

PADDFISH STRIKE AT ARTIFICIAL DIPOLES SIMULATING THE WEAK ELECTRIC FIELDS OF PLANKTONIC PREY

WINFRIED WOJTENEK, XING PEI* AND LON A. WILKENS‡

Center for Neurodynamics and Department of Biology, University of Missouri-St Louis, 8001 Natural Bridge, St Louis, MO 63121-4499, USA

*Present address: 300 Rolling Oaks, Apartment 145, Thousand Oaks, CA 91361, USA

‡Author for correspondence (e-mail: lon_wilkens@umsl.edu)

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Summary

The freshwater paddlefish *Polyodon spathula* (Polyodontidae) feeds primarily on the water flea (*Daphnia* sp.), and previous studies suggest that these fish detect their planktonic prey using their rostral electrosensory system. Zooplankton produce direct-current and oscillating alternating-current electric fields containing multiple frequencies and amplitudes. We asked whether an inanimate electric field is sufficient to elicit paddlefish strikes equivalent to their feeding behavior.

Juvenile paddlefish respond to artificial dipole stimuli by investigating the electric field and striking at the dipole electrode tips. These behavioral responses, scored as strikes, exhibit a bandpass characteristic with a maximum response between 5 and 15 Hz. Responses were less frequent at higher (20, 30, 40, 50 Hz) and lower (0.1, 0.5, 1 Hz) test frequencies, with a steep drop-off below 5 Hz. Strike rates also varied with stimulus intensity. Response frequency was greatest at 0.25 μ A peak-to-peak amplitude,

with reduced responses at lower and higher amplitudes (0.125 and 1.25 μ A). Striking behavior was also influenced by water conductivity: strike rate was reduced at higher water conductivity. Dipole-elicited strikes exhibit behavioral plasticity. Fish habituate to repetitive dipole stimuli that are not reinforced by prey capture, and they dishabituate after food reinforcement. These experiments characterize paddlefish feeding strikes towards dipole electrodes at signal frequencies and intensities simulating the electric fields of zooplankton, their natural prey, and demonstrate that electric fields are sufficient to elicit feeding behavior. The results support the conclusion that paddlefish use their passive electrosensory system for planktivorous feeding.

Key words: paddlefish, *Polyodon spathula*, electrosensory feeding, ampulla of Lorenzini, habituation, mimic natural stimuli, plankton, novelty.

Introduction

The freshwater paddlefish *Polyodon spathula* is an ancient chondrosteian fish found throughout the Mississippi River basin. An outstanding feature of the paddlefish is its elongated rostrum, which is covered by clusters of ampullae of Lorenzini (Jørgensen et al., 1972). Ampullae of Lorenzini are known primarily as electroreceptors, but they also respond to mechanical stimuli and to changes in temperature and salinity (Murray, 1967).

Of additional interest, paddlefish are plankton feeders, as shown by stomach content analyses (Rosen and Hales, 1981; Michaletz et al., 1982), and large fish strain suspended zooplankton from the water in enormous quantities. However, juvenile paddlefish feed selectively, capturing zooplankton individually, primarily the slow-moving water flea *Daphnia* sp. (Michaletz et al., 1982). During swimming, plankton that pass within a few centimeters of the rostrum trigger an abrupt change in the swimming path, after which the fish opens its mouth wide and gulps in the small prey. This behavior has been studied extensively using infrared illumination and other

procedures to exclude visual, chemical and hydrodynamic means of prey detection (Wilkens et al., 1997; Russell et al., 1999; Wilkens et al., 2001). These results suggest that paddlefish detect their planktonic prey electrically using the ampullary electroreceptor system of the rostrum. In the present study, we test the hypothesis that pure electric fields elicit feeding strikes by paddlefish. Specifically, we examine how the properties of the electric stimulus (frequency, amplitude, water conductivity) and stimulus repetition influence striking responses to electric dipole sources.

Materials and methods

Juvenile paddlefish (*Polyodon spathula* Walbaum) 10–12 cm in length were obtained from the Blind Pony Fish Hatchery (Missouri Department of Conservation). Fish were maintained in large holding tanks of 12001 and 6001 capacity. Water temperature in the aquarium room was kept between 21 and 26 °C, and lighting was provided on a 14 h:10 h light:dark

cycle. Water conductivity in the holding tanks was 4 mS cm^{-2} . Fish were fed pelleted fish food (Nelson, Silver Cup), frozen bloodworms and live brineshrimp.

Videotaped experiments were performed either in small aquaria (100 or 400 l) containing four fish or in the large holding tank (1200 l) using 13 fish. Experiments were performed at conductivities of $390\text{--}631 \mu\text{S cm}^{-2}$ in the aquaria and at 4 mS cm^{-2} in the large tank, both at a temperature of $21\text{--}23^\circ\text{C}$. For experiments at low conductivity, fish were transferred from the holding tanks to the experimental tank 4–6 h prior to the recordings to allow them to acclimate to the experimental environment. All experiments were performed at night to minimize disturbances, starting between 18:00 and 20:00 h and lasting 9–12 h. Stimuli were delivered according to the protocol given below. Fish behavior was videotaped under infrared illumination (880 nm) with an infrared-sensitive CCD camera (Cohu Solid State or Hitachi KP 160 CCD cameras) using a time-lapse video recorder (Toshiba, KV-6110A) set to extended long-play mode.

To elicit electrically mediated responses, stimuli were delivered through a pair of Ag/AgCl dipole electrodes separated at the tips by 10 mm. Each electrode (0.25 mm diameter silver wire Teflon-coated to within 1 mm of the tips) was connected in series with a $20 \text{ M}\Omega$ resistor and $1 \mu\text{F}$ capacitor and was placed 15 cm away from the wall of the tank, 5–10 cm below the water surface. Stimuli were generated under computer control using a function generator (Global Specialties, synthesized function generator, model 2003) set for zero offset. Stimuli were constant-current since the series resistors greatly exceeded the resistivity of the water and were effectively isolated from ground by the capacitors.

Blocks of stimuli at frequencies of 0.1, 0.5, 1, 5, 10, 15, 20, 30, 40 and 50 Hz and a 0 Hz control were delivered continuously, with the stimulus sequence in each block randomly ordered, to investigate whether the striking response was influenced by stimulus repetition. Stimulus duration at each frequency was 120 s, with a rest interval of either 5 or 126 s between stimuli. Thus, depending on the duration of the rest interval, 22–30 stimulus blocks (5 s delay between stimuli) or 12 blocks (126 s delay) were delivered to the dipole during a nightlong experiment. Stimulus intensity was presented at 0.125, 0.25 and $1.25 \mu\text{A}$ peak-to-peak (p-p) amplitude.

Responses to dipole stimuli were examined for two groups of fish. Experiments performed in the small tanks were based on a cohort of juvenile fish less than 1 year old (14–27 cm total length) with four fish in each experiment. Experiments performed in the large tank used fish from a cohort of 13 1-year-old paddlefish (24–45 cm total length). The behavioral response was characterized as a strike at the dipole electrodes or as an avoidance. If a fish struck more than once during a single approach, we regarded this as a single strike event. Avoidance was coded for fish whose swimming path approached the electrodes but abruptly turned away.

The electric fields of the planktonic water flea (*Daphnia* sp.) were measured with drift-free Ag/AgCl electrodes embedded in agar-filled pipette tips. Plankton were cemented to a fine

monofilament line and lever system and advanced by micromanipulator so that the carapace surface was adjacent to the active electrode (see also Wilkens et al., 1997; Wojtenek et al., 2001). The reference electrode was located at the side of the chamber. Oscillating potentials were recorded with the plankton held stationary.

Statistical analyses

To determine whether the occurrence of striking responses towards an oscillating dipole depended on stimulus frequency, we pooled the data for seven experiments, each representing the behavior of four initially naive fish. We used a nonparametric analysis of variance (PROC GLM on the ranks of strike rate; SAS Institute Inc., 1998), with the dependent variable as strike rate and the independent variables stimulus frequency and stimulus block. Strike rate refers to how often any of the four paddlefish struck at the stimulus. All fish were similar in size, and single strikes could not, therefore, be associated with an individual fish. Because the data are unbalanced, the results are given as sum of squares Type III.

The effects of stimulus amplitude on striking activity were analyzed with a nonparametric two-way analysis of variance (ANOVA). The dependent variable was strike rate and the independent variables were stimulus amplitude, stimulus frequency and stimulus block (PROC GLM on the ranks of strike rate; SAS Institute Inc., 1998). Thirteen fish were used for experiments studying the effects of stimulus amplitude.

Paddlefish swimming was also analyzed to determine whether the fish were attracted into the vicinity of the electrodes, a space defined as an 18 cm diameter cylinder centered on the electrodes. Incursions relative to stimulation frequency and intertrial interval were analyzed by a nonparametric analysis of variance (PROC NPAR1WAY ANOVA, Kruskal–Wallis; SAS Institute Inc., 1998), with stimulus frequency as the independent variable. Incursion frequencies during stimulation (120 s) and during intertrial intervals (126 s) were compared (PROC NPAR1WAY; Wilcoxon). By noting whether paddlefish entered the 18 cm diameter stimulus zone, strike probability could be determined as the number of strikes per incursion. Strike probability was analyzed according to ranks using ANOVA (PROC GLM).

To test the effects of water conductivity, a Kolmogorov–Smirnov two-sample test was applied to compare striking activity in low-conductivity water with that in high-conductivity water (PROC NPAR1WAY; Kolmogorov–Smirnov).

To examine changes in striking activity in response to repetitive stimulation (measured as strikes per successive stimulus blocks), we applied regression analysis to the results of 19 experiments (PROC REG; SAS Institute Inc., 1998). The dependent variable was the number of strikes per stimulus block, with the independent variable as the number of blocks. Where brineshrimp were used, we pooled the data separately for strikes before and after adding *Artemia salina* and compared strike rates using the Wilcoxon sign rank test (PROC UNIVARIATE; SAS Institute Inc., 1998).

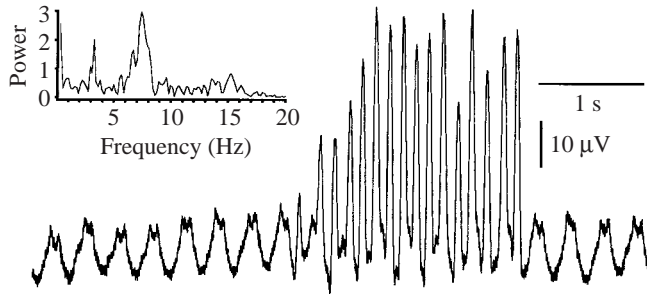


Fig. 1. Electric field oscillations of the water flea (*Daphnia* sp.) recorded with a monopolar electrode at the surface of the carapace relative to a distant reference electrode. Amplitude modulations include both small (approximately $10\ \mu\text{V}$ peak-to-peak) and large oscillations that correspond, respectively, to thoracic and antennal feeding and swimming movements. The inset shows the power spectrum (in arbitrary units) of the electric field oscillations with maxima at 3 and 7 Hz. Water temperature was $23\ ^\circ\text{C}$ and conductivity $400\ \mu\text{S cm}^{-2}$. The direct-current component of the electric field is not shown.

Results

As planktivorous feeders, paddlefish are thought to rely on the detection of the electric fields of their prey for capture. Planktonic prey, here the water flea *Daphnia* sp., produce weak electric fields with both direct-current (d.c.) and alternating-current (a.c.) components. The direct-current component can be characterized as an electric dipole of up to 1 mV measured at the surface of the carapace (Wilkens et al., 1997; Wojtenek et al., 2001). As reported previously (Wilkens et al., 1997), alternating-current oscillations correspond to the muscle field potentials of the antennae, thoracic appendages and abdomen. As shown here (Fig. 1), oscillation frequencies are low, ranging from 2 to 15 Hz. The power spectrum (Fig. 1 inset) shows peaks at 3 and 7 Hz. To simulate the natural field potentials of *Daphnia* sp., we introduced sinusoidal electric fields (see Materials and methods) with a frequency range bracketing those of *Daphnia* sp. Actual signals from the

plankton will have additional dynamic components caused by the motion of the fish relative to the stationary plankton. The paddlefish is a ram ventilator (Burggren and Bemis, 1982) and is, therefore, in continuous swimming motion (at approximately $10\ \text{cm s}^{-1}$). Thus, a stationary dipole stimulus source in the tank with a swimming fish effectively mimics the signals of the relatively stationary planktonic prey.

Here, we report the results of 25 experiments examining the strike behavior of paddlefish to electric signals simulating their planktonic prey. A feeding response or strike is elicited when a paddlefish detects an appropriate stimulus signal. This electrical ‘feeding’ response is equivalent to the feeding strike analyzed extensively in the capture of live planktonic prey (Wilkens et al., 1997; Russell et al., 1999; Wilkens et al., 2001). During an approach, as the rostrum nears the dipole electrodes, the fish will turn abruptly towards the stimulus source, open its mouth wide and strike at the dipole wires as if gulping planktonic prey (Fig. 2). The dipole strike response depends on a number of factors. Important variables are the oscillation frequency, the stimulus amplitude, the water conductivity and the repetition of stimulus presentations.

Feeding strikes at the artificial electrical field depend on stimulus frequency, occurring primarily in response to sinusoidal frequencies in the range 5–15 Hz (Fig. 3). There is a graded decrease in strike rate from peak to baseline at higher frequencies, whereas strike rate falls abruptly between 5 and 1 Hz. Nonparametric analysis of variance shows that strike rates in response to dipole stimuli vary significantly with stimulus frequency ($P < 0.0001$), and there was no significant interaction between stimulus frequency and block of stimulation. Duncan’s multiple-range test showed that strike responses in the 5, 10 and 15 Hz group differed significantly from those for all other stimulus frequencies ($P = 0.05$). Thus, paddlefish responses to artificial stimuli fall into the range of frequencies exhibited by their planktonic prey.

In one experiment, the strike response was analyzed in terms of strike probability ($N = 148$ strikes). Whether or not paddlefish swam near the dipole electrodes, i.e. within the 18 cm diameter

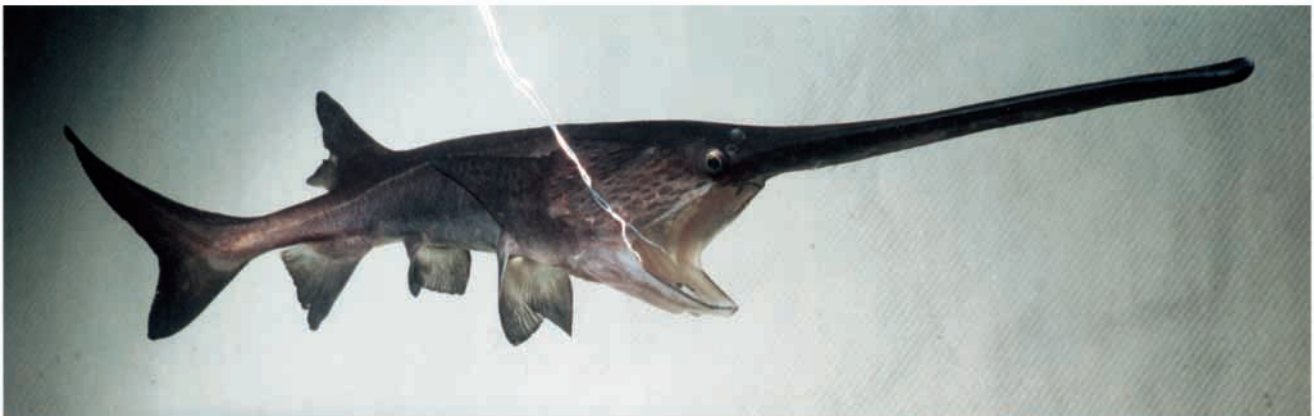


Fig. 2. A typical paddlefish strike at the dipole electrodes ending at the corner of the mouth. The twisted leads can be seen extending obliquely across the operculum of the fish. Strikes at artificial electric fields are comparable with paddlefish strike captures of their natural planktonic prey. This fish is 18 cm, total length.

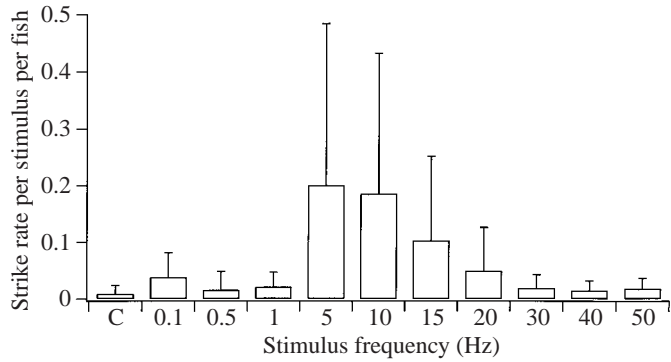


Fig. 3. Paddlefish feeding strikes elicited by sinusoidal electrical signals. Strike preference is for stimulus frequencies of 5, 10 and 15 Hz. The results are based on seven experiments, each with four paddlefish in the tank. Values are means + S.E.M. Stimulus amplitude was 0.25 μ A. Note that the x-axis is not linear. C, control in the absence of electrical signals.

criterion zone (filled columns, Fig. 4A), was independent of stimulus frequency (Kruskal–Wallis test, $\chi^2_{10}=9.2099$, $P<0.51$). Similarly, the number of incursions into the dipole vicinity did not change during the 126 s non-stimulus intervals (open columns, Fig. 4A) (Kruskal–Wallis, $\chi^2_{10}=7.1557$, $P<0.7107$). Finally, the relative number of incursions during and after stimulation was not significantly different for the two groups (Wilcoxon two-sample test, $Z=1.53037$, $P<0.1259$). Thus, paddlefish encounter the dipole stimulus on a random basis. However, strike probability, based on how often the paddlefish were within the criterion distance from the dipole electrodes, depended significantly on stimulus frequency ($F_{11,252}=45.61$, $P<0.0001$). Duncan’s multiple-range test generated a peak group for frequencies of 5, 10 and 15 Hz ($P=0.05$) (Fig. 4B).

In addition to frequency, striking behavior also varies with the strength of the electric field (Fig. 5). In experiments performed at a conductivity of 4 mS cm⁻², using 13 fish in a large tank, the highest strike rate occurred at the intermediate stimulus intensity (0.25 μ A p-p). Both lower and higher stimulus intensities (0.125 and 1.25 μ A p-p) resulted in a lower

Fig. 5. Intensity-dependence of strike activity. The intermediate stimulus amplitude (0.25 μ A) elicited the most strikes. Low (0.125 μ A) and high (1.25 μ A) stimulus amplitudes elicited fewer strikes. Experiments were performed with 13 fish at a water conductivity of 4 mS cm⁻²; two experiments for each amplitude. C, control in the absence of electrical signals.

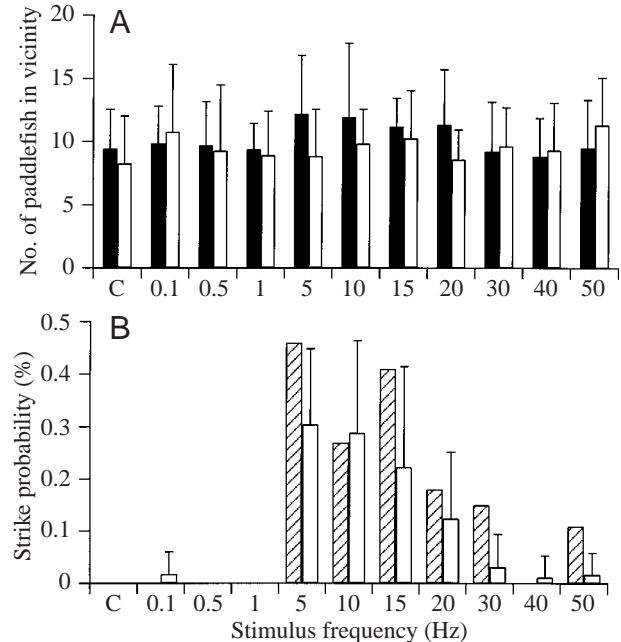
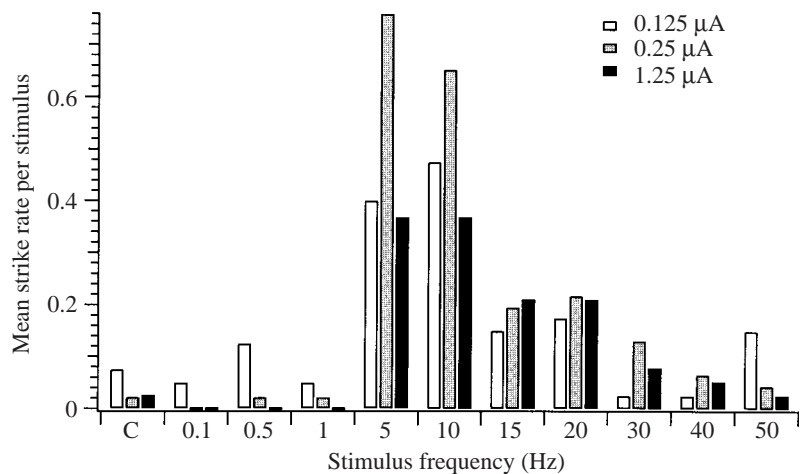
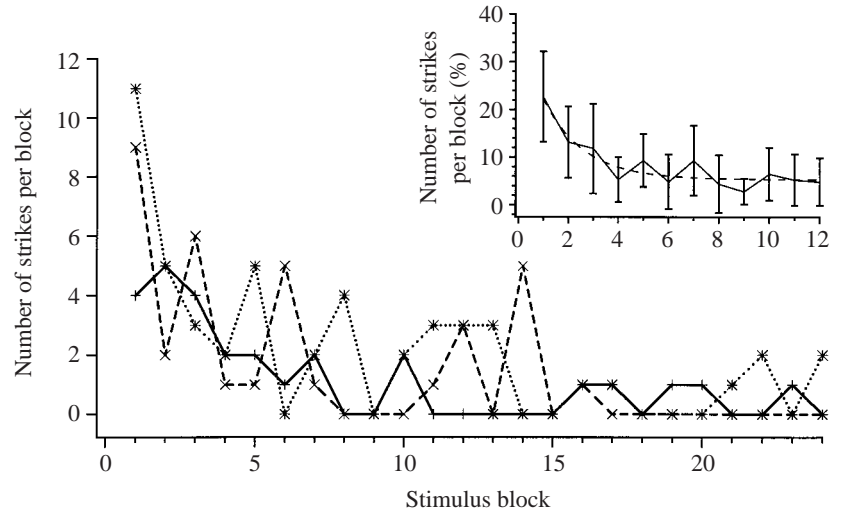


Fig. 4. Relative paddlefish occurrence in the vicinity of the stimulus dipole and strike probability. (A) An index of nearness to the electrodes shows no difference for dipole frequencies during stimulation (filled columns) or without stimulation (open columns) ($P<0.17$). C, control in the absence of electrical signals. (B) The probability of feeding strikes elicited by sinusoidal signals shows that there is a preference for stimulus frequencies of 5–15 Hz. Strike probability is greatest during the first block (hatched columns, except for 10 Hz) in comparison with subsequent stimulus sequences (open columns). Stimulus duration was 120 s, with a pause between stimuli of 126 s. Each stimulus frequency was presented 12 times, with four fish in the tank. Values are means + S.E.M. C, control in the absence of electrical signals.

strike rate. We statistically analyzed differences in strike rate as a function of stimulus frequency, amplitude, stimulus block and the interactions between those variables. We found significant differences in strike rate for amplitude, frequency and stimulus block and for the interaction between frequency and amplitude. This interaction shows that the effects of

Fig. 6. Strike response decrement with repetitive stimulus presentation. Results are presented for three experiments showing that, for repetitive stimulus blocks (containing all frequencies at $0.25\ \mu\text{A}$), strike activity is high initially but drops off rapidly with continued stimulation. Delays between stimuli were 5 s; total time per experiment was 552 min. Inset: strike responses for 12 consecutive stimulus blocks show an exponential-like decline. Values are means \pm S.E.M. Results are pooled for seven experiments using four fish (none of the 28 fish had been exposed previously to the electric stimulation).



amplitude depend on frequency. In addition, stimulation at $1.25\ \mu\text{A}$ p-p in fresh water occasionally triggered an avoidance response, in which the fish abruptly changed direction and swam away excitedly. Stimulus intensities of $1.25\ \mu\text{A}$ p-p or greater in fresh water occasionally produced a saccade-like shaking of the rostrum, a response not observed in plankton-feeding fish and, therefore, considered to be a non-physiological response. Regardless of the stimulus intensity, the frequency tuning of striking behavior is little changed (cf. Figs 4 and 5), with a peak strike response at 5–10 Hz.

Changes in strike behavior with repetitive stimulation were marked by a decline in response frequency during the course of an experiment. Counting strikes at all stimulus frequencies for each stimulation block, responses were highest for stimuli presented early in the experiment, as illustrated for three experiments in Fig. 6. Regression analyses for 19 experiments (delay time of 5 or 126 s) resulted in a significant decline in strike response in eight of 11 experiments (73%) for which the rest interval was 5 s and three of eight experiments (37%) for which the rest interval was 126 s (regression, $P < 0.05$). Frequently, the decline in the number of strikes appeared to be exponential, as illustrated in the normalized data from seven experiments with a 5 s rest interval (inset, Fig. 6) (regression $F_{1,10} = 19.161$, $r^2 = 0.6571$, $P < 0.0014$).

To test whether the decline in strike rate could be offset, a food reward was presented as a dishabituating stimulus. In three experiments, brineshrimp (*Artemia salina*) were added to the experimental tank in three aliquots during the last half of the experiment. As shown in Fig. 7A, the number of 'electrical' strikes increased dramatically following each of the brineshrimp additions. All live plankton were consumed before the addition of successive aliquots. Following the last addition of prey, the response rate again declined. The mean strike rate at the dipole electrodes differed significantly between the non-reinforced and brineshrimp-reinforced portions of the experiments (Fig. 7B) ($N = 3$, 28 blocks; Wilcoxon sign rank test, $P < 0.0001$).

In nature, paddletfish encounter waters of varying conductivity. Electrical signals will change in intensity relative

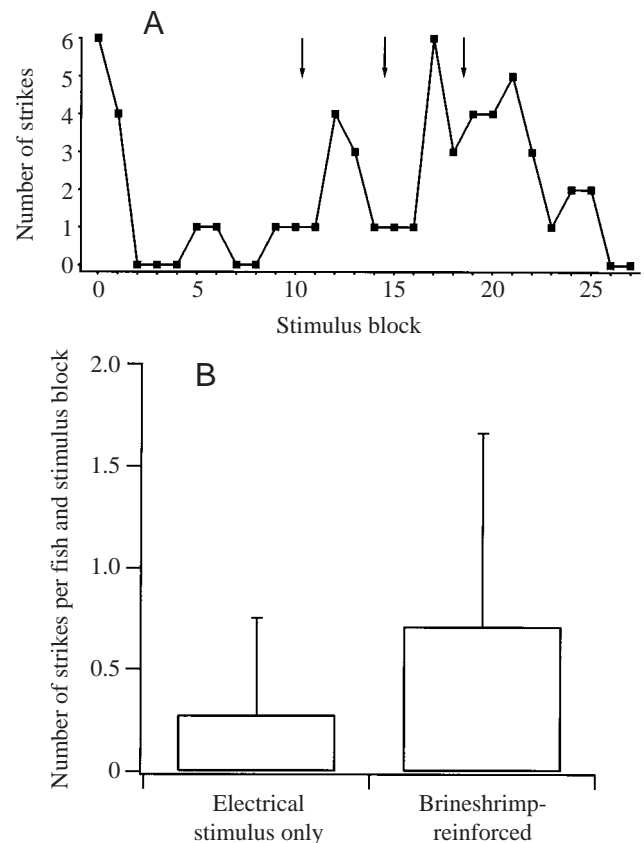


Fig. 7. Dishabituation of the strike response. (A) The decrease in strike activity is offset by the addition of live brineshrimp (at the three arrows). (B) Pooled values of the strike rate for three experiments comparing strikes with and without brineshrimp reinforcement show a significant increase in electrical strikes in response to the addition of brineshrimp (Wilcoxon sign rank test, $P < 0.0001$). Values are means \pm S.E.M.

to the conductivity of water, being attenuated at higher conductivity values. Following this assumption, we tested whether water conductivity influenced the striking activity. Four fish were tested on successive days using brackish water

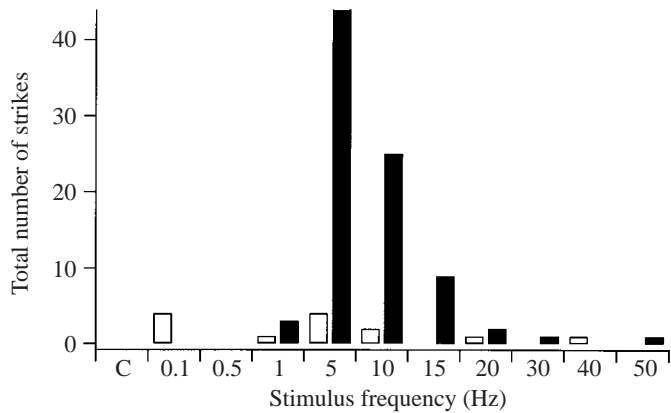


Fig. 8. Effects of water conductivity on the strike rate of paddlefish. A comparison of the strike frequency in water with a conductivity of 4 mS cm^{-2} (open columns) and the strike rate in water with a conductivity of $400 \mu\text{S cm}^{-2}$ (filled columns) shows more strikes in low-conductivity water. The results are based on equivalent tests using the same four fish. C, control in the absence of electrical stimuli.

(4 mS cm^{-2}) and then fresh water ($400 \mu\text{S cm}^{-2}$). A comparison of strike frequency shows that fish rarely strike at the dipole electrodes in high-conductivity water (Fig. 8) and that the strike rate distributions differ significantly (Kolmogorov–Smirnov two-sample test, $P < 0.0233$). This suggests that the detection of electric fields by paddlefish is constrained by the reduced signal strength in higher-conductivity water.

Discussion

The present study examines the passive detection of electrical signals and its role in the feeding behavior of juvenile paddlefish and supports the working hypothesis that pure electric fields are sufficient to elicit feeding strikes. We have shown that paddlefish strike readily at the tips of dipole electrodes delivering weak current oscillations as if they were capturing their natural planktonic prey. In a freshwater environment, effective electric field stimulus variables were in the range 5–15 Hz and $0.25 \mu\text{A}$. The stimulus evoking this behavior is purely electrical since all experiments were performed in the dark, to eliminate sight-induced responses, and no chemical or hydrodynamic signals can be attributed to the stationary dipole electrodes.

The passive electrosense in feeding

Paddlefish feeding behavior elicited by electrical dipole stimulation exhibits a low-frequency bandpass characteristic, with strikes primarily in response to oscillating frequencies of 5–15 Hz. Although strike rate varied slightly from experiment to experiment, the bandpass characteristics were constant. In addition to frequency, striking behavior also varies with the strength of the electric field. Under the present experimental conditions involving random swimming, strike threshold is approximately $0.125 \mu\text{A p-p}$. This value corresponds to $310 \mu\text{V cm}^{-1}$ at the stimulus electrodes in 4 mS cm^{-2} water, but

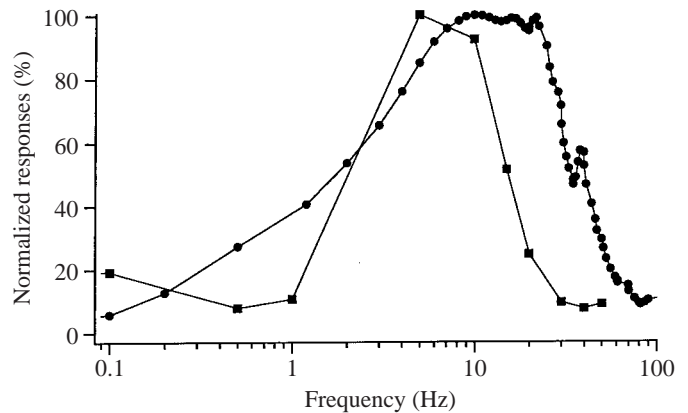


Fig. 9. Striking responses (squares) relative to the tuning curves of primary afferent neurons (circles). The normalized responses show considerable overlap, although primary afferent sensitivity extends to higher frequencies by 10–20 Hz. Data for neuronal frequency sensitivity were calculated from the data ($N=10$ neurons) of Pei et al. (1998).

does not necessarily reflect detection threshold since electric field intensity will depend on the distance and orientation of the paddlefish relative to the dipole field. Thus, one must distinguish between detection and strike thresholds.

Daphnia sp. exhibit direct-current electric potentials of 0.5–1 mV measured at the surface of the carapace, similar in origin to those previously reported for macroscopic organisms in the aquatic environment (Barham et al., 1969; Peters and Bretschneider, 1972; Kalmijn, 1974; Scheich et al., 1986). Strikes in response to 0.125 and $0.250 \mu\text{A}$ stimulus intensities are representative of these potentials. Striking falls off dramatically at $1.25 \mu\text{A}$ (Fig. 5), a stimulus intensity (and corresponding electric potential) several times greater than that of the plankton, presumably a less attractive feeding stimulus and also one producing occasional escape responses. In contrast, paddlefish detect and capture plankton at distances of up to 9 cm from the surface of the rostrum (Wilkens et al., 1997; Wilkens et al., 2001). Since potentials decrease as the third power of distance, threshold detection will undoubtedly be several orders of magnitude more sensitive than our test stimuli. Unfortunately, unconstrained ram swimming makes it difficult to evaluate detection distances, so it is not possible to compare artificial stimulus and prey threshold intensities. Nevertheless, the strike intensities compare favorably with the near-field potentials exhibited by the plankton.

The stimulus frequencies most effective in eliciting feeding strikes also compare favorably with the electrical signals of their planktonic prey. As illustrated in Fig. 1, *Daphnia* sp. produce an oscillating field potential with maximum power peaking at 3 and 7 Hz. With the movement of the prey relative to the predator, planktonic direct-current field potentials will also be affected, adding to the low-frequency alternating-current components of the live signal (Tricas and New, 1998). Thus, paddlefish strikes at artificial dipole signals correspond to the frequency characteristics of their planktonic prey. The

present results showing feeding strikes in response to these frequencies and amplitudes provide strong support for the conclusion that the paddlefish rostrum and passive electrosensory system are unique and highly specialized adaptations for planktivorous feeding.

The weak electric fields of plankton and the dipole stimuli used here are necessarily near-field signals. As a result, strike responses are subject to the proximity of the fish to the stimulus source. Since the continuously swimming paddlefish is a ram ventilator and does not establish a home territory, there is no regularity in stimulus presentation, i.e. the fish encounter a given stimulus on a random basis, independent of stimulus frequency and previous stimulation, as shown in Fig. 4. Thus, the feeding strike, in nature or as studied in these experiments, is associated to a large extent with undirected swimming and chance detection of planktonic electric fields, a distinct difference from the feeding strategies based on other sensory cues.

In the Midwestern rivers of the United States, paddlefish encounter waters of different conductivity. For example, water conductivity in the Missouri River varies dramatically, both seasonally and daily, depending on residence time in reservoirs and local precipitation. Local values (P. Keck, personal communication, St Louis County Water Co.) vary from 175 to 600 $\mu\text{S cm}^{-2}$, with readings up to 843 $\mu\text{S cm}^{-2}$ elsewhere in Missouri (J. Grady; US Fish and Wildlife Service). The strength of an electrical signal will vary in intensity with the conductivity of the water and will be greater at lower conductivities. Our experiments showed higher strike activity in water with lower conductivity, suggesting the likelihood that the detection of planktonic electric fields by paddlefish would vary accordingly. As a result, feeding efficiency may vary with natural changes in environmental water quality. This may be a critical feature for newly hatched paddlefish. After depletion of the yolk sac, small paddlefish must feed continuously as a result of their high ram-ventilating activity levels. Paddlefish spawning is triggered by high spring waters (Russell, 1986), conditions that would provide low conductivity and maximum plankton detectability.

The present study examines the electrically evoked feeding response with greater resolution than experiments reported previously (Wilkens et al., 1997). Strike frequency was highest for stimulus frequencies of 5, 10 and 15 Hz, decreasing in a graded fashion to baseline levels at 30–40 Hz. In a previous study, strike frequencies were also greatest at 10 Hz, but intermediate frequencies between 10 and 40 Hz were not tested. Intensity/response results, although similar in range in both studies, also differed somewhat. In the present study, responses dropped off at values above 0.5 $\mu\text{A p-p}$, intensities that previously remained attractive. Higher intensities were not tested rigorously since these trigger potential artifacts, including head saccades and avoidance reactions. Such was the interpretation for the phase-locked eye-blink reflex of the shark *Scyliorhinus canicula* for 100 nV cm^{-1} stimuli (Kalmijn, 1982; Kalmijn, 1988). In contrast, the phase-locked electrosensory saccades of the platypus bill were considered to be reflex

responses to physiological stimulus intensities (50 $\mu\text{V cm}^{-1}$ to 1 mV cm^{-1}) and were used to determine directional sensitivity (Manger and Pettigrew, 1995). In the catfish, avoidance responses were also reported at intensities above 1 μA (cf. attraction to near-threshold currents <1 μA) (Parker and van Heusen, 1917).

Our experiments parallel the classic work of Kalmijn (Kalmijn, 1971) in which he demonstrated the use of the passive electrosense in feeding by the shark and skate. That these fish were able to detect flatfish prey electrically was evident by successful attacks following the elimination of potential visual, chemical and mechanical prey signals. In addition, sharks and skates attacked hidden (buried) electrodes simulating the electric fields of prey, ignoring visible bait nearby. These results were confirmed in the field where sharks, lured by chemical scents, attacked electrodes carrying weak (8 μA) direct currents in preference to nearby odor sources and inactive control electrodes (Kalmijn, 1982). Attacks at pure electric field stimuli unequivocally demonstrate an electrosensory role in feeding in sharks, skates and, now, the paddlefish.

Passive electrosensory feeding responses are also known for the freshwater catfish. In the pioneering demonstration of the electrosense, Parker and van Heusen (Parker and van Heusen, 1917) noted that the catfish *Amiurus (Ictalurus) nebulosus* was attracted to metal objects and weak dipole currents (0.67–0.99 μA) and nibbled as if feeding. Stronger currents evoked startle responses. Similar results were reported for the Asian catfish *Parasilurus asotus* (Uzuka, 1934) and in catfish (Peters and Meek, 1973).

In both catfish and elasmobranchs, prey items are macroscopic, and these fish feed naturally as large predators and/or scavengers. However, these fish also rely on other sensory modalities, and their use of olfactory, visual and hydrodynamic senses in feeding is well established (Caprio, 1984; Gerking, 1994). In contrast, the planktivorous paddlefish feeds near the bottom of the food chain and seemingly relies more heavily on the electrosense, perhaps exclusively so in turbid, murky conditions. As a planktivore, the paddlefish occupies the opposite end of the food spectrum from the carnivorous fish mentioned above in terms of prey size, yet both use the electric sense as a highly sensitive mechanism for prey detection, i.e. both 'hunt' electrical signals in their near-field environment. The functionality of the electrosense in the aquatic environment is further illustrated by its use in feeding by amphibians (Himstedt et al., 1982) and in mammals by the platypus (Manger and Pettigrew, 1995).

Decline in strike response: habituation

Electrically evoked feeding strikes declined during the course of long behavioral experiments (Fig. 6): significant declines occurred in 11 of 19 experiments. The decline depended on the experimental protocol, i.e. the length of the rest intervals between stimuli. In experiments with a shorter rest interval, strike rate decrement was more pronounced than in experiments with a longer rest interval, suggesting rest time

effects. Depending on rest time, the probability that paddlefish would encounter a specific stimulus frequency differs, i.e. longer rest times reduce the exposure to any given stimulus frequency. Thus, stimulus experience appears to influence whether paddlefish regard the artificial electric signal as attractive and strike at the electrodes. The novelty of a stimulus is a paddlefish concern.

In these experiments, paddlefish strike behavior was not rewarded. The presentation of a planktonic food reward after a strike at the dipole would have been technically difficult for a fish in continuous swimming motion, and food presentation in close association with the electrical stimulus would have interfered with the test signal. It is likely that the decrement in response to ongoing dipole stimulation is a learned behavior, i.e. habituation. With no food reinforcement, paddlefish lose interest in the artificial electric fields. As evidence for learning, a food stimulus of live brineshrimp was used to dishabituate the electric strike response. Therefore, paddlefish appear to learn that the pure electric signals are not real food but just simulate prey, illustrating behavioral plasticity in paddlefish feeding.

Comparison of behavior and primary afferent tuning

The frequency/response tuning curve for the simulated feeding behavior is in agreement with the response characteristics of the electrosensory primary afferent neurons of the paddlefish. The paddlefish ampullae of Lorenzini are low-frequency electroreceptors (Wilkens et al., 1997; Pei et al., 1998) covering a frequency range characteristic of ampullary electrosensory systems in general. Paddlefish primary afferents show peak sensitivities ranging from 3 to 20 Hz, with a sharp drop off in response to signals above 20 Hz. A replot of the data from Pei et al. (Pei et al., 1998) is shown in comparison with the electrically evoked strike responses from the present study (Fig. 9). The physiological response of the primary afferents shows considerable overlap with the behavioral response in the range 3–10 Hz, but the behavioral response drops off more rapidly above 10 Hz. Receptor sensitivity not only matches but exceeds the range of the behavioral response, providing somewhat of a safety factor at the level of the receptors. This comparison indicates that computational mechanisms for higher-order central processing are required for the behavioral selectivity of relevant electrical signals. For example, changes in tuning selectivity in the ascending lateral line pathways of the goldfish *Carassius auratus* have been demonstrated by Wojtenek et al. (Wojtenek et al., 1997). It is likely that sharpening of neuronal tuning properties in the ascending electrosensory pathway contributes to paddlefish striking preferences and decision-making neuronal computations.

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