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Plasticity of the electric organ discharge waveform of the electric fish *Brachyhypopomus pinnicaudatus* I. Quantification of day-night changes

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Abstract The electric organ discharge of the gymnotiform fish *Brachyhypopomus pinnicaudatus* is a biphasic waveform. The female's electric organ discharge is nearly symmetric but males produce a longer second phase than first phase. In this study, infrared-sensitive video cameras monitored the position of unrestrained fish, facilitating precise measurement of electric organ discharge duration and amplitude every 2 h for 24 h. Males ($n = 27$) increased electric organ discharge duration by 37 ± 12% and amplitude by 24 ± 9% at night and decreased it during the day. In contrast, females ($n = 8$) exhibited only minor electric organ discharge variation over time. Most of a male's increase occurred rapidly within the first 2–3 h of darkness. Electric organ discharge values gradually diminished during the second half of the dark period and into the next morning. Modulation of the second phase of the biphasic electric organ discharge produced most of the duration change in males, but both phases changed amplitude by similar amounts. Turning the lights off at mid-day triggered an immediate increase in electric organ discharge, suggesting modification of existing ion channels in the electric organ, rather than altered genomic expression. Exaggeration of electric organ discharge sex differences implies a social function. Daily reduction of duration and amplitude may reduce predation risk or energy expenditure.

Key words Gymnotiformes · Weakly electric fish · Electric organ discharge · Rapid modulation · Action potential plasticity

Abbreviations *11KT* 11-ketotestosterone · *Amax* maximum EOD amplitude · *Amin* minimum EOD amplitude · cAmp cyclic adenosine 3′,5′-monophosphate · *Dmax* maximum EOD duration ·*Dmin* minimum EOD duration · *EOD* electric organ discharge · *GnRH* gonadotropin-releasing hormone · *GRIF* gonadotropin-releasing inhibiting factor

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Introduction

The gymnotiform fish of South America produce weak electric discharges to communicate and to perceive objects in the dark (Lissmann 1958, 1961; Bastian 1986; Hagedorn 1986). In the adults of each species, the waveform of the electric organ discharge (EOD) has characteristic features such as the number and polarity of phases, the duration and relative amplitude of the phases, and the frequency content of the signal (Bass 1986). In some species, sexually mature males and females show additional differences in EOD waveform (Landsman 1995). Smaller differences among individuals' EOD waveforms may signal individual identity if the waveform remains constant over time (Westby 1975; McGregor and Westby 1992).

Those studies that report individual EOD waveform stability in gymnotiforms recorded EODs from restrained individuals in the laboratory, during daylight hours over 1–4 days (Bullock 1969; Westby 1975; Mills and Zakon 1987; McGregor and Westby 1992; Rasnow et al. 1993). In the context of steroid hormone treatments, where results typically require several days to manifest, investigators measured the EOD once per day at the same time each day (Meyer 1983; Hagedorn and Carr 1985; Meyer et al. 1987; Mills and Zakon 1987; Ferrari et al. 1995). Although these studies controlled for potential circadian fluctuation in the signal, none of them investigated directly the possibility that the EOD waveform changes over a 24-h time scale.

Several species within the hypopomid family have been observed to change EOD waveform from day to night. In restrained *Brachyhypopomus occidentalis*, EOD amplitude increased each night by 36 ± 10% compared to daytime values ($n = 7$; Hagedorn 1995). Captive, but

free-swimming *B. brevirostris* males in breeding condition increased EOD duration at night, especially when in the presence of other males (W. Crampton, personal communication). The EODs of a sexually mature male *B. diazi*, recorded in a Venezuelan stream around midnight, were 50% longer in duration than they were the following noon, immediately prior to capture; the following night in captivity, the EOD failed to increase to the duration observed in the stream, suggesting that stress may affect the day-night change (P. K. Stoddard and E. O. Setteducati, unpublished data).

The EOD waveform is a composite function of the individual action potentials produced by the electrocytes in the electric organ (Bennett 1961). In hypopomids, long columns of electrocytes stretch from the body cavity to the tip of the filament-like tail (Sullivan 1997). Because a longer fish can accommodate more electrocytes in series, EOD amplitude tends to increase with total fish length (Coates and Cox 1945; Brown and Coates 1952; Bennett 1971; Hopkins et al. 1990). Most hypopomids produce a biphasic EOD waveform from two sequential action potentials (Bennett 1961, 1971). The pacemaker nucleus in the hindbrain triggers action potentials across the caudal surfaces of the electrocytes. These action potentials sum to create a headward current flow and generate a head-positive EOD phase outside the body. This current in turn triggers action potentials across the rostral surfaces of electrocytes, reversing the current flow and creating a head-negative EOD phase. The duration and amplitude of each phase of the EOD therefore depend on the duration and amplitude of the action potentials across each electrocyte surface (Bennett 1961, 1971; Hagedorn and Carr 1985; Macadar et al. 1989; Ferrari and Zakon 1993). Other factors, such as the time interval between action potentials in each face and the degree of synchrony among electrocytes also affect the total EOD waveform. The shape of each action potential is determined by the properties of the ion channels in each active electrocyte surface. The electric organ of *Sternopygus* spp. (family Sternopygidae) has been a productive model for the classic effects of steroid hormones on action potentials (Zakon 1996). However, the natural behavior of these species has been described only briefly (Hopkins 1974), and only juveniles are used in the laboratory due to the large size of this fish at maturity.

B. pinnicaudatus (formerly *Hypopomus*; Hopkins 1991) breeds readily in captivity, reaches maturity in 6 months (Franchina 1997a), and displays well-characterized sex differences in anatomy and EOD waveform (Hopkins et al. 1990) (Fig. 1). Juveniles and females produce EODs of ~1.1 ms in duration with two fairly symmetric phases. In contrast, mature males produce EODs in which the second phase exceeds the first, thereby extending total EOD duration (Franchina 1997a; Hopkins et al. 1990). In pilot studies of captive breeding fish, *B. pinnicaudatus* males increased EOD duration at night and decreased it during the day, while female EOD duration remained constant (Franchina 1993). We chose this species as a potential model for studies of the mechanism underlying daily changes in EOD waveform.

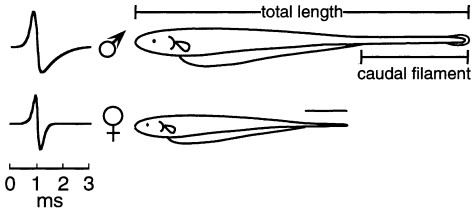

Fig. 1 Schematics of our study organism, sexually mature male and female *B. pinnicaudatus*, shown alongside their electric organ discharges (EODS). On average, total fish length of males exceeds that of females at sexual maturity (Hopkins et al. 1990). Furthermore, the caudal filament of a male is thicker and proportionately longer than that of a female, and it sometimes has a flared, spade-like tip. The first phase of the EOD is similar between the sexes but the second phase is significantly longer in males (amplitudes are re-scaled to the same height for comparison)

Our goal in this study was to quantify the day-night changes in EOD duration and amplitude in unrestrained *B. pinnicaudatus*. To judge the usefulness of this species for physiology studies, we also tested the effects of handling and sudden changes in light levels on EOD modulation. Based on our data and many other areas of research, we propose a cascade mechanism that could explain rapid day-night changes in EOD waveform.

Materials and methods

Environmental conditions

Laboratory-raised *B. pinnicaudatus* lived in mixed-sex groups of 10–30 adults in outdoor pools (~150 cm in diameter and 25 cm deep), which were covered with water hyacinths *(Eichornia crassipes)*. The average conductivity of the pools was 117 ± 30 μS cm^{-1} (8.5 kΩ cm^{-1}). Day length varied from approximately 14.5 h on 18 July to 12.1 h on 13 October. The natural rainy season in Miami, Florida stimulated breeding throughout the experiments; all subjects were in reproductive condition. We identified sex based on morphology of the caudal filament (Fig. 1) (Hopkins et al. 1990).

Experiments took place in two identical tanks (120 × 44 × 44 cm; 232 l) within an indoor light-tight chamber. Recirculated water falling on the surface (artificial rain) was sufficient to induce spawning indoors, so we kept conductivity constant during each experiment (102 ± 2 μS cm^{-1}; 9.8 kΩ cm^{-1}). Six 40-W fluorescent bulbs above each tank were on from 0600 to 2000 hours (14 h), causing the temperature to rise during the day from 27.5 °C to 29.0 °C, on average; a tank heater kept the temperature above 27 °C at night. The faintest visible light suppressed nocturnal activity in this species, so an array of infrared LEDs (OP293A, Newark Electronics) backlit the tank at night for infrared-sensitive CCD video cameras (Cohu 6410; Javelin JE7862). Oligochaete "blackworms" were available ad libitum as food.

EOD Recording

In gymnotiforms the EOD waveform and amplitude change, based on the orientation and distance of the fish relative to the recording electrodes (Hopkins 1986). Therefore, repeated measurements of the EOD may be compared only if the position of the fish is constant with respect to the electrodes. However, restraining the fish prevents normal behavior and may increase stress. We took

advantage of the natural tendency of *B. pinnicaudatus* to seek dark, confined spaces during the day to limit the position of the test fish without restraining it.

Plastic mesh partitions divided the tank into three compartments, with the sides joined by a 22-cm-long, narrow mesh tube (Fig. 2). A grid on the back of the tank indicated the lengthwise position of a fish in the tube to the nearest centimeter. During the day, test fish preferred to rest in the tube, which was narrow and shaded by surface plants. Therefore, the test fish was approximately centered between two recording electrodes located on the far ends of the tank (120 cm apart). This geometry proved the least sensitive to movement (Franchina 1997b). A High-8 VCR (Sony EVO-9500A) recorded both the video camera image of the tank and the amplified electric signal (Charles Ward Electronics amplifier BMA-200) on one digital audio channel at 48k samples s^{-1}. A timer flashed an LED and created a brief click on the second audio channel every 2 s for synchronization of the visual and EOD records. At night, we videotaped as the test fish swam through the tube, then used frame-by-frame analysis of the videotape to select the EOD produced when the fish was centered.

Experiments

We tested 27 males (total fish length 21.6 ± 2.6 cm) and 8 females (15.6 ± 2.2 cm), because pilot data indicated that only male EODs change significantly from day to night (Franchina 1993). Nineteen other adults "stimulated" test fish to swim through the mesh tube frequently (at night). Around 0900 hours, we placed the test fish in a side compartment and a stimulus fish (male, female, or none) in the middle, then waited 30–60 min for them to return to a resting state. Every 2 h during the day, we videotaped resting fish for a few seconds and measured water temperature. At night, we videotaped as the test fish swam through the tube, roughly once every 2 h. We took more frequent records around the transition of light to dark and during special experiments (below). Records covered 24 h, but for statistical analysis we used only data from when the fish entered the tank until 0600 hours the next morning.

To simulate the potentially stressful effect of transfer to the tank, 4 of the 27 males were "captured" briefly, or taken out of the water and held in a small mesh net in the tank for an hour on either their first day or night in the tank. We also kept one male in the tank for an additional 24 h, during which we moved him to a bucket for an hour at night. To test how quickly the EOD would respond to an unexpected transition to dark or light, we recorded the EODs of four other males for a second 24-h period, during which the lights either turned off early at 1300 hours, or came on early at 2230 hours.

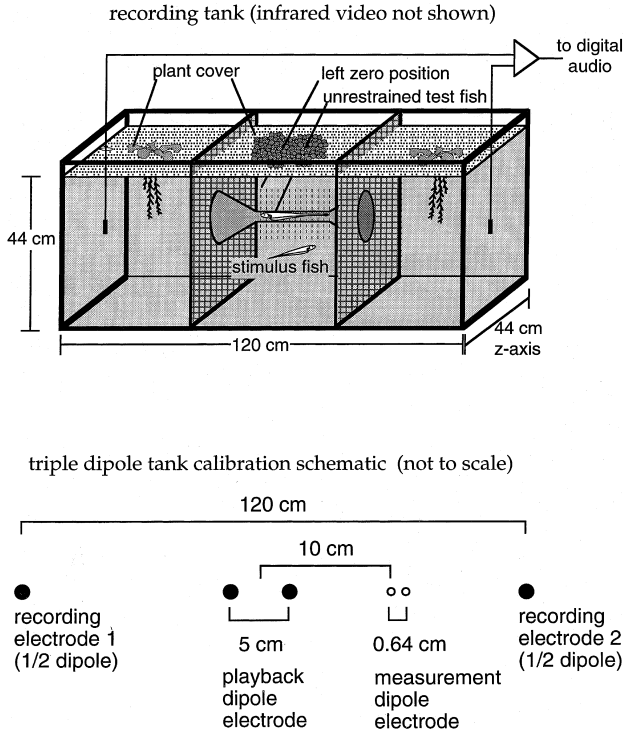

Fig. 2 The experimental tank and EOD amplitude calibration apparatus. The test fish could cross the middle compartment through a mesh tube, while the stimulus fish was confined to the middle compartment by electrically transparent mesh panels. A grid on the back wall indicated lengthwise position in the tube to the nearest centimeter. The triple dipole calibration apparatus (*lower figure*) was placed in the tank only briefly for calibration of the main recording electrodes. A 5-cm dipole playback electrode, centered in the tank, simulated the electric organ of a *B. pinnicaudatus* while we measured the signal amplitude from (a) the recording electrodes on the tank walls and from (b) the measurement dipole located 10 cm from the playback dipole. The ratio of these amplitudes (b/a) was used to convert test fish EOD amplitude (mV) across the recording electrodes to an equivalent electric field intensity (mV cm^{-1}) 10 cm from the source

EOD measurement and analysis

We devised a new method to estimate the field intensity (mV cm^{-1}) of a fish's signal at a distance of 10 cm referenced to the recorded potential difference (mV) between two electrodes 120 cm apart. Direct measurement of the field intensity requires recording electrodes to be placed a precise distance from the dipole electric source. This method is difficult except in an anesthetized fish because slight changes in electrode or fish position result in large errors. Furthermore, the dipole generated by the electric organ of many species moves along the fish's length during the discharge (e.g. Stoddard et al. 1995; Assad et al. 1998).

The effective dipole separation of a mature *B. pinnicaudatus* electric organ is 4–7 cm (P.K. Stoddard, B. Rasnow, C. Assad, unpublished observations); we used a 5-cm carbon rod dipole as a fish mimic. We played a single-period sine wave from the fish mimic at an amplitude similar to that of a real fish. We recorded the signal amplitude across two silver wire electrodes (0.64 cm apart) located 10 cm from the center of the fish mimic, as well as the amplitude across the normal recording electrodes (120 cm apart) located on either side of the mimic (Fig. 2). We used the ratio of the two values to convert all recorded potential differences (mV) into field intensities (mV cm^{-1}) at 10 cm from the source (= amplitude from here on). We measured EOD duration at 5% of the peak-to-peak amplitude (Fig. 3A) (after Hopkins et al. 1990).

To determine the random variation in measurements caused by a.c. line noise and digital quantization, we measured ten playback EODs with the lights on and ten with lights off in each tank. The coefficient of variance (standard deviation/mean) of EOD duration was usually ±1% (±0.02 ms). The coefficient of variance of EOD amplitude was ±0.6% (±0.0026 mV cm^{-1}). EODs from stationary test fish over a 2-s period showed similar variance.

To quantify the effect of variation in the fish's lengthwise position, we selected a series of EODs from positions 1.7 cm apart as three different males (17, 21, and 26 cm in length) swam through the tube in each direction (Fig. 3B). For all three fish, the coefficient of variance of duration or amplitude over a 10-cm movement range was ±2%, or only slightly more than the random variance. Although the fish's position was not identical during every recording, we only analyzed EODs recorded when the fish was ≤6 cm away from center.

We measured the duration and amplitude of a single EOD from each record, or 14–17 EODs for each fish. From this set of measurements, we chose the minimum and maximum EOD duration values (Dmin, Dmax) and minimum and maximum EOD amplitude values (Amin, Amax). We considered all values within 0.04 ms or 0.005 mV cm^{-1} of the minima and maxima to be equivalent and chose the earliest value for statistical analysis. (Random variance was ±0.02 ms, ±0.0026 mV cm^{-1}). ANOVA revealed no signifi-

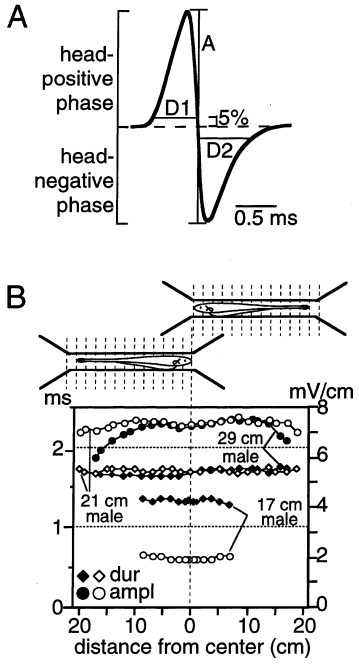

Fig. 3 A Duration of each phase (*D1*, *D2*) was measured at 5% of the total peak-to-peak amplitude then summed. The a.c. line noise and digital quantization error caused ≤1% random variation in the duration and amplitude of ten playback EODs. **B** A fish in the tube with its nose at one end, as shown, was approximately centered in the tank. EOD measurements from three males (17, 21, and 29 cm) are shown for multiple positions in the mesh tube. As long as the fish's body remained straight, large changes in position did not significantly alter EOD duration. Movement up to 10 cm from the center caused ≤2% variance in amplitude. Only EODs recorded from fish positioned within 6 cm of the center were used for analysis

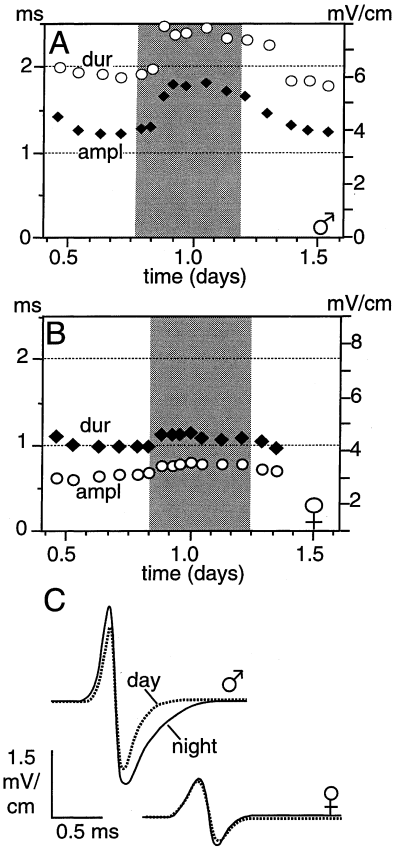

Fig. 4 Male EOD duration and amplitude changed in a cyclic pattern over 24 h (**A**), while female EOD values changed very little (**B**). Each interval on the *x*-axis represents 2 h. **C** This male's maximum nighttime EOD was 40% longer and 21% larger than his minimum daytime EOD. However, the 14% and 12% differences in duration and amplitude in this female's EOD are barely noticeable and probably due to random error or movement of the fish

cant difference in EOD minima, maxima, or amount of change between the four males "captured" during their first period in the tank and 23 males left undisturbed, so all 27 males were lumped for analysis.

We tested for a relationship between EOD waveform and discharge rate by measuring one interval between successive EODs (interpulse interval) of two test males at each recording. In addition, we chose two sequences during which a stationary female changed rate and measured the interpulse interval once per second for 15 s.

Results

Magnitude and timing of EOD change

Over 24 h the male EOD waveform changed so that duration and amplitude were dramatically larger at night than during the day, while the female EOD waveform changed very little (Fig. 4). In males, maximum values could be predicted from daytime minima (duration $r^2 = 0.75$; amplitude $r^2 = 0.89$; $P < 0.001$; all *P*-values two-tailed, $n = 27$). Male EOD duration correlated weakly with total fish length (Dmin: 0.038 ms cm^{-1}, $r^2 = 0.16$; Dmax: 0.051 ms cm^{-1}, $r^2 = 0.17$; $P \leq 0.02$). Male EOD amplitude increased with total fish length (Amin: 0.28 mV cm$^{-1} \cdot$ cm^{-1}, $r^2 = 0.41$; Amax: 0.41 mV cm$^{-1} \cdot$ cm^{-1}, $r^2 = 0.46$; $P \leq 0.001$), but the relationship was much weaker than that found in a daytime study of the same species in the field ($r^2 = 0.84$; $n = 15$; Hopkins et al. 1990). While the amount of duration change in each male (Dmax–Dmin) was unrelated to total fish length ($P = 0.29$), the daily change in amplitude (Amax–Amin) increased with length (0.13 mV cm$^{-1} \cdot$ cm^{-1}, $r^2 = 0.28$, $P = 0.003$).

Male EOD waveforms underwent an average change of 0.51 ± 0.17 ms in duration, or 37% (range 21–65%), and 1.26 ± 0.64 mV cm^{-1} in amplitude, or 24% (range 12–57%) during a 24-h period (Fig. 5A). The second, head-negative phase of the EOD accounted for most of this change in duration as well as most of the inter-individual variation; both phases contributed to the change in amplitude (Fig. 6). The increase in duration and amplitude was clearly associated with the day-to-night transition in males (Fig. 5B, C). In contrast, the differences between minimum and maximum EOD values in females were absolutely and proportionately smaller: only 0.16 ± 0.05 ms (16%) for duration and 0.48 ± 0.32 mV cm$^{-1} \cdot$ cm^{-1} (16%) for amplitude (Fig. 5A). Bartlett's test indicated that the variation in

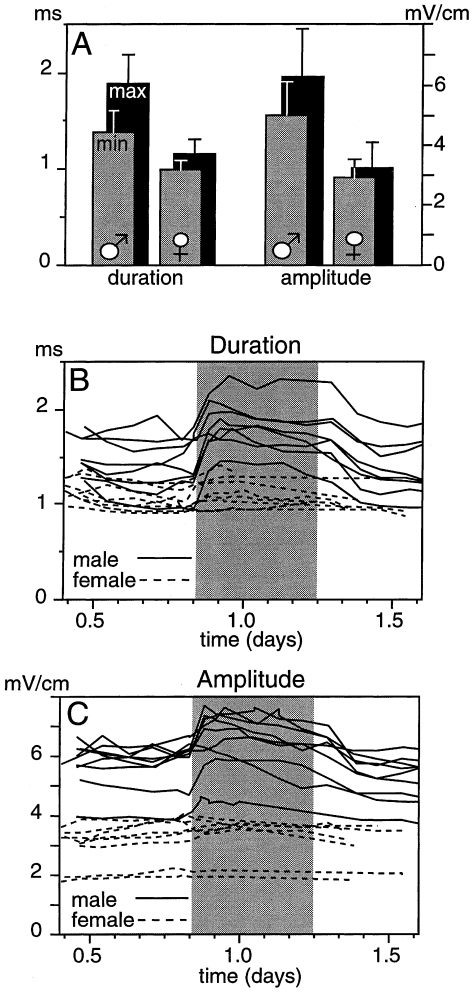

Fig. 5A Twenty-seven males increased EOD duration 37 ± 12%, from a daytime minimum of 1.39 ± 0.23 ms to a maximum of 1.89 ± 0.30 ms at night. These males increased EOD amplitude 24 ± 9%, from 5.14 ± 1.14 mV cm^{-2} during the day to 6.40 ± 1.61 mV cm^{-1} at night. In eight females, the differences between minimum and maximum EOD duration (0.16 ± 0.05 ms; 16 ± 5%) and amplitude (0.48 ± 0.32 mV cm^{-1}; 16 ± 7%) were absolutely and proportionately smaller. **B, C** The EOD values over time of eight males (*solid lines*) and eight females (*dashed lines*). While male EOD duration and amplitude show a sharp increase around the light to dark transition, the slight variation in female EOD values usually appears to be unrelated to the time of day

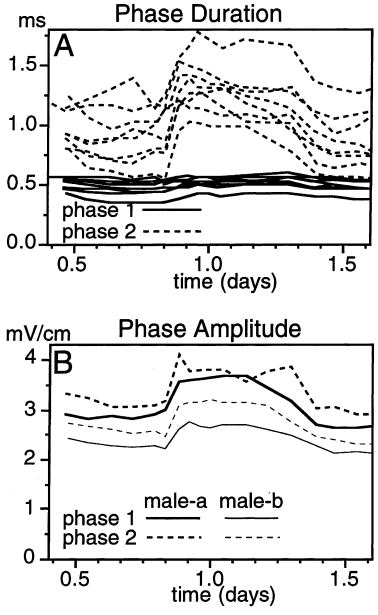

Fig. 6A The second, head-negative phase of the EOD accounted for most of the individual variation in males as well as most of the change in total duration over time (eight males shown). These data suggest that only the anterior faces of the electrocytes are involved in the mechanism for changing duration. **B** Both EOD phases changed in amplitude (two males shown), suggesting a mechanism for changing amplitude that involves the whole electrocyte or electric organ

each female's EOD duration over time was no different than that of a constant playback signal ($P > 0.4$); in fact, for four of eight females the maximum duration value occurred before the minimum (Fig. 5B). On the other hand, in all females the minimum amplitude occurred before the maximum; in three of eight females a small increase occurred near dusk (Fig. 5C).

Male EOD duration and amplitude started to increase rapidly about 1 h before lights out (2000 hours) (Fig. 4A, 5B, C) and reached maxima by 2130 hours, on average (Fig. 7). Therefore, most of the change occurred within 2–3 hours. Usually EOD duration and amplitude began to decrease slowly during the second half of the dark phase and continued to decline after lights on. On

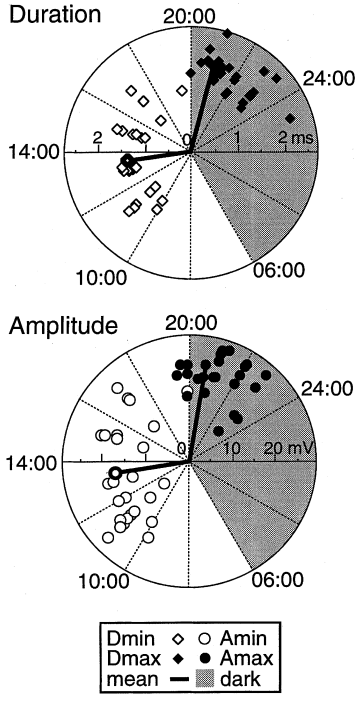

Fig. 7 Timing of minima and maxima. The distance of each point from the center represents the minimum or maximum EOD value of one male; angular location represents the time of day. *Thick lines* indicate the mean vectors. Timing of minima occurred midway through the light phase, on average, but varied greatly because EOD values were relatively constant during most of the day. In contrast, maxima clustered in the first 3 h of the dark phase. Data include from 1000 to 0600 hours of the first 24-h period only

average, male minimum EOD values occurred midway through the light phase (1300 hours) (Fig. 7), but the timing was broadly scattered because waveform usually changed little over most of the day.

Effect of temperature and discharge rate on the EOD

Minimum EOD duration among 27 males was negatively but weakly correlated with water temperature ($Q_{10} = -0.061$ ms °C^{-1}, $r^2 = 0.11$, $P = 0.047$); minimum amplitude was not related to temperature ($r^2 = 0.05$, $P = 0.13$). Thus, a nocturnal drop in water temperature of 1.5 °C could account for a 6% increase in EOD duration, much less than the observed 21–65%, but would not account for the change in amplitude.

Neither EOD duration nor amplitude correlated with the interpulse interval in two fish of each sex, so changes in discharge rate cannot be responsible for the changes in waveform. Even lowering the discharge rate with anesthetic does not alter EOD duration in any species of gymnotiform with a myogenic organ (Mills and Zakon 1987; Assad et al. 1994). However, in certain courtship signals, when males increase EOD rate up to six times the resting rate, EOD amplitude does diminish (Stoddard et al. 1996).

Response to handling

Transferring the fish from the outdoor pools to the tank seemed to disturb fish only temporarily. Fish quickly returned to a resting state, and at least half of the males with stimulus females performed some courtship that night; one pair even spawned after the female escaped the middle compartment. Of the five males that we "captured", two decreased EOD duration but returned to about their previous duration within an hour; the other three males' EOD duration seemed unaffected (Fig. 8). All five males showed virtually no difference in EOD amplitude before and after capture (Fig. 8).

Response to unexpected light or darkness

The two males exposed to early darkness immediately increased EOD duration and amplitude and exhibited normal nocturnal activity. EOD values of one male continued to increase throughout the early dark period (Fig. 9A); for the other, EOD values increased, decreased, then increased again just before darkness normally would have begun (Fig. 9B). Despite the prolonged dark phase, the overall changes in duration and amplitude were no greater than on the previous night. Early termination of darkness did not appear to alter the pattern of EOD change in two males (Fig. 9C), although the duration change of one fish was ambiguous (Fig. 9D). These data imply that a light-to-dark transition at any time triggers a physiological process leading to increased EOD values, while the dark-to-light transition is relatively unimportant. Therefore, physiological exploration of the hormonal and cellular mechanisms of EOD change might be conducted after exposing the fish to a relatively brief period of darkness to trigger the EOD change.

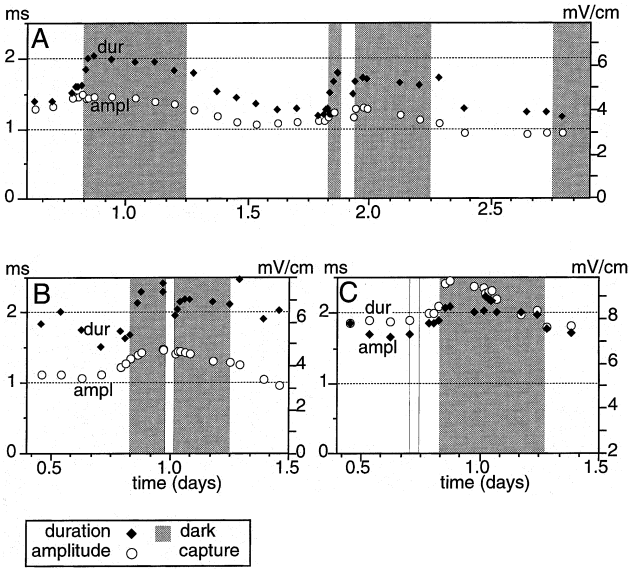

Fig. 8A–D Data from three males that were netted and restrained for 1 h (capture). Males in **A** and **B** showed a small temporary decrease in EOD duration and amplitude. **C** Capture did not seem to affect EOD waveform in this male or two others (not shown)

Discussion

Behavioral significance of day-night EOD changes

The techniques developed in this study allowed precise quantification of nocturnal increases in EOD duration (37%) and amplitude (24%) in free-swimming male *B. pinnicaudatus* (Fig. 5A). Female EOD amplitude, but not duration, may change in a cyclic fashion, but such changes are much smaller on both an absolute and a relative scale than those of males. Thus, the sexual dimorphisms in the EOD waveform are accompanied by striking sex differences in the day-night modulation of these differences.

The EOD is a signal used primarily at night for electrolocation and communication by both sexes. The fact that cyclic changes occur only in males, exaggerating sex differences in the waveform, implies that the changes evolved in the context of sexual selection rather than for electrosensory functions common to both sexes. Courtship and spawning begin between 1 and 3 h after dark (P.K. Stoddard, M.D. Kilburn, K.H. Patterson, unpublished data), about the same time that male EODs reach their maximum amplitudes and durations (Fig. 7). Furthermore, observations of day-night EOD changes in other hypopomids also involved fish in reproductive

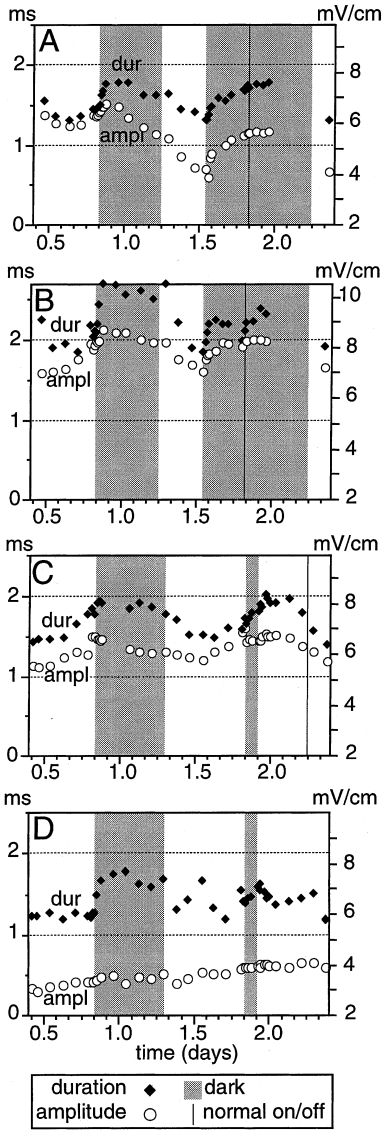

Fig. 9A–C Four males were exposed to a normal 10-h dark phase for one period, then to a long or short dark phase during a second period. **A, B** Males immediately responded to the early onset of darkness with rapid increases in EOD duration and amplitude. **C, D** Early onset of light did not appear to alter the pattern of EOD change, although the duration change of male D was ambiguous. These data imply that a light-to-dark transition at any time triggers a physiological process leading to increased EOD values, while the dark-to-light transition is relatively unimportant. The amplitude axis has been shifted vertically for better point separation

condition (Hagedorn 1995; W. Crampton, personal communication; P.K. Stoddard, E.O. Setteducati, unpublished data).

The decrease in EOD duration and amplitude during the day suggests that the nocturnal increases are somehow costly. A larger amplitude EOD might increase the risk of detection by predatory gymnotiforms, such as the electric eel *(Electrophorus electricus)*, *Gymnotus* spp., and *Magosternarchus duccis*, which tend to be active during the day as well as at night (Westby 1988; M. Hagedorn, personal communication; Lundberg et al. 1996). The fact that the second, head-negative phase of the EOD becomes even longer than the first phase at night increases the net negative d.c. component of the male signal (Stoddard 1994). Such a d.c. component should be more detectable by the ampullary electroreceptors of catfish, some of which specialize in eating gymnotiforms (Reid 1983), as well as by the ampullary electroreceptors of gymnotiform predators. The energetic cost of EOD production has never been measured, so the relative cost of a longer, larger nighttime EOD is unknown. By reducing EOD duration and amplitude while resting during the day, males may improve survival, conserve energy, or both.

A model for cyclic EOD change

We combined evidence from several areas of investigation to illustrate a cascade mechanism that could cause day-night changes in the EOD waveform (Fig. 10). The central nervous system of all vertebrates contains one or more "master" pacemakers, which are entrained by environmental cues and control circadian rhythms throughout the body (Menaker and Binkley 1981; Jacklet 1985). Indirect evidence suggests that cyclic changes in EOD waveform could be driven by an endogenous pacemaker. In *B. occidentalis*, amplitude modulation persisted for two periods in constant darkness (Hagedorn 1995). Three *B. pinnicaudatus* males kept in constant darkness for three periods increased duration at the normal time (Franchina 1997b). In this study, the EOD usually began to change before lights out, despite the absence of cues such as slowly falling light levels (e.g. Fig. 8A).

In teleosts, the master pacemaker is the pineal gland, which synthesizes melatonin rhythmically even in vitro under constant conditions (Zachmann et al. 1992; Bolliet et al. 1995, 1996; Cahill 1996). Melatonin affects the pulsatile release of gonadotropin-releasing or -inhibiting hormones (GnRH, GRIF) from cells in the pre-optic area and/or hypothalamus (Peter 1983; Senthilkumaran and Joy 1995; Khan and Thomas 1996). Melatonin and neural output from the pineal gland may also affect other areas of the brain and peripheral organs directly. GnRH and GRIF control the pituitary's release of gonadotropins, which regulate steroid production by the gonads (Bhattacharya et al. 1994). Steroid hormones fluctuate in circadian cycles in nearly all teleost species examined (Hannes and Franck 1983; Bieniarz et al. 1986; Matsuyama et al. 1988, 1995; Emata et al. 1991; Singh and Singh 1991). In particular, baseline levels of the primary teleost androgen, 11-ketotestosterone (11KT), are reliably associated with male-typical behavior and secondary sex characters (Zakon et al. 1991; Brantley et al. 1993; Borg 1994).

The classic mechanism of steroid action is "slow" alteration of gene expression (Paul and Purdy 1992). Treating adult or juvenile gymnotiforms with androgens increases EOD duration in most species, but only after

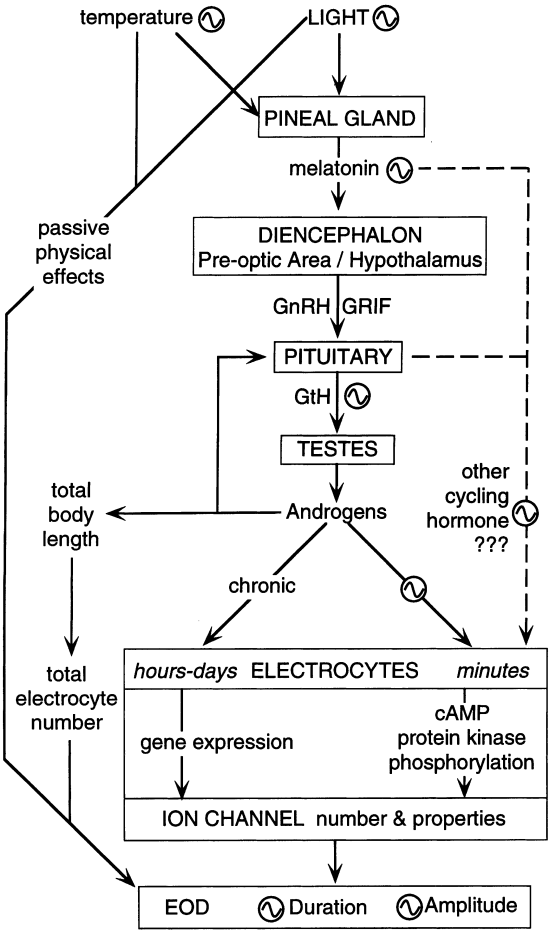

Fig. 10 A mechanism to explain modulation of the EOD waveform; *circled sine waves* represent circadian fluctuation. EOD duration and amplitude are affected directly by the properties and numbers of ion channels in the electrocytes, as well as by other factors. Chronic implants or daily injections of steroids alter EOD waveform after several days, presumably by affecting gene expression in the electric organ. In addition, hormone levels fluctuate throughout the day in response to signals from the pineal gland. (This pattern, common to several teleosts, still has to be demonstrated in the gymnotiforms.) Hormones may also have immediate, non-genomic effects on cell physiology through second messenger systems, such as a release of cAMP leading to phosphorylation of ion channels. This model predicts that applying the appropriate hormone or intracellular messenger to electrocytes will cause rapid changes in the action potentials, which can be extrapolated to changes in EOD waveform. See text for citations of contributing work. *GnRH* gonadotropin-releasing hormone; *GRIF* gonadotropin release-inhibiting factor; *GtH* gonadotropin; *11KT* 11-ketotestosterone; *cAMP* cyclic AMP

several days (Hagedorn and Carr 1985; Ferrari et al. 1995; Landsman 1995). Genomic steroid effects probably account for sex differences in total fish length, caudal filament morphology, and EOD duration in *B. pinnicaudatus* (Hopkins et al. 1990). However, steroids can alter gene expression in as little as 3 h in vitro (Paul and Purdy 1992). In this study, male EODs changed from minimum to maximum values over a 3- to 6-h period (Fig. 7). However, males responded to the onset of darkness at mid-day by increasing EOD duration and amplitude dramatically in as little as 1 h (Fig. 9A, B), and measurable changes have recently been induced in less than 15 min (P.K. Stoddard, V. Salazar, unpublished data). Therefore, a genomic steroid mechanism is not sufficient to explain the response to unexpected darkness.

In contrast to classic genomic mechanisms, steroid hormones and other neuromodulators can alter existing ion channels within minutes through a second messenger cascade (Erulkar and Wetzel 1987; Schumacher 1990; McEwen 1991; Paul and Purdy 1992; Wehling 1997). For example, steroids can affect the activity of adenyl cyclase, which regulates the level of cyclic adenosine 3′, 5′-monophosphate (cAMP) (Erulkar and Wetzel 1987; Harrelson and McEwen 1987). Cyclic AMP promotes the activity of protein kinase A, which phosphorylates ion channels. Phosphorylation changes the duration of some ion currents by changing the inactivation time constant or voltage dependence of the channel (Hille 1992). In other cases, the amplitude of an ion current changes because phosphorylation alters the probability of channel opening (Levitan 1988). Steroids may also recruit existing channels into the membrane to increase ion current amplitude. While rapid plasticity is apparently an inherent property of all ion channels, the behavioral consequences of phosphorylation in the nervous system are still mostly unknown (Huganir 1988; Levitan 1988; Hille 1992; Wehling 1997).

In weakly electric fish, phosphorylation of ion channels in the electric organ should affect EOD waveform directly. Cyclic AMP applied to the electrocytes of *Sternopygus* spp. increases the amplitude of the sodium current within 20 min in vitro (McAnelly and Zakon 1996). (Rapid modulation of EOD waveform has not yet been demonstrated in intact *Sternopygus* spp.) In the electrocytes of *Electrophorus electricus*, phosphorylation reduces the sodium current by 80% within 30 s (Emerick et al. 1993). Thus, Fig. 10 illustrates one potential pathway by which environmental cues could cause daily fluctuation in steroid levels and concurrent change in EOD waveform characters.

Our data suggest that EOD duration and amplitude are controlled separately in *B. pinnicaudatus*. The first and second phase of the EOD correspond to action potentials in the caudal and rostral surfaces of the electrocytes, respectively. The second EOD phase fluctuated much more in duration than the first EOD phase did (Fig. 6), suggesting that kinetics of ion channels in the rostral surface are more affected by the modulating hormone(s) than those in the caudal surface. Because the amplitude of both phases fluctuated about equally, total EOD amplitude may be controlled by a single function, such as changing the number of active ion channels or even the number of electrocytes that respond to the pacemaker. The studies of *Sternopygus* spp. electrocytes also suggest independent mechanisms for altering EOD duration and amplitude (Ferrari et al. 1995; McAnelly and Zakon 1996).

The EODs of weakly electric fish play an essential role in communication. At the cellular level, the electric

organ is the sum of two action potentials, each created by localized ion channels. Thus *Brachyhypopomus* spp. could be an excellent model for investigating rapid hormone effects at both a molecular and behavioral level.

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References

Assad C, Rasnow B, Stoddard PK, Bower JM (1994) Maps of the electric organ discharges of several gymnotiform fish. Soc Neurosci Abstr 24: 370

Assad C, Rasnow B, Stoddard PK, Bower JM (1998) The electric organ discharges of the gymnotiform fishes: II. *Eigenmannia* sp. J Comp Physiol A 183: 419–432

Bass AH (1986) Electric organs revisited. In: Bullock TH, Heiligenberg W (eds) Electroreception. Wiley, New York, pp 13–70

Bastian J (1986) Electrolocation: behavior, anatomy, and physiology. In: Bullock TH, Heiligenberg W (eds) Electroreception. Wiley, New York, pp 577–612

Bennett MLV (1961) Modes of operation of electric organs. Ann NY Acad Sci 94: 458–509

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

Bhattacharya S, Halder S, Manna PR (1994) Current status of endocrine aspects of fish production: an overview. Proc Indian Natl Sci Acad Part B 60: 33–43

Bieniarz K, Sokolowska M, Fostier A, Epler P (1986) Daily changes in blood serum levels of 17 beta-estradiol and 11-ketotestosterone in the mature carp *Cyprinus carpio* L. Chronobiologia 13: 23–27

Bolliet V, Falcón J, Ali MA (1995) Regulation of melatonin secretion by light in the isolated pineal organ of the white sucker (*Catostomus commersoni*). J Neuroendocrinol 7: 535–542

Bolliet V, Ali MA, Lapointe FJ, Falcón J (1996) Rhythmic melatonin secretion in different teleost species: an in vitro study. J Comp Physiol B 165: 677–683

Borg B (1994) Androgens in teleost fishes. Comp Biochem Physiol C 109: 219–245

Brantley RK, Wingfield JC, Bass AH (1993) Sex steroid levels in *Porichthys notatus*, a fish with alternative reproductive tactics, and a review of the hormonal bases for male dimorphism among teleost fishes. Horm Behav 27: 332–347

Brown MV, Coates CW (1952) Further comparisons of length and voltage in the electric eel, *Electrophorus electricus* (Linnaeus). Zoologica 37: 191–197

Bullock TH (1969) Species differences in effect of electroreceptor input on electric organ pacemakers and other aspects of behavior in electric fish. Brain Behav Evol 2: 85–118

Cahill GM (1996) Circadian regulation of melatonin production in cultured zebrafish pineal and retina. Brain Res 708: 177–181

Coates CW, Cox RT (1945) A comparison of length and voltage in the electric eel, *Electrophorus electricus* (Linnaeus). Zoologica 30: 89–93

Emata AC, Meier AH, Hsiao SM (1991) Daily variations in plasma hormone concentrations during the semilunar spawning cycle of the gulf killifish, *Fundulus grandis*. J Exp Zool 259: 343–354

Emerick MC, Shenkel S, Agnew WS (1993) Regulation of the eel electroplax Na channel and phosphorylation of residues on amino- and carboxyl-terminal domains by cAMP-dependent protein kinase. Biochem 32: 9435–9444

Erulkar SD, Wetzel DM (1987) Steroid effects on excitable membranes. In: Strauss III JF, Pfaff DW (eds) Current topics in membranes and transport, vol 31. Academic Press, New York, pp 141–190

Ferrari MB, McAnelly ML, Zakon HH (1995) Individual variation in and androgen-modulation of the sodium current in electric organ. J Neurosci 15: 4023–4032

Ferrari MB, Zakon HH (1993) Conductances contributing to the action potential of *Sternopygus* electrocytes. J Comp Physiol A 173: 281–292

Franchina CR (1993) The waveform of the weakly electric fish *Hypopomus pinnicaudatus* changes daily in the male. J Comp Physiol A 173: 742

Franchina CR (1997a) Ontogeny of the electric organ discharge and the electric organ in the weakly electric pulse fish *Brachyhypopomus pinnicaudatus* (Gymnotiformes, Hypopomidae). J Comp Physiol A 181: 111–119

Franchina CR (1997b) Ontogenetic, day-night, and socially mediated changes in the electric organ discharge waveform of a weakly electric fish (Gymnotiformes; Hypopomidae). PhD dissertation, Cornell University

Hagedorn M (1986) The ecology, courtship, and mating of gymnotiform electric fish. In: Bullock TH, Heiligenberg W (eds) Electroreception. Wiley, New York, pp 497–526

Hagedorn M (1995) The electric fish *Hypopomus occidentalis* can rapidly modulate the amplitude and duration of its electric organ discharges. Anim Behav 49: 1409–1413

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

Hannes RP, Franck D (1983) Diurnal variation of blood androgen and corticoid levels in male swordtails (*Xiphophorus helleri*). Zool Jb Physiol 87: 337–341

Harrelson A, McEwen B (1987) Steroid hormone influences on cyclic AMP-generating systems. In: Strauss III JF, Pfaff DW (eds) Current topics in membranes and transport, vol 31. Academic Press, New York, pp 217–247

Hille B (1992) Ionic Channels of excitable membranes, 2nd edn. Sinauer, Sunderland, MA

Hopkins CD (1974) Electric communication in the reproductive behavior of *Sternopygus macrurus* (Gymnotoidei). Z Tierpsychol 35: 518–535

Hopkins CD (1986) Temporal structure of non-propagated electric communication signals. Brain Behav Evol 28: 43–59

Hopkins CD (1991) *Hypopomus pinnicaudatus* (Hypopomidae) a new species of gymnotiform fish from French Guiana. Copeia 1991: 151–161

Hopkins CD, Comfort NC, Bastian J, Bass AH (1990) Functional analysis of sexual dimorphism in an electric fish, *Hypopomus pinnicaudatus*, order Gymnotiformes. Brain Behav Evol 35: 350–367

Huganir RL (1988) Regulation of the nicotinic acetylcholine receptor channel by protein phosphorylation. In: Agnew WS, Claudio T, Sigworth FJ (eds) Current topics in membranes and transport, vol 33. Academic Press, New York, pp 147–163

Jacklet JW (1985) Neurobiology of circadian rhythms generators. Trends Neurosci 8: 69–73

Khan IA, Thomas P (1996) Melatonin influences gonadotropin II secretion in the Atlantic croaker (*Micropogonias undulatus*). Gen Comp Endocrinol 104: 231–242

Landsman R (1995) Sources of plasticity in behavior and its physiology: sex, hormones, environment and the captivity model. In: Moller P (ed) Electric fishes: history and behavior. Chapman and Hall, London, pp 303–344

Levitan IB (1988) Modulation of ion channels in neurons and other cells. Annu Rev Neurosci 11: 119–136

Lissmann HW (1958) On the function and evolution of electric organs in fish. J Exp Biol 35: 156–191
Lissmann HW (1961) Ecological studies on gymnotids. In: Chagas C, Paes de Carvalho A (eds) Bioelectrogenesis. Elsevier, Amsterdam, pp 215–226
Lundberg JG, Fernandes CC, Albert JS, Garcia M (1996) *Magosternarchus*, a new genus with two new species of electric fishes (Gymnotiformes; Apteronotidae) from the Amazon River basin, South America. Copeia 3: 657–670
Macadar O, Lorenzo D, Velluti JC (1989) Waveform generation of the electric organ discharge in *Gymnotus carapo* II. Electrophysiological properties of single electrocytes. J Comp Physiol A 165: 353–360
Matsuyama M, Adachi S, Nagahama Y, Matsura S (1988) Diurnal rhythm of oocyte development and plasma steroid hormone levels in the female red sea bream, *Pagrus major*, during the spawning season. Aquaculture 73: 357–372
Matsuyama M, Yoneda M, Takeuchi H, Kawaga H, Kashiwagi M, Tabata K, Nagahama Y, Ijiri S, Adachi S, Yamauchi K (1995) Diurnal periodicity in testicular activity in the Japanese flounder, *Paralichthys olivaceus*. Fish Sci (Tokyo) 61: 17–23
McAnelly L, Zakon HH (1996) Protein kinase A activation increases sodium current magnitude in the electric organ of *Sternopygus*. J Neurosci 16: 4383–4388
McEwen BS (1991) Non-genomic and genomic effects of steroids on neural activity. Trends Pharmacol Sci 12: 141–147
McGregor PK, Westby GWM (1992) Discrimination of individually characteristic electric organ discharges by a weakly electric fish. Anim Behav 43: 977–986
Menaker M, Binkley S (1981) Neural and endocrine control of circadian rhythms in the vertebrates. In: Aschoff J (ed) Handbook of behavioral neurobiology, vol 4. Biological rhythms. Plenum, New York, pp 243–255
Meyer JH (1983) Steroid influences upon the discharge frequencies of a weakly electric fish. J Comp Physiol A 153: 29–37
Meyer JH, Leong M, Keller CH (1987) Hormone-induced and maturational changes in electric organ discharges and electroreceptor tuning in the weakly electric fish *Apteronotus*. J Comp Physiol A 160: 385–394
Mills A, Zakon HH (1987) Coordination of EOD frequency and pulse duration in a weakly electric wave fish: the influence of androgens. J Comp Physiol A 161: 417–430
Paul SM, Purdy RH (1992) Neuroactive steroids. Fed Am Soc Exp Biol 6: 2311–2322
Peter RE (1983) The brain and neurohormones in teleost reproduction. In: Hoar WS, Randall DJ (eds) Fish physiology, vol IX. Academic Press, New York, pp 97–135
Rasnow B, Assad C, Bower JM (1993) Phase and amplitude maps of the electric organ discharge of the weakly electric fish, *Apteronotus leptorhynchus*. J Comp Physiol A 172: 481–491
Reid S (1983) La biologia de los bagres rayados *Pseudoplatystoma fasciatum y P. tigrinum* en la cuenca del rio Apure, Venezuela. Rev UNELLEZ Cienc Tecnol 1: 13–41
Schumacher M (1990) Rapid membrane effects of steroid hormones: an emerging concept in neuroendocrinology. Trends Neurosci 13: 359–362
Senthilkumaran B, Joy KP (1995) Effects of melatonin, *p*-chlorophenylalanine, and alpha-methyparatyrosine on plasma gonadotropin level and ovarian activity in the catfish, *Heteropneustes fossilis*: a study correlating changes in hypothalamic monoamines. Fish Physiol Biochem 14: 471–480
Singh A, Singh TP (1991) Circadian variations in circulating plasma sex steroids in female catfish, *Clarias batrachus*. Indian J Exp Biol 29: 864–866
Stoddard PK (1994) Low frequency electric field production and concealment by gymnotiform fish with pulsed electric organ discharges. Neurosci Abstr 20: 370
Stoddard PK, Rasnow B, Assad C (1995) Electric organ discharges of the gymnotiform fish *Brachyhypopomus* sp. In: Burrows M, Matheson T, Newland P, Schuppe H (eds) Nervous systems and behavior, Proceed 4th Intl Congress of Neuroethology, Cambridge, England p 417
Stoddard PK, Kilburn MD, Patterson KH (1996) Complex electric signal structure in reproducing gymnotiform electric fish. Soc Neurosci Abstr 22: 450
Sullivan JP (1997) A phylogenetic study of the neotropical hypopomid electric fishes (Gymnotiformes: Rhamphichthyoidea). PhD dissertation, Duke University
Wehling M (1997) Specific, nongenomic actions of steroid hormones. Annu Rev Physiol 59: 365–393
Westby GWM (1975) Further analysis of the individual discharge characteristics predicting social dominance in the electric fish, *Gymnotus carapo*. Anim Behav 23: 249–260
Westby GWM (1988) The ecology, discharge diversity and predatory behaviour of gymnotiforme electric fish in the coastal streams of French Guiana. Behav Ecol Sociobiol 22: 341–354
Zachmann A, Ali MA, Falcón J (1992) Melatonin and its effects in fishes: an overview. In: Ali MA (ed) Rhythms in fishes. Plenum, New York, pp 149–165
Zakon HH (1996) Hormonal modulation of communication signals in electric fish. Dev Neurosci 18: 115–123
Zakon HH, Yan HY, Thomas P (1991) Electric organ discharge frequency and plasma sex steroid levels during gonadal recrudescence in a natural population of *Sternopygus macrurus*. J Comp Physiol A 169: 493–499