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REVIEW

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# Regulation and modulation of electric waveforms in gymnotiform electric fish

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**Abstract** Weakly electric gymnotiform fish specialize in the regulation and modulation of the action potentials that make up their multi-purpose electric signals. To produce communication signals, gymnotiform fish modulate the waveforms of their electric organ discharges (EODs) over timescales spanning ten orders of magnitude within the animal's life cycle: developmental, reproductive, circadian, and behavioral. Rapid changes lasting milliseconds to seconds are the result of direct neural control of action potential firing in the electric organ. Intermediate-term changes taking minutes to hours result from the action of melanocortin peptides, the pituitary hormones that induce skin darkening and cortisol release in many vertebrates. Long-term changes in the EOD waveform taking days to weeks result from the action of sex steroids on the electrocytes in the electric organ as well as changes in the neural control structures in the brain. These long-term changes in the electric organ seem to be associated with changes in the expression of voltage-gated ion channels in two gene families. Electric organs express multiple voltage-gated sodium channel genes, at least one of which seems to be regulated by androgens. Electric organs also express multiple subunits of the *shaker* (Kv1) family of voltage-gated potassium channels. Expression of the Kv1 subtype has been found to vary with the duration of the waveform in the electric signal. Our increasing understanding of the mechanisms underlying precise control of electric communication signals may yield significant insights into the diversity of natural mechanisms available for modifying the performance of ion channels in excitable membranes. These mechanisms may lead to better understanding of normal function in a wide range of physiological systems and future application in treatment of disease states involving pathology of excitable membranes.

**Keywords** Androgens · Electrogenesis · Phenotypic plasticity

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## Introduction

Moment to moment an animal's behavior is influenced by the stimuli it encounters. Responses to environmental stimuli are frequently not instantaneous, but vary over a wide variety of timescales. In most species, behavior is expressed within a circadian cycle and suites of behaviors may be further partitioned within a particular circadian phase. Over longer timescales, animals show seasonal changes in behavior. This is most obvious in seasonally breeding species where reproductive behaviors develop and are expressed over many days or weeks.

In keeping with the time courses of these influences on behavior, neurotransmitters, neuromodulators, and hormones have different modes of action in which their effects may be rapid (seconds or minutes), intermediate-term (tens of minutes to hours), or long-lasting (days to weeks) (Fig. 1). While much is known about the actions of these agents on the cellular and molecular levels, the weak link lies in our understanding of how cellular events influence behavior. Electric fish are superb animals in which to study this problem. Their electrical behaviors are simple and easily quantified. The circuits generating these behaviors are known, simple, hierarchically organized, and largely amenable to electrophysiological and molecular analysis. Because these behaviors are in the currency of the nervous system—electricity—it is easy to generate realistic and testable hypotheses about how behavior is generated and regulated at the level of membrane biophysics. In

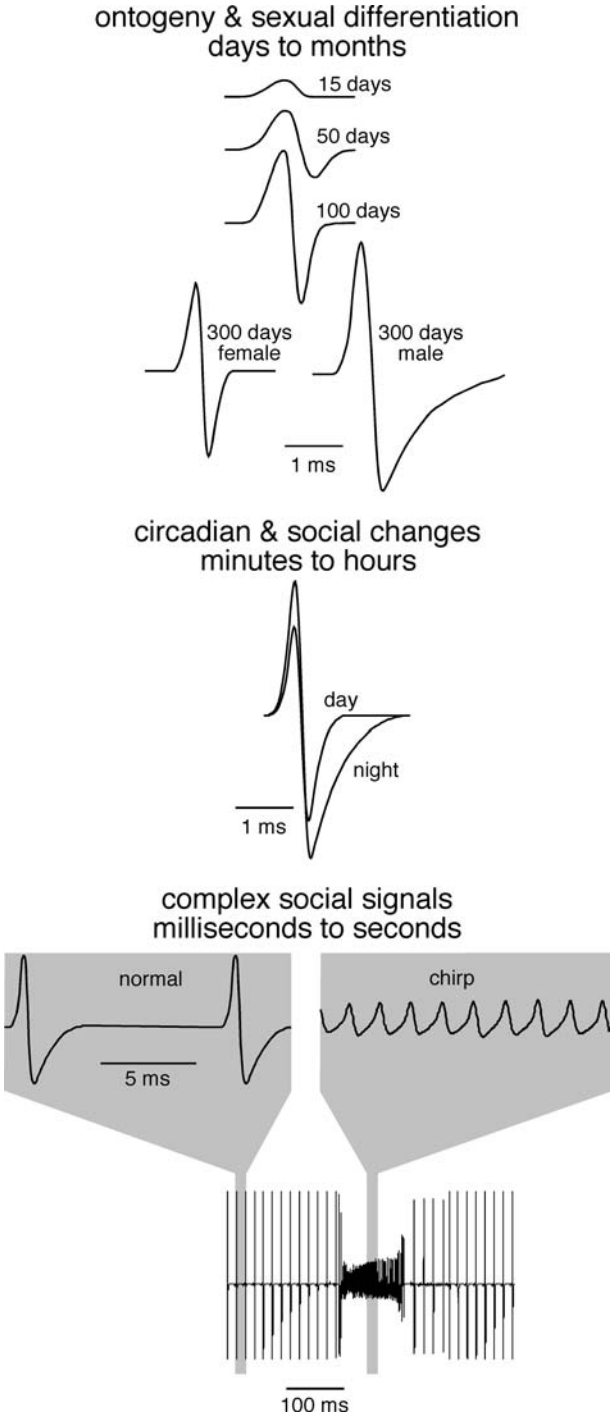


**Fig. 1** EOD waveforms change over timescales spanning ten orders of magnitude. Shown here are three general classes of waveform plasticity in the EOD of *Brachyhypopomus pinnicaudatus*. Developmental changes and sexual differentiation are the *slowest*, driven by growth factors and sex steroid hormones. Circadian and rapid social changes are *intermediate*, driven by monoamines and melanocortin peptides. Complex social signals are the *fastest*, driven by direct neural control

this paper, we discuss how behaviorally relevant variations in the waveform of the electric organ discharge (EOD) of the electric fish are modulated by peptide neuromodulators and steroid hormones, and how these agents modulate ion currents to cause the changes in behavior. Much of the work on hormonal regulation of the EOD centers on two species of South American gymnotiform fish, *Sternopygus macrurus*, the gold-lined or black knife fish, and *Brachyhypopomus pinnicaudatus* (Hopkins 1991), the feather-tail knife fish or "pinni;" so we focus our review on these species.

## The EOD-generating circuitry

EODs are triggered by a midline medullary nucleus called the pacemaker nucleus. Within the nucleus are 50–100 intrinsically active pacemaker neurons. Pacemaker neurons make electrotonic and chemical synapses on each other, and on a second group of neurons within the pacemaker nucleus called relay cells (Bennett et al. 1967; Elekes and Szabo 1981). Pacemaker neurons are spontaneously active and fire at high frequencies with highly regular rates (hundreds of Hz) in the wave fish, and fire at slower and less precise rates in the pulse fish (tens of Hz; Spiro 1997). These oscillatory neurons maintain their rhythmicity even when removed from a fish and placed in a slice chamber (Dye 1991; Smith and Zakon 2000).

The extensive interconnections among and between the pacemaker and the relay neurons cause them to fire synchronously. The relay cells project out of the nucleus and down the spinal cord to synapse on the specialized spinal motor neurons called electromotoneurons. These innervate the muscle-derived cells of the electric organ, the electrocytes. The electrocytes are usually arranged into rows in which they are oriented in the same axis and ensheathed in electrically resistive connective tissue. The resistive sheathing channels current along the axis of the electric organ, out into the water, and back into the other end of the electric organ (Bennett and Grundfest 1959; Bennett 1961; Bell et al. 1976).

The waveforms of EODs differ among species in the number and polarity of their phases and by differences in the amplitude and duration of each phase. The number of phases and their polarities are determined by fixed morphological parameters, such as the site of innervation, whether individual electrocytes have single or multiple electrically excitable surfaces, the geometry of the electrocytes, and their location in the body (reviews Bass 1986; Caputi 1999; Hopkins 1999). Variation in waveform within a species, such as sex differences or individual differences, depends on modifiable physiological properties of the electrocyte such as the duration and amplitude of the action potentials (APs) generated by each face (see below).

The simplest EOD is a monophasic pulse, such as that produced by *S. macrurus*, which is generated by an electrocyte with one electrically excitable face and another face that is electrically inexcitable (Keynes and Martins-Ferreira 1953; Bennett 1961). Upon depolarization by synaptic input, the excitable face

fires an AP during which a positive current ($Na^+$ ions) enters that face and a positive current (probably $K^+$ ions) exits the opposite face through leak channels. In other words, if the posterior face of an electrocyte is excitable, then current flows in that face and out the anterior face; the net positive current from all the simultaneously active electrocytes flows headward along the length of the electric organ into the water, and back into the fish's tail. This results in a head-positive pulse.

Both faces of the electrocyte are excitable in those species that produce a biphasic discharge, such as *B. pinnicaudatus*. First, the innervated face fires an AP during which the current flows toward the head. Then, the flow of current through the other membrane depolarizes it causing an AP to be generated with the current flowing toward the tail. The alternate flow of the current headward and then tailward gives the EOD its biphasic shape (Bennett 1961).

Some species, the "wave fish," generate sine wave-like EODs. Wave-type EODs are essentially pulse-type EODs generated at an extremely regular rate. If the durations of the EOD pulses are about the same as those of the inter-pulse intervals, the EOD will be periodic and nearly sinusoidal. In wave fish of the family Sternopygidae, the EOD pulse is monophasic because the simple electrocytes have single active faces. The frequency of a wave-type EOD is determined by the firing frequency of the pacemaker nucleus. However, for the waveform to approximate a sine wave, the EOD pulse duration must also vary with the pulse rate. So, for example, the EOD frequency of *S. macrurus* varies from 50 to 200 Hz and EOD pulse duration varies from 14 to 4 ms (Fig. 2). These two parameters co-vary so that the fish with the lowest EOD frequency has the longest pulse (Mills and Zakon 1987, 1991). These two independent parameters may be manipulated by hormonal modulation (see below).

## The EOD as a communication signal

The EOD serves as a multi-purpose system: it is used to sense objects around the fish and also for communication among conspecifics. EOD parameters are sexually dimorphic and often individually distinct. Sex differences in the waveform appear related exclusively to communication. Though male and female *Brachyhypopomus*

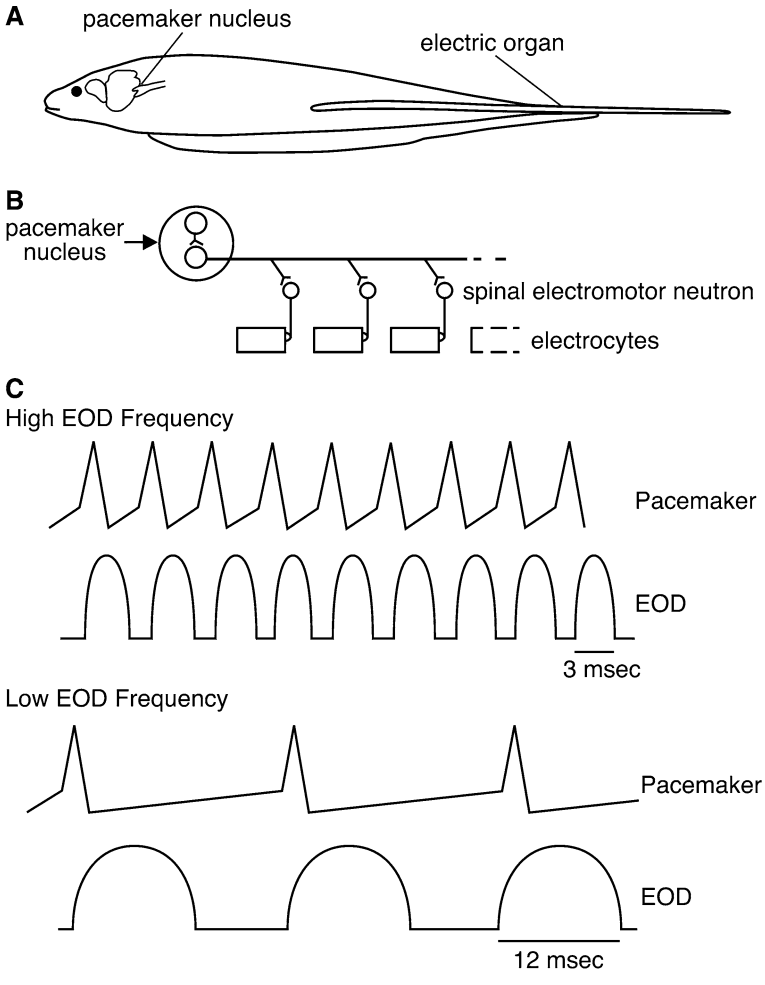


**Fig. 2** EOD-generating circuitry in *Sternopygus*. **a** The EOD is triggered by neurons of the pacemaker nucleus in the hindbrain, which activate the electric organ via **b** descending projections to spinal electromotor neurons. Each EOD pulse is the summation of the action potentials of the cells of the electric organ, the electrocytes. **c** Each action potential in the pacemaker nucleus is followed by a pulse from the electric organ. In a wave fish, such as *Sternopygus*, the pacemaker neurons of females discharge at a high rate and their electrocytes produce a brief action potential to generate a sinusoidal EOD at a high frequency. **d** The pacemaker neurons in males, on the other hand, fire at lower rates, and the EOD pulse is of longer duration to preserve the sinusoidal nature of the EOD (McAnelly and Zakon 2000)

have strikingly different waveforms, limited testing shows their acuity in electrolocation to be identical (Fig. 3; Olman 2001).

One commonality of pulse and wave fish is that the EOD pulses of mature males are frequently longer than those of mature females or juveniles. In some species of pulse fish with biphasic EOD pulses, both phases of the male EOD pulse are longer in duration than the equivalent phases in females of the species; while in others, only one phase of the EOD is sexually dimorphic. The first phase of the EOD pulse is similar in duration in male and female *B. pinnicaudatus*, but the second phase is longer in males than in females (Hopkins et al. 1990; Hopkins 1991). As discussed above, the EOD pulse also varies in duration in the wave fish *Sternopygus*. Mature males discharge at low frequencies and have long EOD pulses, while mature females discharge at higher frequencies and have short EOD pulses. Behavioral studies indicate that fish discriminate between male and female EODs of their species. For example, male *Sternopygus* will court dipole playback electrodes emitting synthetically generated female EOD mimics (Hopkins 1974). Likewise, female *B. occidentalis* responded differentially to single period sine waves that match male-typical and female-typical spectral peaks (Shumway and Zelick 1988). *Eigenmannia* have been conditioned to make similar discriminations in synthetic waveforms (Kramer and Zupanc 1986).

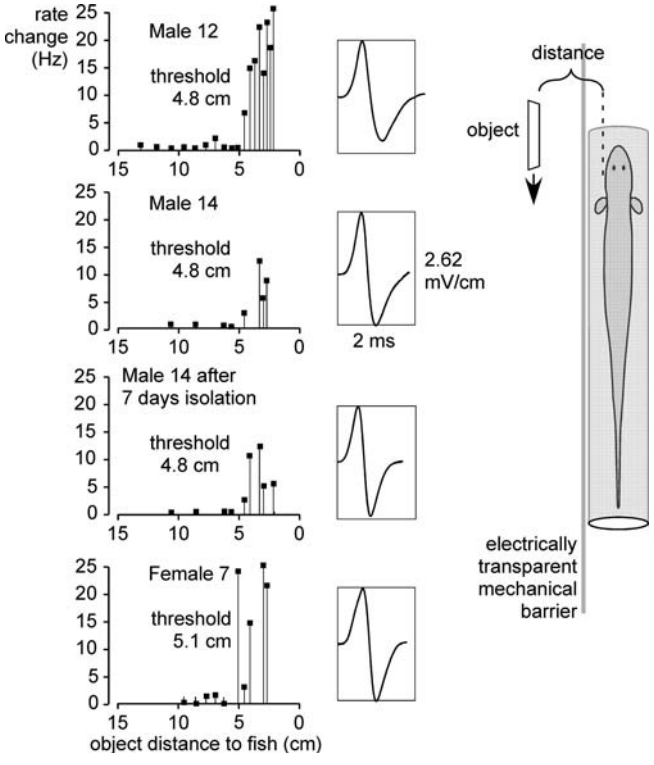


**Fig. 3** Active electroreception thresholds of *B. pinnicaudatus* for moving objects are not affected by amplitude or second phase duration of the EOD (Olman 2001). To determine object detection thresholds, fish were placed in a screen tube located behind a rigid but porous polyethylene barrier permeable to electric signals, but impermeable to mechanical waves that might activate the lateral line system. A 3 cm square sheet of plastic 1 mm thick was moved at 10 cm $s^{-1}$ along a 50 cm track parallel to the fish's head–tail axis, at decreasing distances lateral to the fish. The fish's EOD rate was monitored throughout. Plots show the change in maximum discharge rate from that during a control period immediately prior to the test run. The fish increased its discharge rate when it noticed the object, a reflex in pulse fish known as the novelty response. The detection threshold was considered to be the greatest distance with a sharp increase in EOD rate. EOD waveforms of subjects plotted in *boxes* sized to that of the second fish, male 14. Neither lower amplitude (e.g., male 12) nor lower second phase duration (e.g., male 14 after 1 week isolation) affected the object detection threshold. Insensitivity to waveform differences should not be surprising if one realizes that the EOD is variable primarily at the tail whereas the EOD varies little at the head (Stoddard et al. 1999), and the head harbors the most sensitive electroreceptors (Yager and Hopkins 1993) at the greatest density (Szabo 1974)

## Rapid changes in the EOD

Rapid changes in the EOD waveform result when the medullary pacemaker nucleus drives the EOD faster than the electrocytes in the peripheral electric organs can continue to produce full APs. At these times, the EODs may diminish in amplitude or fail entirely. These amplitude modulations make up the basis of dynamic courtship signals (review Stoddard 2002).

## Intermediate-term changes in the EOD

The genus *Brachyhypopomus* appears to be a specialist in intermediate-term modification of its EOD waveform occurring over the course of minutes to hours. Mature individuals of both sexes display day–night changes in the amplitude of the EOD and the duration of the second phase (Hagedorn 1995; Franchina and Stoddard 1998), the same features that are sexually dimorphic. These rhythms are much larger and more consistently expressed by males (Franchina and Stoddard 1998; Silva et al. 1999). The day–night changes are true circadian rhythms that free-run under conditions of constant light or constant dark, constant temperature, and random feeding (Fig. 4; Stoddard et al. 2003; P.K. Stoddard et al., unpublished data). Increases in amplitude and second phase duration of the EOD waveform (enhancement of the sexual dimorphisms) generally begin in the late afternoon. By sunset when the fish become active, the changes are in rapid swing. An hour after dark when the initiation of courtship is most frequent, the EODs are fully enhanced. After midnight, the EOD cycle begins to wane.

In addition to circadian oscillation, EODs of *Brachyhypopomus* of both sexes undergo rapid enhancement in response to potentially aggressive social contacts (Fig. 5). When a conspecific is introduced to another individual's resting place, the EODs of both individuals undergo rapid enhancement, first evident in 5–10 min, and increasing over the next 45 min (Franchina et al. 2001; Stoddard et al. 2003). After competing for a resting place, winners

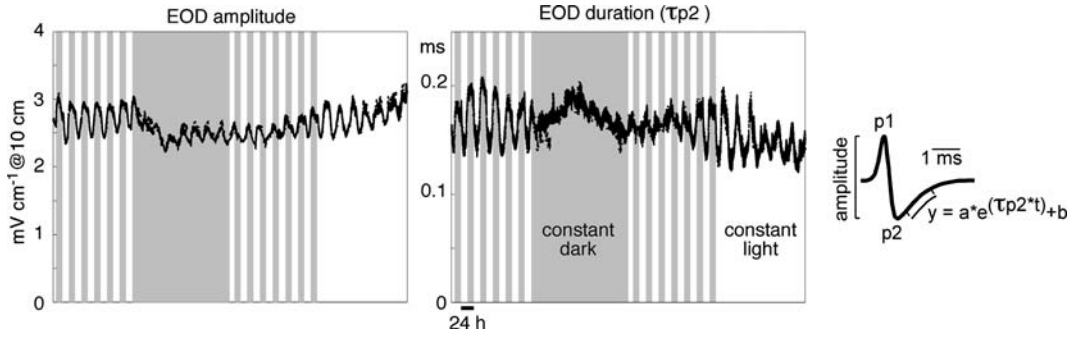


**Fig. 4** The amplitude and duration of EOD waveforms of *B. pinnicaudatus* increase at night. These day–night changes free-run under both constant darkness and constant light, and thus constitute true circadian rhythms. EODs are recorded automatically in the EOD machine (Stoddard et al. 2003). Repeatable recordings of amplitude and waveform shape are obtained by recording only when the fish is oriented lengthwise in an electrically transparent shelter tube. τ-p2 is the time constant of the exponential decay of second phase of the EOD, a parametric quality that tracks the duration of the second phase. The *gray bars* are periods of darkness

retain more masculine EOD characteristics than losers (Hagedorn and Zelick 1989).

When *B. pinnicaudatus* dyads interact at night, one or both may show rapid EOD enhancement (Franchina et al. 2001) but not always. The change depends on the prior state of the individuals, their sex, and their competitive status. Generally dominant males show and retain the greatest increase in EOD parameters (S. Allee and V.L. Salazar, unpublished data), but when animals are allowed to interact through electrically transparent mesh the responses are variable: in some instances a smaller resident male enhances his EOD while a larger male, introduced to the tank for a few hours, reduces his EOD. We have seen similar effects in limited trials with *Sternopygus*. Studies are currently underway to clarify the nature of the responses to individuals of different competitive status.

Social isolation of sexually mature male *B. pinnicaudatus* predictably results in a steady loss of EOD masculinity and circadian rhythmicity over a week's

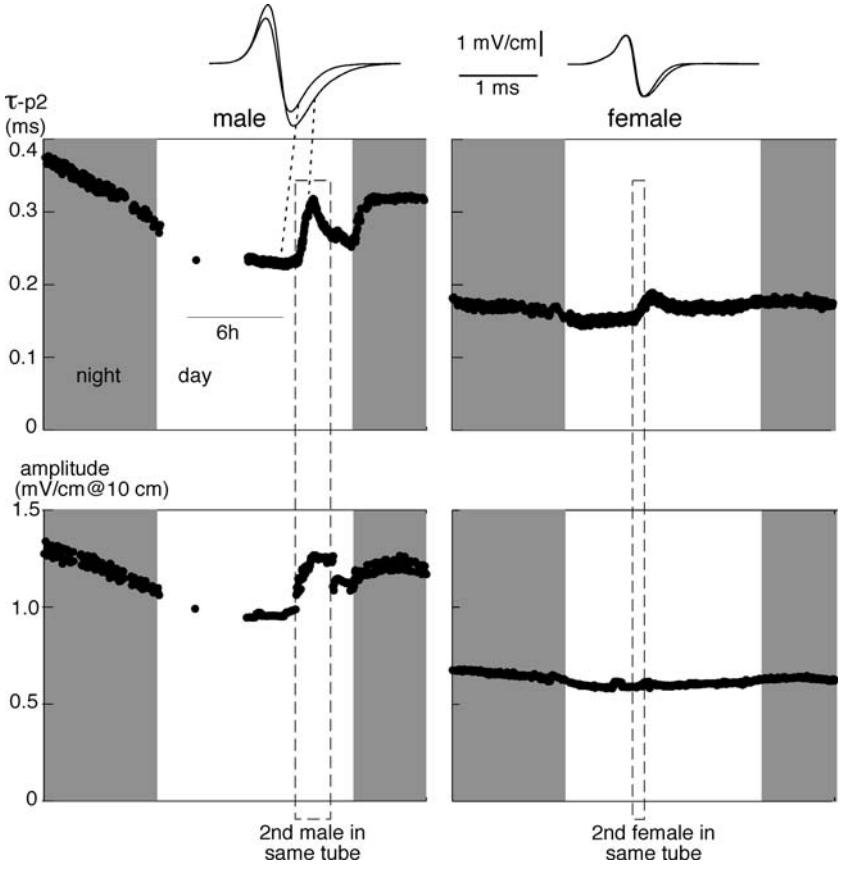


**Fig. 5** Stressful social stimuli induce rapid EOD enhancement in both sexes of lab-reared *B. pinnicaudatus*. Shown here are typical data from a male (*left*) and female (*right*) forced to share a hiding tube during the day with a same sex conspecific (females respond similarly to intrusion by males). The EOD waveforms shown above correspond to the time before the intrusion and the peak of the response. Females' EOD enhancements are smaller than males' and are restricted to changes in duration of the EOD's second phase (measured here as τ-p2), which increases power in the low end of the frequency spectrum (Stoddard 2002)

time. Restoration of a social environment, likewise, restores the masculinity of the EOD and its circadian rhythmicity. Restoration of EOD masculinity appears on two distinct timescales. Partial restoration occurs over an hour or two, while full restoration is slower and takes 2 days to 1 week, depending on the sexes and sizes of the social stimuli (Franchina et al. 2001). Thus we expect to find two distinct biochemical processes involved in the regulation of the waveform, one fast-acting (e.g., minutes) and a second slow-acting (e.g., days).

The more rapid masculinization of the EOD is produced by peripheral action of melanocortin peptide hormones, including adrenocorticotropic hormone (ACTH) and α-melanocyte stimulating hormone (α-MSH). Intramuscular injection of either hormone in vivo, or direct application to electrocytes in vitro, results in rapid increase in amplitude and second phase duration of the EOD of *Brachyhypopomus* on the same timescale (6–45 min) as observed during social interactions (Markham and Stoddard 2005; Fig. 6). Melanocortin peptides are different cuts of the same pro-hormone, produced by the intermediate lobe of the pituitary. These peptides initiate their actions via five membrane-bound melanocortin receptor subtypes (MC1R–MC5R) with diverse roles and patterns of distribution (review Hadley and Haskell-Luevano 1999).

The α-MSH analog SHU9119, which is a potent antagonist of mammalian MC3R and MC4R but an agonist at MC1R and MC5R (Hruby et al. 1995), masculinizes single-electrocyte potentials in a manner similar to ACTH (Fig. 6). Caution is in order when interpreting the effects of ligands across taxa, but these results at least suggest that modulation of electrocyte APs is mediated by activation of a receptor pharmacologically similar to the mammalian MC1R or MC5R. Involvement of either isoform is plausible in the modulation of electrocyte APs. In addition to inducing cortisol release, another major function of melanocortin action in the periphery is MC1R-mediated melanosome dispersal within melanocytes as part of the stress-induced skin-darkening response of fish and amphibians. The MC5R, widely distributed in peripheral tissues and the primary isoform expressed in skeletal muscle (Fathi et al. 1995), could be

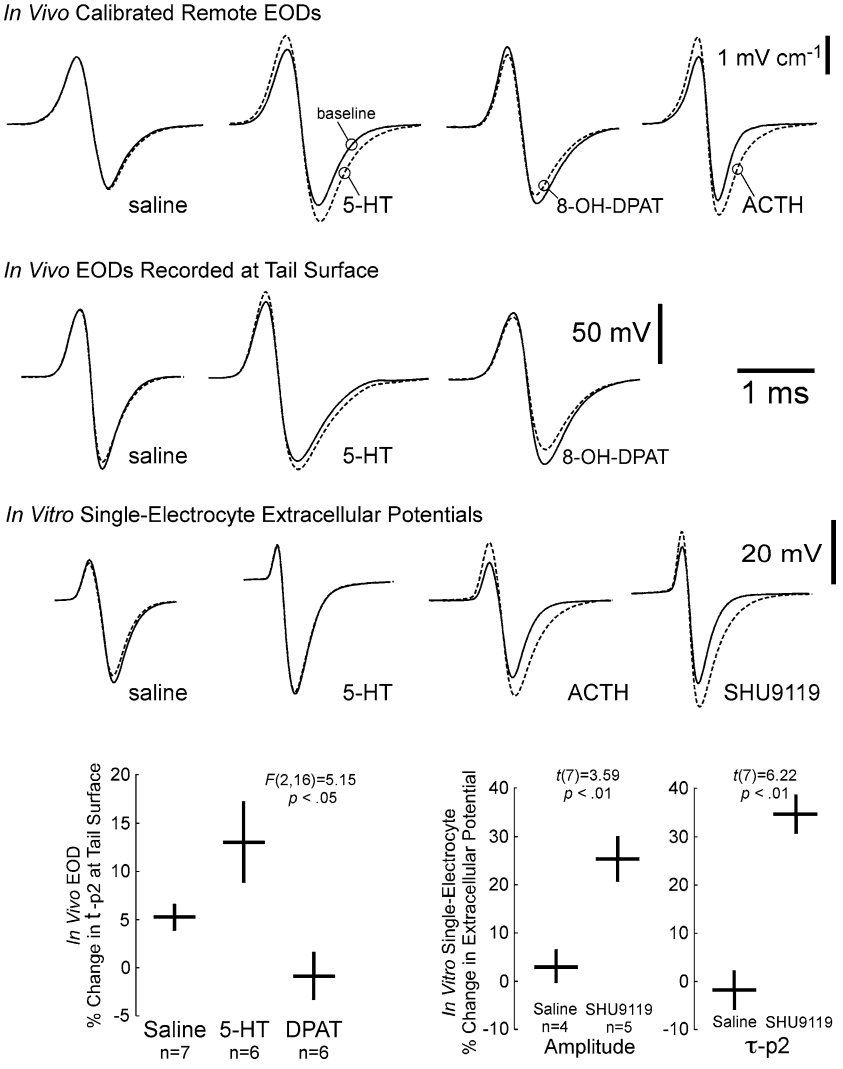


**Fig. 6** The EOD of *B. pinnicaudatus* is modulated rapidly by serotonergic and melanocortin agents. *Solid lines* represent baseline recordings. *Dashed lines* represent recordings 60 min after pharmacological treatment. Intramuscular injections of serotonin (5-HT) and melanocortin peptides such as adrenocorticotropic hormone (ACTH) enhance both amplitude and second phase duration (τ-p2) of the EOD in *B. pinnicaudatus*. Saline injections performed quickly and gently have no effect, and the serotonin 1A receptor agonist 8-OH-DPAT reduces both amplitude and τ-p2 of the EOD. These effects are apparent in calibrated remote EODs measured 50 cm from the fish and in EODs recorded by miniature electrodes at the tail surface. Additionally, the melanocortin ACTH enhances amplitude and τ-p2 of single-electrocyte potentials in vitro, whereas serotonin does not. The effect of ACTH on single-electrocyte potentials in vitro is mimicked by application of SHU9119, a selective agonist of the mammalian melanocortin receptor isoforms MC1R and MC5R. *Bar charts* present means, with *error bars* indicating SEM

retained during the developmental conversion of myocytes to electrocytes.

All melanocortin receptors are positively coupled to adenlyate cyclase, which often modifies cellular physiology through intracellular activation of protein kinase A (PKA). Application of the membrane permeable cAMP analog 8-bromo-cAMP induces rapid enhancement of the EOD amplitude in vivo and in vitro in *Sternopygus* and *Brachyhypopomus*, and prior blockage of PKA prevents these actions (McAnelly and Zakon 1996; McAnelly et al. 2003; Markham and Stoddard 2005).

In *Sternopygus*, the enhancement of EOD amplitude is due to an increase in the magnitude of the $Na^+$ current which affects the EOD amplitude in two ways. First, this increase insures that each AP reaches the sodium equilibrium potential, $E_{Na}$, so that each electrocyte generates a maximum amount of current. Secondly, the increase in the number of open $Na^+$ channels during the AP decreases the internal resistance of the electric organ. If the electric organ is viewed as the battery of a Thevenin equivalent circuit, and the resistances of this circuit are simplified to the internal resistance of the electric organ and the resistance of the water around the fish (Bennett 1970), a drop in the internal resistance of the organ increases the voltage drop across the resistance of the water (McAnelly et al. 2003). In *B. pinnicaudatus* electrocytes treated with melanocortins or 8-bromo-cAMP, amplitude of the intracellular AP is almost invariant while the extracellular potential increases in amplitude. As before, extra current is passed outside the cell, increasing the voltage drop across the media outside the cell. Unlike the case in *Sternopygus*, however, the intracellular AP does not approach $E_{Na}$ (Markham and Stoddard 2005). To maintain a constant intracellular peak while increasing the voltage outside the cell, the equivalent circuit must require the resistance of the other excitable membrane face to decrease simultaneously with the inward $Na^+$ current. Further work will reveal whether this model is correct.

Serotonin (5-hydroxytryptamine or 5-HT) appears to be a significant central regulator of rapid EOD masculinity, with a time course close to that following melanocortin injections. Intramuscular injection of serotonin causes a rapid enhancement of the EOD of *B. pinnicaudatus* (Stoddard et al. 2003), but a direct application to the electrocytes in vitro or the spinal cord in vivo has little effect (Markham and Stoddard 2003, 2005; Fig. 6). We conclude its action lies upstream, probably in the pituitary and/or hypothalamus. Unpublished pharmacology studies have identified two distinct serotonin receptors involved in dynamic (~30–60 min) regulation of the EOD (Stoddard 2006). A receptor with the drug response profile of a 5HT2A receptor enhances the EOD, consistent with the effects seen following 5HT injections. A second receptor, matching the profile of a 5HT1A receptor, exerts rapid control over EOD masculinity, but the response is inverted: antagonists enhance the EOD over an hour's time and agonists cause EOD masculinity to wane (Fig. 6). Interestingly, activation of the 5HT1A system is typical of subordinate individuals of many taxa. In salmonids, for instance, 5HT1A activation darkens the skin of individuals on dark backgrounds, making them visually cryptic (Hoglund et al. 2002a, b). Similarly, 5HT1A agonists in *B. pinnicaudatus* reduce EOD amplitude and duration, making the individual electrically cryptic. What appears different is that 5HT1A activation in salmonids *enhances* melanocortin release to darken the skin, whereas in the electric fish *B. pinnicaudatus*, it probably *reduces* the melanocortin release to diminish the EOD. This proposed inversion of the serotonin–melanocortin connection begs further investigation.

## Long-term changes in the EOD

Changes in EOD waveform lasting days to months may be activated by environmental stimuli that initiate breeding, such as increased food availability, decreased water conductivity, or social stimuli, such as the introduction of a mate or a competitor into a tank. We do not yet know how these stimuli are "transduced" by the animals, but these stimuli are known to regulate the levels of various hormones, such as androgens or glucocorticoids, which in turn initiate long-term effects via their actions on steroid receptors.

Beginning with the work of Meyer (1983) numerous studies have demonstrated the masculinizing effects of androgens on the EOD waveforms of various electric fish. 5α-dihydrotestosterone (5α-DHT) has been a valuable tool in elucidating responses to androgens, because it binds androgen receptors and it cannot be aromatized to estradiol. Slow-release implants of 5α-DHT in adult female *B. pinnicaudatus* masculinize the response of the EOD waveform to social challenges, serotonin, and ACTH. These implants also masculinize (increase) circadian rhythms in EOD duration, but fail to masculinize the circadian rhythm in EOD amplitude (S. Allee et al., unpublished data; Fig. 7). Together, these results suggest a strong peripheral effect of androgens on regulation of the EOD, though the incomplete masculinization of female circadian rhythms in EOD amplitude could result from the maturity of the subjects or perhaps imply additional contributing factors.

5α-DHT implants in adult *Sternopygus* lower EOD frequency, presumably by acting on the firing frequency of the neurons in the pacemaker nucleus, and lengthen the EOD pulse by broadening the electrocyte AP (Few and Zakon 2001). The variation in EOD pulse duration of *Sternopygus* measured in the water outside the animal is reflected in differences in duration of APs measured intracellularly: long duration APs in fish with low EOD frequencies and short duration APs in fish with high EOD frequencies (Fig. 2). Androgen treatment broadens the AP much as it does the whole EOD pulse recorded in vivo (Mills and Zakon 1991). The *Sternopygus* electrocyte has three ionic currents involved in generation of the AP: an inward rectifier that sets resting potential, a $Na^+$

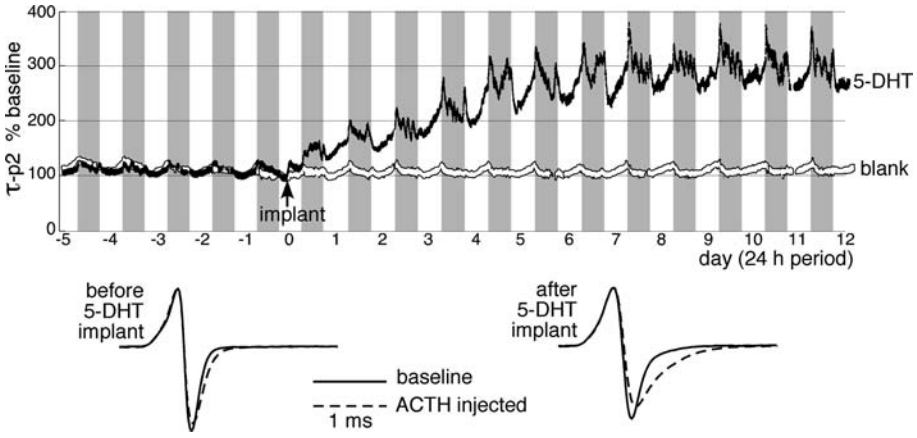


**Fig. 7** Sexually mature female *B. pinnicaudatus* develop male-like circadian rhythms in EOD duration (τ-p2) when implanted with the non-aromatizable androgen 5α-dihydrotestosterone (5α-DHT). Further, the 5α-DHT implants augment the response to the injections of the melanocortin ACTH. EOD waveforms in this figure are normalized to the magnitude of the first EOD phase

current ($I_{Na}$) that generates the rising phase of the AP and whose inactivation initiates the falling phase of the AP, and an outward rectifier ($I_K$) that repolarizes the AP (Ferrari and Zakon 1993). In this system $I_{Na}$ and $I_K$ are uniquely co-regulated with great precision to generate the fourfold variation in AP duration seen in *Sternopygus*. Fish with long APs have slowly inactivating $I_{Na}$ and slowly activating $I_K$, thereby broadening the AP, while fish with short APs have rapidly inactivating $I_{Na}$ and rapidly activating $I_K$, producing a shorter AP (Ferrari et al. 1995; McAnelly and Zakon 2000; Fig. 8).

The Zakon lab has cloned $Na^+$ and $K^+$ channel genes from the *Sternopygus* EO in order to understand how these currents are regulated. One major family of voltage-dependent $K^+$ channels, the *shaker* or Kv1 family, has eight members in tetrapods, and at least a dozen in teleost fish (H. Zakon et al., unpublished data). Kv1 subunits are often associated with a delayed rectifying current such as we observed in the electrocytes. Three different Kv1 genes are expressed in the *Sternopygus* EO: Kv1.1a, Kv1.2a, and Kv1.2b (P. Few and H. Zakon, unpublished data.). Kv1.2b does not show any variation in expression across the EOD frequency range, whereas Kv1.1a and Kv1.2a are expressed in high levels in fish with high EOD frequencies, low levels in fish with low EOD frequencies, and intermediate levels in fish with intermediate EOD frequencies. Furthermore, if females (who have high EOD frequencies) are treated with 5α-DHT, Kv1.1a and Kv1.2a are suppressed, but Kv1.2b levels are unaffected (Fig. 9).

Potassium channels form as a tetramer, and different subunits from the same channel family may co-associate within a tetramer. Let us assume that the levels of protein of each subunit reflect the levels of its RNA and that there is no preferential association for particular pairs of subunits. Then we imagine that the $K^+$ channel tetramer in fish with high EOD frequencies is formed primarily of Kv1.1a and/or Kv1.2a and that these subunits have rapid inactivation kinetics. Conversely, $K^+$ channels from fish with low EOD frequencies are formed primarily by tetramers composed of Kv1.2b subunits and have slow activation kinetics. Finally, $K^+$ channels from fish with intermediate EOD frequencies have Kv1 tetramers with a mix of all the three subunits (Fig. 9).

These genes have not yet been studied in an expression system. However, pharmacological dissections of the $K^+$ current in the native environment of the electrocyte are largely in agreement with the above scenario. The electrocyte $K^+$ current from fish with high EOD frequencies activates most rapidly and is sensitive to the $K^+$ channel blocker tetraethylammonium (TEA); the $K^+$ current from fish with low EOD frequencies activates slowly and is less sensitive to TEA, whereas $K^+$

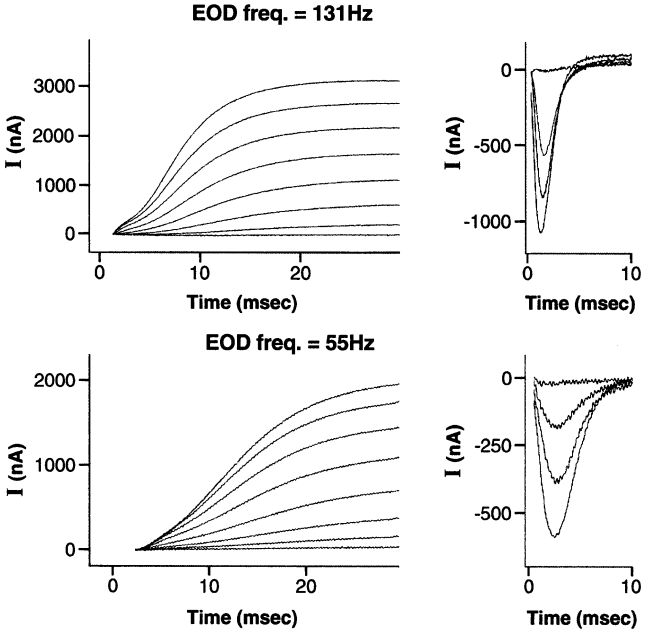


**Fig. 8** Kinetics of $Na^+$ current inactivation and $K^+$ current activation vary with EOD frequency and sex in *Sternopygus*. *Top traces* are $Na^+$ and $K^+$ currents recorded under voltage clamp from the electrocytes of a fish with a high EOD frequency (female). *Bottom traces* are these currents recorded from a fish with a low EOD frequency (male). Note that the rates at which the $Na^+$ current inactivates and the $K^+$ current activates are faster in females than in males (McAnelly and Zakon 2000)

currents from fish with intermediate EOD frequencies are intermediate in both properties (L. McAnelly and H. Zakon, unpublished data). In mammals, Kv1 homomers are highly TEA sensitive, Kv1.2 channels are far less so, and Kv1.1/1.2 heteromers are intermediate in their sensitivity.

Sodium channels may be comprised of multiple subunits, but the conducting α-subunit is one large protein, which is a fully functional channel on its own. Thus, there is no way for two α-subunit gene products to interact. All the voltage-gated $Na^+$ channel genes have been cloned from *Sternopygus* and eight genes have been found (Lopreato et al. 2001; A. Novak et al., unpublished data). Two of these genes are orthologs of the mammalian gene Nav1.4, a gene expressed only in muscle in mammals. The *Sternopygus* genes, initially named sterNa1 and sterNa6, are now named Nav1.4a and Nav1.4b, respectively. Nav1.4a is expressed in the muscle and the EO, whereas Nav1.4b is expressed only in the EO. In the EO, levels of Nav1.4b do not vary across the EOD frequency, but those of Nav1.4a are higher in fish with a high EOD frequency (Fig. 10). When fish are treated with 5α-DHT, levels of Nav1.4b are unaffected whereas levels of Nav1.4a are suppressed (Liu et al. 2005).

These genes are now being studied in an expression system so that their biophysical properties will soon be known. Given that Nav1.4a is in muscle, which presumably makes a brief AP, and is abundant in the EO of high-frequency female fish, which makes a short AP, we predict that Nav1.4a generates a $Na^+$ current that inactivates "normally." Nav1.4b, on the other hand, is expressed only in the EO and shows many amino acid substitutions suggesting that it has undergone significant evolution for a role in social signaling (H. Zakon et al., unpublished data). We suggest that it is slowly inactivating, but this must be verified by expression studies.

While the differential expression of these two genes might, at the first glance, seem sufficient to explain variation in $Na^+$ currents, that is not the case based on theoretical grounds. If $Na^+$ currents are the result of current flowing through two different channels, then the kinetics of the inactivation of the currents will be best fit by a bi-exponential decay time constant, each exponent reflecting the exponential decay of each current. The magnitudes of each exponential term rather than the rate of decay should vary with EOD frequency. However, what is observed is that the inactivation time constant is well fit by a single decay function whose time constant varies across the EOD frequency range.

Ion channels have accessory proteins that are involved in either targeting the channels to the membrane or modifying the channels' properties. Sodium channels in mammals have four different accessory proteins, called β subunits, and one of these, β1, is expressed in muscle (Isom 2001). The addition of the β1 subunit increases the rate of $Na^+$ current inactivation when the β1 subunit is co-expressed with cRNA for mammalian $Na^+$ channel in frog oocytes. Electric fish have a β1 subunit and it is expressed in brain and muscle as well as in electric organ (Liu and Zakon 2004). Since the β1 subunit speeds inactivation, we would predict that it is more abundant in the electrocytes of fish with high EOD frequencies. That is, in fact, the case. However, the situation is further complicated by the fact that the β1 subunit is alternatively spliced in teleosts—a similar β1 subunit found in zebrafish is spliced identically (Hobson and Isom 2003). One of the splice forms, the "short form," is in greater abundance in the EO of fish with high EOD frequencies, whereas the "long" form does not vary with EOD frequency (Liu et al. 2005). To date, only Nav1.4a has been expressed in *Xenopus* oocytes. As expected, it generates a fast $Na^+$

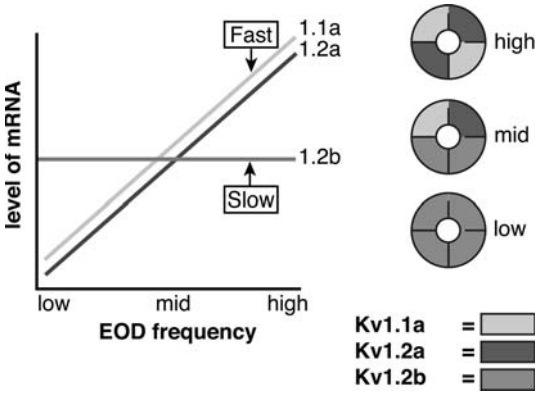


**Fig. 9** Schematic diagram of the relationship between Kv1 gene expression and EOD frequency in *Sternopygus*. "Fast" and "slow" refer to the presumed rates of activation of each subunit in a homomer. The colored doughnuts on the *right* illustrate hypothetical subunit composition of the electrocyte Kv1 channel of fish with high-, mid- and low-frequency EODs in accordance with the relative abundance of the mRNAs of each subunit in fish across the EOD frequency range (P. Few and H. Zakon, unpublished data)

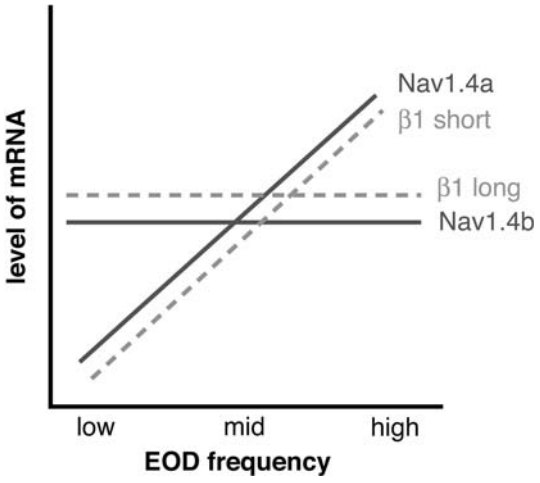


**Fig. 10** Schematic diagram of the relationship between $Na^+$ channel gene expression and EOD frequency in *Sternopygus*. $Na_v1.4a$ and the short isoform of the β1 subunit are expressed in higher levels in fish with high EOD frequencies and short EOD pulses. We do not yet know how the two splice forms of the β1 subunit associate with the two $Na^+$ channels (Liu et al. 2005)

current, and co-expression of Nav1.4a with either the short or the long form of the $\beta$1 subunit speeds up current inactivation. It will be interesting to test whether Nav1.4b inactivates more slowly on its own, and how this is influenced by the $\beta$1 subunits. Finally, it will be intriguing to learn how the interactions of two $Na^+$ channels and two splice forms of the $\beta$1 subunit give rise to a $Na^+$ current with a simple mono-exponentially decaying inactivation.

## Biomedical implications of EOD modulation

Understanding the long-term and short-term mechanisms by which gymnotiform electric fish modulate their EODs has a number of important biomedical implications. Because changes in the EOD waveform result directly from changes in the intrinsic excitability of electrocytes, these fish offer exemplary systems to investigate both long- and short-term modulations of intrinsic membrane excitability. Such intrinsic plasticity of excitable cells has a widespread importance for both normal and pathological functioning in a range of systems. As we have discussed here, electric fish offer a model system wherein intrinsic excitability is modified through at least two distinct classes of mechanisms. For long-term EOD modulations, hormones control the remodeling of the excitable membrane by changing the ion channel populations. These effects may also serve to potentiate the signaling pathways involved in short-term EOD modulation. In the case of short-term EOD modulation, membrane-bound receptors initiate intracellular cascades that target working ion channels. In both cases, these changes in intrinsic excitability are accomplished while maintaining a homeostatic balance that enables ongoing function of the electrolocation and electrocommunication systems.

Together with the synaptic plasticity of neural networks, intrinsic plasticity is critical for proper functioning in most neural systems. The important relationship between synaptic plasticity and intrinsic plasticity is especially evident in the neural networks controlling rhythmic motor systems underlying behaviors such as respiration, locomotion, and sleep rhythms (Calabrese 1998). In other areas, experience-dependent intrinsic plasticity plays an essential role in learning and memory (review Zhang and Linden 2003), and intrinsic plasticity of neurons within the prefrontal cortex has even been identified as potentially contributing to drug-seeking during cocaine withdrawal (Dong et al. 2005). Further investigations of the rapid and reversible modulation of electrocyte excitability, as well as the remarkable co-regulation of multiple ionic currents to achieve a precise state of membrane excitability will likely lead to additional insights on mechanisms of intrinsic plasticity.

Inherited pathologies of intrinsic neural excitability are clearly associated with many forms of epilepsy (e.g., Lossin et al. 2002, 2003), and similar disorders of intrinsic excitability in peripheral tissues lead to disease states in cardiac and skeletal muscle, and endocrine systems (Ashcroft 2000). Gymnotiform electric fish have evolved mechanisms for changing the performance of homologous channels to alter the meaning of the resultant communication signals. In so doing, these fish use a range of mechanisms to effectively transform ion channels in the electric organ between physiological states. One possible approach to treating such disorders in future could be replacing pathological channels with normal ones, but doing so presents the problem of maintaining functionality during such channel substitutions. The electric fish's ability to remodel pathological channels in situ suggests the possibility of alternative treatment tactics.

In addition to being strong model systems for studying intrinsic plasticity, the circadian rhythms in EOD waveforms of electric fish present unique opportunities for investigating the neuroendocrine systems involved in circadian rhythms and the factors that may lead to disruption or resilience of these systems. The normal functioning of circadian systems is readily observed in an organism's sleep/wake cycles, the timing of mealtime hunger, and cycles of overall activity. Less obvious examples include circadian fluctuations in physiological parameters associated with the onset and severity of many medical conditions, such as dermatitis (e.g., Gelfant et al. 1982; Pigatto et al. 1985), asthma (e.g., Smolensky et al. 1999; Burioka et al. 2000; Panzer et al. 2003), and cancer (Keith et al. 2001; Filipski et al. 2002; Fu et al. 2002). Circadian rhythms also are responsible for variations in the effectiveness of many medications (Andreotti et al. 1997) such as local anesthetics (e.g., Bruguerolle et al. 1991) and chemotherapy agents (e.g., Levi 2001). Perhaps most importantly, circadian rhythms are observed in numerous immune system functions (revs. Haus and Smolensky 1999; Roberts 2000). Disturbances in the circadian rhythms, at any level of organization, have serious consequences for illness, health, and well-being.

While the pacemaker components of circadian systems are relatively well understood, much less is known about regulation of the output pathways in which the cycles of oscillator activity are ultimately expressed as circadian rhythms in specific physiological or behavioral processes (reviews Dunlap 1999; Hardin 2000; Allada et al. 2001; Reppert and Weaver 2001). Understanding circadian output pathways is of particular importance in light of the mounting evidence that disruptions in circadian rhythms often result not from problems with the pacemaker, but instead from modulation or attenuation in particular circadian output pathways. This is the case for some circadian disturbances associated with normal aging (review Monk and Kupfer 2000) and almost all stress-related disturbances in circadian function (e.g., Richter 1967; Meerlo et al. 1997, 2002).

The EOD of weakly electric fish is a strong model system for exploring the neuroendocrine mechanisms of modulation in circadian output systems. The EOD waveform and the amplitude of its circadian rhythms

can be altered by changing the social or physical environment in an aquarium and can be induced chemically by injecting certain neuroactive compounds. Because the EOD is itself a socially significant behavior, changes in EOD waveform and rhythmicity are not simply assays of circadian rhythmicity; these changes directly impact the organism's interaction with and fitness within its social environment. Thus, this system allows us to investigate the significance and mechanisms of modulation in the circadian EOD rhythms at all levels of analysis ranging from social interaction to neuroendocrine events to ion currents in isolated membranes of dissociated cells.

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