Temporal Coding of Species Recognition Signals in an Electric Fish

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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 electrodes 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 neurophysiologic 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. 1 A)—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 (6). We therefore decided to determine whether these fish could discriminate different EOD's having electrical power spectra.

This study was conducted in the interior of Gabon, West Africa (0.34°N, 12.32°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 Brichyrhynchus brachyiustus triphasic (Fig. 1C), 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 poikiliotic electric fish (7).

During the rainy season, daily census-es 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. brachyiustus triphasic while others are solitary (presumed nonmat-

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 "ramp" (burst of EOD's at high frequency) in the presence of a more distant female. (C) Brichyrhynchus brachyiustus triphasic. (D) Playback of EOD's differing in waveform but not in power spectra to three solitary male B. brachyiustus triphasic in the field. The sequence of pulse intervals, the same for all stimuli, comes from a normal female sequence. The ordinate is the mean increase in numbers of raps produced by the resident male during the 2-min playback compared with the 2-min period before the playback. Stimuli are either normal female EOD's phase-shifted by 0° or a time-reversed EOD. N is the number of trials. Vertical bars are standard errors of the mean. The number of raps responses to 0° do not differ from that at 0° = 180° (Mann-Whitney U test, U < 54); nor does it differ from that at 0° = 45° (U = 90). Fewer responses occur to 0° than to 0° U = 24, P < .05), and fewer responses occur to time-reversed EOD's than to 0° U = 37, P < .05. (E) Playback of examples of different duration and the female EOD to one solitary male B. brachyiustus triphasic in the field. Sequence of intervals and the order 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 = 3, 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 number of raps 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 trichopsis, digitized at 100 kHz, was subjected to Fourier analysis to obtain the power and phase spectra. Phase angles were advanced by a constant angle, $\Psi$, where $\Psi = 9^\circ$, $45^\circ$, $60^\circ$, $90^\circ$, and $180^\circ$, 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 recomposed an EOD with an altered waveform. One additional stimulus, produced by inverting 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 scanning an SPI from a female B. brachyistius trichopsis 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 raps to normal female EOD’s ($6 = 0^\circ$) and the reverse polarity ($6 = 180^\circ$), which is normal for a fish facing in the opposite direction (Fig. 1D). Responses dropped as $6$ 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 nongyrids (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 701). Knollenorgans produce spike-like 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 $\rightarrow$ P), and the spike is phase-locked to the stimulus (Fig. 2A). Outside P $\rightarrow$ 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 (11). Figure 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 $N \rightarrow P$ transition occurs between peak II and peak III of the EOD. For the reversed polarity (Fig. 2C), there are two main $N \rightarrow P$ 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 II-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.
Phase-shifted EOD's are cooled 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 differ 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 of 140 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 generated by the scanned 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 rectangular wave from a female's EOD and concluded that species-specific EOD. Additional studies indicate that EOD's sympatric species do not elicit rasps, nor do they evoke the same neural code from Knollenbelaer (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.

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References and Notes
7. Separated specimens were identified as Brachydeutera hageni by J. Tavener of the Marine Biological Laboratory, Woods Hole, Massachusetts, but we have recently distinguished distinct morphologically similar species on the basis of dish pattern, antennal pattern, and mouth parts. Further work is needed to separate these species. Males, in the 2:3 phase-present, have no phase shift of their waves and female waves.
8. Differences in the EOD's of elasmobranch fishes were also found in the 2:3 phase-presentation of waves with second order multiples. In particular, the EOD's of Indi- 
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