

An Electric Sense in Crayfish?

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*A variety of aquatic vertebrates, including teleost and non-teleost fish, amphibians, and monotreme mammals, are sensitive to low-frequency electric signals with thresholds of low nanovolts per centimeter to high microvolts per centimeter, have specialized detectors for these signals, and use electroreception to locate food or orient in their environment (1–9). Although invertebrates would similarly benefit from an electric sense (10), none are known to use it. A recent report concluded that the freshwater crayfish species *Cherax destructor* responds to electric fields (11). Some years ago, we investigated whether another species of freshwater crayfish, *Procambarus clarkii*, has an electric sense and uses it to find prey. Weak fields were produced across water-bridge electrodes spaced 1–5 cm apart, and exploratory feeding behaviors such as touching, grabbing, and tugging at the electrodes using the claws, legs, and mouthparts were examined. Our results show that *P. clarkii* responds to fields with intensities of 20 mV/cm and greater. We also recorded from sensory neurons in *P. clarkii* legs and claws and found that the only field-sensitive cells had similarly high thresholds and were also responsive to chemical and mechanical stimuli. We conclude that *P. clarkii* does not have a high-sensitivity, specialized electric sense used in locating food.*

Male and female freshwater crayfish (*Procambarus clarkii*), 55–80 mm total length, were shipped from Atchafalaya Biological Supply Co. (Louisiana), kept in our laboratory, and fed shrimp pellets. A week before behavioral assays, animals were isolated without food in holding aquaria under a photoperiod of 12 h light to 12 h dark. We assumed that if *P. clarkii* has an electric sense, these crayfish would use

it in a context in which many electroreceptive animals normally use it—to identify prey animals on the basis of small, weak dipole fields generated by those prey species. Thus, we examined the feeding behavior of the crayfish when we presented such fields. Animals were tested in a 40- × 40- × 20-cm aquarium (Fig. 1A). Dipole electric fields were generated by applying DC or sinusoidal voltages across a pair of water-bridge electrodes. Field strength and direction were measured with a pair of electrodes held a fixed distance apart and moved and rotated to determine field strength and orientation around the dipole source (Fig. 1B; Supplemental Fig. 1, <http://www.biolbull.org/supplemental/>). Animals were tested in the dark phase of the light:dark cycle under dim red light, to which they are minimally sensitive. Each animal was tested twice, first in the absence of an electric field (control) and then a few days later in the presence of an electric field. At the beginning of a trial, the animal was secured in a shelter located 3 cm from the electrodes. After 45 min, the shelter door was opened and the behavior of the animal was videotaped for 2 h.

Behaviors elicited close to the dipole source (*i.e.*, within 0.5 cm of the electrodes spaced 1 cm apart or within 2.5 cm of the electrodes spaced 5 cm apart) were quantified by an observer unaware of the experimental treatment. Behaviors analyzed were exploratory and appetitive feeding behaviors typical of crustaceans (12), including the following: (1) *Pass-by*: crayfish passed close to the electrodes with its first two pairs of legs or claws. (2) *Touch*: crayfish repeatedly touched the electrodes with its first pair of legs or kept its first pair of legs in contact with the electrodes. (3) *Active behavior on the electrodes with claws down*: crayfish touched the electrodes and brought one or both claws down close to them. (4) *Tug*: crayfish tugged the electrodes with its mouthparts. (5) *Grab*: Crayfish grabbed the electrodes with one or both claws. The cumulative duration of all four “active” behaviors in proximity to the electrodes (*i.e.*, touches, claws down, tugs, and grabs) was also used as a

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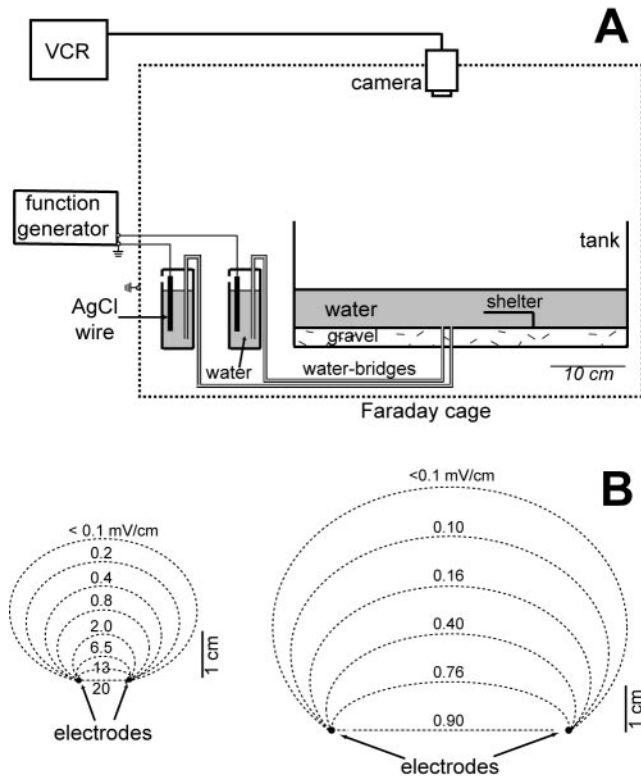


Figure 1. Experimental setup. (A) Apparatus for generating electric fields and testing behavior. The aquarium was constructed of darkened acrylic plastic. The bottom was covered with a 2-cm-deep layer of black gravel. Animals were videotaped from above. Electric fields were generated using a pair of water-bridge electrodes (ethanol-soaked Nalgene tubes, outside diameter 3.2 mm, inside diameter 1 mm) to prevent any effects of electrolysis. The water-bridge electrodes were located in the center of the aquarium, 1 cm or 5 cm apart from each other, ends flush with the top of the gravel. The water-filled electrodes extended from the aquarium to a 500-ml beaker of water containing a coil of Ag/AgCl wire. Electric fields were produced using a function generator to pass DC or sine wave currents through the Ag/AgCl wire and water-bridge electrodes (resistance $8.0 \pm 1.2 \text{ M}\Omega$). Water in the aquarium and in the water-bridge electrodes was regularly changed and had a resistivity of $8.6 \pm 1.2 \text{ k}\Omega \cdot \text{cm}$. The test aquarium and the water-bridge electrodes were surrounded by a Faraday cage to minimize 60-Hz noise. (B) Measurement of electric fields. Voltage produced by a function generator was adjusted to generate field intensities between $10 \mu\text{V/cm}$ and 100 mV/cm as measured along the median axis between the two water-bridge electrodes. For each field intensity, the total current flowing through the water-bridge electrodes was also calculated on the basis of voltage applied at the function generator and the resistivity of the water-bridge electrodes (Supplemental Fig. 1, <http://www.biolbull.org/supplemental/>). The electric fields produced in the aquarium were measured using a pair of Teflon-coated Ag/AgCl wires (tip of $0.3 \times 0.3 \times 0.3 \text{ mm}$) connected to a differential amplifier and oscilloscope. The electrodes were mounted on a rotating cylinder and secured to a micromanipulator that provided precise positioning and orientation of the electrodes in the field. The two measuring electrodes were positioned 0.5 cm apart from each other, exactly between the two water-bridge electrodes about 1 mm above the gravel surface. Fields were also measured by positioning the measuring electrodes at various distances from the median axis between the water-bridge electrodes and by orientating them to achieve the maximum field intensity. The field strength dropped off quickly, within a few centimeters of the median axis, especially when the water-bridge electrodes were 1 cm apart. To estimate the current density, measurements were also taken close to the water-bridge electrodes where all currents converge. For this purpose, the measured elec-

measure of responsiveness. For each animal, the number of each of these behaviors that occurred in the electric field versus in the control was compared pairwise using Wilcoxon matched pairs tests.

Our results for dipole electric fields produced by electrodes 1 cm apart showed that *P. clarkii* responded only to high-intensity fields (20 mV/cm or greater). For DC, 4-Hz, 10-Hz, and 100-Hz fields of 20 mV/cm , the animals spent significantly more time producing active behaviors than in control experiments with no fields (Table 1; Supplemental Fig. 2, <http://www.biolbull.org/supplemental/>). Crayfish did not orientate within these fields. Rather, they showed interest in an electrode when a leg contacted or was within a few millimeters of it. They did not prefer one electrode over the other, even for a DC field. Crayfish tended to respond most intensely to lower frequency sinusoid fields. The numbers of touches and active behaviors with the claws down were significantly higher for 4-, 10-, and 100-Hz fields than in the controls (Table 1; Supplemental Figs. 3 and 4, <http://www.biolbull.org/supplemental/>), and the numbers of tugs and grabs were significantly higher than controls for DC and 4-Hz fields (Table 1; Supplemental Fig. 5, <http://www.biolbull.org/supplemental/>). Overall, 4-Hz fields elicited the strongest responses. The behaviors involving tugs or grabs lasted significantly longer within fields of 20 mV/cm ($20.6 \pm 24.5 \text{ s}$ per behavior) than those involving touches and claws down ($11.0 \pm 9.4 \text{ s}$ per behavior) or touches ($3.3 \pm 5.0 \text{ s}$ per behavior) (Table 2). The duration of tugs and grabs was also significantly longer within fields of 20 mV/cm ($20.6 \pm 24.5 \text{ s}$ per behavior) than in the respective controls ($6.5 \pm 5.3 \text{ s}$ per behavior) (Table 2), whereas the duration of active behaviors involving touches or claws down was not significantly different in field-on versus control experiments (Table 2). These results suggest that grabs and tugs constitute the most significant and strongest behaviors related to the fields.

Once crayfish left the shelter and began walking through the aquarium, they tended to show active behaviors, particularly touches, sooner when the field was on than when it was off (Table 2). However, none of the active behaviors evoked by electric fields, including tugs and grabs, systematically occurred during the first encounters with the electrodes (Table 2). Some animals first responded to the field in the 19th encounter with the field. The number of encounters

trodes were 0.2 cm apart and positioned at various locations and orientations around the water-bridge electrodes (Supplemental Fig. 1, <http://www.biolbull.org/supplemental/>). These measurements showed that for electrodes 1 cm apart, the total current flowing through the source electrodes was (mean \pm SD) $0.14 \pm 0.02 \mu\text{A}$ for the 1-mV/cm field, $1.29 \pm 0.18 \mu\text{A}$ for the 10-mV/cm field, and $2.89 \pm 0.55 \mu\text{A}$ for the 20-mV/cm field. For electrodes 5 cm apart, the total current was $2.6 \mu\text{A}$.

Table 1

Summary of behavioral responses of crayfish in electric fields vs. their respective controls

	Field Frequency and Strength (mV/cm)												
	DC*		4 Hz*			10 Hz*			100 Hz*			1000 Hz*	4 Hz†
	10	20	1	10	20	1	10	20	1	10	20	20	1
# of animals (<i>n</i>)	13	12	16	14	11	11	13	15	12	9	13	14	10
Duration of active behaviors	0.41	0.05	0.33	0.04	0.01	0.17	0.89	0.02	1.00	0.78	0.02	0.11	0.39
# of touches	0.84	0.69	0.30	0.10	0.03	0.29	0.76	0.05	0.29	0.76	0.05	0.06	0.86
# of active behaviors w/claws down	0.29	0.09	0.14	0.20	0.03	0.07	0.80	0.03	0.07	0.80	0.03	1.00	0.04
# of tugs and grabs	0.25	0.03	0.59	0.72	0.02	—	0.60	0.20	—	0.60	0.20	—	0.20

Values are the probability that crayfish responded similarly in electric field vs. control (*i.e.*, no field). Results are presented for four behaviors: (1) cumulative duration of active behaviors at the electrodes; (2) number of touches; (3) number of active behaviors with claws down; (4) number of tugs and grabs. (For a more complete presentation of the data, see Supplemental Figs. 2–5.) Probabilities in **bold** indicate that crayfish responded with **higher intensity** in the field than in the control, and probabilities in *italics* indicate that crayfish responded with **lower intensity** in the field than in the control (Wilcoxon matched pairs test, $P < 0.05$). Number of animals tested (*n*) is shown in the top row.

* Distance between electrodes is 1 cm.

† Distance between electrodes is 5 cm.

with electrodes before first responding did not differ between field-on and control experiments.

A group of crayfish was also tested in an aquarium in which the dipole source electrodes were 5 cm apart and produced a field of about 1 mV/cm between the electrodes (Fig. 1B). This field, even at 4 Hz, did not elicit any significant active behaviors (Supplemental Fig. 6, <http://www.biolbull.org/supplemental/>).

We expect that if crayfish have an electric sense, then they will have specialized and sensitive sensory cells that detect electric fields and no other sensory modalities, as is

the case for all other animals with an electric sense (1–6, 10) and for the vast majority of animals with other sensors. Consequently, we examined the physiological responses of sensory neurons in the legs and claws of *P. clarkii*, using a preparation that allows single-unit recordings (13). Legs and claws are the appendages closest to the electric fields when animals encountered the fields, and they were directly involved in the behavioral responses that we observed. While recording single-unit activity, the sensory portion of the preparation was stimulated with chemicals such as food odors; mechanical stimuli such as touch and changing water

Table 2

Summary of behavioral responses of crayfish in 20-m V/cm electric fields vs. controls (no field)

	Touches		Claws down		Tugs and Grabs	
	No field	Field	No field	Field	No field	Field
Duration of behaviors (s)						
# of behaviors (<i>n</i>)	76	111	7	22	4	36
Mean ± Std. Dev.	3.0 ± 2.9	3.3 ± 5.0	8.4 ± 8.2	11.0 ± 9.4	6.5 ± 5.3	20.6 ± 24.5
Median (Min—Max)	2 (1–18)	2 (1–36)	6 (2–26)	8 (2–37)	6 (2–12)	15 (2–136)
# encounters w/electrode before 1 st active behavior						
# of behaviors (<i>n</i>)	42	50	6	15	4	25
Mean ± Std. Dev.	6.0 ± 4.9	4.5 ± 3.8	9.2 ± 6.4	6.1 ± 3.2	4.3 ± 3.9	16 ± 4.9
Median (Min—Max)	5 (1–22)	3 (1–19)	7 (2–19)	6 (1–11)	3 (1–10)	5 (1–19)
Time out of cage before 1 st active behavior (s)						
# of behaviors (<i>n</i>)	42	50	6	15	4	25
Mean ± Std. Dev.	2637 ± 2153	1360 ± 1465	4035 ± 2446	2682 ± 1865	2467 ± 2911	2230 ± 1804
Median (Min—Max)	3301 (1–6895)	883 (1–6405)	4522 (150–7013)	2820 (1–6455)	1637 (1–6592)	2335 (1–6528)

The data were pooled from all fields of 20 mV/cm, *i.e.*, DC and 4, 10, 100, and 1000 Hz. Columns with **bold values** indicate that the behavior is significantly different in the electric field vs. the control (no field) (Wilcoxon matched pairs test, $P < 0.05$).

flow; or electric fields. Despite searching in many preparations, we did not find sensory neurons specifically and highly responsive to electric fields, but we did find many examples of chemo- or mechanosensory neurons that were also activated by electric fields of the intensity that stimulated behavioral responses in our experiments. We conclude that stimulation of these chemo- and mechanosensory neurons by strong electric fields is the likely basis for the behaviors that we observed.

In a recent paper, Patullo and Macmillan (11) concluded that another freshwater crayfish, *Cherax destructor*, responds to electric fields. Their study differed from ours in a number of ways, including the types of electric fields generated and the types of behaviors analyzed. We designed our study to examine whether *P. clarkii* detects and responds with typical feeding behaviors to electric fields that mimic those produced by their intact or wounded prey. Such signals are typically a DC signal with a superimposed low-frequency component often related to biological rhythms such as breathing and moving (5–9). These fields are almost always less than 1 mV/cm at the animal's surface (5–9). Furthermore, field strength decreases quickly with distance from the source, even in freshwater where that medium's higher resistivity can result in stronger fields more distant from the source, as we show in Figure 1B. Signals from animate objects are typically 10–100 $\mu\text{V}/\text{cm}$ within 1 cm of animate sources (5–9). Thus, in our experiments with *P. clarkii*, we created small (1–5 cm) dipole fields near the substrate surface and examined the feeding behavior of the crayfish because, if they use an electric sense, we expect that they will use it to detect and locate prey as is typical of passive electroreceptive animals. In contrast, most of the experiments by Patullo and Macmillan on *C. destructor* used two 13.5- × 10-cm stainless steel plate electrodes placed 14–15 cm apart to generate uniform DC fields at one end of an aquarium, with the intention of mimicking large fields generated at the physicochemical boundaries of large inanimate objects. Inanimate objects in natural environments can generate electric fields—for example where current flows between objects differing in chemical composition, concentration, or temperature (5)—creating an electrical landscape that can be used by animals in orienting in their environment (1, 5–8). Such fields are reported to be of strengths up to 0.3 mV/cm, but are typically much less (5, 8). Patullo and Macmillan (11) reported that the stimuli they produced had current densities of 0.2 to 0.4 $\mu\text{A}/\text{cm}^2$ in water with conductivity of 55–65 $\mu\text{S}/\text{cm}$, which equates to field intensities of about 3 to 7 mV/cm. The behaviors that they analyzed were time spent in a prolonged DC electric field or a transient change in movement during presentation of a brief (~1.3-s) field. For example, they reported that during a 5-min trial, animals spent more time at the end of the aquarium with the electric field. They also reported that animals that had been motionless when the field was off subsequently increased the duration of movement

of their claws, antennae, or legs during presentation of the brief field. They concluded that *C. destructor* responds to DC fields of about 3 to 7 mV/cm and may be attracted to them. They also produced 14–15-cm fields across their large plate electrodes; these fields had waveforms aimed to mimic the muscle potentials of animals and of about 7 mV/cm, and they reportedly evoked responses. Finally, the authors reported producing a signal of about 1.3 s, through dipole electrodes spaced 2.5 cm apart and 4 cm above the bottom of the aquarium, to animals about 5 cm distant. In response, animals that were walking showed a decline in how long they moved their legs, chelae, and body. The strength of the field about 5 cm from the electrodes was not reported, so the field strength that the animals experienced cannot be determined.

Our study and that of Patullo and Macmillan (11) demonstrate that two species of freshwater crayfish can detect and respond to DC and low-frequency electric fields. The behavioral thresholds reported in the two studies are within an order of magnitude (~20 mV/cm for *P. clarkii* and ~3–7 mV/cm for *C. destructor*) and thus, in our view, are relatively similar. Reported differences in behavioral thresholds might result from differences in species, behaviors analyzed, field shape, and other methodological details. In any case, these behavioral thresholds are extremely high compared to typical fields of animate and inanimate objects in their natural environment and compared to the thresholds of animals known to have an electric sense. Furthermore, our electrophysiological study failed to identify specialized electroreceptors, suggesting that the ability of *P. clarkii* to detect and respond to these strong electric fields is not based on a specialized electric sense. We conclude from our work that *P. clarkii* does not use electroreception in locating or identifying its prey in its natural environment. We believe that it remains to be shown that any crayfish species, or for that matter any invertebrate, has the electrosensitivity required to respond in adaptive ways to electric fields of the types that they would encounter in their natural environments.

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