaria at a temperature of 25–28 °C and at a pH of 7.0. Our general recording methods, used throughout this study, are outlined below, followed by protocols for the maturational observations (experiment one) and hormonal implants (experiment two).

Recording methods

i) EOD recordings. The small amplitude EODs of juvenile fish were obtained by aspirating the fish into a 6 mm diameter plastic tube into which recording electrodes 1.5 cm apart had been placed. The EOD was amplified by a Grass P-15 amplifier and then passed to a DEC PDP 11/40 for frequency determinations (average frequency determined over 165 successive EOD cycles). The frequencies of the larger fish (with higher amplitude EODs) were determined by holding the fish in a net and then placing a dipole recording electrode next to the fish. After amplification, the EOD frequency was determined using a Data Precision 5740 frequency counter. In repetitive recordings from the same animals or where comparisons between animals were necessary, EOD frequencies were adjusted to values which would be obtained at a temperature of 27 °C using a Q_{10} of 1.5 (Enger and Szabo 1968).

ii) Electroreceptor tuning. Two methods were used in this study for recording electroreceptor tuning characteristics: impulse-evoked receptor oscillations from electroreceptor organs, and single-unit recordings from primary afferent fibers. Young fish proved quite fragile upon surgical manipulation; it was not possible, therefore, to record directly from their primary afferent fibers. Thus, only receptor oscillations were used to determine tuning characteristics in these animals, as well as in the majority of adult animals (total number of animals recorded from in experiments one and two = 43). In 4 adult animals, however, both single unit and oscillation parameters were recorded. The techniques used in single unit recordings and oscillation recordings are discussed below:

a. Receptor oscillations: Individual adult *Apteronotus* were placed in a recording tank and allowed to adapt for 15 to 30 min. The recording tank was maintained at a temperature of 26 ± 0.5 °C, a resistivity of 5 kΩ·cm, and at a pH of 7.0. After the adjustment period, the fish's EOD frequency was determined, and the animal was then immobilized by giving an injection of Flaxedil (circa 10 µg). The neurogenic EOD of *Apteronotus* is not silenced by Flaxedil. To record uncontaminated oscillations, it was thus necessary to electrically isolate the fish's head (where recordings were made) from its tail (wherein the electric organ resides). Each fish was thus placed in a divided plastic chamber in the center of the recording tank. This chamber had a double layer of thin rubber sheets between the front and rear compartments, with silicone grease between the two sheets. The fish's head was passed through thin vertical slits in each of the two sheets so that the animal's head rested in the front compartment and was isolated from the tail in the rear compartment. Attenuation of 60 dB between compartments was achieved by this method.

Recording tank water was used to fill the two compartments of the chamber as well as to respirate the fish via a glass tube placed in the fish's mouth. Square wave electrical impulses (0.5 ms duration, 10 mV/cm amplitude) from an Exact function generator were passed via a stimulus isolation unit to a dipole stimulating electrode placed in the front compartment straddling the fish. A micropipette (10 µm tip diameter) placed over a patch of electroreceptors on the animal's head

was used to record the impulse-evoked oscillations. Oscillations were amplified (WPI M-707A or Biomedical Engineering NB-100-1 amplifiers) and the responses to multiple stimulus presentations were averaged ($n=512$ to 2048; stimulus repetition rate 10 to 15 Hz; Tracor Northern 1505 or Nicolet 1070) with a sampling rate of 50 kHz. Determination of the number of peaks and time intervals between peaks in the oscillation was done directly for recordings made with the Nicolet averager, but required the Tracor recordings to be passed to a DEC 11/40 computer for subsequent analysis. The frequency of the oscillation was calculated by dividing the time interval between the first and last peaks in the oscillation by the total number of cycles in that oscillation. Oscillations were recorded from at least 5 different sites over the surface of the animal's head and then averaged to obtain a mean oscillation frequency for that animal. As with EOD frequencies, oscillation frequencies were adjusted to control for temperature differences during repetitive recordings from the same animal or in comparisons between animals. For oscillations a Q_{10} of 1.45 was used. This Q_{10} was determined by altering the water temperature while recording oscillations from an individual fish (see Results).

Small juvenile fish (<3 cm) were immobilized by being placed in water containing Flaxedil (5 µg/ml). The animals were then placed between layers of thin gauze in the center of a 10 cm diameter petri dish located in the recording tank. The head was electrically isolated from the tail by using a barrier constructed of silicone grease. A small diameter glass tube was placed in the animal's mouth and tank water was used to both respirate the fish and to fill the petri dish. Oscillations were subsequently recorded using procedures similar to those outlined for adult animals.

b. Single unit recordings: Each adult *Apteronotus* was placed in the recording tank and allowed to equilibrate for 1/2 to 1 h, after which its EOD frequency and oscillation frequency were determined using the above methods. To eliminate the animal's EOD for single unit recordings, it was necessary to destroy the animal's spinal cord. Each animal was thus removed from the chamber, placed on a surgical tray, and respirated with a weak anaesthetic solution of MS 222. A wire was subsequently passed up the vertebral canal to destroy the spinal cord. The animal was then returned to the recording tank and held in a foam-lined forceps at a roll angle of 45°. The posterior branch of the anterior lateral line nerve was exposed and penetrated with a glass micro-electrode (25–50 MΩ tip resistance). The animal was stimulated by means of continuous sine waves which were produced by an Exact function generator and passed into the tank via a stimulus isolation unit to two carbon electrodes placed transversely to the fish. The stimulus amplitude was varied between 0.1 and 10 mV/cm, while the stimulus frequency used to search for single unit activity was set to a value near to the animal's original EOD frequency (600 to 1,000 Hz, dependent on the animal). Upon isolating a unit, the firing probability of the unit relative to each stimulus cycle was determined via on-line monitoring by a DEC 11/40 computer. The frequency of the stimulus was varied, and the frequency at which the unit fired with a maximal probability was determined by visual inspection of a computer-generated display of the unit's firing probability. In cases where the unit was saturated, the stimulus amplitude was decreased so that only a single frequency would elicit a maximal response. The repeatability of this method was ± 10 Hz. Ten to 20 individual units were recorded from each animal.

Experiment one: maturational changes

Male and female *Apteronotus* were placed in large 200–800 l aquaria (4 to 6 animals per tank) and maintained at 26–28 °C. Using methods detailed by Kirschbaum (1975, 1979) and Hagedorn and Heiligenberg (1985), the animals were induced to spawn. Eggs were removed from the large aquaria the day after spawning and placed in small petri dishes floating on the surface of 75 l aquaria. Three to 4 days later, viable eggs hatched. After 5 to 6 additional days, larvae were released into the 75 l aquaria for 1 to 2 days. Fish were then individually placed in half-liter plastic floating containers with nylon screen bottoms. The juveniles were fed daily with plankton obtained from local ponds until they were large enough (approximately 2 cm) to be fed chopped tubificid worms. EOD frequencies and receptor tuning characteristics were determined on individual animals at different times after hatching using the above outlined techniques. To fully explore the relationship between the process of maturation and EOD/receptor tuning properties, larger adult animals (16–21 cm), purchased from local dealers, were also used. Recordings were made from a total of 30 animals. In three cases, recordings were made from the same animals at different maturational stages.

Experiment two: hormonal changes

Individual adult *Apteronotus* were placed in electrically-isolated compartments of 75 l aquaria (individual compartments ranging in size from 15 to 25 l). The aquaria were maintained at a temperature of 25 to 27 °C, and at a pH of 6.0 to 7.0. Fish were fed on a daily basis and their EOD frequencies monitored at intervals of 1 to 2 days. After allowing 1 to 5 days for the fish to adjust to their compartments, animals were removed from the tank and anaesthetized with a weak solution of MS222. While still being respirated with the MS222 solution, fish were given implants in the posterior abdominal cavity of either estradiol 17β ($n=7$), 5α-dihydrotestosterone (DHT; $n=6$), or blanks (empty capsules; $n=6$). Implants were prepared using the methods detailed in Keller et al. (1986) and contained less than 0.5 mg of steroid. EOD frequencies were subsequently followed at intervals of 1 to 2 days for 10 days.

Animals used to assess the effects of steroid upon electroreceptor tuning were placed in individual compartments, and after an adjustment period of 1 to 5 days, their EOD frequencies and oscillation frequencies determined using the above techniques. Three days afterwards, the animals were given blank ($n=4$), DHT ($n=5$) or estradiol ($n=4$) implants. EOD frequencies were followed for 12 days, after which oscillation frequencies were again determined.

Results

Experiment one: maturational changes

i) EOD. EODs of juvenile fish were recorded within 1 to 2 days after the animals first started to discharge, which normally occurred at 6 to 8 days after hatching. The regularity at which the EODs were emitted was slightly less for newly-discharging animals than for adults: coefficients of variation (determined over 165 successive EOD cycles) for fish discharging 1 week or less were typically in the range of 1%, while those of adults typically were 0.1% or less. The frequencies of juvenile fish's EODs also differed from adults (Fig. 1a). There was a steady increase in the discharge frequencies

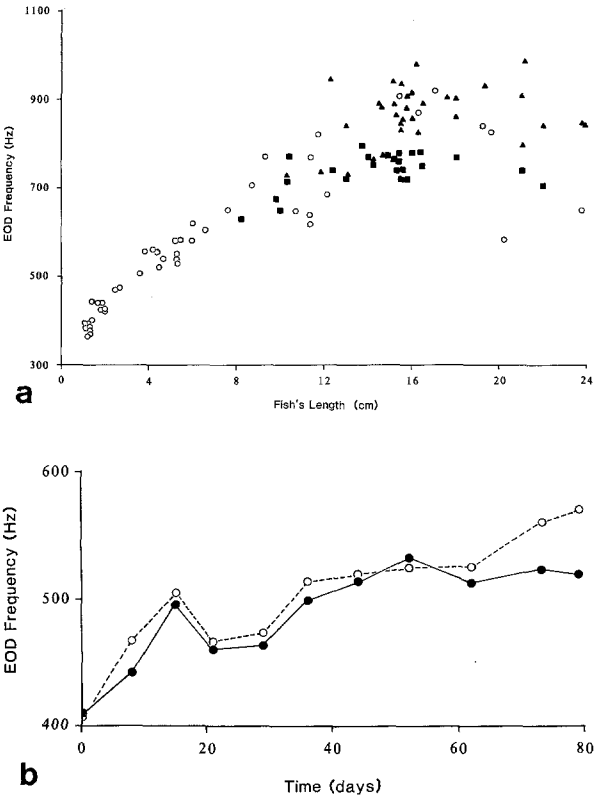

Fig. 1. a EOD frequencies as a function of fish size for laboratory maintained *Apteronotus*. All EOD frequencies have been adjusted to a value expected at 27 °C using a Q_{10} of 1.5. Males: filled triangles; females: filled squares; sex unknown: open circles. **b** EOD frequencies versus time for two newly-discharging *Apteronotus* juveniles. EOD values have been adjusted to 27 °C

of animals with increasing size: young juveniles started to discharge at approximately 400 Hz, with very little variation between the discharge frequencies of individual animals, while adult animals had EOD frequencies which ranged from 600 to 1,000 Hz. This age-dependent rise in EOD frequency is further demonstrated in Fig. 1b, which presents discharge frequencies as a function of time for two juveniles. Discharge frequencies rose most rapidly in the first several days after the EOD onset. Occasional large EOD frequency rises or even frequency declines were noted. Since these almost always occurred synchronously among the individuals in a tank, it is likely that such fluctuations represent environmental influences rather than changes intrinsic to a given animal.

Though the sexes of fish less than 8 to 9 cm could not be determined, the observation that discharge frequencies increased in all animals suggests that increases occur in both males and females. For fish between 9 and 15 cm, there is considerable overlap in the discharge frequencies of the two sexes, while in larger fish females consistently have lower discharge frequencies than males. Figure 1a thus suggests that both males and females increase their discharge frequencies early in maturation, and that while male fish continue to increase their discharge frequencies with maturation, female fish maintain their discharge frequencies at lower values.

ii) Electroreceptor tuning: The majority of single afferent units were spontaneously active. Most of these units discharged at irregular rates, but infrequently units were encountered which discharged at constant frequencies ranging from 700 to 1,200 Hz. These units had higher best frequencies and lower stimulus thresholds at best frequency (BF) than the irregular units. This difference resulted in a considerable range in the receptor best frequencies for any given animal. Despite this, the animal's mean BF correlates well with that animal's EOD frequency, with the mean BF being somewhat less than the EOD frequency (Fig. 2).

As indicated earlier, single unit recordings required the destruction of an animal's spinal cord, thus rendering these individuals unsuitable for repetitive recordings. Likewise, single-unit recordings were impractical in small juveniles. It was therefore necessary to utilize oscillation recordings, of which a typical recording is shown in Fig. 3. Oscillations in *Apteronotus* were similar to those that have been recorded in other species of gymnotoids but typically had a greater number of cycles than oscillations seen in *Sternopygus* or *Eigenmannia*: in *Apteronotus*, 7 to 10 cycles were often observed, while 2 to 3 cycles are usual in *Sternopygus* oscillations (Meyer et al. 1984). As in *Sternopygus* (Zakon and Meyer 1983; Meyer et al. 1984), mean oscillation frequencies for individual animals were consistently higher than their single unit best frequencies (Fig. 4). Despite this difference, the strong correlation ($r=0.98$, $n=4$) between mean oscillation frequency and mean single unit best frequency indicates that mean oscillation frequencies serve as a good measure of electroreceptor tuning properties.

To compare oscillations recorded at different temperatures, a Q_{10} value for oscillation recordings was experimentally derived. This was done in one animal by altering the temperature in the recording chamber while the electrode was left stationary and recording the oscillations at various temperatures. A value of 1.45 was determined ($n=$ 10 temperature values; range 25.5–28.5 °C; $r=$ 0.96 for log oscillation frequency versus temperature) and subsequently used in cases where it was

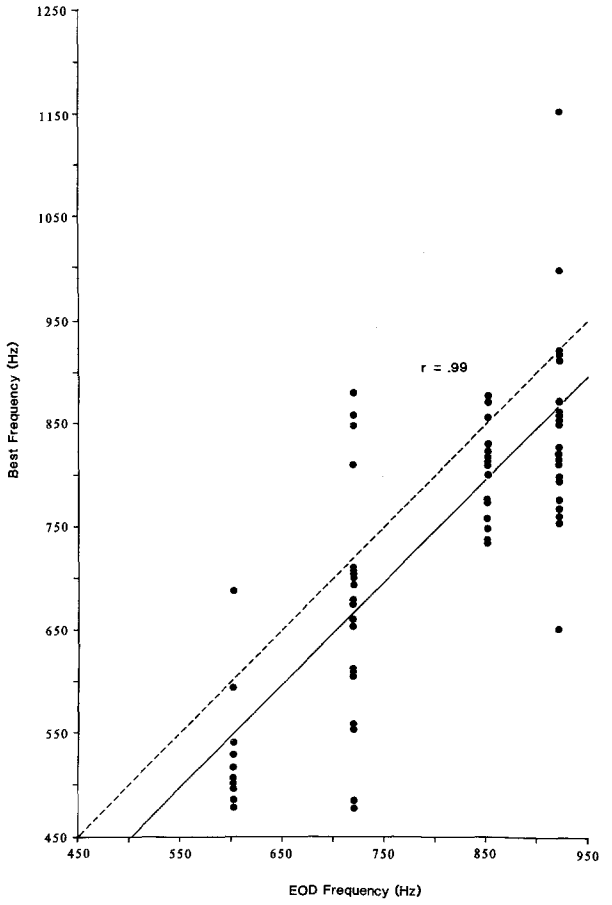

Fig. 2. Single-unit best frequencies versus EOD frequencies for four adult *Apteronotus*. Filled circles indicate individual afferents' best frequencies. The solid line indicates the regression line based on the mean best frequency for each of four animals. The dashed line indicates the line of one-to-one correspondence between single-unit best frequencies and EOD frequencies

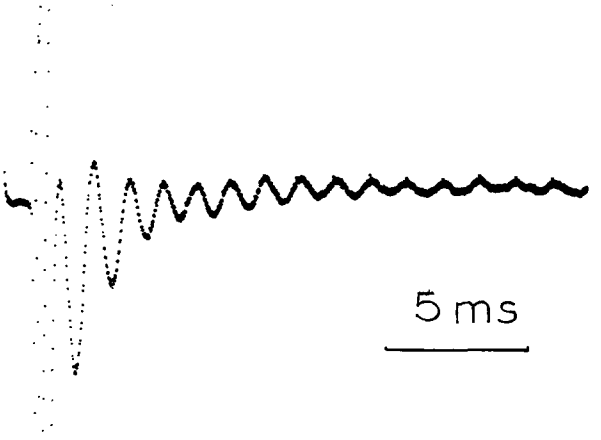

Fig. 3. Impulse- evoked electroreceptor oscillation from adult *Apteronotus*. This averaged response is based upon 1024 stimulus repetitions, with a sampling rate of 50 kHz. Stimulus duration was 0.5 ms and the amplitude was 10 mV/cm

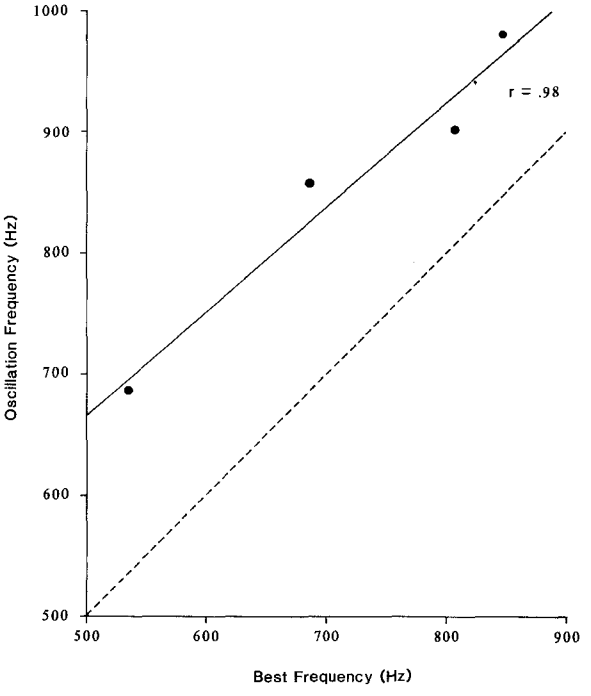

Fig. 4. Mean oscillation frequency versus mean single-unit best frequency for the same four adult *Apteronotus* presented in Fig. 2. The solid line represents the regression line; the dashed line indicates the line of one-to-one correspondence

necessary to adjust oscillation frequencies for differing temperatures.

The frequency for the oscillation shown in Fig. 3 was 827 Hz, whereas the fish's EOD frequency was 755 Hz. Oscillation frequencies higher than the EOD frequency were typical for *Apteronotus* and have also been noted in other gymnotoids (Zakon and Meyer 1983). Despite this fact, oscillation frequencies were highly correlated with discharge frequencies ($r=0.94$; $n=33$). Thus, adult *Apteronotus*, like other species of wave-type discharging gymnotoids, display individual matching between EOD frequencies and electroreceptor oscillation frequencies.

Oscillations recorded from juvenile animals were similar to those of adults in their waveform, and in the fact that oscillation frequencies were close to but higher than the EOD frequencies. For example, in four newly discharging fish (<2 cm length, recording 13 to 25 days after discharge-onset), oscillation frequencies ranged from 500 to 617 Hz, while EOD frequencies ranged from 400 to 500 Hz. However, these fishes' individual oscillation frequencies and the same fishes' EOD frequencies correlated poorly ($r=0.25$). This contrasts with the strong correlation found in adults

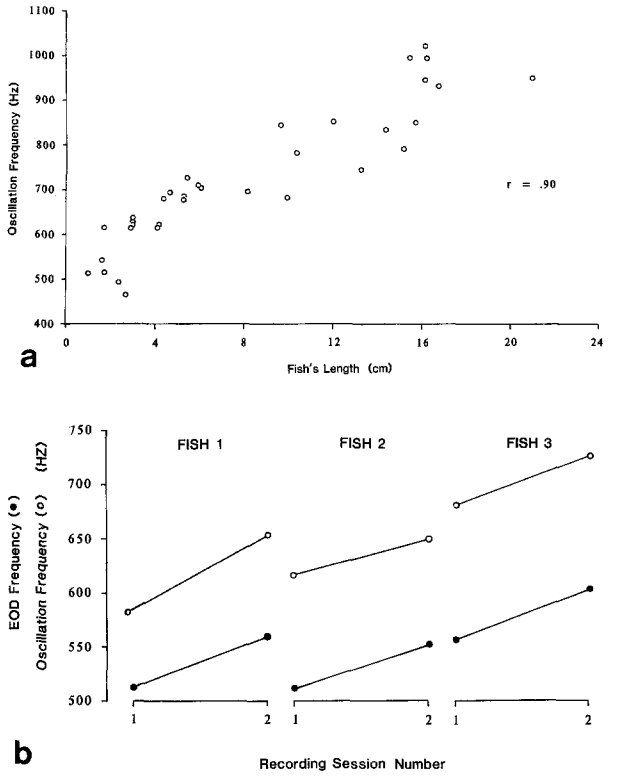

Fig. 5. a Oscillation frequency versus fish's length. These oscillation frequencies are adjusted to 27 °C using a Q_{10} of 1.45. **b** Repetitive oscillation (open circles) and EOD (filled circles) frequencies for three juvenile *Apteronotus*. These recordings were made 21 to 22 days apart in which time each fish grew 11 to 12 mm in total length. All oscillations and EOD frequencies are adjusted to 27 °C using Q_{10}s of 1.45 and 1.5, respectively

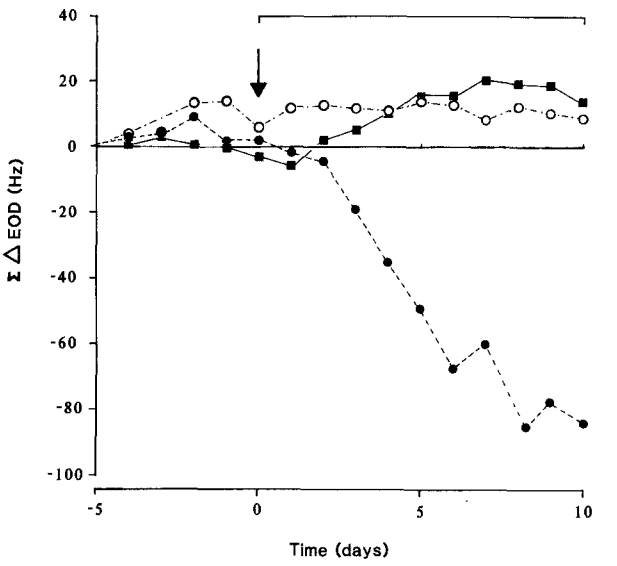

Fig. 6. Cumulative mean change in EOD frequency over 10 days versus time for implanted *Apteronotus*. Filled squares indicate DHT-implanted animals ($n=6$), filled circles indicate estradiol-implanted animals ($n=7$), open circles indicate control (blank-implanted; $n=6$) animals. Implantations were performed on day 0 as indicated by the arrow above the trace. The experimental period was from day 0 to day 10, as indicated by the bar above the trace

($r=0.94$) and in young fish 2 to 3 months after discharge onset ($r=0.98$ for 6 animals).

The higher range of oscillation frequencies of adult animals (from 600 to 1,000 Hz; Fig. 5a) implies that oscillation frequencies must shift as the animals mature. This is confirmed in Fig. 5b, which presents successive EOD and oscillation frequencies recorded from three individuals (sizes 4.0 to 6.0 cm), and which also shows that changes in EOD frequencies are paralleled by changes in oscillation frequencies.

Experiment two: hormonal changes

i) EOD. In the period prior to hormone implantation, EOD frequencies of individual animals tended to fluctuate about a mean value which was relatively stable over periods of days to weeks (Fig. 6). Following implantation, mean EOD frequencies of the blank-implanted control animals and the DHT-implanted animals showed little change. In contrast, EOD frequencies declined sharply following implantation of estradiol. These changes were apparent within 3 days after implantation and continued throughout the 10 day experimental period, with the majority of the decline occurring in the first 6 days. At the end of the ten day experimental period, estradiol-implanted animals ($n=7$) had decreased their EOD frequencies by a mean value of $10.23 \pm 1.64\%$ (SEM), while the DHT-implanted animals ($n=6$) increased their EOD frequencies by $1.45 \pm 1.43\%$. The mean change exhibited by the blank-implanted control animals ($n=6$) of $0.04 \pm 0.73\%$ was not a significant change by itself, but was significantly different from the changes seen in the estradiol-implanted animals ($P<0.01$, Mann Whitney U test). The change in EOD frequency in DHT-treated animals, however, was not significantly different from controls ($P>0.05$, Mann Whitney U test). Amongst the estradiol-implanted animals, higher-frequency fish showed larger decreases than lower frequency fish. In several animals, implants were removed after the experimental period. In two of these cases, discharge frequency subsequently increased substantially. While not noted in all animals over the duration of the monitoring period, these increases suggest that the effect of the implanted estrogen may not be permanent.

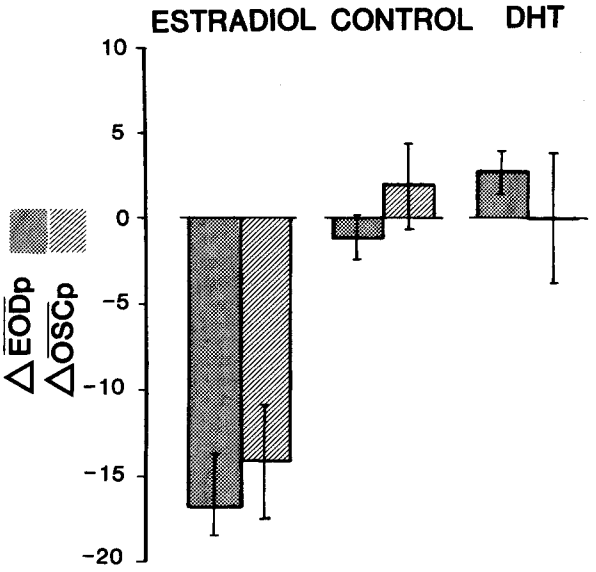

Fig. 7. Mean percentage change in EOD frequency ($\Delta\overline{EODp}$; stippled bars) and oscillation frequency ($\Delta\overline{OSCp}$; hatched bars) over 12 days for estradiol, DHT and control animals. $n=5$ for the DHT-implanted group; $n=4$ for both the estradiol- and blank-implanted groups. The vertical lines indicate standard errors

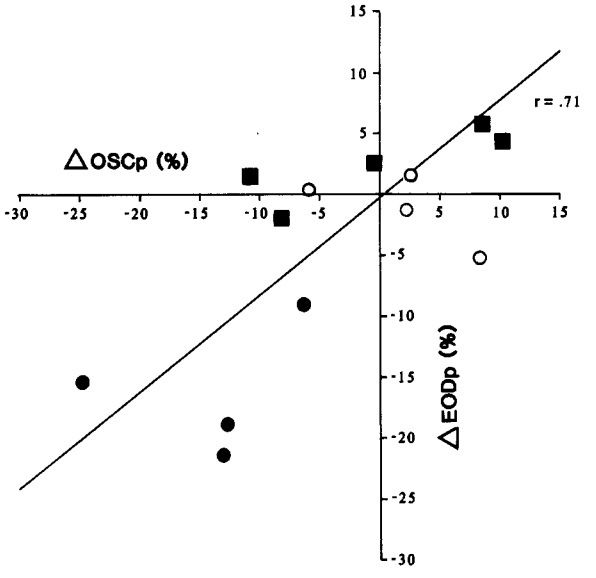

Fig. 8. Percentage change in EOD frequency (ΔEODp) versus percentage change in oscillation frequention (ΔOSCp) for individual *Apteronotus*. The filled squares indicate DHT-implanted fish, filled circles indicate estradiol-implanted fish and open circles indicate control fish. The solid line is the regression line

ii) Electroreceptor tuning. Similar to the findings noted above, electroreceptor oscillation frequencies were well correlated with, though higher than, discharge frequencies. Following DHT implants, no significant changes were seen in either EOD frequency or oscillation frequency when compared to blank implants (see Fig. 7; $P>0.05$, Mann Whitney U test). Amongst the estradiol-implanted animals, however, EOD frequencies following implantation decreased significantly ($P<0.01$), and these decreases were accompanied by significantly lower oscillation frequencies ($P<0.01$). Figure 8, which presents changes in EOD frequencies as a function of changes in oscillation frequencies for control, DHT and estradiol-implanted animals, illustrates that the two changes are well-correlated. The slope of the regression line was not significantly different from 1.0 (*t*-test of slope compared to 1.0, alpha = 0.05). Thus, under the influence of estrogen, electroreceptor tuning plasticity and EOD frequency alterations in *Apteronotus* are closely linked.

Discussion

In the present study, we have examined the characteristics of maturational and steroid-induced EOD alterations in *Apteronotus* and have used these two different types of EOD alterations to determine the generality of matched EOD-receptor tuning changes.

EOD frequency alterations

The first type of EOD change studied was a maturational shift in EOD frequencies. Kirschbaum (1983) has characterized the EODs of *Apteronotus* at discharge onset as low-frequency (200 Hz) myogenically-derived sinusoidal signals. In the first weeks of discharge, the EOD frequency rises rapidly and the relative importance of the neurogenic electric organ gradually increases. Frequencies typical of adult fish (approximately 800 Hz for males and 700 Hz for females) are reached over a period of months. We have confirmed many of these findings in our study, noting that juveniles originally had discharge frequencies in a narrow range centered around 400 Hz, which rapidly increased in the first week after discharge onset and then gradually increased over the next months to reach adult frequencies of greater than 600 Hz, with females tending to have lower discharge frequencies than males. Maturational changes have been noted in other wave-type gymnotoids. *Eigenmannia*, for example, undergoes early EOD frequency changes (Kirschbaum 1977), but in this species, EOD frequencies achieve adult levels within days after discharge onset (Kirschbaum and Westby 1975; Dye and Meyer 1986). In *Sternopygus*, early matura-

tional changes are presently unknown, but a later gradual divergence in male and female discharge frequencies occurs such that males have lower discharge frequencies than females (Hopkins 1974; Meyer 1983). Thus, maturational changes in EOD frequency exhibit wide species-specific variations, and further exploration of these differences may provide a greater understanding of those factors responsible for regulation of the discharge frequency.

In addition to the changes in discharge frequency, changes in the regularity of EODs also occurred with maturation: individual juveniles discharged with greater variation in EOD frequency than did individual adults. This difference might be attributed to a change in the electric organ as it shifts from a myogenic to a neurogenic state (Kirschbaum 1983). The greater regularity in the EODs of older animals might also be attributed to a change in the pacemaker nucleus which establishes the discharge frequency. This medullary nucleus consists of pacemaker cells whose rates of spontaneous depolarization set the EOD rate, and relay cells which are electrotonically coupled to the pacemaker cells (Bennett et al. 1967). These latter cells synapse onto the electromotor neurons that produce the EOD. Since each pacemaker cell spontaneously depolarizes at a certain frequency and is electrotonically coupled to the other cells in the nucleus it acts as an element in an oscillating network. An increase in the number of elements in an oscillating circuit will result in greater regularity in the overall output of the circuit (Enright 1980). Thus, maturational changes in pacemaker cell number, though as yet not verified, might serve to stabilize the rate of discharge in adults. Conversely, the individual pacemaker cells themselves might be less regular in juveniles than in adults, thus causing the output of the entire nucleus to be less stable.

The second type of EOD alteration examined in this study was the significant frequency decrease noted in adult animals treated with estradiol 17β. This effect appears specific to estrogens, since the androgen 5α-DHT had no significant effect upon discharge frequencies. The actions of estradiol therefore may account for the dimorphism found in *Apteronotus* in which females have lower discharge frequencies than males. In other gymnotoid species, androgens and not estrogens induce discharge frequency decreases (Meyer 1983; Hagedorn and Carr 1985; Leong, unpubl. data) and can thus explain the dimorphisms noted in these species in which males have lower EOD frequencies than females (Hopkins 1974; Westby and Kirschbaum 1981; Meyer 1983; Hagedorn and Carr 1985). Thus, different classes of gonadal steroids in a specific manner induce similar behavioral actions in different members of the same order. This can be contrasted with the mormyrids in which androgens and estrogens each can induce EOD frequency decreases within the same species (Bass and Hopkins 1985). This contrast may reflect phylogenetic differences or perhaps differences in the site of hormonal action, since steroids may act centrally in wave-type gymnotoids (Meyer et al. 1984) but peripherally in mormyrids (Bass and Hopkins 1983).

A previous study of the effects of steroids upon discharge frequencies of *Apteronotus* found only short-term pharmacological effects of steroids upon discharge frequencies. Estrogen caused slight, but non-significant long-term decreases in discharge frequencies (Meyer 1984). The differing results in the present study might be attributed to the different modes of steroid administration used in the two studies. In the earlier study, using a protocol found to be effective in other species of gymnotoids (Meyer and Zakon 1982; Meyer 1983), steroids were administered via daily injections. Since saline injections alone, however, significantly decreased discharge frequencies (Meyer 1984), it is possible that any effects of estrogens might have been masked by the effects of injections alone. In the present study, control (blank) implants had no significant effect upon discharge frequencies, and as such would not mask the actions of implanted estrogen.

The mechanism(s) by which the EOD frequency is altered by either maturation or by steroids is unknown, but in some manner must be attributable to changed activity of the medullary pacemaker nucleus. While the pacemaker cells receive synaptic input, this input does not appear to be important for tonic regulation of the rates at which the pacemaker cells depolarize. Isolation of the pacemaker nucleus has no immediate effects upon the discharge rate of the pacemaker (Meyer 1984). Increased discharge frequencies of maturing fish or the decreased discharge frequencies of estrogen-treated animals are thus probably due to changes within the pacemaker nucleus itself. Gonadal steroids are known to modify neuronal excitability and firing characteristics in a number of other systems (see McEwen 1981; Pfaff and McEwen 1983 for reviews). In the isolated pacemaker nucleus of *Apteronotus*, pharmacological doses of androgens induce short-term decreases in the discharge frequency (Meyer 1984). The manner in which physiological levels of estradiol induce long-term de-

creases in EOD frequencies remains to be determined.

Electroreceptor tuning plasticity

All wave-type gymnotoids studied to date have shown individual matching between EOD frequency and electroreceptor tuning in adult animals (Hopkins 1976; Viancour 1979; Meyer and Zakon 1982; Zakon and Meyer 1983). As such, adult *Apteronotus* are no exception in that we noted individual matching in both oscillation and single-unit type recordings. Our findings also indicate that electroreceptor tuning closely approximated EOD frequencies not only in adult animals, but also in juvenile animals shortly after the onset of their discharges. In these newly-discharging juveniles, however, individual fishes' EOD frequencies were not well correlated with their own receptor best frequencies. This poor correlation may be due to the small sample size ($n=4$) and limited range of frequencies for newly discharging fish, or it may indicate that some maturational adjustment is necessary to achieve individual matching, since fish by the age of 2 months (25–45 mm length) had electroreceptors closely tuned to their EOD frequencies. Thus, while electroreceptors achieve gross frequency selectivity early on in development it is unclear whether a maturational change is required to achieve individual matching. It also remains unknown whether the electroreceptors of juvenile animals are tuned prior to discharge-onset. Zakon (1986) has recently reported that regenerating electroreceptors in adult animals do not require EOD exposure to be tuned. Whether such results can be applied to animals which have never been exposed to an EOD remains to be determined.

Electroreceptor organs have many adult features at the stage when fish first start to discharge (Kirschbaum and Denizot 1975), but then undergo an extensive structural modification as animals mature (Zakon 1984a). The present study implies that such modifications are not necessary for frequency selectivity, since electroreceptors of juvenile fish are already tuned. It is possible that the anatomical reorganization seen in developing fish might be associated with changes in the sensitivities of these structures to electric signals. The oscillation-type recordings used for juveniles did not allow determination of electroreceptor thresholds or bandwidths. Single-unit experiments will be necessary to determine these and other functional properties of electroreceptors.

The present study illustrates that electroreceptors of *Apteronotus*, like those of *Sternopygus*, display tuning plasticity. Receptor best frequencies increased in juveniles as animals increased their discharge frequencies during maturation, while receptor best frequencies declined in adults as animals decreased their discharge frequencies during estradiol treatment. Tuning plasticity thus appears to be present from birth and is not solely a property of adult electroreceptors. This finding correlates well with the recent report that newly-regenerated electroreceptors in adult *Sternopygus* also display shifts in their tuning properties, starting at best frequencies lower than the EOD and then gradually increasing to match the frequency of the EOD and of intact electroreceptors (Zakon 1986).

The mechanisms associated with each of these changes, and with tuning plasticity in general, are not clear. Experiments utilizing androgen-treated, electrically-silenced animals (Meyer et al. 1984) suggest that altered electroreceptor tuning is secondary to changes in EOD frequency. A direct effect of androgens also contributed to electroreceptor tuning, but was not predominant. Bass and Hopkins (1984) reached similar conclusions in studies on mormyrids. In more recent experiments, however, Keller et al. (1986) suggest that tuning plasticity arises predominantly from a direct effect of androgens upon electroreceptors. Our results indicate that tuning plasticity occurs under conditions other than just those situations, such as resulting from exogenous steroid administration or breeding season changes, which are associated with elevated androgen levels. Unfortunately, the results do not provide evidence as to the mechanism by which this change in tuning is mediated. The results do, however, indicate the generality of tuning plasticity and different conditions under which electroreceptors can maintain their match to the animal's discharge frequency.

Electrical filters occur not only in electroreceptors but also in hair cells (Crawford and Fettiplace 1981; Lewis and Hudspeth 1983; Ashmore 1984; Fuchs 1985). Since the ionic currents in both electroreceptors and hair cells appear similar (Lewis and Hudspeth 1983; Zakon 1984b), the generality of tuning plasticity as noted in electroreceptors suggests that plasticity in electrical filters of hair cells could also be present. Rubel and Ryals (1983) have reported apparent developmental shifts in the mechanical tuning of hair cells in the chick cochlea; whether or not the electrical filters of these cells (Fuchs 1985) also display developmental tuning alterations is as yet unknown.

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