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REVIEW

Electrocyte physiology: 50 years later

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Summary

Weakly electric gymnotiform and mormyrid fish generate and detect weak electric fields to image their worlds and communicate. These multi-purpose electric signals are generated by electrocytes, the specialized electric organ (EO) cells that produce the electric organ discharge (EOD). Just over 50 years ago the first experimental analyses of electrocyte physiology demonstrated that the EOD is produced and shaped by the timing and waveform of electrocyte action potentials (APs). Electrocytes of some species generate a single AP from a distinct region of excitable membrane, and this AP waveform determines EOD waveform. In other species, electrocytes possess two independent regions of excitable membrane that generate asynchronous APs with different waveforms, thereby increasing EOD complexity. Signal complexity is further enhanced in some gymnotiforms by the spatio-temporal activation of distinct EO regions with different electrocyte properties. For many mormyrids, additional EOD waveform components are produced by APs that propagate along stalks that connect postsynaptic regions to the main body of the electrocyte. I review here the history of research on electrocyte physiology in weakly electric fish, as well as recent discoveries of key phenomena not anticipated during early work in this field. Recent areas of investigation include the regulation of electrocyte activity by steroid and peptide hormones, the molecular evolution of electrocyte ion channels, and the evolutionary selection of ion channels expressed in excitable cells. These emerging research areas have generated renewed interest in electrocyte function and clear future directions for research addressing a broad range of new and important questions.

Key words: electric organ discharge, electroreception, ion channel regulation, molecular evolution, weakly electric fish.

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Introduction

In the late 1700s, scientists were in agreement that certain species of fish such as the electric eel generated strong electric discharges from a readily identifiable electric organ (EO), while a number of smaller fish species possessed ‘pseudoelectric organs’ – tissues that closely resembled the eel’s EO but produced no detectable electric output (Finger and Piccolino, 2011). It was almost 200 years later, when Lissmann amplified and recorded the weak electric signals generated by *Gymnarchus niloticus*, that the weak electrical discharges of these pseudoelectric organs were finally revealed (Lissmann, 1951). The function of these weak electric organ discharges (EODs) was initially in dispute, as the weak EODs were not of sufficient magnitude to be useful for predation or defense. Lissmann went on to prove conclusively that these weak biogenic electric fields were used for active sensing and communication (Lissmann, 1958; Lissmann and Machin, 1958).

These discoveries sparked a period of intensive research on the sensory and electrogenic mechanisms underlying these electrosensory and electric communication systems in weakly electric fish. Research on the sensory processing of electric signals quickly gained traction and expanded rapidly with momentum that continues today (e.g. Chacron et al., 2011; Pereira and Caputi, 2010; Requarth and Sawtell, 2011). Similarly, steady and growing progress is evident in the extensive work on central mechanisms controlling EOD rate, especially in the contexts of social communication (reviewed by Lorenzo et al., 2006; Scheffel and Kramer, 2006). In contrast, less emphasis has been placed on understanding the functional properties of electrocytes – the specialized electrogenic cells that compose the EO. The electrocytes are a crucial link in the electrosensory process: they are the target of central control by the pacemaker nucleus, and the effector tissue for the electric signal that is the primary information carrier in the environment and the input to the electrosensory system. I will review here recent work that has revealed a number of novel and important phenomena in electrocyte function in addition to highlighting numerous standing questions in need of further investigation.

Electrocytes: the cellular basis of electrogenesis

It has been just over 50 years since the first comprehensive investigations of electrocyte physiology and function. The first widely available analysis of electrophysiological mechanisms in weakly electric fish was Bennett and Grundfest’s work on the gymnotid fish *Gymnotus carapo* (Bennett and Grundfest, 1959). This work was soon followed by Bennett’s elegant and comprehensive exploration of the functional physiology of EO cells from mormyrid and gymnotiform electric fish (Bennett, 1961; Bennett and Grundfest, 1961). Bennett’s work in this area became authoritative and continued for another decade, but culminated with his assessment that relatively little was left to be gained from continued investigation of electrocyte physiology (Bennett, 1970).

The assumption widely held at that time was that electrocyte discharges were static and fixed by cellular morphology, rendering the electrocyte’s electrical properties at best no more interesting than those of other excitable tissues such as nerve and muscle. Furthermore, technical obstacles arising from the size and geometry

of electrocytes prevented additional investigation of the ionic mechanisms underlying electrocyte function. Bennett predicted that the main experimental value of EO tissue was likely to be that the sheer size and number of electrocytes would provide sufficient amounts of material for biochemical analyses of key molecules. Indeed, *Torpedo californica* electrocytes proved a rich enough source of acetylcholine receptor to allow the first cloning and sequencing of this receptor (Noda et al., 1982), and *Electrophorus* electrocytes provided sufficient molecular material to allow the first cloning and sequencing of the voltage-gated sodium channel (Noda et al., 1984). Aside from these pursuits, however, interest in the physiology of EOs fell off sharply in the 1970s.

A resurgence of interest in electrocyte physiology began in the early 1980s with the discovery that EOD waveforms were sexually dimorphic and hormonally regulated. This led to the discovery that electrocyte function is regulated and modulated by hormones on multiple timescales. Advances in electrophysiological techniques allowed investigation of the ionic basis of the electrocyte discharge, revealing mechanisms of EOD waveform plasticity and mounting evidence that signal variation across species relies not only on different cellular morphology but also on the recruitment of very different combinations of ion channels. Newly developed molecular analyses have revealed striking rates of molecular evolution in electrocytes as well as hints of the mechanisms by which electrocytes are created from skeletal muscle (Gallant et al., 2012; Kim et al., 2004). Finally, the growing realization that the energetic demands of electrogenesis place important constraints on the function of electrosensory and electrocommunication systems (e.g. Salazar and Stoddard, 2008) places renewed focus on the electrocytes, which are likely to be the key drivers of energetic demand and the target of selective pressures to optimize the energy efficiency of electrogenesis.

Other papers in this special issue address in depth the mechanisms of phenotypic conversion from muscle to EO (Güth, et al., 2013; Unguez et al., 2013) and the energetics of electrical signaling (Salazar et al., 2013). Accordingly, these topics will not be discussed at length here. I will instead begin with a brief overview of electrocyte structure and function, then focus on historical findings and recent developments in understanding the hormonal regulation of electrocyte excitability, the molecular evolution in electrocytes, and the potential role of ion channel diversity in determining EOD waveform diversity.

The structure and function of gymnotiform and mormyrid electrocytes

The EOD of weakly electric fish is produced by coordinated electrocyte action potentials (APs) within the EO. A brainstem pacemaker nucleus synchronizes the discharges of large populations of electroctyes such that their summed APs create current flow through the EO and out into the surrounding water. In ‘wave-type’ fish the pacemaker produces EODs in highly regular trains where the interpulse interval is roughly equal to the EOD duration, thereby creating a sinusoidal wave. ‘Pulse-type’ fish emit EODs with interpulse intervals that are variable and generally much longer than the EOD duration.

In all but one clade of weakly electric fish, adult electrocytes are myogenic (derived from skeletal muscle). The only exception is the South American genus *Apteronotus*, where the adult EO is of neural origin. A major unifying feature of these myogenic electrocytes is that the cells are cylindrical with flattened areas (faces) of electrically excitable membrane oriented such that the membrane currents across these faces are directed along the rostral–caudal axis (Fig. 1). The cell’s width can be much larger than the diameter, resulting in a cigar-shaped cell such as for *Eigenmannia virescens*, whereas in other species the electrocytes are flattened and disc-like with widths being very narrow relative to the diameter. The disc-like electrocytes sometimes also feature stalks that protrude from the flattened membrane surface (Fig. 1).

The simplest EODs in both gymnotiforms and mormyrids are produced by electrocytes where only one face is electrically excitable while the other is electrically passive. Synaptic input initiates an AP on the excitable face where Na^+ current enters the cell, creating an axial current within the EO. If, for example, the active face is the posterior electrocyte membrane, the net positive current is directed headward, creating a monophasic head-positive pulse shaped by the depolarization and repolarization of the posterior membrane. In species with more complex biphasic or multiphasic EOD waveforms, both electrocyte faces are excitable and an AP on the posterior innervated face is followed closely by an AP on the opposite face (Fig. 1). The combination of these successive APs with their ionic currents directed in opposite directions creates a biphasic discharge that is shaped by the waveforms of the two APs as well as the delay between the APs (Bennett, 1961; Hopkins et al., 1990; Markham and Stoddard, 2005).

In some gymnotiform species, such as *G. carapo*, EOD waveform complexity is further increased by the asynchronous recruitment of different electrocyte populations within distinct regions of the EO (Caputi, 1999; Lorenzo et al., 1988; Macadar et al., 1989). The spatio-temporal pattern of activation across these EO regions contributes to the multiphasic EOD waveform measured head-to-tail (Fig. 1) as well as producing large spatial variation in EOD waveforms measured at different locations near the body (Caputi, 1999). Many mormyrid species produce EODs of comparable complexity to *Gymnotus*, but the mechanisms underlying waveform complexity are quite different. Mormyrid EOs are compact and consist of a single, relatively homogeneous population of electroctyes. It is the morphological complexity of these electrocytes that is responsible for producing multiphasic EODs. In species that generate more complex EOD waveforms, electrically excitable stalks penetrate the electrocyte (Fig. 1), a structural feature that enhances waveform complexity when APs are propagated along the stalks toward the electrocyte body (Bennett and Grundfest, 1961) (Fig. 1). As a function of this morphological complexity in mormyrid electrocytes, EOD waveforms in this group are equally, if not more, diverse and complex than gymnotiform EOD waveforms (cf. Arnegard et al., 2010a; Crampton and Albert, 2006).

Larval EOs that precede the adult EO are present in both Mormyrid and Gymnotiform species (Franchina, 1997; Kirschbaum and Schwassmann, 2008). The neurogenic organs of Apteronotids arise after the development and loss of a myogenic larval organ (Kirschbaum, 1983). In many mormyrids with adult organs that possess complex patterns of innervation and stalk morphology, larval organs are observed where electrocytes resemble (and may be homologous to) the structurally simpler electrocytes of *G. niloticus* (Denizot et al., 1978; Hopkins, 1999; Westby and Kirschbaum, 1977; Westby and Kirschbaum, 1978).

All weakly electric fish have developed mechanisms for centering the EOD energy on 0 V DC. Doing so eliminates or attenuates the low frequency energy detectable by electroreceptive predators. Two strategies are employed to accomplish this. In wave-type fish with monophasic head-positive EODs, the electrocytes’ non-innervated anterior faces generate a head-

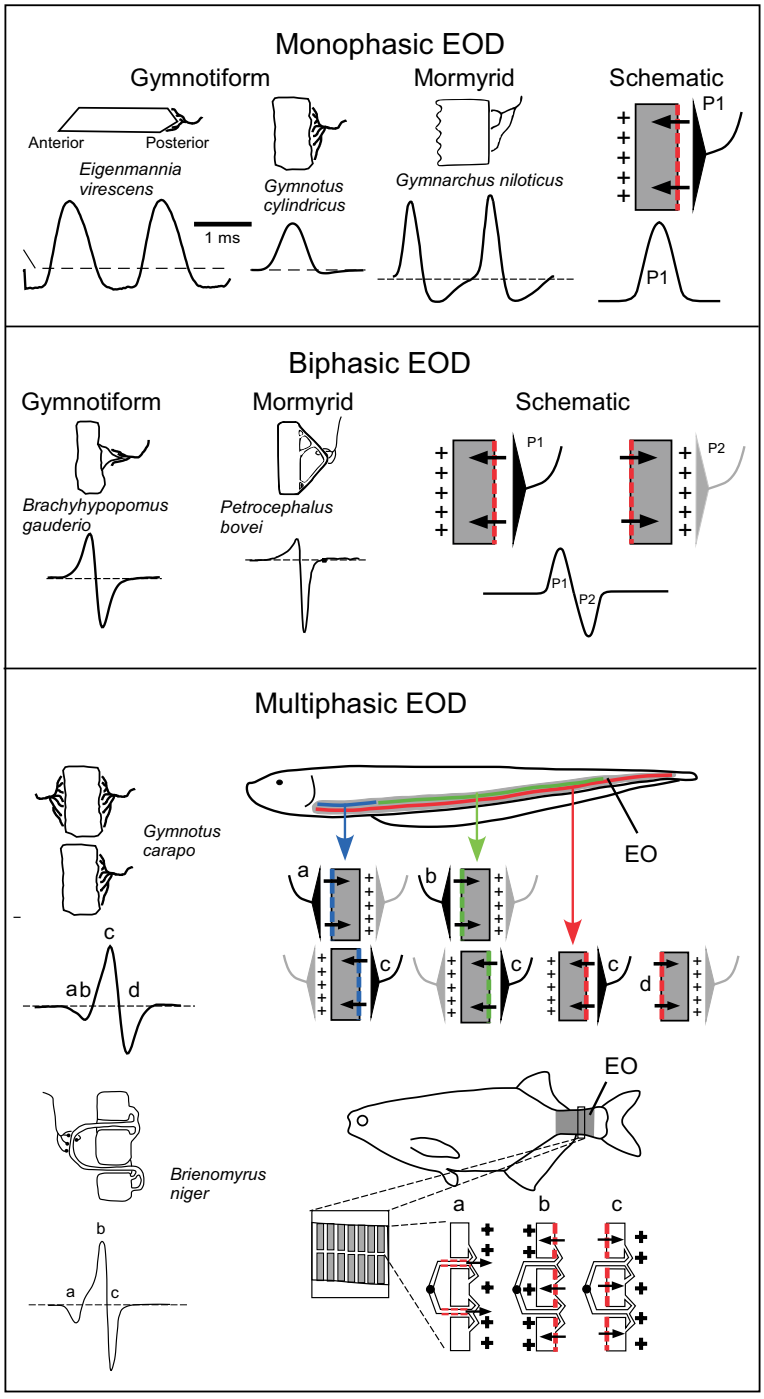

Fig. 1. Mechanisms of electric organ discharge (EOD) generation and EOD waveform diversity in gymnotiform and mormyrid fish. Line drawings show cross-sectional diagrams of representative electrocytes with corresponding EOD waveforms below. In schematic representations of electrocyte function, black triangles represent active synaptic inputs and gray triangles are quiescent synaptic inputs. Bold dashed lines (red, green, blue) represent activated excitable membrane and arrows indicate the direction of membrane current flow. Top panel: monophasic EODs in both gymnotiform and mormyrid fish are produced when electrocytes innervated on the posterior membrane generate a single action potential (AP) on the posterior membrane upon synaptic activation. This AP produces headward current flow and the resulting EOD is a monophasic head-positive pulse. Middle panel: biphasic EODs in both gymnotiforms and mormyrids are produced by disc-shaped electrocytes where both the anterior and posterior membranes are electrically active. Upon synaptic activation, an AP on the posterior membrane creates headward current flow and the head-positive EOD phase (P1). A subsequent AP on the non-innervated anterior membrane produces the head-negative EOD second phase (P2). Bottom panel: generation of multiphasic EOD waveforms occurs through different mechanisms in the gymnotiform *Gymnotus carapo* and the mormyrid *Brienomyrus niger*. In *G. carapo*, the EOD is a multiphasic waveform with two initial head-negative components (a,b) followed by a head-positive phase (c) then a final head-negative phase (d). This waveform is produced by the spatio-temporal activation of three distinct electric organ (EO) regions (shown as blue, green and red) populated by three types of electrocytes. Two populations of electroctyes are innervated on both faces, while a third population is innervated on only the posterior face (Macadar et al., 1989). In one type of doubly innervated electrocytes, synaptic activation produces an AP on both the anterior and posterior membrane (blue region). In the second type of doubly innervated electrocyte, activation of the anterior synapse produces only a postsynaptic potential and activation of the posterior synapse produces an AP (green region). For electrocytes innervated only on the posterior membrane (red region), activation of the synapse produces an AP on the posterior membrane followed by an AP on the anterior membrane. The asynchronous activation of these different EO regions produces the complex multiphasic EOD. *Gymnotus carapo* EO and electrocyte schematics adapted from Caputi (Caputi, 1999). In *B. niger*, the EOD is a multiphasic waveform with an initial head-negative component (a) followed by a head-positive phase (b) then a final head-negative phase (c). The EOD of *Brienomyrus* is generated by the near-simultaneous activation of a single population of electrocytes. The electrocytes are innervated on a stalk that begins anterior to the electrocyte then penetrates through the electrocyte to join the posterior membrane. Synaptic activation initiates an AP in the stalk and the propagation of this AP through the electrocyte penetration produces the initial head-negative phase (a). The initiation of an AP on the posterior membrane face produces the head-positive second phase (b) and the subsequent AP on the anterior membrane face produces the final head-negative phase (c). Mormyrid electrocytes and EOD waveforms adapted from Hopkins (Hopkins, 1999). Electrocyte discharge schematic diagrams adapted from Bennett and Grundfest (Bennett and Grundfest, 1961).

negative DC current that sums with the head-positive APs (Bennett, 1961) to center the EOD energy around 0 V. This occurs in *G. niloticus* through the passive discharge of the anterior membrane's sizable capacitance. In wave-type gymnotiforms such as *Eigenmannia* and *Sternopygus*, the cellular mechanism underlying this head-negative DC current remains unknown despite being first described over 50 years ago (Bennett, 1961).

The remaining gymnotiform and mormyrid species also reduce low frequency spectral energy in the EOD, but accomplish this with a different mechanism at the level of individual electrocytes. To each head-positive pulse, they add a trailing head-negative pulse. The net EOD has roughly as much energy above 0 V DC as below, so the waveform is a biphasic pulse centered on 0 V DC which nulls the signal's DC component and attenuates low frequency energy, thereby 'cloaking' the signal from electroreceptive predators sensitive to low frequencies (Stoddard and Markham, 2008).

Recent progress in electrocyte physiology

Hormonal modulation

The discovery of sexual dimorphism in the EOD frequency of the gymnotiform *Sternopygus macrurus* (Hopkins, 1972) led to the identification of sexually dimorphic EOD waveforms in many gymnotiforms (Hagedorn and Carr, 1985; Hopkins et al., 1990; Kramer, 1985) and mormyrids (Hopkins, 1980; Hopkins, 1981). In

subsequent experiments, administration of exogenous androgens masculinized the EOD frequency and/or waveform of juvenile and female fish in gymnotiforms (Bass and Hopkins, 1983; Mills and Zakon, 1987). Changes in EOD frequency were traced to changes in the firing frequency of the central pacemaker nucleus, but indirect evidence in these studies suggested that changes in EOD waveform occurred in the peripheral EO. This hypothesis was confirmed in both mormyrid (Bass and Volman, 1987) and gymnotiform species (Hagedorn and Carr, 1985; Mills and Zakon, 1991) through studies demonstrating that hormonal regulation of EOD waveform stemmed from regulation of the AP waveforms in individual electrocytes.

The development of voltage-clamp techniques for *S. macrurus* electrocytes (Ferrari and Zakon, 1993) opened the door to analyzing sexual dimorphism and hormonal regulation of EOD waveforms at the level of the electrocyte's ionic currents. Exogenous androgens increase EOD duration by slowing the kinetics of the electrocyte's voltage-gated Na^+ current (Ferrari et al., 1995) and estrogen treatment feminized (shortened) the EOD waveform by speeding up the inactivation kinetics of the electrocyte's voltage-gated Na^+ current (Dunlap et al., 1997).

At the same time that the hormonal modulation of electrocyte physiology over time scales of days was coming into focus, evidence was accumulating that changes in electrocyte discharge waveform could occur on much faster time scales – over minutes to hours. Several behavioral experiments demonstrated that the EOD waveform in some gymnotiforms changed on a diurnal rhythm (Franchina, 1993; Franchina and Stoddard, 1998; Hagedorn, 1995) and within minutes of certain social encounters (Franchina et al., 2001). These findings collectively pointed to the possibility that the electrophysiological properties of electrocytes were modified on a moment-to-moment basis. These findings were consistent with reports that uncovered cellular mechanisms for rapid changes in electrocyte ion currents and APs. In *Sternopygus*, electrocyte Na^+ current magnitude and thereby the AP amplitude increase within minutes of activating the intracellular cAMP/protein kinase A (PKA) pathway (McAnelly et al., 2003; McAnelly and Zakon, 1996) but the upstream regulators of this action were unknown.

Research on the neuroendocrine regulation of EOD waveform in *Brachyhypopomus gauderio* (Allee et al., 2008; Markham et al., 2009a; Stoddard et al., 2003) ultimately led to identification of melanocortin peptide hormones as factors producing rapid changes in electrocyte discharge waveform. These peptide hormones such as adrenocorticotropic hormone or α-melanocyte stimulating hormone bind to G-protein-coupled receptors in the electrocyte membrane and activate a cAMP/PKA pathway that modulates both the AP waveforms and the delay between the APs, producing changes in EOD duration and amplitude, respectively (Markham and Stoddard, 2005). The same neuroendocrine axis is responsible for rapid circadian- and socially controlled changes in EOD amplitude in *Sternopygus*, where circulating melanocortin peptides regulate the trafficking of voltage-gated ion channels into the electrocyte membrane within minutes of social encounters (Markham et al., 2009b). This process is remarkable for its speed as well as for its clear connection of a vertebrate communication signal to its underlying molecular events.

Most recently, a number of studies have shown that steroid and peptide hormones have interactive effects on electrocyte excitability and EOD waveform. In addition to modulating the baseline EOD characteristics, steroid hormones also regulate the plasticity of EOD waveform changes in response to social interactions and injections of melanocortin hormones (Allee et al., 2009; Gavassa et al., 2011; Goldina et al., 2011; Salazar and Stoddard, 2009). Taken together, these outcomes strongly suggest that electrocytes are the cell-autonomous point of convergence where long-term effects of steroid hormones shape the nature of short-term EOD waveform regulation by peptide hormones. The cellular-level interaction of slow steroid effects and faster peptidergic regulation appears to be the primary mechanism through which EOD waveform carries important information about the sex, identity, social history and condition of the signaler. Gavassa and colleagues in this issue (Gavassa et al., 2013) address in detail the endocrine mechanisms that encode and integrate ecological history, seasonal variables, social cues and current conditions to determine relevant EOD waveform characteristics. No studies to date, however, have directly investigated at the electrocyte level how exactly steroid and peptide hormones interact to co-regulate the electrocyte discharge.

Recent research on the hormonally regulated plasticity of electrocyte function has uncovered a number of phenomena not anticipated by early work on electrocyte physiology, and has raised a number of important questions still in need of resolution at the cellular, ecological and evolutionary levels. For example, in all known cases of rapid EOD modulation, activation of PKA is the key intracellular signal driving the underlying changes in electrocyte excitability (Markham and Stoddard, 2005; McAnelly et al., 2003). The exact phosphorylation targets, however, are not yet known and could include direct phosphorylation of ion channels, vesicular trafficking components or other regulators of ion channel function. In addition, while the ionic mechanisms of rapid EOD modulation have been characterized in *S. macrurus*, corresponding mechanisms have not yet been identified in biphasic pulse fish such as *B. gauderio*, an apparent specialist in rapid EOD modulation. Because the electrocytes of biphasic or multiphasic gymnotiforms possess two distinct areas of excitable membrane, currents are likely expressed differentially on the electrocyte's two excitable membrane faces, precluding analysis by whole-cell voltage clamp. In my own work, gigaseal cell-attached patch recordings have proven impossible in these tissues because of excessive adherent tissue near the electrocyte membrane. However, I have recently recorded fast voltage-gated currents from each face of *B. gauderio* electrocytes by using an enhanced cell-attached loose patch technique (Almers et al. 1983; Stuhmer and Almers, 1982) (Fig. 2). Planned application of these procedures should allow analysis of the exact ion current modulations that produce rapid changes in EOD amplitude and duration in biphasic gymnotiforms such as *B. gauderio*.

A particularly interesting phenomenon that begs for additional experimentation at the level of single electrocytes is the EOD waveform's sensitivity to temperature in some temperate-zone gymnotiform species. In both *Brachyhypopomus pinnicaudatus* and *G. carapo* fish that are not in reproductive condition show a dramatic decrease in the amplitude of the EODs head-negative phase when water temperature is raised from ~20°C to above 28°C (Ardanaz et al., 2001; Caputi et al., 1998). The time course of this change and its sometimes transient nature shows that these temperature effects are not simply a function of temperature-dependent kinetics. Most importantly, sexually mature fish in breeding condition and non-differentiated fish given testosterone implants show little to no temperature-induced change in EOD waveform (Caputi et al., 1989; Quintana et al., 2004; Silva et al., 1999). Analyzing the cellular mechanisms of temperature sensitivity and its regulation by androgens is an important area for future experimental work. Additional comparative studies are

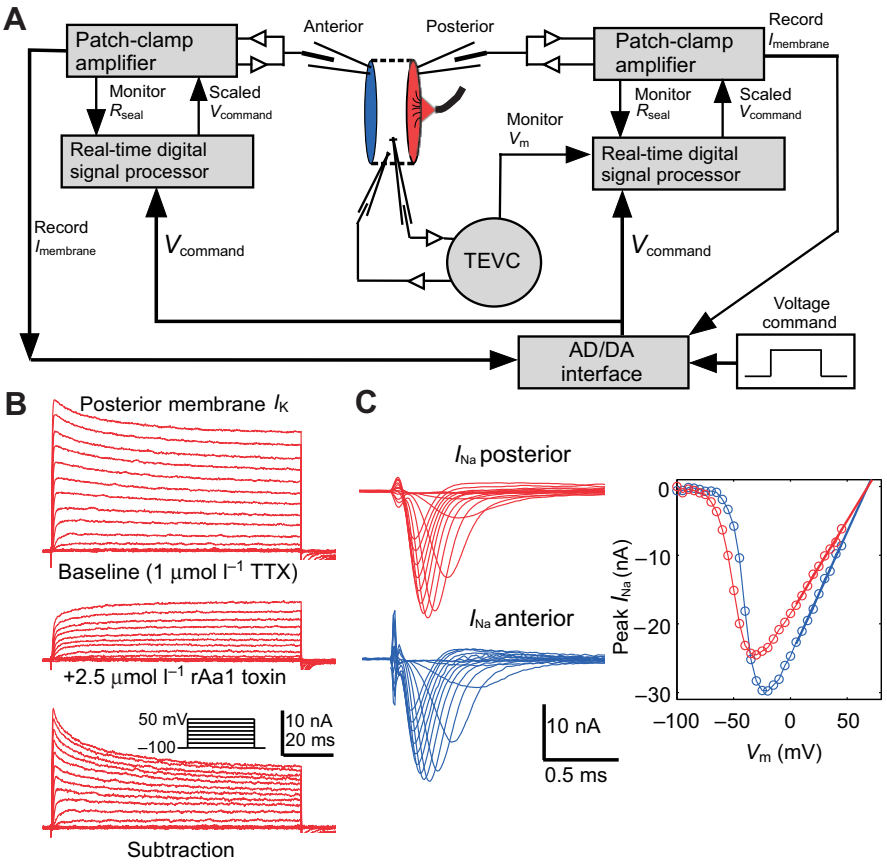

Fig. 2. (A) Schematic representation of loose-patch recording preparation and representative data. The essential feature of this technique is a low seal resistance (R_{seal}) of just a few MΩ. The pipette resistance together with the seal resistance form a non-negligible voltage divider, such that the command voltage ($V_{command}$) applied to the pipette internal solution produces an attenuated voltage at the pipette tip. We measure pipette resistance before approaching the cell, monitor total resistance (pipette + seal resistance) after contacting the cell, then calculate a correction factor and scale the applied voltage commands to produce the desired membrane potential at the pipette tip. Recorded patch currents ($I_{membrane}$) are scaled to compensate for leakage current through the seal resistance. A dual-patch recording preparation allows simultaneous recording of ion currents from each face of the electrocyte. Once both patch pipettes are positioned on the cell, we impale the cell with two pipettes within 100 µm of the patch pipettes and clamp the cell to the desired intracellular potential *via* two-electrode voltage clamp (TEVC), ensuring that the voltage commands and currents in the patch pipette do not affect the membrane potential outside the patches and maintaining a known intracellular potential, necessary for scaling the patch pipette voltage commands to achieve the desired membrane potential across the patches. A real-time signal processor monitors seal resistance and membrane potential for each pipette before each trial, then scales the voltage-command waveforms in real-time to achieve the desired membrane potentials. Capacitative and leak currents are subtracted using a p/6 subtraction protocol. (B) Pharmacological isolation of A-type K^+ currents and delayed-rectifier K^+ currents in an anterior membrane patch. Recordings in 1 µmol l^{-1} tetrodotoxin (TTX) produce total K^+ currents (I_K). Application of the scorpion toxin rAa1 selectively blocks the A-type K^+ current. Subtraction of these records from total K^+ current records reveals the A-type K^+ current. (C) Simultaneous recordings of Na^+ current (I_{Na}) from anterior and posterior membranes. I_{Na} shows identical reversal potential at +60 mV in both patches, strong evidence of accurate membrane voltage control (M.M., unpublished).

needed to address the broader question of whether androgens also confer resistance to EOD temperature sensitivity in species from tropical or sub-tropical climates where temperatures are more stable.

Finally, it is noteworthy that both mormyrid and gymnotiform fish show sexual dimorphism in their EOD waveforms under the control of steroid hormones as well as socially induced EOD waveform changes that occur over the course of days. However, only a subset of gymnotiform species exhibit rapid circadian and socially induced EOD modulations (A. Goldina, M.M. and P. Stoddard, unpublished data). If this pattern proves to be reliable, it raises the interesting question of why rapid social and circadian EOD modulations are not present in the mormyrid fish as well as why only some gymnotiform species show this rapid EOD waveform plasticity.

Molecular regulation

Electrocyte morphology plays a clear role in shaping the EOD waveform for both gymnotiform and mormyrid fish, and alongside these morphological effects, molecular composition of the electrocyte's ion channels is also a major factor determining EOD waveform. Changes in ion channel subunit composition, novel splice variants of channel proteins, and molecular evolution in important functional regions of the channel protein all can change the voltage dependence and/or kinetics of the electrocyte ion channel, potentially having profound effects on the EOD waveform.

In *S. macrurus*, EOD and electrocyte AP durations vary over a fourfold range between individuals, determined by steroid hormones that co-regulate Na^+ channel inactivation kinetics and K^+ channel activation kinetics (Ferrari et al., 1995; McAnelly and Zakon, 2000). Faster kinetics produce shorter EOD durations in

female fish with high EOD frequencies, while slowing channel kinetics increases EOD duration in males with low EOD frequencies.

At least three different K^+ channel genes from the Kv1 family are expressed in *Sternopygus* electrocytes. One of these genes, *Kv1.2b*, shows no difference in expression levels across individuals, while expression of *Kv1.1a* and *Kv1.2a* is inversely correlated with EOD duration. Treatment with steroid hormones that change EOD duration produce corresponding changes in expression levels of these genes (Few and Zakon, 2007). Voltage-gated K^+ channels are formed as tetramers of channel subunits, either homotetramers of a single subunit or in heterotetramers where different subunits from the same family associate to form the functional channel. In the case of heterotetrameric channels, the channel's functional properties are usually intermediate between the properties of the various subunits. Thus, changing the relative representation of Kv1.1a and/or Kv1.2a subunits in the electrocyte's voltage-gated K^+ channels is potentially the mechanism underlying the effects of steroid hormones on electrocyte AP duration in *Sternopygus*, a prediction that awaits experimental confirmation.

A similar picture emerges for the voltage-gated Na^+ channels in *Sternopygus* electroctyes. Sodium channels consist of a single α-subunit that by itself can form a functional channel, with the addition of accessory β-subunits that can alter the functional properties of the channel. *Sternopygus* electrocytes express two α-subunit genes, *Na_V1.4a* and *Na_V1.4b*, with the *Na_V1.4b* gene having both long (*Na_V1.4bL*) and short (*Na_V1.4bS*) splice variants. *Na_V1.4b* is expressed both in muscle and in electrocytes while *Na_V1.4a* is expressed only in electrocytes (Zakon et al., 2006). Expression levels of *Na_V1.4a* in electrocytes are constant and independent of EOD duration, but expression of *Na_V1.4bL* is negatively correlated with EOD duration, and androgen treatment suppresses *Na_V1.4bL* expression levels in electrocytes. Expression levels of a sodium channel β1-subunit expressed in electrocytes are also negatively correlated with EOD duration and suppressed by androgen treatment (Liu et al., 2007). The role of both the Na_V1.4bL and β1-subunits in accelerating Na^+ channel inactivation were confirmed in heterologous expression systems, establishing regulation of their expression levels in electrocytes as the most likely mechanism by which androgens control EOD duration at the level of electrocytes (Liu et al., 2007; Liu et al., 2008). An important area for future investigation will be to determine whether similar mechanisms of ion channel regulation are responsible for sexual dimorphism of EOD waveform in the numerous species of gymnotiform and mormyrid fish where sexual dimorphism is observed.

Molecular evolution

The whole-genome duplication that preceded the radiation of teleosts (Hurley et al., 2007) provided these fish with two paralogs of every gene. In both gymnotiforms and mormyrids, the presence of this second gene copy allowed the sequestration of one paralog in the evolutionarily novel EO where functionally significant mutations that would otherwise be disadvantageous might be freed from this negative selection in the EO. The best example is the *Na_V1.4* sodium channel gene which is expressed only in EO while its paralog *Na_V1.4b* is expressed in both muscle and EO. This arrangement is a convergent adaptation common to both gymnotiform and mormyrid fish (Arnegard et al., 2010b; Zakon et al., 2006).

Once freed from negative selection pressures in skeletal muscle and subject to positive selection forces likely tied to EOD waveform divergence, the Na_V1.4a channels expressed only in gymnotiform and mormyrid electrocytes have rapidly accumulated mutations at locations in the channel gene known to affect channel kinetics (Arnegard et al., 2010b; Zakon et al., 2006). Interestingly, some of these mutations that confer presumably adaptive signal diversity in the EO are associated with disease states when they occur in human sodium channels (Zakon et al., 2006). While the rapid evolution of sodium channel genes in electrocytes is the only case studied to date, it seems likely that similarly rapid evolution of key signaling and regulatory molecules in EO would have accompanied the broad divergence of EOD waveforms and waveform regulation mechanisms. The identification and characterization of additional molecules subject to rapid evolution in electrocytes offers an extremely fertile area for future investigation.

Changing channels

With the adaptation of patch- and voltage-clamp procedures for recording ion currents in electrocytes, it became possible to begin dissecting the ionic components of the electrocyte discharge. In addition to uncovering how electrocyte ionic currents are subject to slow modulation by steroid hormones (Dunlap et al., 1997; Ferrari et al., 1995) and rapid modulation by peptide hormones (Markham et al., 2009b), another outcome of this line of research has been the finding that electrocytes in different species express a strikingly varied complement of ion currents.

The first data on ionic currents underlying electrogenesis came from patch-clamp studies of *Electrophorus* electrocytes (Shenkel and Sigworth, 1991), which showed that the dominant ionic currents were voltage-gated Na^+ currents and inwardly rectifying K^+ currents. Application of two-electrode voltage clamp techniques later produced the first recordings of isolated ionic currents in electrocytes of a weakly electric fish, *S. macrurus*. These electrocytes expressed voltage-gated Na^+ currents, inward rectifier K^+ currents and delayed rectifier K^+ currents (Ferrari and Zakon, 1993). Many years later, voltage-clamp analysis of ionic currents in *G. carapo* revealed a broader complement of ion currents, including two functionally distinct inward rectifiers, a transient Na^+ current, a delayed rectifier K^+ current, an inactivating A-type K^+ current and a persistent Na^+ plateau current (Sierra et al., 2005; Sierra et al., 2007). Most recently, we have found that *Eigenmannia virescens* electrocytes express an inward rectifier K^+ current, an ultra-rapid transient Na^+ current and, surprisingly, the predominant repolarizing ion current is not a delayed-rectifier K^+ current, but instead a Na^+-activated K^+ current (Markham et al., 2013).

This sample across just four of the 175+ gymnotiform species reveals a striking diversity of ion channel mechanisms in gymnotiform electrocytes, suggesting that recruitment of different ion channel mechanisms is a major driver of signal diversity in this clade. No data are currently available on the ionic currents expressed in mormyrid electrocytes. The complex, multiphasic EOD waveforms in many mormyrid species are attributable primarily to electrocyte morphology and multiple patterns of stalk penetration (Alves-Gomes and Hopkins, 1997; Bennett and Grundfest, 1961; Gallant et al., 2011). However, the extreme diversity of EOD durations, ranging from hundreds of μs to more than 10 ms (Hopkins, 1999), is more likely a function of the electrocyte ion channel kinetics. The anatomy of the mormyrid EO with its tightly packed pancake-like electrocytes creates steep technical challenges for voltage- or patch-clamp recordings of ionic currents in these cells. These obstacles, however, likely can be overcome and the resulting data would provide important insights

on the parallel evolution of electrical signaling mechanisms in mormyrid and gymnotiform species. One intriguing possibility is that selective pressures promoting EOD diversity produced the recruitment of diverse ion channel combinations in gymnotiform electrocytes, while in mormyrids signal diversity arose by increased variability in electrocyte morphologies.

Conclusions

The first detailed analyses of electrocyte physiology appeared just over 50 years ago. Soon thereafter, Mike Bennett, a pioneering researcher on electrocyte physiology, saw little prospect for future gains from studying electrocyte physiology *per se*. As is often the case, researchers discovered unexpected features of electrocyte physiology and function that have produced important advances and new questions on several fronts.

There is little doubt that the greatest progress toward understanding electroreception and communication has come from stunning advances in the areas of central electrosensory processing and central regulation of EOD rate as a social communication signal. Nonetheless, a full understanding of the electrosensory and electric communication systems in weakly electric fish will also require a complete detailing of the evolution, development and regulation of electrocyte function. Beyond the world of electric fish, investigation of electrocyte physiology stands to contribute significantly to the understanding of more general biological principles. Electrocytes represent the point of convergence for steroid and peptide hormone regulation in animal communication, a crucible of molecular evolution, the primary driver of energetic demand in an animal communication system, and a model for the evolutionary selection of ion channels in excitable cells. It is clear that research on the physiology of electrocytes still has much to offer, and many questions with broad relevance for understanding the physiology and evolution of excitable cells and animal communication systems remain to be answered.

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Competing interests

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