Temporal Coding of Species Recognition Signals in an Electric Fish

Abstract. *An electric fish in the African family Mormyridae recognizes members of its own species by "listening" to electric organ discharges, which are species-specific signatures. Reactions of fish in the field and of individual electroreceptors to both normal and modified computer-synthesized discharges emphasize the importance of the waveform (time-domain cues) in species recognition.*

We have found behavioral and neurophysiological evidence for a novel mechanism of temporal coding of species recognition signals in a mormyrid electric fish, which differs from more typical frequency-domain (power-spectral) mechanisms for discriminating species-specific sounds by vertebrate auditory systems (*1*).

Electric fish are useful for studying species recognition: They produce a wide variety of electric signals used in social communication (*2*), the entire electric communication channel can be characterized in terms of natural signal variation and channel noise, signals are easy to generate and modify, and the sensory and motor pathways for communication are distinct (*3*).

Two components characterize the electric discharges of mormyrids. The stereotyped part—the pulsatile waveform of the electric organ discharge (EOD) (Fig. 1A)—is fixed by the anatomy, organization, and physiology of excitable cells in the electric organ (*4*). The variable part, the sequence of pulse intervals (SPI), by contrast varies with social context (Fig. 1B). The EOD's are diverse among coexisting mormyrids and are generally species-specific (*5*).

We were concerned that EOD's of mormyrids show too much overlap in power spectra to permit species recognition using these cues only, (*5*). We therefore decided to determine whether these fish could discriminate different EOD's having identical power spectra.

This study was conducted in the interior of Gabon, West Africa (0.34°N, 12.52°E) during the October–December rainy season. Previous fieldwork demonstrated that more than 20 species of mormyrids coexist in the Ivindo River and surrounding streams, many with distinctive EOD's (*5*). We concentrated on one species, known tentatively as *Brienomyrus brachyistius triphasic* (Fig. 1C) (*6*), which inhabits shallow streams and seems to breed during the rainy season. Reproductively mature males and females differ in their EOD's (Fig. 1A). To our knowledge this is the first field case of a sexually different EOD in a pulsing electric fish (*7*).

During the rainy season, daily censuses showed that males are stable in their positions, spaced every 5 to 20 m in streams 1 to 3 m wide and 10 to 30 cm deep. Some males are associated with female *B. brachyistius triphasic* while others are solitary (presumed nonmated). When a discharging female *B. brachyistius triphasic* passes a solitary male's hiding place (dense vegetation or an undercut bank), the male produces a high-frequency burst of EOD's sounding like a "rasp" on the audio monitor (Fig. 1B). Rasps apparently function in courtship calling in this species, as they are not elicited by passing males or other species, and they are heard only during the rainy season. Solitary males were ideal subjects for EOD-playback experiments, their rasps being easily quantified as responses to playbacks.

We presented tape-recorded EOD's to solitary males through Ag/AgCl electrodes placed near the stable hiding place of a male at night. Two minutes of playback were preceded and followed by 2

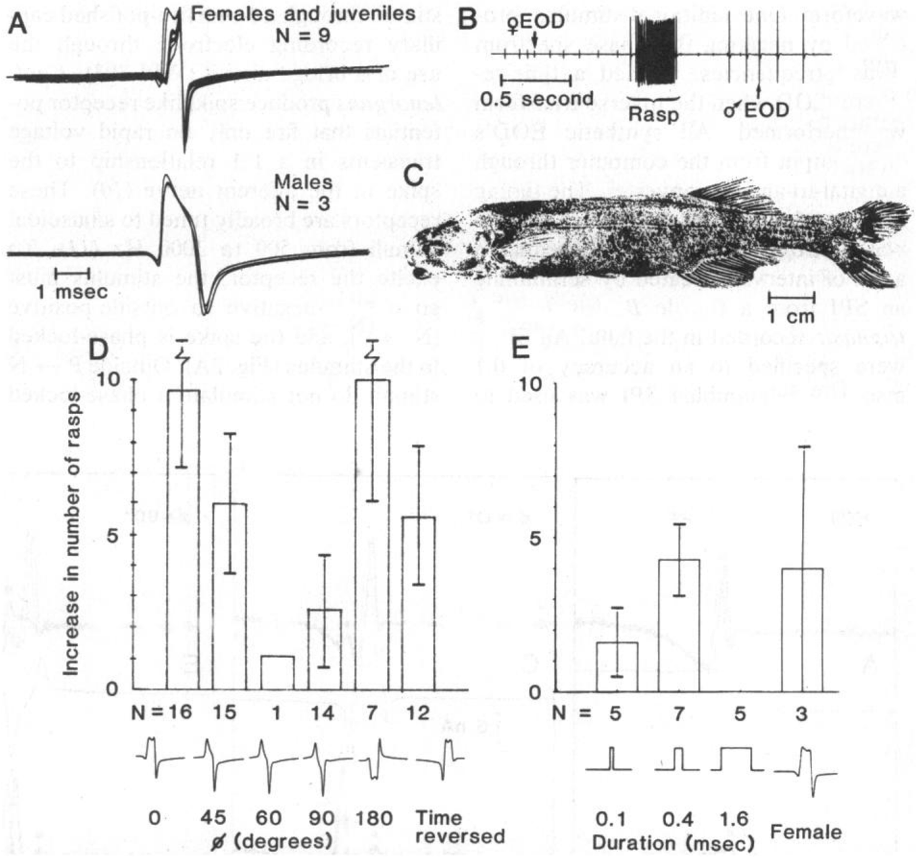

Fig. 1. (A) Oscilloscope tracings of EOD's. The tracings illustrated characterize the general pattern, which fits several hundred additional specimens of the same species recorded during two field seasons. (B) Oscillograph of the discharge of a male producing a "rasp" (burst of EOD's at high frequency) in the presence of a more distant female. (C) *Brienomyrus brachyistius triphasic*. (D) Playback of EOD's differing in waveform but not in power spectra to three solitary male *B. brachyistius triphasic* in the field. The sequence of pulse intervals, the same for all stimuli, comes from scrambling a normal female sequence. The ordinate is the mean increase in numbers of rasps produced by the resident male during the 2-minute playback compared with the 2 minutes before the playback. Stimuli are either normal female EOD's phase-shifted by ϕ, or a time-reversed EOD. N is the number of trials. Vertical bars are standard errors of the mean. The number of rasp responses to $\phi = 0°$ does not differ from that at $\phi = 180°$ (Mann-Whitney U test; $U = 54$); nor does it differ from that at $\phi = 45°$($U = 56$). Fewer responses occur to $\phi = 90°$ than to $\phi = 0°$ ($U = 24$, $P < .001$), and fewer responses occur to time-reversed EOD's than to $\phi = 0°$ ($U = 37$, $P < .05$). (E) Playback of rectangles of different duration and the female EOD to one solitary male *B. brachyistius triphasic* in the field. Sequence of intervals and ordinate are as in (D). The responses to the female EOD do not differ from the response to the 0.4-msec rectangle ($U = 7$). The responses to the 0.4-msec rectangle differ significantly from those to the 0.1-msec rectangle ($U = 5$, $P = .02$) and to the 1.6-msec rectangle ($U = 0$, $P < .01$).

minutes of control listening. A 2-minute pause separated successive trials. We counted numbers of rasps produced by the resident during the control and playback periods.

Experiment 1 (Fig. 1D) demonstrated that males discriminate between EOD's with different waveforms but identical power spectra. The stimuli were generated by digital computer (*8*). An EOD from a female *B. brachyistius triphasic*, digitized at 100 kHz, was subjected to Fourier analysis to obtain the power and phase spectra. Phase angles were advanced by a constant angle, ϕ, where ϕ = 0°, 45°, 60°, 90°, and 180°, for all positive frequencies, and retarded by the same angle for all negative frequencies to maintain the required odd symmetry of the phase spectrum. The inverse Fourier transform reconstituted an EOD with an altered waveform. One additional stimulus, produced by negating the phase spectrum for all frequencies, yielded a time-reversed EOD when the inverse transform was performed. All synthetic EOD's were output from the computer through a digital-to-analog converter. The timing of intervals between successive EOD's was controlled by computer according to a list of intervals created by scrambling an SPI from a female *B. brachyistius triphasic* recorded in the field. All SPI's were specified to an accuracy of 0.1 msec. The scrambled SPI was used to eliminate species-specific sequences of EOD's.

Males produced the greatest number of rasps to normal female EOD's (ϕ = 0°) and the reverse polarity (ϕ = 180°), which is normal for a fish facing in the opposite direction (Fig. 1D). Responses dropped as ϕ approached 90° and for time-reversed EOD's. We conclude that males can discriminate EOD's on the basis of phase-spectral cues alone, when power spectral and SPI cues are held constant.

To uncover a neural basis for phase sensitivity to such short pulses, we examined the responses of *Knollenorgans*, the putative electric communication receptors of mormyrids (*9*), to our synthetic stimuli. *Knollenorgans*, easily located on the skin as large unpigmented spots, can be studied noninvasively by applying stimuli through a 1-mm fire-polished capillary recording electrode through the use of a bridge circuit (WPI 707). *Knollenorgans* produce spikelike receptor potentials that fire only on rapid voltage transients in a 1:1 relationship to the spike in the afferent nerve (*10*). These receptors are broadly tuned to sinusoidal stimuli from 500 to 3000 Hz (*11*). To excite the receptor, the stimulus must go outside-negative to outside-positive (N → P), and the spike is phase-locked to the stimulus (Fig. 2A). Outside P → N stimuli do not stimulate a phase-locked response but may raise the receptor's excitability after a 1- to 2-msec delay (Fig. 2B) (*12*).

Computer-synthetic EOD's were applied to *Knollenorgans* through the bridge circuit while recording from single receptors (*13*). Fig. 2 (C and D) shows the strict phase relationship between EOD and spike responses. The female EOD evokes only one spike for the polarity shown in Fig. 2C and only one for that in Fig. 2D. The spike latency differs by 0.4 msec for the two polarities. For polarity 2D, the main N → P transition occurs between peak II and peak III of the EOD. For the reversed polarity (Fig. 2C), there are two main N → P transitions: peak I to II and peak III to baseline. Spikes are triggered only on the I-II transition, as the receptor is refractory during the III-baseline transition.

By presenting both polarities of a single waveform to a receptor, we imitated what happens in nature. The receiver senses current flowing in one side of its body and out the other, so that patches of skin on the two sides receive the two representative polarities of a signal. This dual polarity feature is probably unique to electroreception. We hypothesize a central mechanism that integrates responses from different patches of skin to yield a two-spike code for the female's signal. The interval between the two spikes is 0.4 msec for this signal.

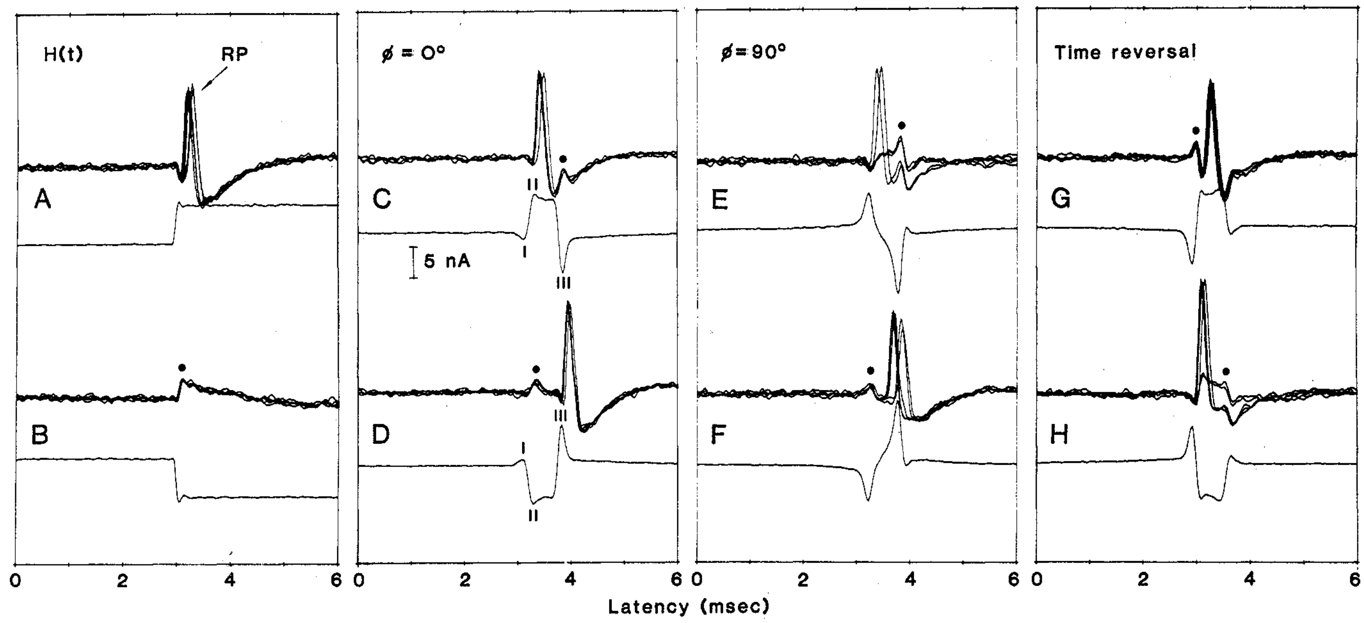

Fig. 2. Spikelike receptor potentials of a single *Knollenorgan* (upper traces) from a juvenile *B. brachyistius triphasic* to steps and EOD's (lower traces). Pairs of stimuli, of normal and reversed polarity, are shown for each waveform. Filled circles are stimulus artifact. Outside positive goes upward in stimulus traces; calibration of the stimulus is as in (C). Water resistivity was 4.1 kilohm-cm. (A) The positive-going step function, $H(t)$, generates a spike with an 0.1-msec latency. (The stimulus appears distorted because of the tape recorder used to record the experiment.) (B) On the negative-going step, no spike is generated, but many receptors fire a delayed spike after 1 to 2 msec. (C and D) Normal female EOD presented to the receptor in both polarities. In (C), the spike fires on the first (I → II) transition; in (D), it fires on the II → III transition. The difference in spike latency for (C) and (D) is 0.4 msec, a fixed interval characteristic of this EOD. (E and F) By phase-shifting the EOD 90°, the waveform now evokes spikes with more jitter, at a higher threshold. The latency between spikes in (E) and (F) differs by less than that in (C) and (D). (G and H) Time reversal of the EOD evokes spikes with a latency difference of only 0.1 msec.

Phase-shifted EOD's are coded differently from normal ones (Fig. 2, E and F). As ϕ approaches 90°, the threshold for 1:1 firing increases, jitter in spike latency increases, the latency difference between one polarity and the reverse decreases slightly, and the difference in threshold for 1:1 firing for the two polarities decreases. Time-reversed EOD's evoked spikes differing in latency by only 0.1 msec (Fig. 2, G and H). Any or all of these changes may render phase-shifted EOD's less effective during playback experiments.

We tested the behavioral significance of the 0.4-msec difference in spike latency in one additional playback experiment by presenting a solitary male with rectangular waves (*14*) of variable duration (Fig. 1E). At the receptor level, rectangles evoked a single spike on the leading edge when outside became positive; inversion of the polarity shifted the spike to the trailing edge. All stimuli were driven by the scrambled female SPI. This male produced rasps for rectangular waves of 0.4 msec but not for 0.1 msec nor for 1.6 msec. For this paradigm, he failed to discriminate an 0.4-msec rectangle from a female's EOD. We conclude that species recognition depends on a two-spike code evoked by the species-specific EOD. Additional studies indicate that EOD's of sympatric species do not elicit rasps, nor do they evoke the same neural code from *Knollenorgans* (*11*).

To have species recognition rely upon a simple code comprising only two spikes separated by the appropriate interval may be unique for electric communication, where the channel is exploited by fewer users than auditory channels. Temporal coding may be especially advantageous for the electric modality, where signal waveforms are invariant because signal conduction is instantaneous and there are no echoes. Temporal cues are also regarded as important in recognition of auditory signals, especially in invertebrates (*15*).

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6. The preserved specimens were identified as *Brienomyrus brachyistius* by L. Taverne at the Musée Royal de l' Afrique Centrale, Tervuren, Belgium, but we later distinguished four morphologically similar species on the basis of discrete differences in EOD's. The temporary name, *triphasic*, refers to the three-phase waveform of the EOD typical of both males and females.
7. Sex differences in the EOD's of electric fish were first discovered in the quasi-sinusoidal (wave) discharge of *Sternopygus macrurus*. [C. D. Hopkins, *Science* **176**, 1035 (1972)].
8. W. Heiligenberg and R. A. Altes, *Science* **199**, 1001 (1978). We used a PDP 11/34 computer. Time series analyses were performed through the use of the DEC program "Sparta."
9. Mormyrids have three receptor types for electroreception—*Knollenorgans*, mormyromasts, and ampullary receptors. *Knollenorgans* function solely in communication. They are adapted to receiving weak electric signals from other fish; they are the most sensitive class of tuberous receptors; and the responses are always phase-locked to the pulse stimulus, thereby preserving temporal information. Most important, *Knollenorgan* afferents project to the nucleus of the lateral line lobe, where interaction with a corollary discharge from the electric organ pacemaker inhibits signals to the mesencephalon [B. Zipser and M. V. L. Bennett, *J. Neurophysiol.* **39**, 713 (1976)]. Thus, the fish never "hears" its own EOD on its *Knollenorgan* system, hence its putative role in communication.
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13. The fish was gently restrained with smooth Plexiglas rods. We played EOD-like stimuli to 30 *Knollenorgans* in six *B. brachyistius triphasic* and found no response variation over the body surface.
14. The power spectrum of a rectangle wave differs from that of the female's EOD.
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16. We thank the French CNRS [Centre National de la Récherche Scientifique (France)] for generous support during our visit to the field station in Makokou, Gabon, and G. Michaloud and S. Michaloud for local logistic help. R. Lewis helped with the fieldwork, V. M. Wong drew the fish, J. Bastian and N. Viemeister helped improve the manuscript, and C. Knox thought of time-reversing the EOD. Supported by NIMH grant MH26140, NIH postdoctoral program NS06309, the National Science Foundation, the National Geographic Society, and the Graduate School of the University of Minnesota.

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Visual Claustrum: Topography and Receptive Field Properties in the Cat

Abstract. *A region containing visually responsive cells was found in the dorsocaudal claustrum. This area contains a single orderly map of the contralateral visual field. Like cortical cells, most claustral cells are selective for stimulus orientation. They are binocular, and they respond to either direction of movement and to a broad range of velocities. Their most striking property is a marked preference for very long stimuli.*

The claustrum is a narrow strip of gray matter in the forebrain of mammals, sandwiched between the putamen and the insular cortex. It is reciprocally connected with the entire neocortex, the corticoclaustral projection arising from pyramidal cells in cortical layer VI and the return pathway terminating most densely in layer IV (*1*). In the cat, a visually responsive region at the caudal end of the claustrum has been described by the use of evoked potentials (*2*). We have studied the organization of this region using standard single-unit recording techniques (*3*), with the hope of casting light on the function of the corticoclaustral loop.

Tungsten electrodes were used to record from the claustral visual area in 13 cats anesthetized with pentobarbital and paralyzed with gallamine. For the mapping of the visual field representation in the claustrum we have relied principally on results from four cats in which 43 vertical electrode penetrations were made through the visually responsive area. In the remaining animals, recordings were made primarily for the purpose of studying receptive field properties or injecting neuroanatomical tracers, but in addition these experiments provided confirmation of the mapping results.

In coronal sections (Fig. 1A, inset) the claustrum is comma-shaped, with an expanded dorsal region and a narrower, laterally curving stem. At the anterior limit of the visual region (about 4.0 mm in front of the caudal tip of the claustrum) the dorsal surface is flat and slanting, while farther back it becomes narrower and rounded. On a typical vertical electrode penetration, the cells first encountered at the dorsal surface of the claustrum had receptive fields in the far periphery of the contralateral visual field; as the electrode was advanced, receptive fields moved rapidly toward the vertical meridian, sometimes even crossing it and ending centered as much as 10° into the ipsilateral visual hemifield. In general this movement of fields toward the vertical meridian was accom-