

Signal Cloaking by Electric Fish

PHILIP K. STODDARD AND MICHAEL R. MARKHAM

Electric fish produce weak electric fields to image their world in darkness and to communicate with potential mates and rivals. Eavesdropping by electroreceptive predators exerts selective pressure on electric fish to shift their signals into less-detectable high-frequency spectral ranges. Hypopomid electric fish evolved a signal-cloaking strategy that reduces their detectability by predators in the lab (and thus presumably their risk of predation in the field). These fish produce broad-frequency electric fields close to the body, but the heterogeneous local fields merge over space to cancel the low-frequency spectrum at a distance. Mature males dynamically regulate this cloaking mechanism to enhance or suppress low-frequency energy. The mechanism underlying electric-field cloaking involves electrogenic cells that produce two independent action potentials. In a unique twist, these cells orient sodium and potassium currents in the same direction, potentially boosting their capabilities for current generation. Exploration of such evolutionary inventions could aid the design of biogenerators to power implantable medical devices, an ambition that would benefit from the complete genome sequence of a gymnotiform fish.

Keywords: biogenerator, electrogenesis, electroreception, Gymnotiformes, melanocortin

If you were to fall into the Amazon River, you would find yourself in a world of darkness. The mixture of rain-water, tannins, and suspended minerals known as “white-water” is virtually opaque. Although the limited penetration of sunlight might be expected to restrict primary productivity, the whitewater rivers of South America are among the world’s richest in terms of fish diversity and abundance. The limited visibility in these rivers has shaped the sensory worlds of their inhabitants, favoring the use of electricity and olfaction for navigation, hunting, and communication signaling. Two sister orders of teleost fishes, the catfishes (Siluriformes) and the knifefishes (Gymnotiformes), can detect electric fields. The catfishes use their olfactory whiskers to track the odor trails of prey (Atema 1971, Pohlmann et al. 2001), then rely on their electric sense to zero in on the minute electric stimuli (microvolts per centimeter) generated by the prey’s muscle contractions and nervous system activity (Finger 1986). The knifefishes use a specialized electric organ to generate comparatively stronger electric fields, in the range of millivolts per centimeter, and “see” their world within half a body length by analyzing distortions in these electric fields caused by nearby objects with different impedances than the surrounding water. The electrogenic ability of the electric fishes has enabled the secondary evolution of communication. Male electric fish sing electric courtship songs to the females and engage in energetically expensive contests of electric one-upmanship with their rivals (Franchina et al. 2001, Salazar 2003). The same story plays out in the rivers of West Africa, where the Mormyridae, an independent lineage of weakly electric fishes, replace the gymnotiforms.

At the mention of electric fish, people inevitably ask about the electric eel (*Electrophorus electricus*), the largest and best known of the Gymnotiformes. The electric eel is the only species capable of producing not only weak signals for navigation and communication but also a strong discharge (hundreds of volts) to stun prey and deter would-be predators. The electric eel is itself a formidable predator, and anecdotal evidence suggests that it relies on its electric sense to “eavesdrop” on weakly electric fish (Westby 1988, Stoddard 1999). After locating weakly electric fish by their electric signals, *Electrophorus* stuns them with its high-voltage discharge and gulps them down before they can recover. Various piscivorous catfish likewise eavesdrop on weakly electric fish. To an electroreceptive predator, the electric fields of a weakly electric fish produce a distinct “eat me” signal. Though we cannot see what goes on below the surface, lab experiments and analysis of stomach contents have borne out the supposition that electroreceptive predators are attuned to the rich foraging opportunities that arise from a shared electric sense and an abundant guild of signaling prey. The stage is set for evolutionary escape from predation, a condition that often precedes adaptive radiation (Ehrlich and Raven 1964), or possibly for an evolutionary arms race if the sensory systems of predators

Philip K. Stoddard (e-mail: stoddard@fiu.edu) is a professor, and Michael R. Markham is a research associate, in the Department of Biological Sciences at Florida International University in Miami. They study the evolution, neurobiology, and behavior of communication. © 2008 American Institute of Biological Sciences.

adapt to keep up with the shifting signal strategies of their electrogenic prey.

Escape from predation

Electric fish can reduce predation risk by inhabiting aquatic refugia where predators cannot enter. Some gymnotiforms can breathe air, allowing them to escape the predatory catfish by inhabiting floating meadows where dissolved oxygen concentrations approach zero (Crampton 1998a, Julian et al. 2003). The electric eel, an obligate air breather, does penetrate the floating meadows, as do some of its piscivorous low-voltage kin in the family Gymnotidae (Crampton 1998b). Other gymnotiforms cannot survive in low-oxygen waters and must run the gauntlet of hungry eavesdropping catfish in the river channels. Gymnotiforms constitute 80% of the stomach contents of the piscivorous catfish *Pseudoplatystoma tigrinum* (Reid 1983), suggesting that there is intense predation pressure on electric fishes in the river channels where these large pimelodid catfish live.

The other key strategy an electric fish can use to lower predation risk is the classic strategy of spectral shifting, or moving the energy in its signals above the sensory range of unwelcome eavesdroppers. Spectral shifting can work only if the signaler already has, or can readily evolve, a sensory range outside that of the eavesdropper. Gymnotiform electric fishes and catfishes share a class of ampullary electroreceptors, similar in physiology to the ampullary electroreceptors of sharks, rays, and other ancient fishes (Zakon 1986). Ampullary receptors detect electric fields in the low-frequency spectral range of 0 to 60 hertz (Hz). Their extreme sensitivity (microvolts per centimeter) allows these receptors to detect the weak electric fields produced by muscle action and by water movements of their prey. A second class of tuberous electroreceptors, derived from the ampullary receptors, is tuned to the higher frequencies of electric signals. Tuberous electroreceptors are less sensitive than ampullary receptors, and are used both for active electrolocation and for communication. As far as we know, tuberous electroreceptors exist only in the electric fishes. One piscivorous Neotropical catfish has been found to have cutaneous receptors on its face whose morphology is similar to that of a tuberous electroreceptor, though the physiology of these receptors has not been confirmed (Andres et al. 1988). Though we should not be surprised to find that some piscivorous catfishes have evolved electroreceptors whose physiology resembles that of the tuberous electroreceptors of the electric fishes on which they prey, the African clariid catfish that prey on mormyrid electric fishes do appear limited in their electrosensory capabilities to the low-frequency spectrum of the ampullary electroreceptors (Hanika and Kramer 2000).

The electric organ discharge (EOD) is a transient electric field with a characteristic voltage waveform at a distance. At the body surface, the waveform is much more variable, and this spatial variability influences the local spectrum. To understand the structure of the electric signal, it is helpful to understand how a gymnotiform fish produces electricity.

Electrogenic cells called electrocytes derive from the conversion of myocytes in the hypaxial muscle (Kirschbaum 1977, Franchina 1997, Zakon and Unguez 1999). Unlike neurons, which are optimized for moving information down axons and dendrites, electrocytes are optimized for moving current out of the cell. Electrocytes are huge cells, up to 0.75 millimeters across in fish of the genus *Brachyhypopomus* (see the photograph in box 1). The active membranes are convoluted, increasing the surface area that bears voltage-gated ion channels. Voltage-gated sodium and potassium channels are proteins that function like transistors to serve as the main gates for the movement of charge in electrocytes. Ion channels expressed in the electrocytes of electric fishes have undergone considerable evolution, including specializations for the production of species- and sex-specific communication signals (Zakon et al. 2006).

Each electrocyte is innervated by a spinal motoneuron that typically forms a synapse on a stalk projecting from the posterior surface. The motoneuron thus initiates an action potential in the innervated, posterior side of the electrocyte (figure 1, box 1). When the electrocytes fire action potentials on their posterior surfaces, positively charged sodium ions (Na^+) flow into the cells from the extracellular space posterior to the cells. As in most action potentials, the initial, inward sodium current is countered by an opposing, outward potassium (K^+) current that repolarizes the cell and closes the sodium channels, thus ending the action potential. The net

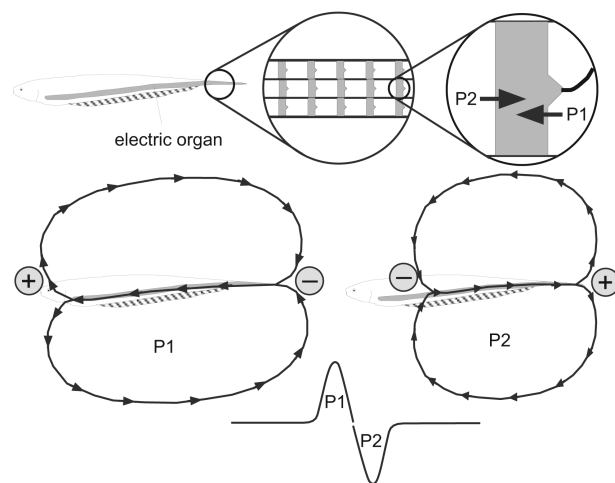


Figure 1. Schematic of biphasic electric organ discharge production by a hypopomid electric fish of the genus *Brachyhypopomus*. The electric organ is composed of rows of electrocytes in series to sum voltages, and multiple series (three shown here) in parallel to sum currents. To create the first phase (P1), the innervated posterior face fires an action potential on every electrocyte, creating a headward current that makes the head positive relative to the tail. Next, the anterior faces of the posterior two-thirds of the electrocytes discharge, creating a tailward current that makes the tail positive relative to the head (P2).

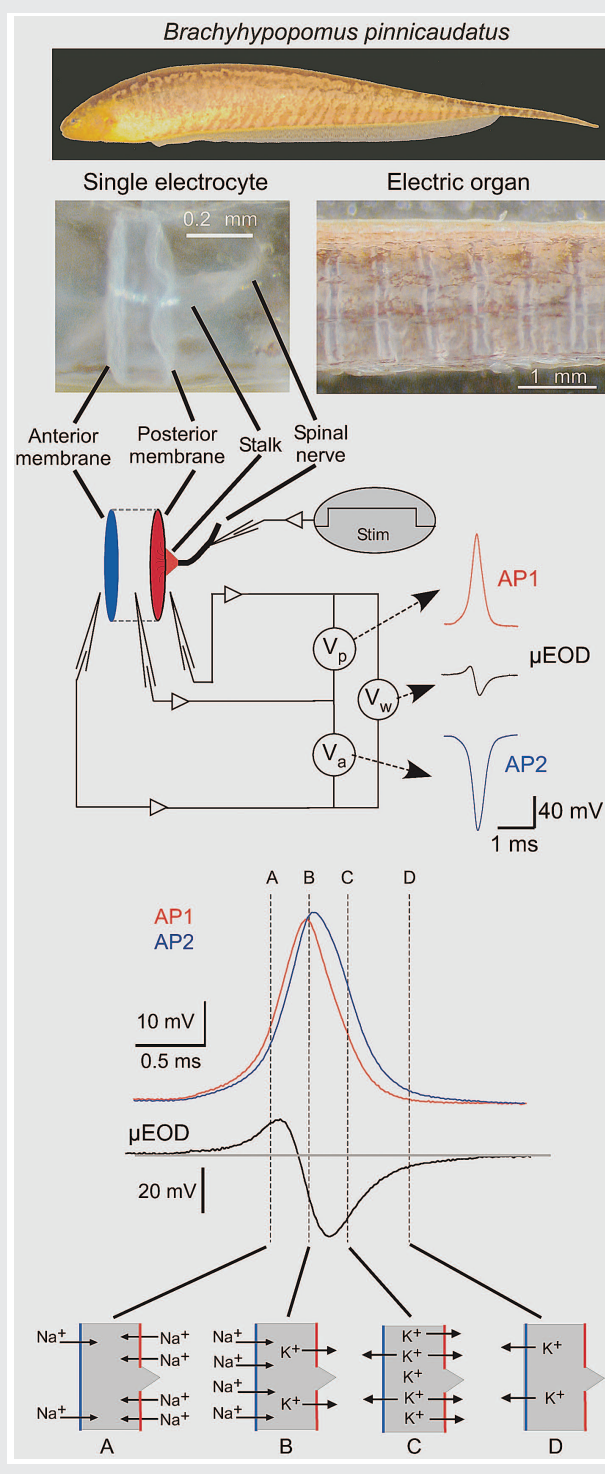
Box 1. Dissecting the electric discharge of a single electrocyte.

The electric organ consists of regularly ordered rows and columns of electrocytes, large disc-shaped cells innervated on a stalklike protrusion of the posterior face. The whole-cell micro-electric organ discharge (μ EOD) is the sum of the action potentials on the innervated posterior face and the noninnervated anterior face.

Procedures pioneered by Bennett (1961) allow researchers to simultaneously record the μ EOD and break it down into its component action potentials. Stimulation of the spinal nerve initiates the μ EOD. One micropipette penetrates the cell and records the intracellular potential, while two extracellular pipettes placed near the anterior and posterior membranes yield recordings of the potentials just outside the anterior and posterior membranes. Offline subtraction of the posterior extracellular record from the intracellular record yields the posterior-membrane voltage (V_p) and the posterior-face action potential (AP1). Subtraction of the anterior extracellular record from the intracellular record yields the anterior-membrane voltage (V_a) and the anterior-face action potential (AP2). Subtraction of the posterior extracellular record from the anterior extracellular record yields the whole-cell voltage (V_w) and the μ EOD.

Superimposing the two action potentials reveals their contributions to the μ EOD. Both membranes begin depolarizing simultaneously, but AP1 rises slightly ahead of AP2 (time point A), producing the head-positive phase of the μ EOD. AP1 peaks and starts to repolarize while AP2 is still depolarizing (time point B), thus beginning the head-negative phase of the μ EOD. The peak head-negative phase of the μ EOD results from the longer duration of AP2 (time point C), and the final resolution of the μ EOD is entirely a function of AP2 (time point D).

Assuming that the depolarizing phase of an action potential is primarily a function of sodium (Na^+) influx, while the repolarization phase is largely a function of potassium (K^+) efflux, leads to the hypothesis that, during the μ EOD, ion flux is sometimes in opposition but cooperative at other times. Particularly interesting is the energy-saving possibility illustrated at time point B, where inward Na^+ flux across the anterior membrane might be summing with outward K^+ flux across the posterior membrane to produce the larger second phase of the μ EOD.



flux of positive charge nevertheless is in the headward direction. Headward positive flux makes the head of the fish positive relative to the tail, and the electric charge in the water outside the fish is likewise positive around the anterior region relative to the posterior region (figure 1). In some species, a

second, delayed action potential fires on the opposing membrane. This delayed action potential directs positive charge in the tailward direction, producing a second phase to the EOD. In this second phase, the tail becomes positive relative to the head. By convention, though, polarity is referenced to the head,

so the two phases are said to be “head positive” and “head negative,” respectively.

A brain-stem pacemaker nucleus coordinates the near-simultaneous discharge of all electrocytes within the electric organ, so the discharges of the electrocytes are summed to create a detectable electric field surrounding the fish. In gymnotiforms, a bilateral pair of electric organs runs from just behind the head to the tip of the tail (figure 1). In the simplest model of an electric fish, electrocytes are arrayed in series within the electric organ like batteries in a flashlight. Multiple series may be arrayed in parallel (e.g., three serial arrays in figure 1). If the serial arrays of electrocytes were contained within an insulating tube, current could not escape the electric organ except at the ends. Only the skin at the two ends of the fish would sense a transdermal electric field. Such a limited area of transdermal electric stimulation would leave much of the fish's body unable to detect objects through active electrolocation. To electrolocate objects lateral to body surface, the fish needs some current to leak out through the sides of its body, and thus, in the case of a fish with a long electric organ, through the sides of the electric organ itself. Accordingly, most gymnotiforms have imperfect insulation around the electric organs, so some current does leak out the sides, allowing the field to extend laterally through the skin and create transdermal electric fields across the entire body surface.

Rostrocaudal differences in the conductive properties of the body, timing of action potentials down the length of the electric organ, and presumed ion-channel distribution within and between electrocytes produce spatial and temporal heterogeneity in the electric fields measured at or close to the skin (Assad et al. 1999, Stoddard et al. 1999). Figure 2 shows the spatiotemporal heterogeneity of the electric field in the lateral plane of *Brachyhypopomus*.

Spectral shifting

The EODs of most gymnotiform fish resemble either continuous sine waves or discrete sinusoidal pulses separated by silent intervals. Whereas a continuous sine or cosine wave (figure 3a) has all its energy concentrated at a single frequency, the single-period sine pulse (figure 3b) and the single-period cosine pulse (figure 3c) have broad spectra, caused by the long silent intervals between pulses. The ancestral EODs, simple monophasic pulses (Alves-Gomes 2001), are still produced by some basal taxa of electrogenic fish (figure 3d). These EODs resemble one period of a cosine pulse. Unlike continuous sine or cosine waves, single-period waveform transients show a flat spectrum with a drop-off at the higher frequencies (figure 3c, 3d). An EOD with this shape will excite ampullary and tuberous electroreceptors alike. Not surprisingly, lab studies have found that catfish and electric eels readily detect playback of monophasic EOD waveforms (Stoddard 1999, Hanika and Kramer 2000).

A continuous sine function has just a single frequency (figure 3a), but if that same function is not centered on 0 volts of direct current (VDC), a second 0-Hz frequency component

appears in the spectrum (figure 3e). Thus, monophasic EODs given in rapid succession would resemble a direct current (DC)-offset sine wave and would have a significant DC component. So-called wave fish do produce EODs in rapid succession. Wave fish in the family Sternopygidae (genus *Eigenmannia*; figure 3f) offset their EODs to center the energy on 0 VDC. In so doing, they eliminate the low-frequency energy detectable by ampullary electroreceptors, thus reducing their conspicuousness to piscivorous catfish. Apparently, the sternopygids' electric organs center their EOD train around 0 VDC by generating a head-negative DC current that sums with the head-positive action potentials (Bennett 1961). Surprisingly, the mechanism underlying this head-negative DC

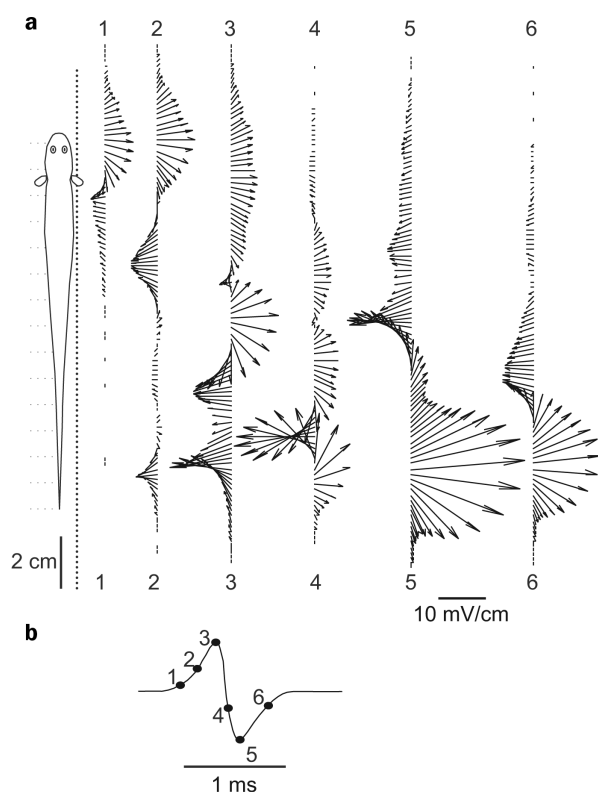


Figure 2. Electric field vector plots of electric organ discharge (EOD) measured along the side of a female *Brachyhypopomus beebei* (a). The vector plots (b), drawn to scale, show the electric field vectors in the lateral plane measured at each of the points on the adjacent dotted line. Vectors are plotted at six time points in the EOD, numbered 1–6. Numbers to the left of each vector row correspond to the times marked on the accompanying EOD waveform. Intensity in each vector row is shown relative to the vertical scale bar. Spinal propagation of the discharge command from the brain appears in the initial strengthening of vectors at the anterior end of the fish (time point 1), whereupon more posterior areas become activated. The tail of the fish produces more intense electric fields than the trunk or head. Adapted from Stoddard and colleagues (1999).

current remains a mystery almost 50 years after its discovery. During brief cessations of the EOD given during courtship (Hopkins 1974, Hagedorn and Heiligenberg 1985), the negative DC offset remains. This transient DC offset generates a low-frequency pulse readily detected by *Eigenmannia*'s ampullary electroreceptors (Naruse and Kawasaki 1998) and has been proposed to function as a mate attraction signal (Hagedorn and Heiligenberg 1985).

Other families of gymnotiforms and most of the mormyrids have also minimized their low-frequency spectral energy, but have done so using a different mechanism. To each head-positive pulse, they add a trailing head-negative pulse. The net EOD has as much energy below 0 VDC as above, so the EOD (figure 3g) resembles a sinusoidal pulse (figure 3b). Balancing energy around 0 VDC, seen as equal area above and below 0 amplitude in the voltage waveform, nulls the DC component and suppresses energy at low frequencies.

The single pulse and the continuous train of pulses constitute the ends of a spectral continuum from broadband to narrowband. What happens at intermediate frequencies? So-called pulse fish, such as *Brachyhypopomus*, discharge at variable rates from 4 to 100 Hz, with silent intervals between the EODs that are longer in duration than the EODs themselves. In practice, repetition rate contributes negligible power to the overall spectrum until the silent interval approaches the pulse duration.

The electric eel is an interesting predator because it has both ampullary (low-frequency) and tuberous (high-frequency) electroreceptors. In laboratory playback experiments, *E. electricus* detected monophasic pulse trains much better than DC-symmetric pulse trains with twice the amplitude (Stoddard 1999). This finding suggests that predation risk can be reduced more effectively by limiting the low-frequency spectrum than by limiting the overall waveform amplitude.

Electric cloaking

High-frequency sounds show greater excess attenuation (more than spherical spread) than low-frequency sounds, a property that may allow small animals such as mice to communicate with ultrasound over short distances without alerting their predators. Unlike acoustic signals, however, bioelectric signals show no frequency-dependent attenuation over distance (Brenowitz 1986). Nonetheless, electric fish in the genus *Brachyhypopomus* restrict the low-frequency spectrum of their electric fields to an area within a few

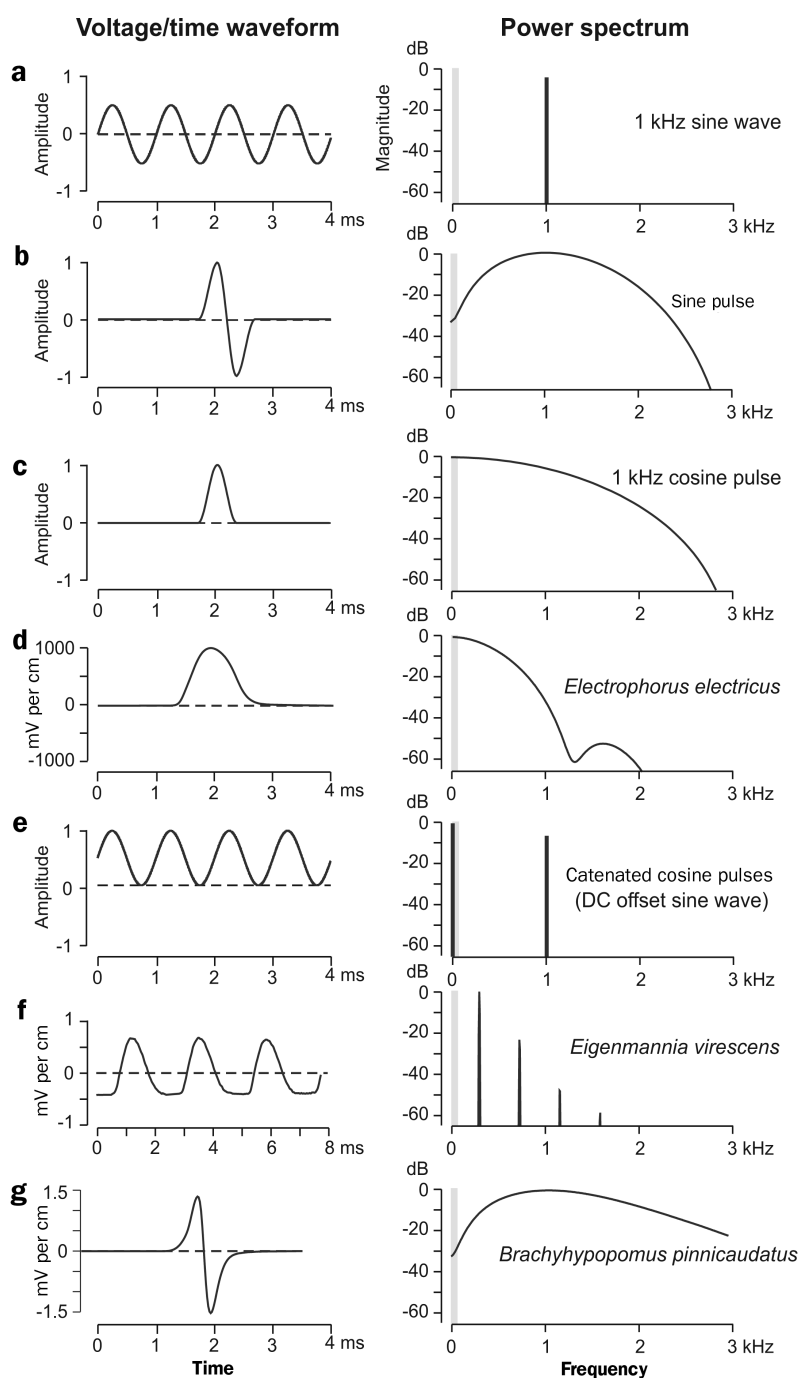


Figure 3. This collection of signals, a mix of synthesized waveforms and digitized electric organ discharges (EODs), shows the importance of symmetry around the zero ordinate value for suppressing low-frequency energy. On the left are synthetic signals and their counterparts from gymnotiform electric fish. On the right are the corresponding power spectra. The gray bars span 0–60 hertz (Hz), the spectral sensitivity of ampullary electroreceptors, which are used by predators to “eavesdrop” on the EODs of weakly electric fish. Those signals that show symmetry around zero in the voltage/time waveforms also show spectral suppression of energy in the range of ampullary electroreceptors. Continuous waveforms (a, e, f) have narrow spectra, in contrast to transient “pulse” waveforms (b–d, g), which have broad spectra.

centimeters of their bodies while allowing greater spread of the higher frequencies (Stoddard et al. 1999). This mechanism necessarily makes the signal less detectable by predators with ampullary electroreceptors (Stoddard 1999, Hanika and Kramer 2000), and we have argued that this mechanism evolved specifically to reduce hostile eavesdropping by predators (Stoddard 2002). We refer to this spatially restricted spectral shift as “cloaking,” after the science fiction technology that conceals the presence of an object from sensors.

To understand how *Brachyhypopomus* achieves spatial restriction of the spectrum, we begin with a simple model of how spatial heterogeneity can cloak the low-frequency spectrum. Recall that asymmetry of the waveform with respect to 0 VDC produces low-frequency spectral energy. If the EOD local to any part of the body is DC asymmetric, the electric field at that point will contain low-frequency energy. Let us begin with a hypothetical electric organ that produces monophasic local EODs, head positive in the anterior half of the body, and head negative in the posterior half. If the local EODs were simultaneous, they would cancel each other entirely at a distant point lateral to the fish (figure 4a). Electric communication would not be possible with this arrangement. But if the local fields were temporally offset, they would merge at a distance to produce a waveform like a single-period sine pulse (figure 4b). Close to the fish's body, the spectrum of the EOD is broad, with significant energy in the low-frequency range because the waveform is asymmetric with respect to 0 VDC. But distant from the fish's body, the merged waveform has equal energy above and below 0 VDC, suppressing the low-frequency energy. Suppression of low-frequency energy at a distance would cloak the fish's signal from predators that eavesdrop on the low-frequency channel.

To produce the local EODs in figure 4b, the fish would need to array anterior and posterior electrocytes in opposition. The anterior set would fire first, creating a headward current (positive at the head). The posterior set would fire afterward, creating a tailward current (negative at the head). In *Brachyhypopomus*, by contrast, all of the electrocytes discharge an action potential on the posterior membrane, producing

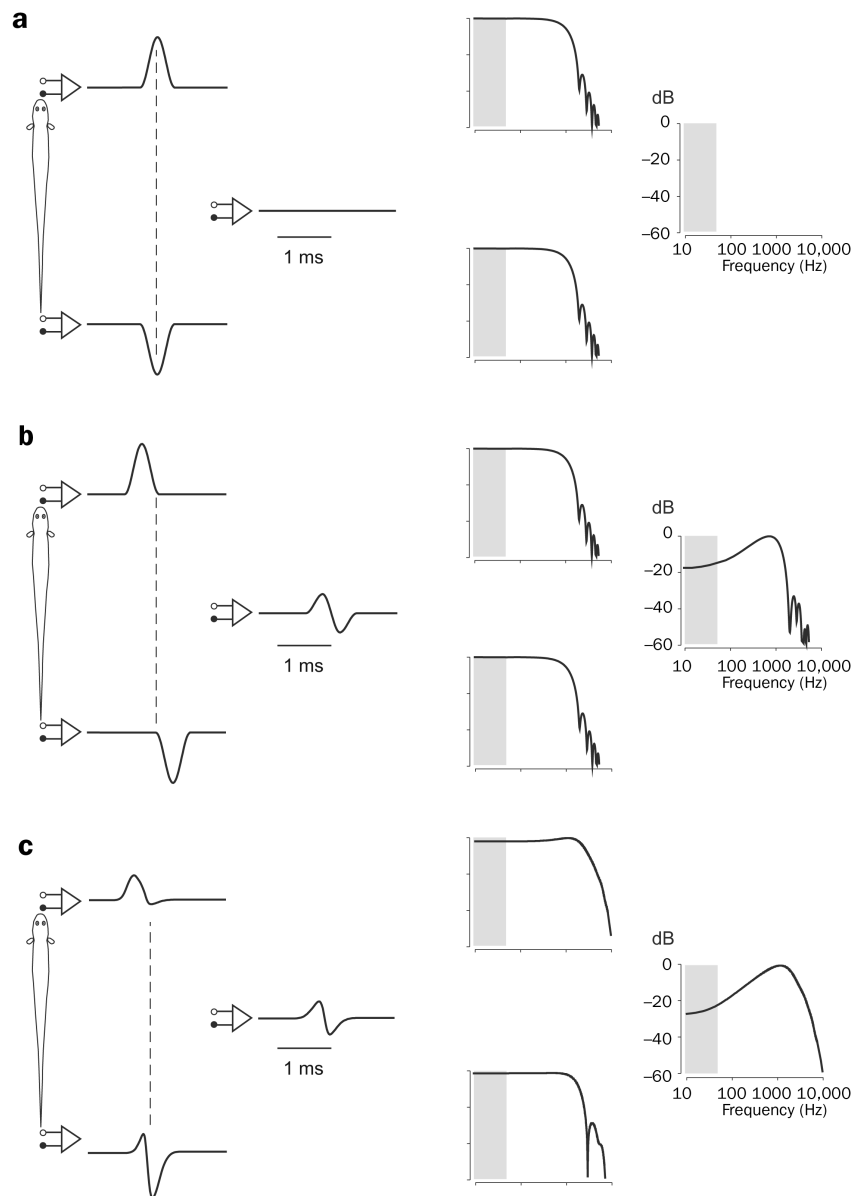


Figure 4. Three models of how local electric organ discharges (EODs) sum to cloak the low-frequency spectrum at a distance. EOD waveforms are shown as they would be detected by differential recording electrodes placed at the paired dots. The dashed lines show time alignment between the local EODs. Associated power spectra are shown to the right. The gray areas in the power spectra represent the sensory range of ampullary electroreceptors. In the first model (a), monophasic EODs local to the head and tail are emitted simultaneously. Opposite in sign, they null the entire waveform at a distance. In the second model (b), the same local EODs are temporally offset. They sum at a distance to produce a sinusoid that lacks the low-frequency power present in the monophasic local EODs. The third model (c) includes actual recordings from a female *Brachyhypopomus pinnicaudatus* (timescale is correct; amplitudes have been changed for visual clarity). The EOD local to the head is nearly monophasic (the small trace of a second phase is spread from the tail). The EOD local to the tail is asymmetrically biphasic (see box 1 for the mechanism). The local EODs sum to produce a direct current—symmetric remote EOD. Thus, the fish generates broad-spectrum electric fields close to the body, while cloaking the low-frequency spectrum at a distance.

Na^+ currents that enter the cell across the posterior membrane. Because these Na^+ currents are in the direction of the head, the head becomes positive relative to the tail. In addition, electrocytes in the back half of the animal fire a second, delayed action potential from their anterior, noninnervated surfaces, producing inward Na^+ currents across the anterior membranes, toward the tail. This delay appears to be intrinsic to the anterior membrane of the electrocyte; experimental depolarization of the entire cell at once still results in the spike on the anterior membrane following that on the posterior membrane (Bennett 1961, Markham and Stoddard 2005).

The tailward Na^+ current from the second action potential makes the tail positive relative to the rest of the animal (by convention a “head-negative” pulse), though this current is local to the back half of the animal. This second action potential overlaps and swamps the first (box 1). Thus, the head of the animal, with its single action potential, produces a monophasic local EOD. The tail of the animal, with its two partially overlapping action potentials, produces a biphasic local EOD in which the second phase is much larger than the first (figure 4c). The local electric fields sum over a distance to produce the symmetric, DC-balanced waveform with little energy in the spectrum of the ampullary electroreceptors (Bastian 1977, Caputi et al. 1998, Stoddard et al. 1999). By generating a second action potential from a subset of electrocytes, the fish eliminates the spread of low-frequency energy that would otherwise attract electroreceptive predators. Without this compensation, we estimate that the signal would be detectable by electroreceptive predators at a distance of one meter, a figure compatible with data from playback experiments in the lab (Stoddard 1999, Hanika and Kramer 2000).

Channel surfing

The asymmetric discharge of the tail electrocytes is key to the cloaking mechanism of *Brachyhypopomus*. We refer to the discharge of a single electrocyte here as a micro-EOD (μEOD), a discharge that resembles the EOD in miniature. The asymmetry of the μEOD results from differences between the two extracellular action potentials produced by each electrocyte in the tail (see box 1).

How does an electrocyte create a much bigger extracellular potential from the action potential on the noninnervated anterior face (AP2) than from that on the innervated posterior face (AP1)? The two action potentials are approximately the same voltage magnitude, so the difference could lie in the timing and duration of the spikes, as well as the amount of current generated by each. AP1 and AP2 overlap temporally, and so partially cancel each other outside the cell (Bennett 1961, Hagedorn and Carr 1985). But AP2 has a longer duration than AP1 and so persists longer outside the time of overlap (Markham and Stoddard 2005). This overlap appears to be a bizarre waste of energy, especially given that EODs have been found to expend significant fractions of the animal's energy budget (Salazar 2003).

We suspect that *Brachyhypopomus* offsets this wastefulness with a novel trick to exploit another, preexisting source of energy. The duration of an action potential is commonly regulated by an outward flow of K^+ ions that oppose and quench the inward Na^+ current. The electrocytes of *Brachyhypopomus* could be harnessing the K^+ current to boost the overall current of the EOD's second phase. The two spikes are offset in time, so the outward K^+ current in AP1 can occur at the same time as the inward Na^+ current in AP2. AP1 and AP2 are generated by the two planar membranes on opposite faces of the flattened cells (posterior and anterior, respectively). Thus, the outward K^+ current in AP1 is tailward, as is the inward Na^+ current in AP2. In a typical neuron, these currents oppose one another. But in these electrocytes, the two currents sum, because they are produced by separate and opposite excitable membrane faces. Moving the K^+ ions across the electrocyte membrane costs little or nothing, because these membranes at rest are highly permeable to K^+ . After an action potential, K^+ balance within the cell should be partially restored by pumping out the Na^+ with the ion exchanger $\text{Na}^+\text{K}^+\text{ATPase}$. Any remaining K^+ imbalance is restored by passive diffusion as the membrane approaches the equilibrium potential for K^+ .

We do not know how the fish achieves waveform symmetry of the whole electric field when it never experiences the field in this way, but we have looked at individual parts that make up the balance. Most interesting to us are electrocytes in the tail that generate the sharply asymmetric μEODs . How does one cell generate two action potentials precisely separated in time? We have shown that significant changes in the μEOD waveform occur with changes of only 10 to 20 microseconds in the timing of these two action potentials (Markham and Stoddard 2005). One might imagine that a single action potential would propagate around the edges of the cell, from the innervated posterior face to the anterior noninnervated face. This idea, however, turns out to be wrong. Visualization of voltage-gated sodium channels by antibody labeling reveals that electrocytes have none of these channels on the edges between the two main faces. Thus, the action potential cannot advance slowly from the posterior to anterior surface by progressive propagation (Stoddard 2006). Apparently, the two phases of the μEOD are based in two completely independent action potentials generated by the opponent faces of the electrocyte.

Another hypothesis would be that the first action potential (AP1) is initiated by the synaptic potential generated in the stalk. AP1, in turn, could depolarize the cell sufficiently to initiate AP2, thus causing the delay. But this hypothesis is also wrong. Careful electric dissection of the EOD (box 1) reveals that both membranes begin depolarizing simultaneously, but that AP1 spikes first. The delay could be intrinsic to threshold differences between ion channels in the two membranes, or it could be caused by the greater capacitance (and thus the longer charging time) of the more convoluted anterior membrane.

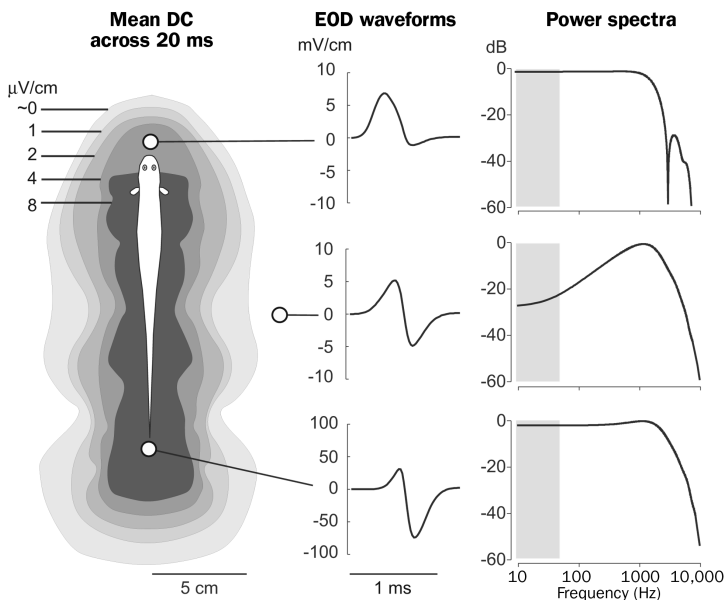


Figure 5. A female *Brachyhypopomus beebei* generates low-frequency energy within approximately 5 centimeters of its body (left). Isolines represent the 0-hertz (direct current) spectral component of electric field vectors averaged across 20 milliseconds. Electric organ discharges (EODs) close to the body have different, asymmetric waveforms that merge to produce a more symmetric EOD farther from the body (center). Spectra of these EODs (right) show that merging asymmetric waveforms suppresses low-frequency energy at a distance from the body. The ampullary electroreceptor range is shown in gray. Thus, the fish occupies a private bubble of low-frequency energy that is not available to “eavesdropping” predators relying on ampullary electroreceptors to locate prey. *Brachyhypopomus beebei* is a sister species of *Brachyhypopomus pinnicaudatus*—its EOD is 25% shorter but otherwise similar. Source: Stoddard and colleagues (1999).

Sensory consequences of cloaking

Because the asymmetric waveforms near the fish’s body surface are not DC-balanced, the fish is enveloped in a low-frequency electric field bubble (figure 5). The fish’s own ampullary electroreceptors, which would not be stimulated by a symmetric waveform, reside within local electric fields that contain ample energy in their ranges of spectral sensitivity. The widely held assumption that the ampullary system of weakly electric fish is not affected by self-generated electric fields must surely be wrong in the case of *Brachyhypopomus pinnicaudatus* and its relatives. To the contrary, any outside stimulation of the ampullary electroreceptors must ride on top of the self-generated fields. Modulations in discharge rate vary the time-averaged potential to which these receptors are acutely sensitive (Naruse and Kawasaki 1998), which could enable ampullary electroreceptors to be used for active electroreceptive imaging on a fine spatial scale. Likewise, fish engaging in close social interactions would enter the low-frequency fields of their companions, possibly overwhelming both of their sensitive ampullary systems with unpredictably intense low-frequency energy.

Cloaking and uncloaking

Brachyhypopomus pinnicaudatus makes its first EOD, a monophasic pulse, six days after hatching (Franchina 1997). As the fish grows, its EOD changes from a monophasic pulse to a nearly symmetrical biphasic pulse (figure 6a). Accordingly, low-frequency energy is gradually suppressed as the waveform approaches symmetry. At sexual maturity, the EOD of a female *B. pinnicaudatus* achieves nearly perfect symmetry. The second phase of the male’s EOD, on the other hand, becomes greatly extended (Hopkins et al. 1990). The male waveform departs radically from DC symmetry, uncloaking the low-frequency spectrum (figure 6b). EODs of mormyrids with the same shape as those of male *B. pinnicaudatus* are readily detected by the predatory African catfish *Clarias gariepinus* (Hanika and Kramer 1999, 2000), a confirmed predator of mormyrid electric fish (Merron 1993). The male *B. pinnicaudatus* greatly extends the second phase of its EOD at night when courting females and when confronted by conspecific males (Kawasaki and Heiligenberg 1989, Franchina et al. 2001). This phase extension, which takes 30 to 45 minutes to complete, increases energy in the spectrum of the ampullary electroreceptors by 12 to 15 decibels and exhibits a true circadian rhythm that persists in constant light or constant darkness (Stoddard et al. 2007). We speculate that low-frequency signals are attractive to females, though this has yet to be shown experimentally. These extended signals surely increase the risk of predation. One theory of sexual selection posits that males display their quality by bearing a proportional cost or “handicap” such as increased predation risk (Zahavi 1975, 1977).

We set out to determine how the fish modified its electric waveform. If the dynamic extension of the EOD were caused by direct neural stimulation, we would expect changes in discharge rate to influence the waveform. Indeed, *B. pinnicaudatus* triples its discharge rate as night approaches, but changes in discharge rate have no effect on the shape of the EOD waveform (Franchina and Stoddard 1998). We took an educated guess that changes in the waveform were instead mediated by the direct action of some hormone or neuromodulator in general circulation. We initiated a screening program to identify the chemical modulator, injecting candidate modulators and monitoring the EOD waveform parameters with a real-time recording and analysis system we named the “EOD machine.” Androgens are known to masculinize the waveform, but injection of various androgens (testosterone, 11-ketotestosterone, 4-dihydrotestosterone) elicited no rapid alterations (i.e., within minutes) of the EOD waveform. Neither did the steroids estradiol, progesterone, pregnenolone, or DHEA (dehydroepiandrosterone). We tried cortisol over a range of concentrations, because its effects are

typically dose dependent, but we saw no effect. Next, we tried the peptide hormones GnRH (gonadotropin-releasing hormone) and AVP (arginine vasopressin), both of which elicit male-typical behavior in a variety of vertebrates. Again, there was no effect. We tried the bioamines adrenaline, noradrenaline, dopamine, melatonin, and serotonin.

Ultimately, it was serotonin (5-hydroxytryptamine) that elicited waveform changes consistent with those seen in social interactions and day-night transitions (Stoddard et al. 2003). We found that serotonin can either reduce or increase the extension of the second EOD phase, depending on the sub-population of serotonin receptors that are activated (Stoddard et al. 2003, Stoddard 2006, Allee et al. 2008). Serotonin activity has been generally thought to be higher in subordinate animals than in dominants, but newer data on lizards indicates that serotonin activity is high in the brains of dominant animals shortly after an aggressive encounter (Korzan et al. 2001, Summers and Winberg 2006). After extensive efforts on the physiology rig, we concluded that serotonin had no direct action on the electrocytes, and therefore it must act centrally to release some other substance into peripheral circulation (Markham and Stoddard 2005).

After two years spent building our EOD machine and screening substances, we still had no strong candidates for a peripheral modulator. Clifford Summers, at the University of South Dakota, suggested that we try the melanocortin peptide hormones or their releasing hormones. A review of notes taken 20 years previously, in a graduate-level course Steven C. Woods taught at the University of Washington, indicated that one of the melanocortin peptides, adrenocorticotrophic hormone (ACTH), has a strong circadian rhythm, and though ACTH is best known for releasing glucocorticosteroids, it has other actions as well. We found that ACTH rapidly enhanced the EOD when injected into a free-swimming fish and also enhanced the μ EOD when applied directly to electrocytes in a dish (Markham and Stoddard 2005). The enhancement of the μ EOD is a result of two distinct mechanisms: increasing the delay between the electrocyte's two action potentials and selectively broadening the second action potential.

In addition to ACTH, the family of melanocortin peptides also includes the melanocyte-stimulating hormones (aMSH, bMSH, and gMSH). A growing body of literature shows that these melanocortins regulate energy balance and support a suite of traits involved in reproduction, competition, and emergency strategies for survival. For instance, melanocortins suppress appetite, induce fat burning (lipolysis), facilitate penile erection, promote the release of sex and aggression pheromones in rodents, facilitate aggressive behavior, and rapidly darken the skin of fish, amphibians, and lizards involved in aggressive interactions (Nowell et al. 1980a, 1980b, Castrucci et al. 1997, Strand 1999, Caldwell and Lepri 2002, Huang et al. 2006, Morgan and Cone 2006). These functions are compatible with sex and aggression signaling, the roles expected for exaggeration of sexually dimorphic characters in the EOD.

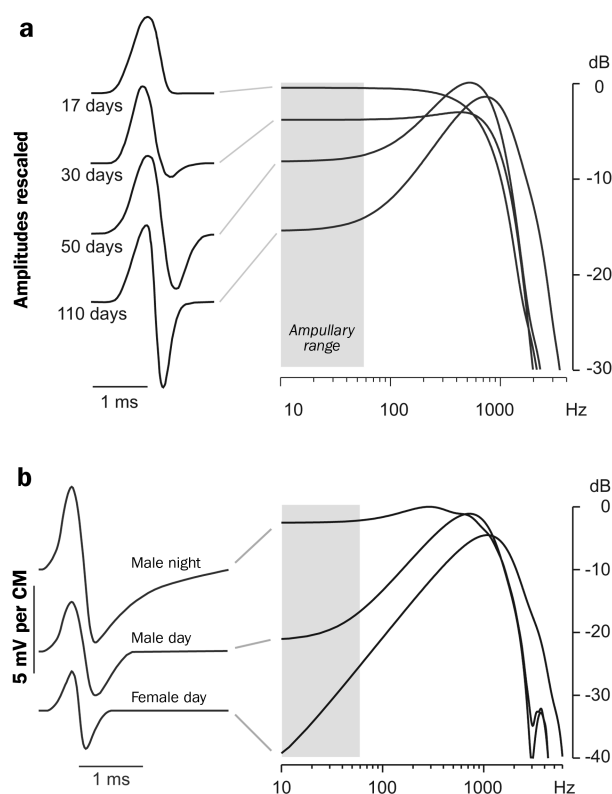


Figure 6. (a) Developmental series of electric organ discharges (EODs) of a *Brachyhypopomus pinnicaudatus*. EOD waveforms have been rescaled to equate the amplitude of the first phase. Age is shown as the number of days postfertilization. When the fish is very small, its EODs are monophasic (day 17), with maximal power in the spectral range of the ampullary electroreceptors (gray bar). As the fish grows, the electric organ produces an increasingly biphasic EOD waveform. By 110 days, energy in the spectral range of the ampullary electroreceptors has been suppressed by 15 decibels, rendering the young fish much less vulnerable to predators than it would have been had it retained the initial monophasic waveform. (b) EOD waveforms and spectra of male and female *B. pinnicaudatus*. At night, the second phase of the male's EOD is considerably extended, which increases spectral energy in the sensory range of the ampullary electroreceptors (gray bar). The female's EOD changes only slightly (not shown).

A need for better field studies of electroreceptive predators

Our understanding of the sensory biology of electroreceptive predation comes from lab studies. Low-frequency suppression clearly hinders detection by some electroreceptive predators in the lab, but we have few supporting field data. Such data would be extremely important for understanding the importance of eavesdropping predators in shaping gymnotiform life history and signal evolution. Part of the problem is logistic:

the large electroreceptive predators are relatively scarce, and the large riverine catfish, in particular, have been depleted by commercial fishing. Electric eels, however, are still reasonably common in some locales, so their predatory habits and sensory physiology could be studied in the field, albeit with care.

Electrocytes as models for biogenerators

Interest has grown around the idea of using the body's own energy to power medical devices and prostheses. In one scheme, a molecular motor in a prosthesis might be powered directly from diffusible chemicals in the bloodstream (Ebron et al. 2006). In one strategy we are exploring, electrocytes could be included in a xenotransplanted biogenerator to power a small medical device. The electrocytes would have to be encapsulated in aerogels to protect them from the host's immune system. Another scheme drawing attention from venture capitalists and the US Department of Energy involves engineered human cells that express ion transporters and channels to create a potential that can be harnessed to power implantable medical devices. If this strategy is used, it will surely benefit from an understanding of the physiology and molecular biology of electrocytes.

Unlike neurons, which evolved to transmit information from cell to cell at tiny synapses, electrocytes evolved to emit power over a broad area. Their many unique adaptations could hold the answers to the design of efficient biogenerators. For instance, electrocytes appear to segregate and anchor different ion channels at different locations within the continuous cell membrane, a feature that orients ion currents in particular directions rather than all inward or all outward. In a neuron, K^+ and Na^+ currents flow in opposite directions, and thus oppose one another, preventing the efficient transmission of power out of the cell. In those electrocytes that generate two action potentials, the hypothesized summation of K^+ and Na^+ currents described above would use no more energy, but could vastly increase the transfer of power out of the cell. To harness these and other adaptations for use in biogenerators, researchers need to understand the molecular structure of the ion channels and transporters, how their subunits interact, and how they are anchored and localized on the cell membranes. A fully sequenced gymnotiform genome would greatly facilitate our ability to work with these molecules. This argument forms the rationale for an ongoing initiative to sequence the genome of a gymnotiform electric fish (Albert et al. 2008).

Acknowledgments

Financial support and equipment were provided by National Institutes of Health grants MBRS GM08205 (P. K. S.) and K01MH064550 (M. R. M.). The data used to generate figure 6 were collected in collaboration with Cheryl Franchina. This paper is contribution 147 of the Tropical Biology Program at Florida International University.

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doi:10.1641/B580508

Include this information when citing this material.