Behavioral Thresholds to Electric Signals in High Frequency Electric Fish

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Summary. 1) The behavioral thresholds of *Apteronotus* and *Eigenmannia* to imposed sine wave electric fields were measured as a function of stimulus frequency using operant conditioning techniques. 2) *Apteronotus* responds to a broad range of frequencies. Its threshold—frequency curve is complex, showing three distinct threshold dips at: <5–15 Hz, 0.6–0.9 μV/cm; 200–300 Hz, 0.9 μV/cm; and 700–2200 Hz to 0.2 μV/cm. The high frequency dip itself is bimodal, with a primary minimum at the fish's electric organ discharge (EOD) fundamental, and a secondary minimum at its second harmonic. 3) The threshold—frequency curve for *Eigenmannia* differs in several respects, being unimodal and sharply tuned to the fish's EOD frequency with a sensitivity of 0.2–0.3 μV/cm. *Eigenmannia* shows a lack of keen low frequency perception, and no marked sensitivity increase at the second harmonic of its EOD. 4) The EOD wave forms of *Apteronotus* and *Eigenmannia* were Fourier analysed: *Eigenmannia*'s EOD approximates a sine wave, while *Apteronotus*' EOD contains considerable power at harmonic frequencies. 5) It is proposed that behavioral thresholds to frequencies within ca. 50–100 Hz of the fish's EOD fundamental or second harmonic are improved by the fish's extraction of, and preference for, low frequency beats of its EOD with the stimulus. This is suggested by the sharp, EOD specific tuning demonstrated by both *Eigenmannia* and *Apteronotus* and the second harmonic sensitivity peak of *Apteronotus*, which are not predicted by known receptor response characteristics. Such a beat extraction capability might provide a mechanism for improving the resolution of conspecific electrocommunication. 6) The influence of water resistivity on the behavioral thresholds of *Apteronotus* was measured. High frequency sensitivity increases as a power function with decreasing water resistivity down to 1 k Ohm·cm, below which it remains constant. Low frequency sensitivity increases progressively from 100 k Ohm·cm down to 10 k Ohm·cm, but decreases again below this value. Thus low frequency electroreceptors are maximally sensitive in 10 k Ohm·cm water, while high frequency receptors function best in water below 1 k Ohm·cm.

Introduction

South American electric fish, of the suborder Gymnotoidei, extract electrical information from their environment with a highly sensitive electrosensory system. Behavioral experiments have shown that a gymnotid fish can evaluate the size, movement, and impedance of nearby

objects by sensing distortions in a self-generated, weak electric field (Bullock, 1968; Caldwell and Germas, 1969; Heiligenberg, 1973; Kalmijn and Adelman, in preparation). It also receives electrically mediated social information regarding the sexual or agonistic disposition of conspecifics, which they broadcast as frequency modulations or cessations of their own electric descharges (Black-Cleworth, 1970; Hopkins, 1972). In addition, electrical cues may inform the gymnotid of an approaching prey (Kalmijn and Adelman, in preparation).

Recognizing an electrical event as a "signal" can be a formidable problem in an electrically noisy environment. Possible sources of water borne electric fields are numerous, especially in tropical regions. Electric fields generated by the numerous species of gymnotid fish (sporadically up to several hundred volts in *Electrophorus electricus*) are common in South American rivers and streams (Lissmann, 1968; Hopkins, 1972). But aquatic animals in nearly every phylum, from coelenterates to vertebrates, produce a.c. and d.c. fields (Kalmijn, 1972). Many of these bioelectric fields have amplitudes in excess of 100 μV, and a few are in the millivolt range. Furthermore, nonbiological sources of electrical disturbance, such as earthquakes, lightning, and other atmospheric ionic fluxes (Storey, 1953; Hopkins, 1973; Kalmijn, in press), are common in South America and create electric fields of considerable magnitude in subjacent bodies of water. These biologically and nonbiologically generated electric fields sum, so that the current flux sensed by electrosensitive fish represents the resultant of many mutually interfering electric fields. Gymnotid fish have solved the problem of signal recognition; and they exploit this capability to occupy successfully a nocturnal niche, in which vision is severely restricted.

Psychophysical measurements of the electrical sensitivity of these fish demonstrate the cooperative sum of the peripheral and central, tuning and sharpening mechanisms, which the fish uses to filter incoming electrical information. The experiments presented in this paper measure the behavioral thresholds of two sympatric species of gymnotid fish to sinusoidal electric fields using operant conditioning techniques in a modified T-maze paradigm.

The two species have very different hunting and social behaviors: the first, *Eigenmannia*, is a gregarious bottom feeder; the second, *Apteronotus*, is a territorial midwater feeder. These behavioral differences are reflected in qualitatively different, frequency specific, electrical sensitivities of the two species.

Methods

A representative species from each of two gymnotid families were trained and tested. They were tentatively identified as *Apteronotus albifrons* (Linnaeus) from

the family *Apteronotidae*, and *Eigenmannia virescens* (Valenciennes) from the family Rhamphichthyidae (Table 1). Each fish was kept in a separate, 5-gal. holding tank, and was transferred with a net to the test aquarium each day for conditioning. Water resistivity in the aquaria was carefully controlled with a salt mixture of $CaSO_4$, $MgSO_4$ and KCl in a weight ratio fo 60:5:4 (recipe from Heiligenberg), at a value of 2 k Ohm·cm for all experiments, except when systematically altered (Fig. 7). Aquaria water was maintained at a temperature of 26–27°C, and a pH of 5.5–6.0. The fish were on a 12 hour day-night cycle: the holding tanks being illuminated from 7 AM to 7 PM.

The test aquarium, which measured $26 \times 120 \times 26$ cm, was divided along its length into three compartments (52 cm; 16 cm; 52 cm) by two electrically transparent plastic screens (Fig. 1A). Holes 4 cm in diameter at the bottom of each screen allowed passage of the fish, and demarked the "decision point" during the trials. Identical feeder-electrode assemblies (Fig. 1A insert) hung into either side compartment. Chloride plated silver wires, which passed down the lumens of a pair of 60×0.7 cm plexiglas tubes and wrapped around their ends, formed the stimulus electrodes. A feeding station was situated between each electrode pair consisting of a short piece of plexiglas tube 4 cm in diameter, that was connected to a surface platform by a small plexiglas tube. Brine shrimp or white worms (*Enchytraea*) were automatically dispensed at the feeding stations through soft plastic catheters, which ran down the lumens of the connecting tubes.

Operant conditioning techniques were used to train the fish, with sinusoidal electric fields serving as the conditioned stimulus. A "response" was scored if the fish's eye appeared on the outside of a passage. If the response were "correct", i.e. made on the side of the field source, a single brine shrimp or white worm "reward" was delivered at the feeding station, and the field was turned off as the fish fed. If the response were "incorrect", the signal was immediately switched off and the fish was driven back into the center compartment with a plexiglas rod. When the signal intensity became low, the fish would occasionally swim back and forth in the center compartment, never making a response. Such a trial was recorded as a "no-go" and indicated the approach of the threshold intensity level, but was not considered in the data analyses.

A test session comprised one hundred trials for the *Apteronotus* and 60 trials for the *Eigenmannia*, being limited in both cases by their respective appetites. Between trials (intertrial interval equaled 60 sec) the fish remained exclusively in the center compartment next to a hand-held plexiglas rod. Removal of the rod signalled the beginning of a trial. A sine wave, generated by a Hewlett Packard 208A test oscillator, was introduced to the tank through one set of electrodes (as predetermined by random, computer selection of "right" or "left") by opening a ganged potentiometer (Fig. 1B). The intensity of the signal was controlled with a Hewlett Packard 350D attenuator. The fish was allowed 60 sec to make a response; during test periods with near threshold stimuli, vacillation for a good part of a minute before deciding was not uncommon.

In searching for "threshold", the signal amplitude was attenuated by 5 dB after each successful trial, until the fish made an incorrect or no-go response. After such a trial, the signal intensity was increased to the previous intensity level, and subsequent trials were conducted by reducing the amplitude in single dB steps. The signal intensity at which more than 70% of the responses made were correct responses, was chosen as the "threshold" intensity (Fig. 2A). The number of trials run at any given threshold intensity was never less than 15 and, at frequencies of special interest, exceeded 50.

Motivation to respond at each frequency was measured in average "response latency" time and in number of reorientations made per trial (Fig. 3). "Response
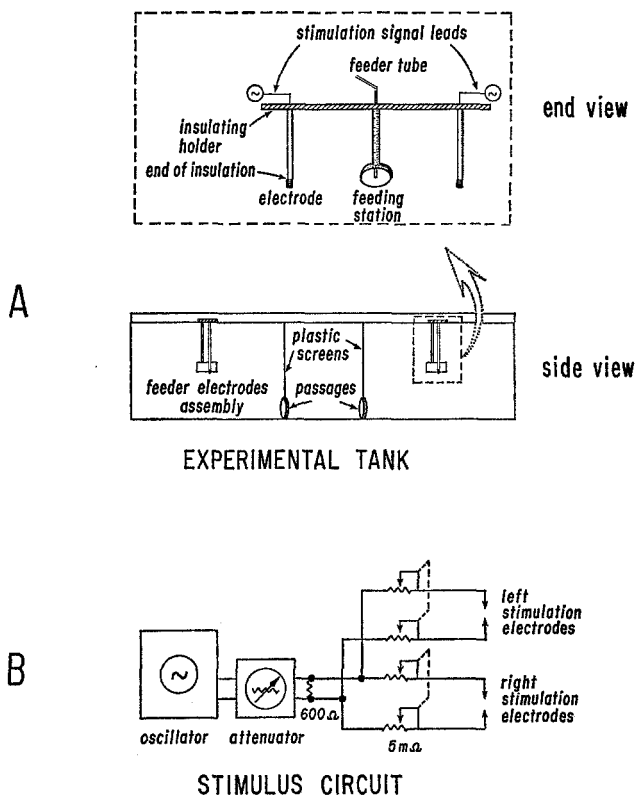
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Fig. 1A and B. The experimental set-up. Insert in A, one of the feeder-electrode assemblies. A, the experimental tank. B, the electronic circuitry used to generate and deliver the electric fields. The dashed lines indicate the mechanical coupling of the ganged potentiometers

latency" was the elapsed time between the beginning of a trial and the first response. A reorientation consisted of the fish "approaching" one screen, then making an "about-face" (AAF) toward the opposite screen. A rise in both of these behavioral measures was observed as the percent of correct responses dropped below the 70% threshold level, corroborating this designation of threshold (Fig. 2B, C).

At the end of each session, the electrode signal intensities at 0 dB were measured on the electrode side of the passages for each frequency used. The calibration was performed with bipolar electrodes set 5 cm apart with a ground electrode in between. Each electrode consisted of an insulated silver wire with a small exposed sphere of silver on its end. No evidence of electrode polarization could be observed down to 5 Hz. The 0 dB signal was amplified with a Grass P9 AC preamplifier and measured on an oscilloscope. The measured voltage was divided by the electrode separation to give the average field gradient, which was then multiplied by the threshold, dB attenuation factor to arrive at the reported threshold field gradient.
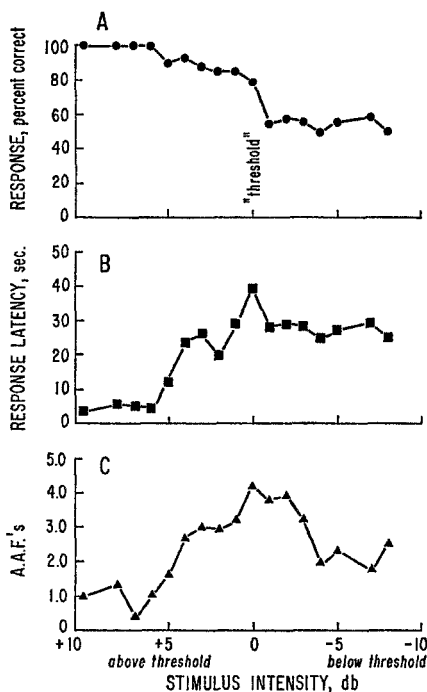
Fig. 2A—C. An example of behavioral response measurements made during a threshold determination of *Apteronotus* No. 1, plotted as a function of stimulus intensity. A, criterion for "threshold" designation: better than 70% correct response. B, response latency as a function of stimulus intensity. C, the number of approach and about-faces (A.A.F.'s) as a function of stimulus intensity. Each point represents the mean value from six sessions at 1200 Hz

Results

The Performance Characteristics of Apteronotus and Eigenmannia

Apteronotus is an aggressive, territorial fish, which readily attacks invading conspecifics. This natural response could be triggered with a dipole electric field and greatly facilitated training *Apteronotus* to the paradigm, since naive fish, placed in the test situation, immediately detected and attacked the active stimulus electrodes. This "attack" behavior was readily elicited with sinusoidal electric fields of 5–3000 Hz, if the field intensity were quite high (1 mV/cm). At lower intensities (10–20 μV/cm), only frequencies of 700–1300 Hz, which correspond to the electric organ discharge [EOD] frequencies of conspecifics (Table 1), would trigger an attack. For this species, training consisted first of shaping

Table 1

Fish	Species EOD frequency range[a] (Hz)	Individual fish		
		number	EOD frequency[a] (Hz)	Length (cm)
Apteronotus[b] *albifrons*	800–1300	1	1000	14.8
(Apteronotidae)		2	900	18.1
Eigenmannia virescens	250– 600	1	290	14.5
(Rhamphichthyidae)		2	400	13.7

[a] Measured at 26° C.
[b] Also classified as *Sternarchus* by some authors.

an association between the source of the electric field and the food reward. Once this was established, the fish learned not to attack the electrodes, but to directly approach the feeding station for a reward. The fish generalized their response to all frequencies with equal motivation, as indicated by uniform response latencies and AAF measurements (Fig. 3). Less than 100 trials were required to achieve 100% correct responses to suprathreshold stimuli for this species.

Eigenmannia proved far more difficult to train. Timid in nature, these fish required a methodical sequence of shaping steps before they would consistently perform the T-maze test. First the fish learned to feed from a hand-held catheter, which was presented directly to it. The fish then learned to follow the catheter to one of the feeding stations where it finally received a food reward. During each shaping trial, a sine wave field was introduced to the tank as the catheter was presented, and turned off as the fish ate. The frequency of the field was changed for each trial. After 50 such trials the fish responded to the electric field by reporting to the feeder. After 50 additional trials, it had generalized its response to both feeders according to the source of the field. Within 300 trials each *Eigenmannia* responded correctly in 20 out of 20 trails to suprathreshold stimuli, and was considered ready for testing.

Apteronotus was a far better test species than *Eigenmannia* because of several behavioral and physiological differences. First, *Apteronotus* is a midwater feeder and is typically very active in comparison to *Eigenmannia*, which is a bottom feeder (Hopkins, 1972). *Apteronotus'* high activity level resulted in rapid sequences of AAF's during a trial, which helped the fish discern the direction of the field gradient quickly and efficiently. In contrast, *Eigenmannia* often hovered at one screen for long
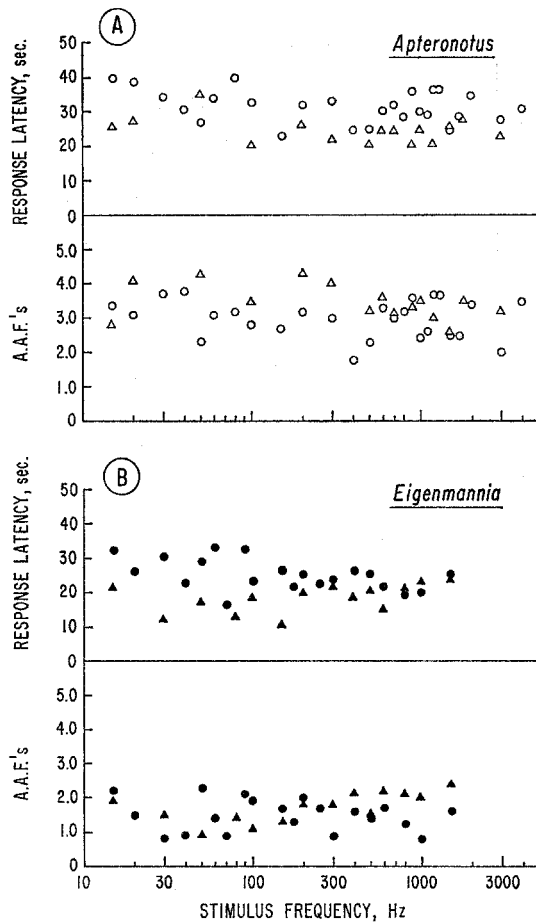
Fig. 3A and B. Behavioral measurements of motivation plotted as a function stimulus frequency. A *Apteronotus* No. 1 (open circles), *Apteronotus* No. 2 (open triangles). B *Eigenmannia* No. 1 (closed triangles), *Eigenmannia* No. 2 (closed circles). Each point represents the mean value for all trials within ± 3 dB of threshold at the designated frequency. Note that motivation appears to be independent of the stimulus frequency

periods of time before swimming to the other. Second, *Apteronotus* is a voracious eater with a tremendous appetite, being capable of well over 100 trials per day, whereas *Eigenmannia's* motivation began to wane after 60–70 trials, even on variable ratio reward. Third, visual distractions from outside the tank, such as lights or hand movements consistently intimidated *Eigenmannia* and hampered its performance, so that a mask
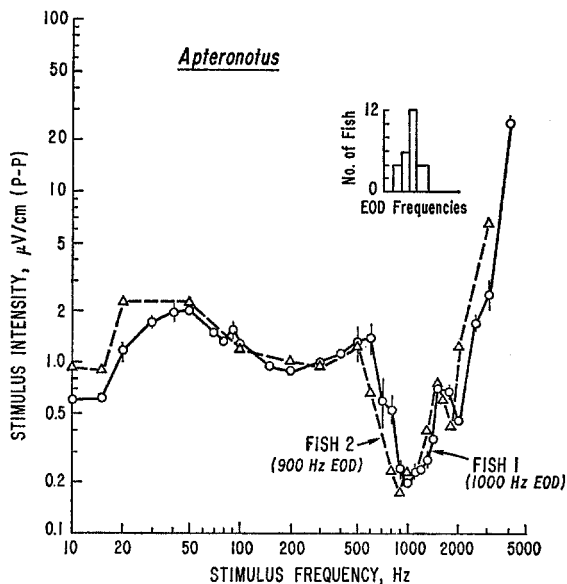
Fig. 4. Behavioral threshold voltage, measured peak to peak, versus stimulus frequency for two *Apteronotus*. *Apteronotus* No. 1 (open circles) has an EOD frequency of 1000 Hz. *Apteronotus* No. 2 (open triangles) has an EOD frequency of 900 Hz. Standard error bars are shown for *Apteronotus* No. 1 only. Insert, a histogram of the number of laboratory *Apteronotus* with a given EOD frequency when measured at 26°C (data from Hopkins)

had to be placed in front of the tank and the trials performed under dim lights. *Apteronotus*, however, seemed completely oblivious to any sort of visual distraction and performed well even under strong flood lights.

Behavioral Thresholds as a Function of Stimulus Frequency

Plotting the threshold stimulus voltage as a function of stimulus frequency for *Apteronotus* reveals a complex, multiphasic sensitivity curve (Fig. 4). Frequency ranges of high sensitivity (threshold less than 1.0 μV/cm) form three distinct threshold dips at < 5–15 Hz, 200–300 Hz, and 700–2000 Hz; the fish's best frequency being that of its own EOD: 0.2 μV/cm. The high frequency threshold dip is itself bimodal, consisting of a primary dip at frequencies close to the EOD of the fish, and a secondary, less sensitive dip at the harmonic of its EOD frequency. This bimodal character of *Apteronotus*' threshold curve in the high frequency range has been previously described by Granath *et al.* (1967).
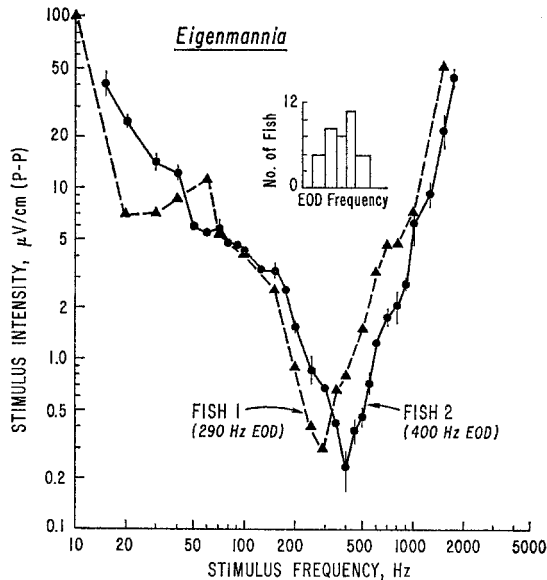
Fig. 5. Behavioral threshold voltage, measured peak to peak, versus stimulus frequency for two *Eigenmannia*. *Eigenmannia* No. 1 (closed triangles) has an EOD frequency of 290 Hz. *Eigenmannia* No. 2 (closed circles) has an EOD frequency of 400 Hz. Standard error bars are shown for *Eigenmannia* No. 2 only. Insert, a histogram of the number of laboratory *Eigenmannia* with a given EOD frequency, when measured at 26°C (data from Hopkins)

Threshold intensities are almost constant from <5 to 15 Hz for a given fish: fish No. 1, 0.6 μV/cm; fish No. 2, 0.9 μV/cm. Sensitivity declines rapidly between 15 and 30 Hz at a rate of approximately —13 dB/octave, where it levels off again at 2.0 μV/cm. Sensitivity gradually increases above 60 Hz, forming a symmetrical, gently sloped threshold dip, the nadir of which lies between 150 and 300 Hz at about 0.9 μV/cm. As its EOD frequency is approached, the fish's sensitivity increases dramatically (+21 dB/octave), peaking at its EOD frequency (0.2 μV/cm). The fish's sensitivity does not immediately decline beyond this frequency, but remains very high (0.25 μV/cm) over a range of several hundred Hz before cutting off at a rate of —25 dB/octave above 1300 Hz. Thus *Apteronotus*, although demonstrating a best frequency at its own EOD frequency, seems to be highly tuned to its entire conspecific frequency range of 800–1300 Hz (Fig. 4 insert). The secondary dip, however, is sharply centered at the second harmonic of the individual's EOD frequency, above which the fish's threshold sharply increases at — 30 dB/octave.

The threshold versus frequency curve for *Eigenmannia* is considerably different from that of *Apteronotus*, being unimodal and markedly tuned to the EOD frequency of the fish (Fig. 5). The response threshold curve is skewed to the lower frequencies and cuts off rapidly at frequencies above the fish's EOD. Neither fish responded to sine wave fields of 100 μV/cm below 10 Hz or above 2000 Hz.

Sensitivity to 10–150 Hz electric fields varied qualitatively between two *Eigenmannia*, with fish No. 1 showing a slight threshold dip at 20–30 Hz, which was not demonstrated by fish No. 2. On the average, however, their sensitivities increase by +6.5 dB/octave up to 150 Hz. The rate of sensitivity increase accelerates above 150 Hz, as the EOD frequency of the fish is approached. Fish No. 1, with an EOD frequency of 290 Hz, showed a more rapid sensitivity increase (+18 dB/octave) than did fish No. 2 (+16 dB/octave), with an EOD frequency of 400 Hz.

The sensitivity of each fish was maximal at its EOD frequency: fish No. 1, 0.3 μV/cm; fish No. 2, 0.2 μV/cm. Both fish were 3–6 dB less sensitive to frequencies, which were 50 Hz above or below their EOD frequencies. When testing in this frequency range, the fish's EOD was measured before and after the session with a Hewlett Packard 5216A 12.5 MHz electronic counter to check for reflex frequency shifts in the fish's output (Bullock *et al.*, 1972). EOD frequency shifts of up to ±5 Hz were recorded when the stimulus frequency approximated the initial EOD frequency.

The fish's threshold increased to frequencies immediately above its EOD frequency, at a rate of −20 dB/octave. No threshold dip could be found at the second harmonic of either fish, although a slight inflection was detectable in this region of the curve.

Fourier Analysis of EOD Wave Forms

It is important to keep in mind that the electric field, which the test fish senses, is not merely the applied sine wave stimulus, but the interaction of this signal with its own EOD field. A stimulus field with a frequency (F_1) which approaches the fish's EOD fundamental frequency or its harmonics (F_2), will result in a signal with a frequency (F_1+F_2)/2 that is amplitude modulated, or beats, at a frequency equal to $|F_1\text{-}F_2|$. The resultant beat amplitude will be the algebraic sum of the stimulus and the respective EOD frequency component amplitudes. To determine what influence frequency components in the fish's EOD may have had on the behavioral threshold measurements, the EOD wave forms of *Eigenmannia* and *Apteronotus* were Fourier analyzed.

To record their EOD's each fish was sewn into a nylon net "sock", and placed in an aquarium containing 26° (C), 2 k Ohm·cm water. Carbon
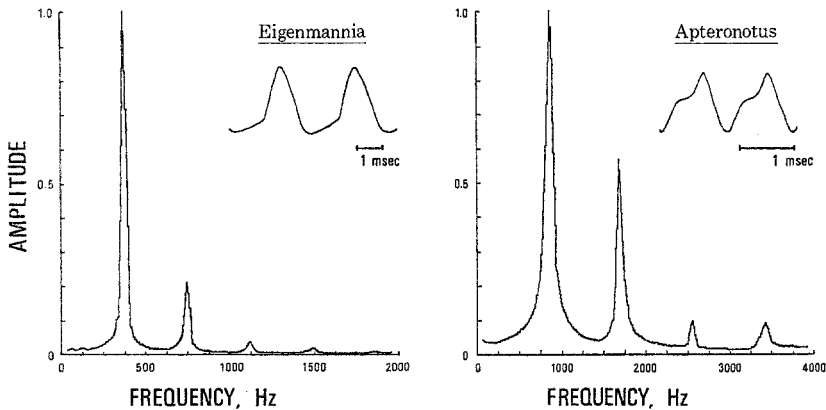
Fig. 6. Fourier analyses of *Eigenmannia* and *Apteronotus* EOD wave forms (shown as inserts). Each analysis is the average of fifteen discontinuous records, with a resolution of eight points per division on the abscissa. The *Eigenmannia* wave form was sampled for a total of 480 msec; the *Apteronotus* wave form was sampled for a total of 240 msec. The EOD's were recorded head to tail in 2 k Ohm·cm water

electrodes were placed at the head and tail of the fish, and the fish's EOD was amplified with a Grass P9 preamplifier and recorded direct on an Ampex SP300 tape recorder. The frequency response of the tape recorder was 50–10 KHz. The EOD wave forms (Fig. 6 inserts) were then digitized and Fourier analyzed with an IBM 1800 computer. Programming and processing of the data were done by T. G. Uter.

The Fourier analyses of *Eigenmannia* and *Apteronotus* EODs (2 k Ohm·cm water) are shown in Fig. 6. The EOD of *Eigenmannia* approaches a pure sine wave; the amplitude at the fundamental is sharply peaked, and harmonic amplitudes are relatively weak (0.2 at the second harmonic). The EOD of *Apteronotus* is a distorted sine wave, containing pronounced harmonic amplitude peaks.

There is a marked correlation between the frequency components of the EOD wave forms and the high frequency behavioral thresholds of the two species: in *Apteronotus* the strong fundamental and second harmonic correlate with the bimodal shape of the high frequency portion of its threshold curve; and in *Eigenmannia*, the nearly pure fundamental correlates with its sharp, unimodal threshold curve.

Behavioral Thresholds as a Function of Water Resistivity

Gymnotid electroreceptors are of two morphologically and physiologically distinct types: "ampullary" receptors connect directly to the skin surface via a short, "jelly" filled canal (Szabo, in press) and respond to very
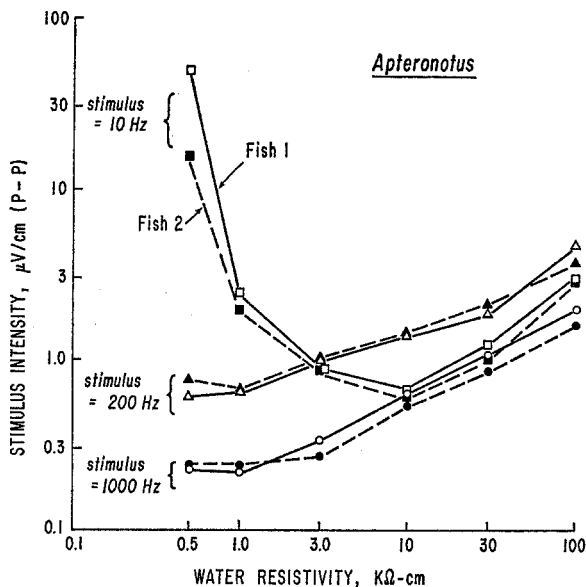
Fig. 7. The influence of water resistivity on the behavioral threshold of *Apteronotus* to given stimulus frequencies. Each point is the mean value of six threshold determinations from two separate graded series on the designated fish

low frequency electric fields; "tuberous" receptors protrude into a subdermal cavity that does not open to the outside, and are sensitive to high frequency electric fields (Bennett, 1967, 1971). The low and high frequency sensitivity of *Apteronotus* (Fig. 4) most likely result from its ampullary and tuberous receptor populations respectively. Because of this functional separation, *Apteronotus* was used to investigate the influence of water resistivity on the sensitivity of ampullary and tuberous receptors.

Two *Apteronotus* were kept in 500 Ohm·cm water for one week prior to the experiment. Their response thresholds were then evaluated at three different stimulus frequencies (10, 200 and, 1000 Hz), corresponding to their best detected frequencies of the three threshold dips described earlier. Following these measurements, the water resistivity in their holding tanks and in the test aquarium were changed to the next value, 1 k Ohm·cm, and the fish were allowed one day acclimation time before being retested. This procedure was followed in two graduated series between 500 Ohm·cm and 100 k Ohm·cm, first with increasing, and a second with decreasing water resistivities. The threshold values measured for a given frequency and water resistivity were consistent for both series; their means are graphed in Figure 7.

The results indicate that the behavioral threshold voltage of *Aptero-
notus* depends, to various degrees, on the water resistivity (Fig. 7); and
that the influence of water resistivity is far more profound on its low
frequency (10 Hz) threshold than on its high frequency (200 and 1 000 Hz)
thresholds. Between 10 k Ohm·cm and 100 k Ohm·cm, the threshold
voltages of all three frequencies increase as a power function of water
resistivity, since each plot approximates a line on the log-log graph
over this resistivity range. At resistivities below 10 k Ohm·cm the voltage
sensitivity of the fish to a 10 Hz electric field declines increasingly
rapidly, so that at 500 Ohm·cm the fish is 30–80 times less sensitive
than it was in 10 k Ohm·cm water. This progressive loss of sensitivity
in water of decreasing resistivity was not observed for 200 and 1 000 Hz
fields. The fish's sensitivities to these frequencies continued to increase
down to 1.0 k Ohm·cm, at which point they leveled off through
500 Ohm·cm.

Discussion

The Biological Importance of Certain Electric Frequencies

Apteronotus and *Eigenmannia* are most sensitive to signal frequencies
which are of particular significance to the individual fish and its species.
Apteronotus senses a broad range of frequencies with enchanced per-
ception of low frequencies (<15 Hz) and of frequencies within several
hundred Hz of its EOD. *Eigenmannia* responds best to frequencies near
that of its EOD; its sensitivity drops sharply and steadily at frequencies
above and below this frequency. These qualitative differences in the
response threshold versus frequency curves of the two species correlate
with differences in their respective biological niches and social behaviors.

Apteronotus is a nocturnal, large gaped, midwater feeder, probably
feeding upon small fish. Ordinary teleost fish generate d.c. fields of up to
500 μV (Kalmijn, 1972). Considering the 0.6–0.9 μV/cm low frequency
threshold of *Apteronotus*, the d.c. field of a fish should be readily
detectable, i.e. *Apteronotus* should be capable of electrically locating prey
as it passes by. Behavioral experiments by Kalmijn and Adelman
(in preparation) substantiate this assertion. In these experiments *Aptero-
notus* located, and attempted to attack goldfish through an agar aquar-
ium bottom, which was electrically transparent. Goldfish extract elicited
no response; but a d.c. dipole field with a magnitude equal to that of an
average goldfish evoked the orientation and attack response. When the
bottom was covered with an electrically insulating, polyethylene sheet,
responses to subjacent goldfish disappeared. Apparently *Apteronotus* can
use its sensitivity to low frequency electric fields to detect and locate
prey. In contrast *Eigenmannia* demonstrates a lack of low frequency
sensitivity, and therefore should be incapable of electrically detecting

prey. However, since *Eigenmannia* is a bottom feeder, which methodically grazes for insect larvae (Hopkins, 1972), the selective pressure to develop keen low frequency reception is probably reduced.

High frequency electroreception is crucial to both *Eigenmannia* and *Apteronotus*. These fish are classified as "wave" fish (Scheich and Bullock, in press) due to their steady EOD's which have a duration about equal to the interval between discharges, as opposed to "pulse" fish that discharge intermittently. Wave fish of a given species discharge within a frequency range which is characteristic of the species (Figs. 4, 5 inserts). Species specific EOD ranges have been exploited by researchers as a means of species identification of electric fish in the field (Lissman, 1958; Hopkins, 1972). Conspecific recognition among wave fish seems also to rely upon EOD frequency (Hopkins, 1972). Moreover, both *Apteronotus* and *Eigenmannia* communicate social information to conspecifics by acutely altering their EOD frequencies (Larimer and MacDonald, 1968; Bullock, 1969; Hopkins, 1972).

Maximal reception of conspecific signals is clearly advantageous, ecpecially for a territorial fish like *Apteronotus*, to detect and warn potential competitors at a distance. However, the fish's EOD serves another function, which is probably of greater importance to the individual's immediate survival: perceiving surrounding objects. The need for unambiguous object "electrolocation" must be weighed against the importance of conspecific recognition and intraspecific communication, since the electric signal of one, is electric noise to the other. Conspecifics, which discharge at frequencies close to that of an individual, will "jam" the individual's reception of its own EOD, causing it to become electrically "blind". Heiligenberg (1973) demonstrated this experimentally with *Eigenmannia* by quantifying its electrokinetic hovering behavior, and measuring the effect of introduced sine wave fields on the fish's ability to sense moving objects. Sine wave fields of frequencies greater than 10 Hz above or below that of the fish's EOD did not affect the fish's performance. As the frequency difference was further decreased, the fish's electrokinetic response deteriorated and the fish became incapable of following the moving objects.

The jamming caused by conspeeific EOD's is a problem that *Eigenmannia* constantly encounters in its natural habitat. Being a gregarious fish, commonly found in large clusters of up to 40 individuals (Hopkins, 1972), the probability of two fish discharging within 10 Hz of each other is high. As one might expect, *Eigenmannia* possesses several mechanisms to minimize the effects of conspecific EOD interference on its electrolocation system. First, the almost pure sine wave form of its EOD (Fig. 6) with relatively slight harmonic frequencies, reduces mutual contamination of individual EOD frequencies. Second, a large portion of

Eigenmannia's high frequency electroreceptors respond best to the fundamental frequency of the fish's EOD (Scheich *et al.*, 1973). These first two properties contribute to a third, which is its highly tuned behavioral sensitivity to its EOD frequency (Fig. 5). Fourth, *Eigenmannia* is capable of reflexly increasing or decreasing its EOD frequency in response to a jamming, extrinsic frequency (jamming avoidance response [Bullock *et al.*, 1972]), so that a sufficient frequency difference is maintained.

Apteronotus may have solved the jamming problem in another way. Its aggressive, territorial nature results in the spatial dispersion of conspecifics, thereby reducing the probability and effect of a jamming signal. Its distorted sine wave EOD and relatively broad high frequency sensitivity (Fig. 4) reflects a diminished selective pressure for a highly tuned electroreception system. The relatively broad band high frequency sensitivity of *Apteronotus* probably increases its susceptibility to jamming signals, which might partially explain its aggressive response to frequencies near its own EOD frequency.

Preferential Extraction of Low Frequency Beats

Many diurnal fish communicate their sexual and agonistic dispositions with visual signals, which involve considerable spatial and temporal complexity. The nocturnal habit of gymnotid fish severely restricts their use of this modality and thus requires a different means of social communication: electrocommunication. Like vision, electrocommunication is relatively instantaneous and can accommodate temporally complex signals, but has the required advantage of being independent of external energy sources. However, electric signals will interact with ambient electric fields, which could obfuscate the information content of the signal. Broad banded, high amplitude electric fields, induced by atmospheric and terrestrial ionic events, are frequent in tropical waters (Storey, 1953; Hopkins, 1973; Kalmijn, in press), as are biogenic electric fields especially from other electric fish (Lissmann, 1968; Hopkins, 1972).

The EOD and electroreceptive system of wave type gymnotid fish are adapted so as to minimize the effect of such extraneous electric fields on communication signals. Their highly regular, quasisinusoidal EOD wave forms contrast sharply with sporadic, atmospheric noise (Hopkins, 1972). Furthermore, the frequencies of their EODs cluster into species characteristic ranges, so that an individual fish could recognize another in its own frequency range either by its frequency, or by the low frequency beat which it produces.

Because of these EOD properties, electroreceptors of wave fish can be narrowly tuned to the EOD frequency of the individual fish to improve

its EOD signal to noise ratio. This has been found to be the case for a class of high frequency receptors, termed "T units" in *Eigenmannia* (Scheich *et al.*, 1973). The frequency response of these units shows a sensitivity increase of 12 dB/octave to frequencies approaching the EOD frequency of the fish, a 100 Hz plateau where all frequencies are passed maximally, then a 40 dB/octave attenuation to higher frequencies. Another class of high frequency receptors, "P units", are also most responsive at the fish's EOD frequency, but their filter properties are less steep. The high frequency electroreceptors of *Apteronotus* that have been examined are P units and are broadly tuned to its EOD frequency. These electroreceptors cut off at a rate of 25 dB/octave to frequencies above the fish's EOD frequency and at approximately 6 dB/octave below 900 Hz (Hopkins, personal communication). Clearly in both genera, considerable stimulus filtering takes place at the receptor level.

To a large extent, the behavorial threshold-frequency curves for *Eigenmannia* and *Apteronotus* are predicted by the frequency response characteristics of their electroreceptors. However certain aspects are not. The sharp tuning of *Eigenmannia* to its own EOD frequency measured behaviorally (Fig. 5), contrasts with the 100 Hz frequency response plateau of its high frequency receptors for this same range (Scheich *et al.*, 1973). In *Apteronotus* (Fig. 4), the behaviorally determined 21 dB/octave attenuation of frequencies below the fish's EOD frequency is 3.5 times sharper than the low frequency cut off of the electroreceptors themselves. Still more striking is the large threshold dip at the EOD second harmonic exhibited by *Apteronotus*.

The correlations between these unexpected threshold-frequency curve characteristics and the Fourier components of the EOD wave forms from these species (Fig. 6) are conspicuous: *Eigenmannia*'s strong EOD fundamental component and weak second harmonic component correlate with its sharp, unimodal threshold curve; *Apteronotus*'s large EOD fundamental and second harmonic components correlate with the bimodal shape of the high frequency portion of its threshold curve. These correlations strongly suggest that the threshold-frequency curve owes certain aspects of its shape to the wave form of the fish's EOD. High frequency electroreceptors have been shown to have a high voltage threshold (70 μV/cm for T units; 700 μV/cm for P units), above which they become very sensitive to voltage changes (10 μV/cm for T units; 25–50 μV/cm for P units; note each value depends on a different criterion for threshold) (Scheich *et al.*, 1973). The fish's EOD establishes an electric field gradient that brings its electroreceptors into this dynamic range of particularly high sensitivity to increments of voltage. Therefore a sine wave stimulus need only be of sufficient intensity to produce amplitude modulations which exceed the fish's threshold to a voltage increment, rather than

its absolute threshold to that frequency alone. This important EOD function maximizes the fish's sensitivity to electric fields in general, but cannot explain the steepness of the behavioral threshold curves or the second harmonic dip in *Apteronotus*, as can be readily shown. One way is electronic simulation with a fixed threshold detector, analogous to the property shown for high frequency electroreceptors (Scheich *et al.*, 1973). Sine wave frequencies mixed with EOD wave forms and passed through a band pass filter approximating electroreceptor properties do not show any amplitude increase at the EOD frequency or its harmonics. The resultant amplitudes can be predicted by the algebraic sum of the component frequencies with respect to their phases. Thus, the increased behavioral thresholds to frequencies near the fish's EOD fundamental and, in the case of *Apteronotus*, its second harmonic may well depend upon some higher order operation.

A capability of *Eigenmannia* and *Apteronotus* to detect low frequency beats of their EODs could explain the steep minima in their threshold-frequency curves. As described earlier in Results, a sine wave stimulus frequency, F_1, will interact with the EOD fundamental and harmonics, F_2, to produce beat frequencies equal to $|F_1 - F_2|$. A stimulus frequency which approaches a fish's EOD fundamental or second harmonic to within ca. 50–100 Hz will have the distinguishing characteristic of causing low frequency beats of the fish's EOD. An interneuron in series with the high frequency electroreceptors could respond preferentially to the beat-induced, low frequency modulations of the primary receptor activity. In this way, stimulus frequencies as much as 50–100 Hz from the fish's EOD fundamental or second harmonic might be selectively sensed. Measurements of another form of behavior, the jamming avoidance response, on these same species (Bullock *et al.*, 1972) demonstrated a maximum sensitivity to beats of low frequency, with the most effective stimulus being one with a frequency ± 3 Hz of the fish's EOD frequency. Scheich and Bullock (in press) report having found a frequency-difference detecting neuron in the torus of *Eigenmannia* which is maximally excited by a 3 Hz beat. Based upon the above arguments, a beat detecting unit can be predicted for *Apteronotus* as well.

This postulated capability of these fish to sense, preferentially, low frequency beats would provide them with a highly sensitive electroreception mechanism that would be tuned to the nearby EOD frequencies of conspecifics. The sensitivity of this species of *Apteronotus* to beats with its second harmonic may play a role in conspecific recognition, since the EOD frequency range of this species overlaps with that of other species (Hopkins, 1972), whereas its wave form may be usefully different. *Apteronotus* might, therefore, be helped in recognizing conspecific social signals by the beats produced from the large second harmonic compo-

nent of their EODs. The frequency range of *Eigenmannia virescens* does
not overlap with that of other species in its habitat (Bennett and
Steinbach, ALPHA HELIX Amazon Expedition 1967, personal communi-
cation; Hopkins, 1972), which reduces the problem of conspecific recogni-
tion for this species. By detecting changes in beat frequency, *Apteronotus*
and *Eigenmannia* could monitor frequency modulated social signals of
conspecifics. Thus a high sensitivity to voltage increments and an appre-
ciation of low beat frequencies could form the basis of a band-pass
electroreceptive system, capable of picking out social signals from a
background of high amplitude noise.

Water Resistivity and Behavioral Threshold

The behavioral threshold of *Apteronotus* to electric fields is greatly
influenced by water resistivity (Fig. 7). The observed decrease in its
voltage sensitivity with increasing water resistivity can be explained on
purely biophysical grounds. The skin of the fish is a relatively high
impedance layer that surrounds low impedance body fluids (skin to inter-
nal tissue resistance ratio equals 1200:1 to 6400:1 when measured at
1 KHz; Scheich and Bullock, in press). Bennett (1967) has shown that the
electroreceptors respond to a voltage drop across the fish's skin, which
is equal to the difference between the potential external to the receptor
and the fish's nearly equal internal potential. So long as the water around
the fish is of low resistivity, the body of the fish will approximate an
insulator in an electric field: the current lines will tend to circumscribe
the fish, and the lines of equipotential will concentrate at the surface
of its skin. This field distortion intensifies the voltage gradient across
the skin and allows the receptors to function most effectively. As the
water resistivity increases, the fish will begin to load the imposed electric
field. Acting now more like a conductor, current lines will bend toward the
fish's body while equipotential lines will tend to disperse around it. Thus
the field distortion caused by the fish's body will diminish the voltage drop
across its skin. The more the fish loads the field (i.e. the higher the water
resistivity), the greater this effect will be, and the greater the applied
voltage required to achieve a threshold drop across the receptors. For
graphic description of this argument see Kalmijn (in press).

The specific loss of low frequency sensitivity in low resistivity water
may not be a biophysical phenomenon, rather a specific susceptibility of
ampullary receptors to high calcium concentrations. Ampullary fibers in
Ictalurus increase their spontaneous rate of firing and decrease their
response to electrical stimulation when the corresponding receptor is
immersed in high calcium solutions (Roth, 1971). The highest calcium

concentrations used by Roth was 10 mM, which equals the calcium concentration in 600 Ohm·cm water in the experiments reported here. Therefore, the loss of low frequency sensitivity of *Apteronotus* in low resistivity water might be due to a similar decrease in ampullary receptor sensitivity in the presence of high calcium concentrations. Since tuberous receptors are anatomically virtually isolated from the external milieu (Bennett, 1971), they may not be directly affected by external calcium concentrations. As shown (Fig. 7), their threshold probably relates more directly to the biophysical properties effecting the voltage drop across the fish's skin.

The threshold voltage gradient for a 200 Hz field was consistently higher than that for a 1000 Hz field, yet qualitatively these thresholds change in the same manner with respect to water resistivity: the plots exhibit similar slopes and inflection points (Fig. 7). One explanation for the enhanced sensitivity to the 1000 Hz field over the 200 Hz field, is that low frequency amplitude modulations, resulting from the interaction of the 1000 Hz stimulus field with the fish's EOD, are sensed by the low frequency, ampullary receptors. If this were the case, however, the discrepancy between the 1000 Hz and 200 Hz threshold values should have disappeared as the ampullary receptors lost sensitivity in low resistivity water. A second, more plausible explanation is that the same population of receptors (the tuberous type) is solely responsible for sensing both signal frequencies, and that the lower threshold to the high frequency field reflects the filter properties of the tuberous receptors. Neurophysiological evidence from tuberous electroreceptors in *Apteronotus* support the latter proposal (Hopkins, personal communication).

Thus, it appears that *Apteronotus* senses low frequency voltage fields most efficiently in approximately 10 k Ohm·cm water (0.6 μV/cm), while its sensitivity to higher frequency voltage fields tends to improve as water resistivity decreases, at least down to 1.0 k Ohm·cm. Water resistivities normally experienced by *Apteronotus* in nature, range from a low of 2 k Ohm·cm during the dry season to a high of 100 k Ohm·cm during the rainy season. An average of 6–15 k Ohm·cm, however, is typical (Hopkins, 1972). Therefore, the water resistivities, which this fish usually encounters, coincide with the water resistivity range of optimal ampullary function, while tuberous receptors must operate at less than maximal efficiency.

Beyond its ethological importance, the demonstrated variation of the behavioral response thresholds with different water resistivities points up an important consideration in threshold measurements. Most previous measurements on other electric fish have been reported without mention of this very influential factor. This makes evaluation of these studies difficult and limits the scope of interspecific comparisons.

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