

Electrosensory optimization to conspecific phasic signals for mating

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Abstract

Ampullary electroreceptor systems in fishes and aquatic amphibians are known to function in prey localization by the movement of the animal through a weak dc field produced by their prey. The round stingray produces an electric field with a complex geometry that is modulated rhythmically by movements of the spiracles and gill slits during ventilation. This weak stimulus is used in the field by reproductively active male stingrays to locate mates, and also by female rays to locate buried conspecifics. Electrosensory primary afferent neurons are most sensitive to stimuli that vary sinusoidally at the same frequency as the natural respiratory movements. The match between primary afferent frequency sensitivity and the ventilatory phasic signals produced by conspecifics indicates that the electrosensory system serves an important biological function in the social behavior of elasmobranchs.

Keywords: Ampullae of Lorenzini; Elasmobranch; Electroreceptor; Frequency response; Localization; Mating

The efficient detection of biologically relevant stimuli is fundamental for the central transfer of information used to mediate motor behaviors such as orientation and localization. The first level of signal processing occurs at the peripheral receptor organ and involves rejection of environmental noise via bandpass filtering [4,8,13]. In elasmobranch fishes (sharks, skates, and rays), some teleosts and aquatic amphibians the ampullary electroreceptors are an important sensory system demonstrated to function in the detection of prey [3,5,6,17]. Electric field stimuli from natural prey were characterized as predominantly static (dc) sources which stimulate the electroreceptor system as a function of the swimming path and velocity of the animal through the standing field [6,7] and are thought to explain the low frequency response properties reported for the system [1,9,14,16]. However, the general lack of opportunity to observe responses of electrosensory animals in natural populations has precluded assessment of the importance of bioelectric stimuli in social behavior. Here, we test the hypotheses that weak bioelectric stimuli are important social signals during reproduction in the stingray, and that the electrosensory system is adapted to

maximize transfer of bioelectric information modulated by conspecific ventilatory movements.

A wild population of round stingrays, *Urolophus halleri*, mates during winter months in clear shallow waters of the Sea of Cortez near Bahia Kino, Mexico, where courtship and mating among many individuals can be readily observed [15]. Each day before sunrise reproductively active female rays (distinguished by mating scars on the body and lack of claspers) move to the shallow habitat along the shoreline and bury under the sandy bottom. Solitary male rays (identified by their external claspers) actively search the area for the visually inconspicuous mates which they successfully locate via some non-visual sensory modality (Fig. 1A).

The bioelectric source in this species, which lacks an active electric organ, originates from differential standing ionic potentials at various sites on the skin and buccal epithelia [2,6] (Fig. 1B). A portion of the standing field is modulated by ventilatory movements of the dorsal spiracles and the ventrally located mouth and gill slits. Electric potentials were recorded from female rays in the laboratory and field site with Ag-AgCl electrodes, differentially amplified at DC-3 kHz bandpass, input to an A/D converter, and stored in digital files on a portable computer.

Negative dc potentials of only a few μV were recorded near the dorsal surface behind the head, fins, and tail in

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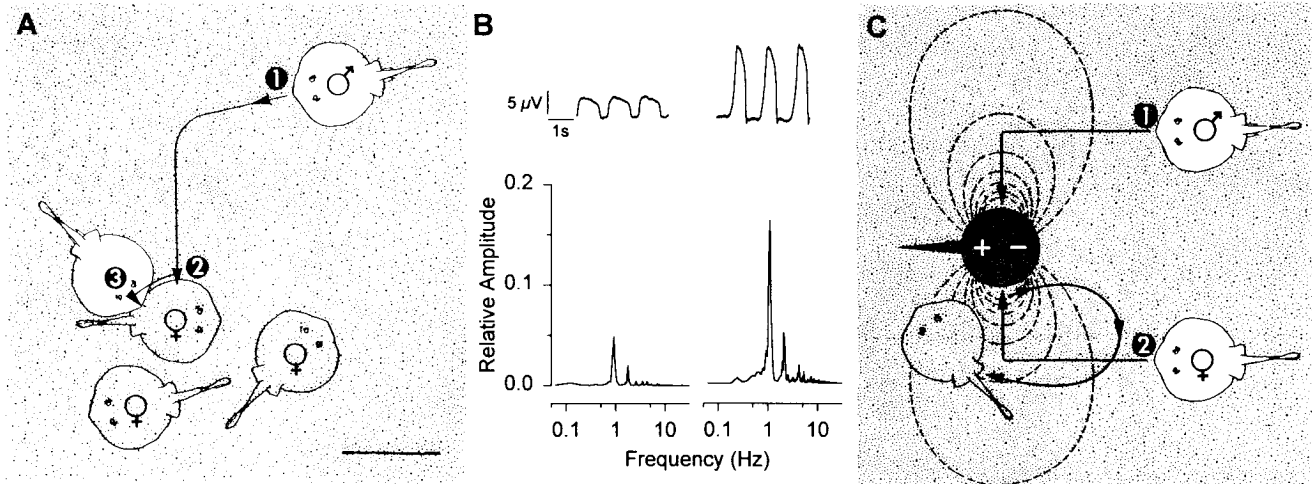


Fig. 1. Orientation responses by round stingrays (*Urolophus halleri*) to buried females and bioelectric stimuli produced by conspecifics during the mating season (January–March, 1989–1993) near Bahia Kino, Mexico. (A) Males localize, orient towards, and inspect buried females in the wild population. Search path of male (1) changes abruptly after detection of and orientation to conspecific female in the sand. Males inspect buried females with the rostrum placed over the margins of her body disc (2), pelvic fins (3), or sometimes the snout. Active courtship and copulation occur after the male excavates the buried female. Scale bar = 25 cm. (B) Female bioelectric potentials recorded on the dorsal surface above the spiracle (top, left trace) and ventral surface at the gill slits (top, right trace). The positive field near the spiracle is often fully modulated relative to a reference point far from the animal. The ventral dc field is negative, much stronger and often >50% modulated (unmodulated dc offset is not illustrated). Recorded potentials were similar for both male and female rays. Scales apply to both top traces. Bottom graphs are Fourier transforms that show the strong frequency components near 1 Hz that result from ventilatory movements. (C) Diagram of orientation responses by round stingrays to the synthesized phasic bioelectric field. Recorded digitized wave forms (e.g. B) were used to simulate the modulated electric field in playback experiments. Male stingrays (1) swim in search of females then orient, approach, and inspect the buried electric model (shown in black). Females (2) orient towards and inspect the electric dipole model but also frequently bury within a 10–100 cm radius of it. Anode (+) and cathode (–) indicate polarities of buried electric dipole model.

reproductive female *Urolophus halleri*. A small localized positive field occurs above the spiracles and is modulated in phase with the ventilatory movements (Fig. 1B, top left). In contrast, the ventral surface shows a much stronger negative peak potential, often more than ten times greater than that of the dorsal surface, and is modulated by 50% (Fig. 1B, top right). To determine the frequency spectrum of these modulated fields the potential wave forms were bandpass filtered at 0.01–100 Hz and analyzed using Fourier transformation. Both the dorsal and ventral fields were found to have a fundamental frequency component at the ventilation rate which is typically near 1 Hz (Fig. 1B, bottom curves).

We tested the hypothesis that this weak modulated bioelectric field can alone provide the primary sensory cue for mate localization. At the field study site the recorded waveform was played back from the computer through a D/A converter and isolation amplifiers (Fig. 1C). Dipole electrodes were positioned under a thin plastic stingray model to distribute the field in a quasi-natural spatial domain, and buried under the sand (cathode rostral, anode caudal) within the mating area. Playback fields were either fully or partially (25–50%) modulated with a peak current of $8 \mu\text{A}$ ([5]; A. Kalmijn, personal communication) which is a reasonable approximation to the natural field strength. Observers recorded 114 stingray orientations to the computer-controlled playback models during 16 h of playback experiments. Male rays searching

the area showed distinct orientation and approach responses to the modulated dipole field model ($n = 92$) that to observers were indistinguishable from those directed towards buried females (Fig. 1C). Female stingrays also oriented to the dipole ($n = 22$) of which one-half subsequently buried next to it. Most orientations to the dipole models occurred at a distance of <1 m from the source, indicating that electrically-mediated localization functions over a relatively short distance. The attraction of rays to the synthesized dipole field confirms that males can use electroreception alone to locate buried females and also that females can use this sense to locate buried conspecifics. Although other sensory systems may also function during mating we found no evidence that chemoreception was involved in this localization behavior (unpublished data).

We next tested the efficiency of the electrosensory periphery for detecting the phasic bioelectric stimulus by neurophysiological recordings of primary afferents in the laboratory. Standard extracellular recording techniques and electric field stimulation were used [9,14]. Resting discharge rate for each neuron at 18°C was recorded and a bipolar transcutaneous sinusoidal uniform field (0.03 – $2.0 \mu\text{V}/\text{cm}$) stimulus applied across the holding tank at frequencies of 0.01–30 Hz. A minimum of 500 spikes were recorded for each stimulus frequency, period histograms constructed off-line, and neural sensitivity (gain) calculated as the net increase in number of spikes/s per

$\mu\text{V}/\text{cm}$. Data were normalized and expressed relative to a value of 0 dB for the frequency of best response for each neuron. The regular resting discharge of electrosensory primary afferent neurons of both male and female rays is modulated by weak sinusoidal electric stimuli (Fig. 2A) with a best response at 1–2 Hz and a rapid decline in neural sensitivity at both higher and lower frequencies (Fig. 2B, upper curve). This peak response of electrosensory primary afferents is at the same frequency as the primary component of the modulated bioelectric source (Fig. 2B, lower curve). Furthermore, the direct encoding of natural phasic signals was confirmed when the bioelectric wave form recorded from wild females was used as the stimulus and its temporal features were encoded by electrosensory primary afferents (Fig. 2C).

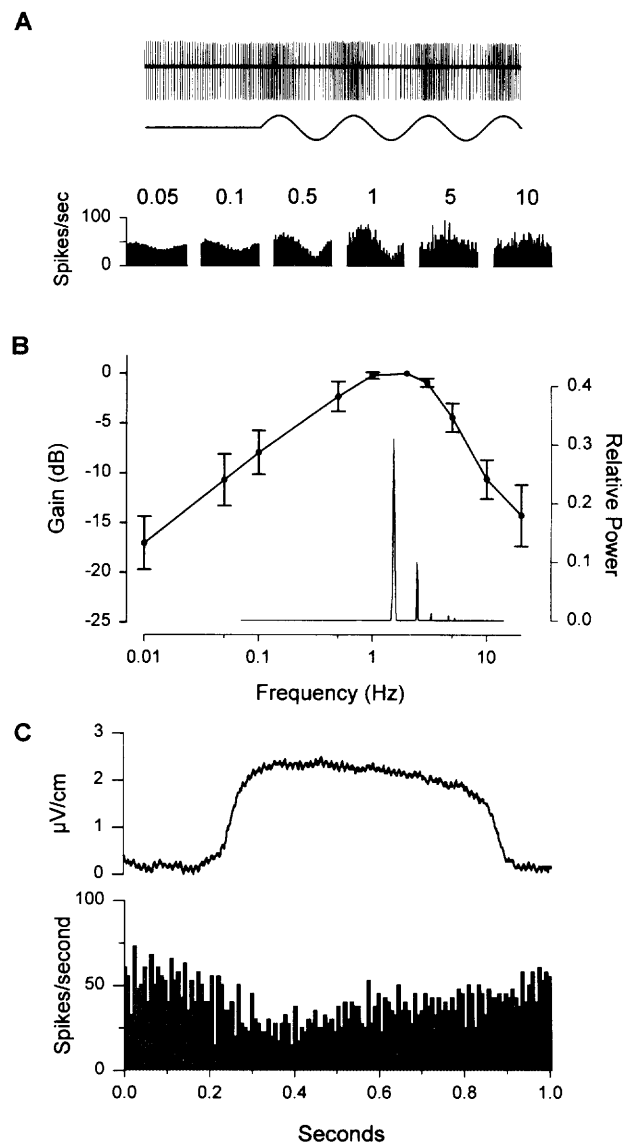
This study demonstrates that weak bioelectric fields can provide the stimulus for conspecific localization, and extends the role of elasmobranch electroreception beyond prey detection [5,17] to include social behavior. Whereas the reception of dc fields may contribute to conspecific localization as suggested for the detection of prey [7], we show that the modulated components are also an effective bioelectric cue. In order to sustain a response to a standing electric field the receptor system must move relative to it because primary afferent discharges adapt to dc stimuli within a few seconds [9,12]. Encoding of a modulated field near best frequency would not require motion of the receptor system relative to the source. The distinct overlap of the electrosensory peak and modulated ventilatory stimulus leads us to suggest that the detection and encoding of phasic bioelectric potentials plays an important function in mate localization when the animal

is at rest relative to the source, such as during inspection behavior or when animals are buried in groups. In situations where neither the source nor the receiver is in motion, modulated stimuli with frequency components near 1 Hz will be best encoded.

Ventilatory modulation of standing electric fields is the major source of sensory reafference and unwanted ‘noise’ in the electrosensory system [2,10]. As shown in this study these same signals from conspecifics form an important use of the electrosense. Since both signal and noise have the same frequency characteristics, bandpass filtering will be unable to separate them and emphasizes the importance for central mechanisms of common mode rejection and adaptive filters [11].

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Fig. 2. Match between the frequency response of electrosensory primary afferent neurons and the frequency spectrum of modulated bioelectric wave forms produced by round stingrays. (A) Primary afferent discharges are modulated by a uniform sinusoidal electric field. Top trace shows resting discharge pattern of an electrosensory primary afferent neuron followed by modulation with a 1 Hz stimulus delivered at a peak amplitude of $0.5 \mu\text{V}/\text{cm}$. Note that resting discharge rate increases during the cathodal (upward) and decreases during the anodal (downward) phases of the bipolar stimulus. Period histograms show discharge modulation for a representative primary afferent across stimulus frequencies from 0.05–10 Hz at a peak amplitude of $0.3 \mu\text{V}/\text{cm}$. (B) The response dynamics of the electrosensory primary afferents in *Urolophus halleri* show best sensitivity at approximately 1–2 Hz with a 3 dB drop at approximately 0.5 and 4 Hz. Data calculated from the period histogram analysis are plotted as the relative mean discharge peak (± 1 SD) for six neurons at ten stimulus frequencies. The dominant frequency component of the bioelectric field (e.g. 0.8 Hz, lower trace, right ordinate) matches the frequency of best sensitivity for the electrosensory system. (C) Response of an electrosensory primary afferent neuron to a waveform recorded from a conspecific. Upper trace shows a single cycle of the waveform recorded near the spiracle used to stimulate the electroreceptors, and delivered at a peak stimulus of $2.5 \mu\text{V}/\text{cm}$ peak and period of 1 s. Period histogram at bottom shows the distribution of neural discharges (transformed to instantaneous spike rate) across 50 consecutive cycles of stimulation with the wave form. Note that the unit discharge is downward modulated by the anodal current field and encodes the inverted amplitude of the waveform.



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