ELECTROSENSORY STIMULUS-INTENSITY THRESHOLDS IN THE WEAKLY ELECTRIC KNIFEFISH *EIGENMANNIA*: REDUCED SENSITIVITY AT HARMONICS OF ITS OWN ORGAN DISCHARGE

IVO KAUNZINGER AND BERND KRAMER

Zoological Institute of the University, D-93040 Regensburg, Germany

Summary

The South American knifefish *Eigenmannia* sp. can detect the electric organ discharges (EODs; 250–600 Hz) of conspecifics when they are superimposed over its own EOD. This study investigates the minimum frequency difference necessary for such signal perception, using the application of sine-wave stimuli. Electrosensory stimulus-intensity thresholds were determined for trained fish using stimuli associated with food rewards. These sine-wave stimuli were 'clamped' to the EOD frequency of the fish. Electrosensory thresholds were also determined for the spontaneous jamming avoidance response (JAR; a change in EOD frequency evoked by a stimulus of sufficiently similar frequency), in this case using unclamped stimuli.

Over the wide frequency range investigated (0.3–3.01 times EOD frequency), the lowest stimulus-intensity thresholds of $0.6 \, \mu\mathrm{V} \, \mathrm{cm}^{-1}$ (peak-to-peak) (0 dB) at a water conductivity of $100 \, \mu\mathrm{S} \, \mathrm{cm}^{-1}$ were found close to (but not exactly at) the EOD fundamental frequency. At exact frequency identity between the EOD and the stimulus, the stimulus-intensity response threshold rose abruptly by more than 10 dB compared with slightly higher or lower stimulus frequencies. A similar 'needle-like' threshold increase was found at exactly two and three times the EOD frequency, but neither at harmonic ratios between stimulus and EOD frequency that represent fractions (e.g. at 5:4=1.25, 4:3=1.33, 3:2=1.5 or 5:3=1.67 times EOD frequency) nor at subharmonics such as half or two-thirds of the EOD frequency. The steepest increase of stimulus-intensity response threshold was in the range 0.998–1.002 times EOD frequency, corresponding to a threshold change, or electrosensory filter slope, of 5000 dB per octave.

For the spontaneous JAR, a similar stimulus-intensity threshold increase was observed when EOD frequency equalled stimulus frequency. Because of the longer rise time for the stimulus amplitude (400 ms rather than 35 ms) the stimulus intensity threshold was higher (up to 32 dB; mean, 20 dB) than in the other experiments (up to 15 dB; mean, 13 dB).

A difference in frequency between the EOD and the applied stimulus as small as 1 Hz (that is, 0.2 % of the EOD frequency) was sufficient for good signal perception in *Eigenmannia* sp. The JAR appears to be useful in avoiding insensitivity at exact integer harmonics of the EOD frequency.

Key words: electroreception, *Eigenmannia* sp., beat analysis, jamming avoidance response, stimulus-intensity threshold, electric organ discharge.

Introduction

The electric organ discharge (EOD) of the South American knifefish *Eigenmannia* sp. is an a.c. wave signal of constant frequency and amplitude, ranging from 250 to 600 Hz (for an oscillogram of the EOD, see Kramer and Otto, 1991, p. 3). Its functions are active electrolocation (see review by Bastian, 1990) and communication (Hagedorn, 1986; Kramer, 1990, 1994, 1995). Any external electric stimulus will therefore be perceived by a fish superimposed over its own EOD. In Sternopygidae, the family which includes the genus *Eigenmannia*, the tuberous electroreceptors are of the P- and T-types (Scheich *et al.* 1973; see reviews by Zakon, 1988; Bastian, 1990).

The superimposed stimulus periodically modulates the EOD 'carrier' signal in its zero-crossings times (that is, the times at which the EOD potential crosses the zero baseline) and amplitude at the frequency difference $\Delta F = F_{\mathrm{EOD}} - F_{\mathrm{stimulus}}$ (for a discussion of the beat physics, see, for example, Scheich, 1977*a*). In *Sternopygus macrurus*, another member of the family Sternopygidae, Fleishman *et al.* (1992) found that the threshold intensity increased by up to 10 dB when a stimulus was applied that exactly matched the EOD frequency, i.e. beats were no longer present in the signal perceived by the fish. Similar results have been obtained for *Eigenmannia* sp.

(Kaunzinger and Kramer, 1993). A lesion of the pacemaker nucleus of the central nervous system (i.e. the fish were made electrically 'silent') caused the threshold intensity to increase by 30 dB in *S. macrurus* (Fleishman *et al.* 1992).

In the present paper, we investigated the following features of *Eigenmannia*. (1) In contrast to *S. macrurus*, *Eigenmannia* species react with a jamming avoidance response (JAR) to a stimulus frequency close to their EOD frequency or its higher harmonics (Watanabe and Takeda, 1963; Bullock *et al.* 1972; Kramer, 1985). Are the stimulus-intensity threshold values obtained from fish trained using food rewards associated with a stimulus comparable to values obtained for the spontaneous JAR? (2) For stimulus frequencies at twice the EOD frequency, Fleishman *et al.* (1992) found only a weak increase in threshold intensity and, for stimulus frequencies of three times EOD frequency, no increase in stimulus frequency compared with those slightly above or below the harmonic was observed. This is surprising, because a signal will apparently vanish, not only at the same frequency as the EOD, but also at all higher harmonics of the EOD. Therefore, if the threshold for stimulus-intensity increases when stimulus frequencies equal the EOD frequency, the same should hold true for stimuli at twice and three times the EOD frequency.

Any errors in initial EOD frequency measurement or due to drifting of the stimulus or EOD frequency during stimulation will magnify a possible frequency mismatch by a factor of two for the second harmonic, a factor of three for the third harmonic, and so on. Therefore, we investigated the stimulus-intensity response thresholds for different frequencies (and EOD frequency harmonics) using stimuli that were precisely frequency-clamped to the EOD.

Materials and methods

Fish ($N=9$, 11–20 cm long) were obtained from a commercial tropical fish dealer who had imported them direct from Colombia, South America; they had not been used in training tests before. They were kept isolated in aquaria at $27\pm1\,°C$ and with a photoperiod of 12 h:12 h L:D. The water conductivity was $100\pm3\,\mu S\,cm^{-1}$. The EOD of a fish was recorded head-to-tail with a pair of vertically oriented carbon-rod electrodes (length, 30 cm; diameter, 6 mm; insulated with plastic except for 4 cm at the tips; distance of electrodes from each other, 70 cm). Another pair of carbon-rod electrodes (of similar design) transmitted a sine-wave electrical stimulus into the water; they were placed at a right angle and centred to the EOD recording electrodes, in order to prevent cross-talk when recording and stimulating simultaneously (distance of electrodes from each other, 37 cm).

Threshold electric field intensities were measured using a small silver-ball dipole (ball diameter, 1 mm; distance from each other, 10 mm) and a differential amplifier, after removing the test fish from the tank. Field intensity was measured half way between the two stimulus electrodes. Because of the unfavourable signal-to-noise ratio in the range of interest, the field intensity was raised by up to 60 dB and values were

extrapolated back to the original threshold levels, after establishing that the system worked linearly.

Intensity thresholds for conditioned fish (stimulus frequency-clamped)

Tank size was 75 cm $\times42$ cm$\times40$ cm. Training of the fish ($N=3$, 16–20 cm long) and definition of a threshold were as described by in Kramer and Kaunzinger (1991). During daylight, the fish usually remained in the shelter provided, a porous pot (length, 20 cm; inner diameter, 6 cm) placed parallel to the long axis of the aquarium with the rear end closed by the glass side of the aquarium. An electrical stimulus was used to indicate the presentation of a food reward (a single *Chironomus* larva). In order to obtain the reward, fish had to swim through a channel of plastic mesh (length, 50 cm; diameter, 6 cm) to the opposite end of the aquarium, where the reward was offered in a small glass Petri dish. The stimulus (duration, up to 20 s) was only presented when the fish was resting in its tube, with its head facing the channel. The interval between two successive trials was at least 90 s. A fish that swam to the feeding station without stimulation was 'punished' using a few air bubbles delivered through a small glass tube or, when this was ineffective, by gentle prodding (without touching) with a metal rod.

The frequency of the amplified EOD (differential a.c. amplifier, 1 Hz to 10 kHz; gain, $\times100$), was averaged over 12–15 EOD cycles using a counter board (Messcomp Co., model A1210; 10 MHz clock frequency) and an IBM-compatible computer. The selected ratio between the EOD frequency and the stimulus frequency was held constant using a custom-designed computer program (G. Stöckl, electronics workshop of the Biology Department). The output frequency of the sine-wave generator (Brüel & Kjær, model 1049; frequency jitter $<0.0025\,\%$), connected to the computer by digital interface (IEEE 488), was corrected approximately eight times per second. The single-ended sine-wave signal passed a manually operated step attenuator (Hewlett Packard, model 350D) and was made symmetrical ($+/-$) and isolated from earth by a custom-built transformer (frequency range transmitted, 100 Hz to 18 kHz). Stimulus intensity and the ratio between the EOD and stimulus frequencies were selected before experiments began. Rise and fall times of the stimulus amplitude were 35 ms. The application of the stimulus was computer-controlled.

Stimulus-intensity responses were determined to ±1 dB using the 'staircase' procedure (see Kramer and Kaunzinger, 1991, p. 46). A threshold response was defined, by interpolation between neighbouring decibel steps, as occurring at stimulus intensities in response to which the fish swam in 70 % of the trials to the feeding station to take the food. At each intensity level in the threshold range, at least 10 trials were performed. For fish nos 1 and 2, 42 frequency ratios, and for fish no. 3, 15 frequency ratios, were investigated over a range 0.3–3.01 times EOD frequency. At frequencies between 0.99 and 1.01 times EOD frequency, fish nos 1 and 2 were tested at six and eight additional frequency ratios, respectively. The effects of the

applied frequency ratio on the threshold intensities were tested using a one-way analysis of variance (ANOVA). Where significant, an *a-posteriori* Student–Newman–Keuls test (N-K-test) was used in order to identify pairs of frequency ratios that were significantly different.

Intensity thresholds of the jamming avoidance response (stimulus unclamped)

The experimental tank ($52\,cm \times 25\,cm \times 28\,cm$) was placed in a larger tank ($80\,cm \times 50\,cm \times 50\,cm$) with its water electrically earthed in order to minimize external electrical noise. A shelter (a porous pot open at one side with holes bored on the other for better water circulation; length, 15 cm; inner diameter, 3.2 cm; wall thickness, 4 mm) was positioned in the centre of the test tank. During trials, the fish ($N=6$, 11 cm long) were held inside the shelter by closing the entrance with plastic mesh. At other times, the fish were freed and were fed on *Chironomus* larvae.

For experiments, fish were selected that gave a good JAR to stimuli of unclamped frequency differences (ΔF values) of -1, 0 and $+1$ Hz ($\Delta F = F_{EOD} - F_{stimulus}$). For experimental details, see Kramer (1987). In this study, no locomotory behaviour was analyzed; only EOD frequency changes were of interest.

In both initial and subsequent test trials, the EOD baseline-frequency (F_{EOD}) was taken as the mean of 120 measurements in the 60 s preceding the stimulus onset. The frequency of the amplified EOD was measured using a Hewlett Packard model 5308A counter (accuracy, 0.0004 %). The applied stimuli were of constant frequency (not clamped to the EOD), in order to allow a fish actively to alter the frequency difference (ΔF), and lasted for 60 s. At each ΔF and each intensity level, 10 trials were carried out; inter-trial interval was 20 min in order to exclude habituation. The stimulus sine-wave was generated by a Hewlett Packard model 3325A synthesizer (frequency jitter, ± 0.0005 %) and had 400 ms rise/fall times for the stimulus amplitude. The stimulus passed an attenuator (HP 350D) and a transformer (details given above) before being transmitted to two vertically oriented carbon rod electrodes, 14 cm apart. Recording the EOD frequency, controlling and adjusting the stimulus frequency, and storage and analysis of data were all automatically performed by a microcomputer using custom-designed software (B. Kramer).

JAR threshold was defined as the lowest stimulus-intensity level that evoked a statistically significant deviation from resting EOD frequency levels within 40–60 s after stimulus onset (two-tailed paired *t*-tests; $P<0.01$). A one-way ANOVA was performed in order to investigate whether the frequency difference, ΔF, significantly influenced JAR threshold level; where significant, subsequent comparisons between certain pairs of sample means were performed using the N-K-test.

Results

Threshold intensities for frequency-clamped stimuli in conditioned fish

Performance of a conditioned behaviour (i.e. approach and capture of a food reward) clearly depended on the ratio

between the EOD and stimulus frequencies: threshold intensities were lowest close to the EOD frequency of a fish (Fig. 1A; lowest threshold intensity, $0.6\,\mu V\,cm^{-1}$ peak-to-peak, p–p). However, when stimulus frequency was exactly equal to EOD frequency, threshold intensities increased up to 12–14 dB compared with slightly higher or lower frequency ratios. For example, when stimulated with 0.99 times F_{EOD} or 1.01 times F_{EOD}, fish no. 1 had extremely low threshold intensity values of approximately $0.6\,\mu V_{p–p}\,cm^{-1}$; however, at a stimulus frequency of 1.00 times F_{EOD}, threshold intensity increased by 14 dB to $3.0\,\mu V_{p–p}\,cm^{-1}$. At lower and higher frequencies than these, threshold intensities increased. A similar 'needle-like' pattern was found for two and three times the EOD frequency.

The ratio between stimulus and EOD frequency had an extremely significant effect on threshold intensity as shown by a one-way ANOVA for the pooled results for all three fish ($F=14.50$; $P<0.0001$; d.f.=44). For all three EOD harmonics studied (the fundamental, the second and the third harmonic), threshold intensities were significantly higher at frequency ratios corresponding to the harmonics than at ratios slightly higher or lower than the harmonics (e.g. $P<0.005$ for the comparison of the thresholds for 0.99 times F_{EOD} and 1.00 times F_{EOD}; N-K-test). At subharmonic ratios, such as at half and two-thirds of the EOD frequency, and harmonic ratios above the fundamental that represent fractions such as major third (5:4 ratio), fourth (4:3 ratio), fifth (3:2 ratio) and major sixth (5:3 ratio), no reduced sensitivity was observed, although 'standing waves' similar to those found at one, two and three times the EOD frequency were observed on the oscilloscope during experiments at these ratios.

The sharp increase in threshold intensity at EOD frequency was confirmed by performing additional trials (Fig. 1B). Threshold intensity increased by a maximum of 5000 dB per octave (10 dB at 0.2 % of an octave) between 0.998 times F_{EOD} and 1.002 times F_{EOD}. In a fish discharging at 500 Hz, this corresponds to a frequency difference between the EOD and the applied stimulus of only 1 Hz. The base of the 'needle-like' threshold intensity increase was approximately 10 Hz wide.

In most cases, the difference between intensities for 'no response' and 100 % response of a fish to a stimulus was only a few decibels. For example, at 0.9 times F_{EOD} and 3 dB amplification (re: $0.6\,\mu V_{p–p}\,cm^{-1}$), fish no. 2 gave 17 % 'correct' responses; this increased to 42 % at 4 dB, 75 % at 5 dB and 89 % at 6 dB.

In Fig. 1C, the threshold curve for *Eigenmannia* sp. is compared with that for *Sternopygus macrurus* (taken from Fleishmann *et al.*, 1992).

JAR thresholds for unclamped stimuli

Threshold intensities of the JAR were determined using stimuli of a constant, unclamped frequency close to, or precisely at, the EOD resting frequency of an individual fish ($\Delta F=0$, $+1$ and -1 Hz). The test fish were previously selected for a good JAR at all three ΔF values. [A JAR at $\Delta F=0$ Hz was first described by Kramer (1987) for half of the juvenile fish
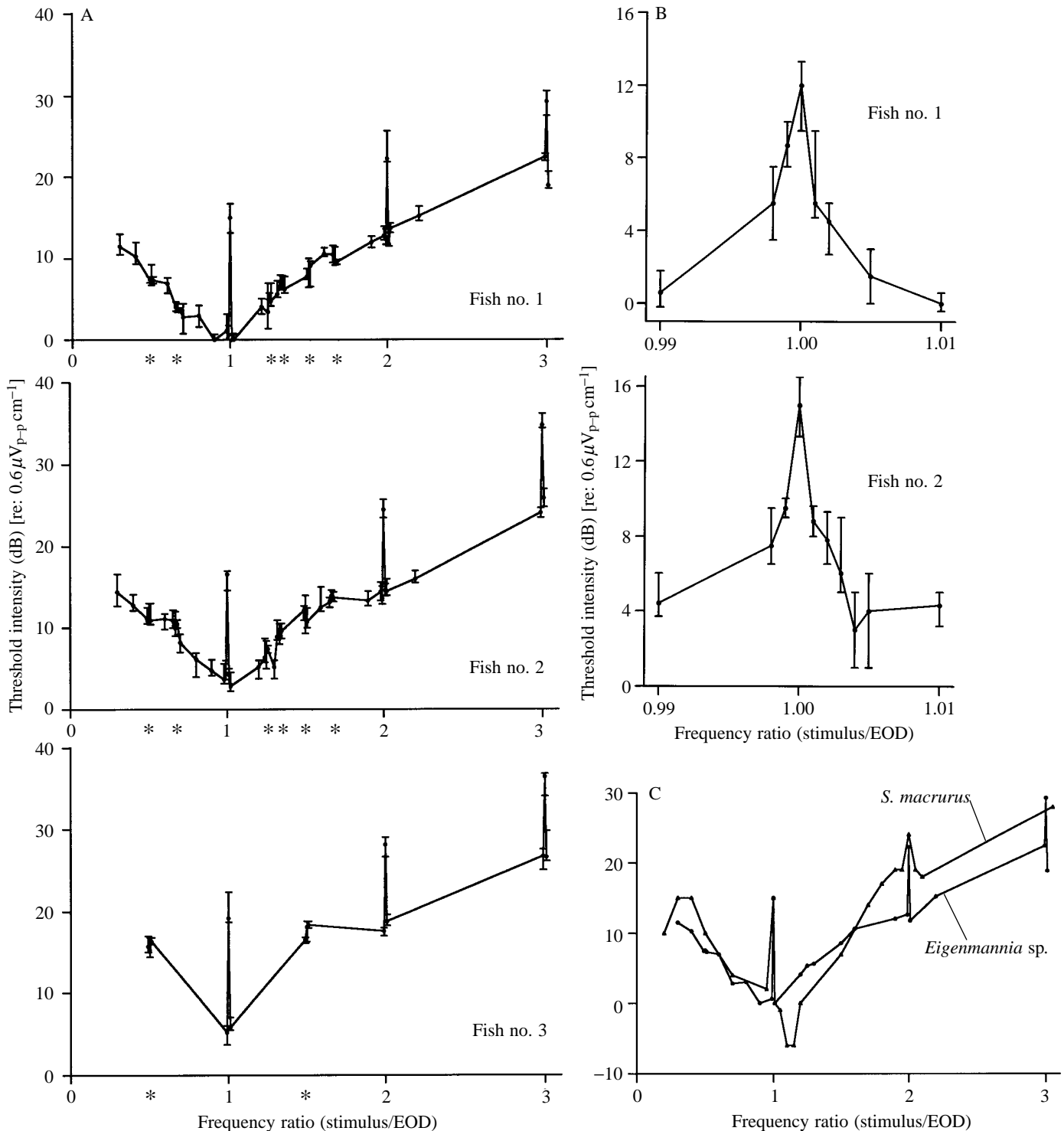
Fig. 1. (A) The dependency of electrosensory thresholds on stimulus frequency in food-rewarded *Eigenmannia* sp. Ordinate, threshold intensity of an applied stimulus at which 70 % of trials resulted in a fish responding (see Materials and methods). Abscissa, ratio of the applied stimulus frequency to the EOD frequency (the stimulus was frequency-clamped to the EOD). $N > 10$ for each data point. Note that for all three fish there is a 'needle-like' threshold intensity increase at the harmonics of the EOD frequency (first to third), but not for subharmonics (half or two-thirds of the EOD frequency), nor for 1.25, 1.33, 1.50 or 1.67 times EOD frequency (see asterisks). The EOD frequency of all three fish tested was approximately 500 Hz. Ranges at which 50–90 % of the trials resulted in a response are shown as 'error bars' (B) Additional results for frequency ratios close to 1.0. (C) Comparison of threshold curves for *Eigenmannia* sp. (fish no. 1 from A; circles) and *Sternopygus macrurus* (triangles) using unclamped stimulation (Fleishman *et al.* 1992; data taken from their Figs 2B and 3).
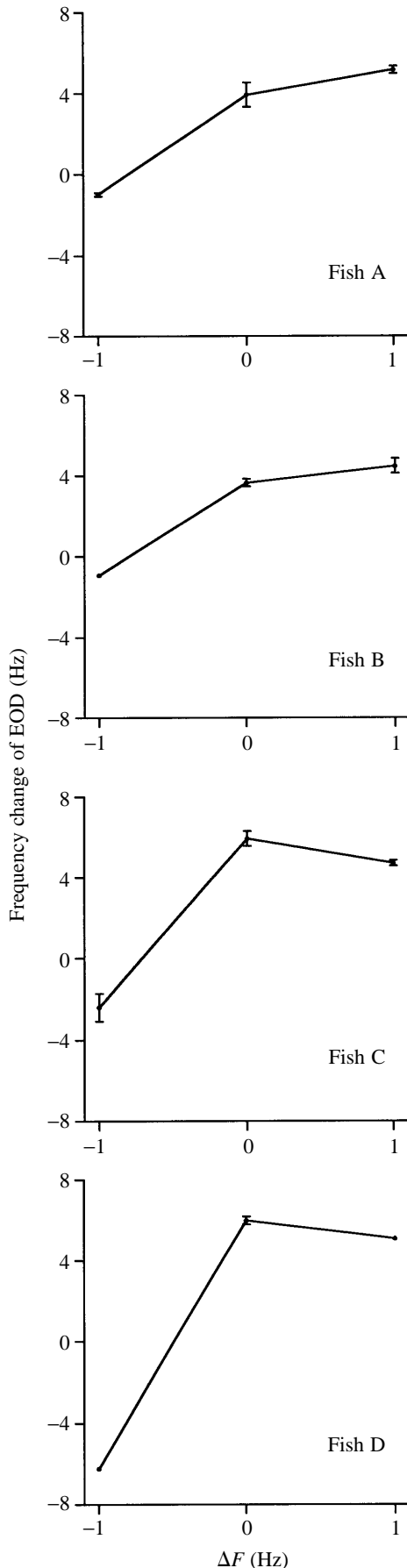
Fig. 2. The frequency change (jamming avoidance response, JAR) in *Eigenmannia* sp. evoked by unclamped electrical sine-wave stimulation at a frequency F_{stim}. The EOD frequency F_{EOD} before stimulation changes as a function of ΔF where $\Delta F = F_{EOD} - F_{stim}$. Stimulus intensity, $80\,\mu V_{p-p}\,cm^{-1}$. Each point is the mean of 10 trials ($\pm$S.E.M.). Note the clear frequency increase at $\Delta F = 0\,Hz$ and $+1\,Hz$ compared with that at $-1\,Hz$.

in his study. Without establishing a JAR at $\Delta F = 0\,Hz$ for each individual, further experiments could not have been performed.]

Fish reduced their EOD frequencies at a stimulus frequency of $\Delta F = -1\,Hz$ (stimulus frequency 1 Hz greater than EOD frequency) and raised their EOD frequency at ΔF values of 0 Hz and +1 Hz (Fig. 2). The strengths of the JARs at $\Delta F = 0$ and +1 Hz were similar; in absolute terms, these JARs were stronger than those for $\Delta F = -1\,Hz$, except in fish D where the frequency change was -6 and $+6\,Hz$ for ΔF values of -1 and 0 Hz, respectively. ΔF had a significant effect on the frequency change of the JAR in all fish combined ($F > 33$; $P < 0.0001$; d.f.$=29$; one-way ANOVA).

At $\Delta F = 0\,Hz$, threshold intensities were, on average, 20 dB above those for a ΔF of $\pm 1\,Hz$ (Fig. 3). Mean threshold intensities ($\pm$ S.E.M.) were $25.07 \pm 11.33\,\mu V_{p-p}\,cm^{-1}$ for $\Delta F = 0\,Hz$, $2.46 \pm 1.03\,\mu V_{p-p}\,cm^{-1}$ for $\Delta F = -1\,Hz$ and $1.27 \pm 0.17\,\mu V_{p-p}\,cm^{-1}$ for $\Delta F = +1\,Hz$; threshold intensity depended significantly on ΔF ($F = 15.154$; $P < 0.0003$; d.f.$=17$; one-way ANOVA). In each individual, threshold intensities at $\Delta F = 0\,Hz$ were significantly higher than at -1 and $+1\,Hz$ ($P < 0.001$; N-K-test), but there was no significant difference between threshold intensities at $\Delta F = -1$ and $+1\,Hz$.

Intensity relationship between EOD and threshold stimulus

The field intensity of a stimulus at lowest threshold levels was approximately $1\,\mu V_{p-p}\,cm^{-1}$ (see Figs 1A, 3, and Kramer and Kaunzinger, 1991). A small silver-ball electrode dipole (electrode distance, 10 mm; see Materials and methods) was used to measure the local EOD field intensity 1 mm from the skin of a fish of typical EOD intensity. Water temperature was $26\,^{\circ}C$; conductivity, $100\,\mu S\,cm^{-1}$. The fish was firmly fixed in a silk sock and the electrode dipole oriented normal to its skin. Between the eye and the origin of the anal fin, which is the region of highest electroreceptor density, the field intensity of the EOD was constant: $3\,mV_{p-p}\,cm^{-1}$.

A stimulus of threshold intensity ($1\,\mu V_{p-p}\,cm^{-1}$) thus modulates the EOD by 0.033 % in intensity in the lateral head region. In order to assess the attenuation of the intensity of the field generated by *Eigenmannia* with distance, the fish was replaced by a dipole connected to a sine-wave generator. The dipole ('model fish') consisted of a pair of vertically oriented carbon-rod electrodes (diameter, 0.5 cm; length, 1.0 cm; separation, 7.8 cm) mounted on a Perspex tube. The dipole was placed close to one end of a large aquarium ($240\,cm \times 50\,cm \times 60\,cm$), in parallel with its long axis. An attenuation of the output field intensity as measured with the silver-ball electrode pair next to the 'model fish' (at 1 mm
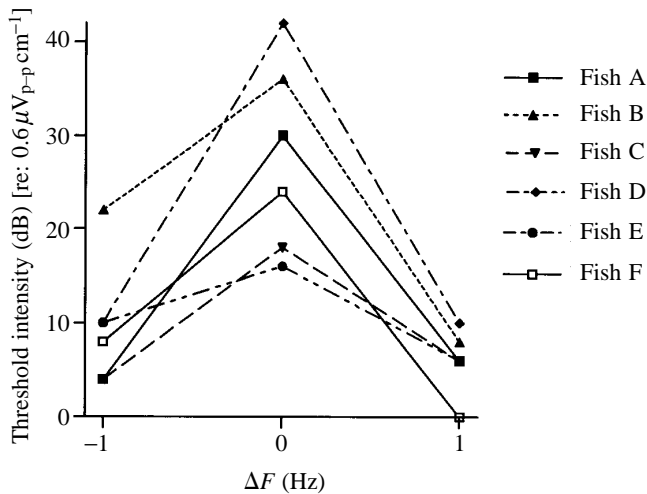
Fig. 3. Stimulus intensity for the JAR threshold depends on ΔF. Threshold intensity values are for statistically significant JARs evoked by unclamped sine waves of constant frequency (for each point, $N=10$, $t>3.36$, $P<0.01$; two-tailed, paired t-test). At frequency identity ($\Delta F=0$ Hz), threshold intensity was significantly higher than at $\Delta F=-1$ Hz and $+1$ Hz ($P<0.001$; one-way ANOVA, N-K-tests).

distance from the electrode facing the far end of the aquarium) of 0.033 % was measured at a distance of 44 cm from the dipole model, following the long axis of the aquarium and taking the output and measuring electrodes facing each other as a reference. ('Model fish' electrodes and measuring silver-ball electrodes were aligned with each other and the long axis of the aquarium.)

Using an equation given by Rose and Heiligenberg (1985) and Carr *et al.* (1986), the above result was used to calculate the peak-to-peak modulation in the zero-crossings time of an EOD superimposed on an electrical signal of $\Delta F \neq 0$ Hz: $D_T=aT/\pi$, where D_T is the maximal time disparity, a is the amplitude ratio between stimulus and EOD and T is the duration of an EOD period. For a 500 Hz fish, we calculated a peak-to-peak modulation of 0.212 μs in zero-crossings time at threshold stimulus intensity. This is in good agreement with previously reported threshold time modulation values for evoking a JAR (Rose and Heiligenberg, 1985; Carr *et al.* 1986).

Discussion

The lowest threshold intensities measured for *Eigenmannia* sp. in this study were observed at stimulus frequencies close to, but definitely not exactly at, the EOD frequencies of the fish (these vary between individuals from 250 to 600 Hz). Like *Sternopygus macrurus* (Fleishman *et al.* 1992), *Eigenmannia* sp. uses its own EOD as a reference for perceiving other electrical signals. Fleishman *et al.* (1992) reported threshold intensities of 0.3–0.6 μV_{p-p} cm^{-1} at a stimulus frequency 10 Hz above EOD frequency and a water conductivity of 700 μS cm^{-1}; the lowest thresholds in the present study on *Eigenmannia* sp. were in the same range, 0.6, 0.9 and

1.1 μV_{p-p} cm^{-1}, in spite of a lower (more natural) conductivity of 100 μS cm^{-1}.

For *Eigenmannia* sp., we found a similar 'needle-like' increase in threshold intensity for frequencies at three times the EOD frequency (the third harmonic) similar to those found for the first and second harmonics. For the first harmonic, the results for *Eigenmannia* sp. and *Sternopygus macrurus* (Fleishman *et al.* 1992) are virtually identical (Fig. 1C). For higher harmonics, however, only the present study found no reduction in the typical 'needle-like' threshold increase. There is a simple explanation for this difference: although *S. macrurus* apparently lacks a JAR (Bullock *et al.* 1975; Matsubara and Heiligenberg, 1978), its EOD 'drifts' (B. Kramer, personal observation), as does any signal source. Any error in the initial measurement of the resting frequency, or frequency drift (of EOD or stimulus) during stimulation, will increase the frequency mismatch between stimulus and EOD harmonic twofold for the second harmonic, threefold for the third harmonic, and so on. Bullock *et al.* (1972), confirmed by Kramer (1985), have demonstrated a constant 'optimum' ΔF for the JAR for all higher harmonics (up to at least the fifth). We believe this is the reason for the apparent fading in the 'needle-like' threshold increase at higher harmonics in *S. macrurus* (Fleishman *et al.* 1992); for the third harmonic, these authors investigated only one point.

Stimulation at 0.5 times EOD frequency or other harmonic ratios, such as the fourth (stimulus frequency=4:3 times EOD frequency) or fifth (3:2 ratio), would result in a pattern where every second or third cycle was identical (seen as 'standing waves', as found for stimulation at integer harmonics). Because the fishes' sensitivity at these frequencies (which were not integer harmonics) was not reduced compared with neighbouring stimulus frequencies (Fig. 1A), this pattern was, apparently, transmitted by the receptors (see, Scheich 1977*b*).

The ratio between the stimulus intensity and that of the EOD was 1/3000 at threshold levels. A similar 0.033 % reduction in the EOD field strength was measured at a distance of 44 cm from the signal source in a rostro-caudal direction; this communication range is much less than that given by Knudsen (1975; approximately 100 cm for a 18.6 cm long *Eigenmannia virescens* in 500 μS cm^{-1} water). This difference is all the more surprising because a high water conductivity, as measured by Knudsen, should severely limit the radius of electrocommunication (see Squire and Moller, 1982).

Results obtained for the spontaneous JAR (using unclamped stimuli; Fig. 3) are similar to our results for trained animals. However, at $\Delta F=0$ Hz, the sensitivity decrease observed in the JAR was more pronounced (up to 32 dB) than for the conditioned behaviour (maximum, 15 dB). The reason for this difference is likely to be the longer rise time of the stimulus in the JAR tests (400 ms *versus* 35 ms in the conditioned tests): a longer rise time will result in adaptation of the electroreceptors and, hence, higher threshold intensities. However, the similarity of our results in both experiments suggests a common mechanism of signal detection. The food-rewarded tests showed 'symmetrical' threshold intensities about the EOD frequency;

JAR threshold intensities, however, seemed to be lower for stimuli 1 Hz above EOD frequency than 1 Hz below it.

According to some authors (see review by Heiligenberg, 1991), *Eigenmannia* sp. does not show a JAR at $\Delta F=0$ Hz. However, this has previously been contradicted (Kramer, 1987), and the existence of a JAR at $\Delta F=0$ Hz is also confirmed in the present study (Fig. 2). The uniform behaviour of our rather small (11 cm), juvenile individuals was noteworthy: for all fish ($N=6$) a positive JAR (frequency increase) was observed with 'unclamped' stimuli at $\Delta F=0$ Hz and a negative JAR (a frequency decrease) at $\Delta F=-1$ Hz (Fig. 3). Thus, the 'change-over' point occurs at negative ΔF values in all our fish. As noted previously by Kramer (1987), at some value of ΔF between 0 Hz and -1 Hz, an individual fish either did not respond or produced equal numbers of positive and negative responses. In this context, the frequency discrimination threshold (frequency difference limen) of 0.6 Hz (Kramer, 1987; Kramer and Kaunzinger, 1991) certainly plays an important role. *Eigenmannia* sp. must avoid frequency identity between its own EOD and that of a stimulus, in order successfully to assess the waveform of the stimulus (Kramer and Otto, 1991) and the frequency difference (see review by Kramer, 1995).

The behaviourally determined 'tuning' curves in our study resemble those of T-receptors more so than those of P-receptors (1) because they are 'V'-shaped rather than more broad-band patterns established for P-receptors, and (2) because T-receptors are more sensitive (by up to 30 dB) than P-receptors (Hopkins, 1976; Fleishman, 1992). P-receptors encode intensity, while T-receptors precisely mark the time of zero-crossings of the received a.c. signal. Tuberous electroreceptors respond to stimuli at twice the EOD frequency only at considerably increased intensity (Hopkins, 1976; Viancour, 1979). Unlike our behavioural 'tuning' curve, not all tuberous electroreceptors are most sensitive close to the EOD frequency; Hopkins (1976) describes a remarkable scatter of 'best' frequencies for P-receptors and Viancour (1979) for tuberous receptors of presumably both kinds, although he made no discrimination, in *Eigenmannia virescens*.

The behavioural response for an intact fish (present study) was evoked at 100–1000-fold lower stimulus intensity than was found to be the threshold intensity of single electroreceptor organs (Hopkins, 1976; Fleishman, 1992; Fleishman *et al.* 1992). Such detection of stimulus intensities far below single receptor thresholds is evidence for the detection and analysis not of the stimulus *per se* but by its effects on modulation of the EOD.

The strategy of signal detection in *Eigenmannia* sp., *Sternopygus macrurus* (Fleishman *et al.* 1992) and probably in other species is obvious. The permanent presence of its own EOD, which is a potential drawback because of signal-masking, is turned into an advantage by the use of specialised beat analysis; the threshold intensities measured in this study are not absolute but are relative, i.e. they are modulation thresholds. Beat analysis is an elegant solution to the problem of signal-masking, but does not work exactly at the EOD frequency or at its higher harmonics. In this case, in *Eigenmannia*, the JAR is of use, making beat analysis independent even of this constraint.

In our experiments, results obtained for $\Delta F=0$ Hz (exact identity between EOD and stimulus frequency) showed greater scatter than results for higher and lower frequencies. A possible reason for this increased scatter could be a dependency of the threshold intensity on stimulus phase (relative to the phase of an EOD cycle; as noted by Kramer, 1987). We will address this problem in our next study.

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