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SIGNAL ANALYSIS IN THE COMMUNICATION OF A WEAKLY ELECTRIC FISH, *EIGENMANNIA VIRESCENS* (GYMNOTIFORMES)

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ABSTRACT

Eigenmannia virescens is a South American freshwater species with an electric organ discharge (EOD) of the wave type. This social fish's EOD is masked by the intense noise from close-by conspecifics. In spite of this, *Eigenmannia* detects external signals at lowest thresholds for a vertebrate octavolateralis sensory system; furthermore, *Eigenmannia* discriminates between stimulus waveforms which is unknown for mechanical sensory modalities. It is suggested that an analysis of beat signals in three steps underlies these sensory feats: (1) stimulus filtering and intensity assessment of a single harmonic of an external signal, in spite of only weak tuning of the high-frequency part of the electrosensory system. (2) Assessment of the frequency difference between a fish's own EOD and that of an external signal. It is shown that temporal cues of the mixed (beat) signal are sufficient, and that beat amplitude cues are not required; nor do they seem to be used by the fish. Steps 1 and 2 completed, a fish may change its EOD frequency (e.g., by a jamming avoidance response) for optimizing (3) the assessment of stimulus waveform from the beat signal, such as external female and male EODs. Sensory models for the assessment of frequency difference and stimulus waveform are presented.

1. Introduction

1.1 *Wave-Type Knifefish Discriminate Between Electrical Waveforms*

Knifefishes (Gymnotiformes) from South American freshwaters generate and sense electric fields. Under central nervous control, electric organs generate a dipole field that is detected by cutaneous electroreceptor organs distributed over wide parts of the skin (reviews on electric organs, Bennett, 1971; Bass, 1986; on electroreceptors, Szabo, 1974; Szabo & Fessard, 1974; Zakon, 1988). Electroreceptor organs form part of these fishes' octavolateralis system, and their afferents are connected to huge, specialized brain areas (reviews, Szabo, 1967; Carr & Maler, 1986; Carr, 1990). These fishes' co-adapted system of electric organ and electroreceptors forms an active electrolocation system (Lissmann & Machin, 1958), making possible a nocturnal life (reviews, Bastian, 1990; 1994).

A second function of the electric system of gymnotiforms is communication (Black-Cleworth, 1970; Hopkins, 1974; Westby, 1975; reviews, Hagedorn, 1986; Kramer, 1990; 1994; 1996). With their three kinds of electroreceptor organs that are specialized in sensitivity and frequency range detected, knifefishes also sense the discharges of other knifefish that are much weaker for a local electroreceptor than the

discharges of its 'own' electric organ. Sensory aspects of communication is the subject of the present paper.

Most of the about 120 or so species of knifefishes discharge their electric organs in a wave-like fashion (Kramer, 1990), generating signals that are exceptionally stable in amplitude and frequency (Bullock, 1970). A strong minority of species generate series of brief pulses, like the well-known electric eel that is both strong and weak electric. Pulse species are not dealt with in this chapter (for reviews, see Hagedorn, 1986; Kramer, 1990, 1996).
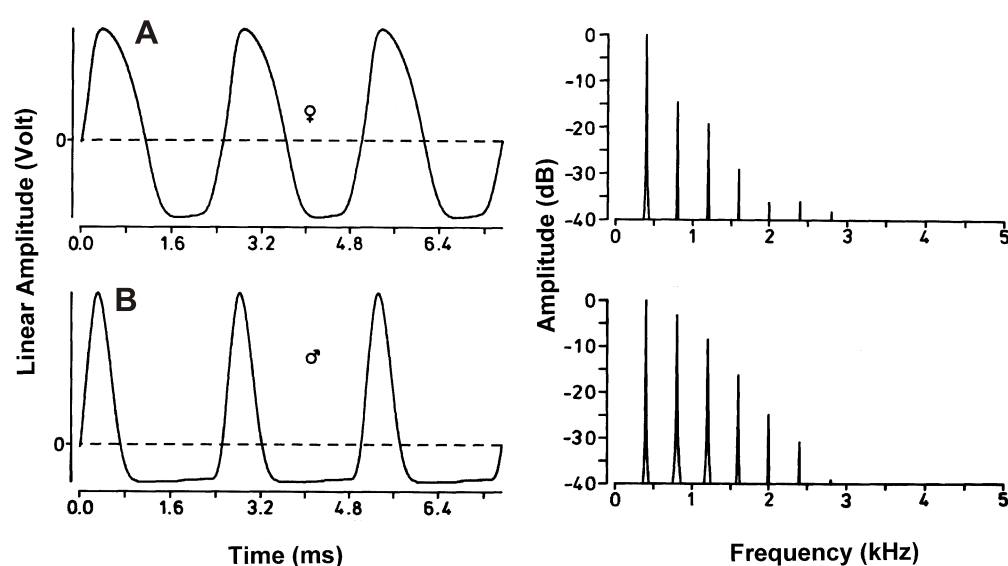

Fig. 1 Waveforms (left) of electric organ discharges in *Eigenmannia*, with their associated Fourier amplitude spectra (right). The ordinates of the left diagrams are arbitrary linear amplitudes (V), of the right diagrams amplitudes expressed as dB attenuation relative to the strongest spectral component of each waveform (which is the first harmonic or fundamental in both cases). (A) female; (B) male. Note the almost sinusoidal waveform of female EOD, the higher harmonics of which are of much weaker amplitude than in the male EOD, whose waveform deviates markedly from a sinusoid (Kramer and Otto 1991).

Wave-type knifefishes of the families Apteronotidae and Sternopygidae (Mago-Leccia, 1994) generate electric organ discharges (EODs) that are species-characteristic, differing widely in waveform (Kramer *et al.*, 1981a; Kramer, 1990, Figs 3.14, 3.18-3.20). Even members of the same species may differ in EOD waveform, as exemplified by *Eigenmannia virescens* (=*lineata*; Planquette *et al.*,

1996). EODs of juveniles and females resemble a weakly distorted sine wave whereas the EOD of males is strongly distorted, with pronounced asymmetry in time and amplitude (Fig. 1; Kramer, 1985). As shown by Fourier analysis, male EODs have stronger overtones (or harmonics of the fundamental frequency) than female EODs. In this regard, female EODs resemble the signal generated by a flute, and male EODs that generated by a violin with its greater "brilliance" due to stronger overtones.

During their nocturnal courtship and spawning (Hagedorn & Heiligenberg, 1986), fish probably analyse and assess EODs for finding a suitable mate. Knifefishes have keen hearing but are not known to produce sound other than that generated by fin movements (von Frisch, 1938; Kramer *et al.*, 1981b; Popper *et al.*, 1988).

Experimental work has confirmed that *Eigenmannia* discriminates between electronically generated, electric signals of different waveforms, including synthetic, conspecific male and female EODs (Kramer & Zupanc, 1986; Kramer & Weymann, 1987). Untrained and unrewarded fish of both sexes demonstrated significant preferences when two signals were played back simultaneously, such as a spontaneous preference for the female rather than the male EOD (Kramer & Otto, 1988). The pairs of signals between which the fish discriminated all differed in both waveform and amplitude spectrum; therefore, it was impossible to decide whether *Eigenmannia* uses temporal waveform or rather spectral amplitude cues (time domain *vs.* frequency domain analysis). In the human, sensitivity for differences in spectral amplitudes (timbre or tone quality) is the basis for discriminating between different voices or musical instruments.

For determining whether an animal discriminates between two stimuli, conditioned discrimination is the method of choice (von Frisch, 1967). Other methods used in the course of the present study include the simultaneous playback of two signals, symmetrically on an untrained, unrewarded fish's right and left, and observing its spontaneous preference as shown by differential approach of signal sources. A further method consisted in determining the strength of jamming avoidance response (JAR) to stimuli of different waveforms. Without any training, juvenile *Eigenmannia* usually lower their discharge frequency to stimuli of slightly higher frequency, and raise their discharge frequency to stimuli of slightly lower frequency (Watanabe & Takeda, 1963; Bullock *et al.*, 1972a,b), whereas especially adult fish may respond differently or not at all (Kramer, 1987).

1.2 Signal Masking By Autostimulation?

In certain insect and frog choruses neighbouring males advertise in alternation, thus minimizing the masking of their vocalizations as received by distant females (Greenfield & Roizen, 1993; Narins, 1995). In contrast to pulse gymnotiforms with their long pauses compared to the short duration of pulses per EOD cycle, the signal

generated by wave fish is always 'on'. Therefore, weakly electric wave fish appear to be insensitive to the signals of conspecifics by masking due to autostimulation - perhaps an inevitable consequence of an adaptation for high electrolocation performance?

This line of thinking was proven wrong by (1) *Eigenmannia*'s low electrosensory threshold to external signals (Bullock *et al.*, 1972a,b; Knudsen, 1974; Kaunzinger & Kramer, 1995) that is about the same compared to pulse species; (2) difference thresholds for frequency and amplitude of an electric stimulus are among the lowest for an octavolateralis sensory system in vertebrates (Kramer & Kaunzinger, 1991). The masking hypothesis was also most clearly refuted in (3) experiments using *Sternopygus macrurus* and *Eigenmannia virescens* (the wave EOD of *Sternopygus* is of lower frequency but otherwise similar to that of *Eigenmannia*):

After silencing the electric organ by destroying the pacemaker nucleus in *Sternopygus*' hindbrain, trained fish were, paradoxically, less sensitive to electrical stimuli by about 30 dB (Fleishman *et al.*, 1992). A loss of sensitivity was also observed in intact *Sternopygus* when the stimulus frequency exactly equalled their EOD frequency. In both experimental settings fish do not experience beats; in fish with a destroyed pacemaker, because their own discharge had been turned off permanently, in intact fish because beats vanish at frequency identity of two signals. Therefore, a wave knifefish appears to sense external signals by their beating against its own 'carrier' EOD that raises weak external signals into the working range of high-frequency (tuberous) electroreceptors.

This idea is supported by the observation of a marked threshold increase when intact fish were stimulated at exactly two times EOD frequency; no increase, however, was observed at three times EOD frequency (Fleishman *et al.*, 1992). If correct, under the above hypothesis we expect a threshold increase at all integer multiples of the EOD frequency, without decrement of the threshold increase at higher harmonics because beats are absent at all of them. This expectation was confirmed in *Eigenmannia* under more rigorous control of stimulus frequency by the use of a frequency clamp and a phase-locking device, maintaining a fixed frequency relationship even when fish showed a JAR (Fig. 2; Kaunzinger & Kramer, 1995). The threshold increase observed at any of *Eigenmannia*'s EOD harmonics investigated (up to the third) is within an extremely narrow, 'needle-like' range of frequencies with filter slopes of up to 5000 dB/octave, resembling a highly selective 'stop-band' filter unmatched by electronic devices. Therefore, even very slow beats (small differences between EOD and stimulus frequency) are detected by the fish. The transition between the sensation 'beat present' and 'beat absent' is an abrupt one like with a switch.

Eigenmannia senses external signals as their beating against its own EOD, therefore, effective sensory and motor mechanisms are required to control beat frequency. For example, at frequency identity (beat frequency too low) fish are
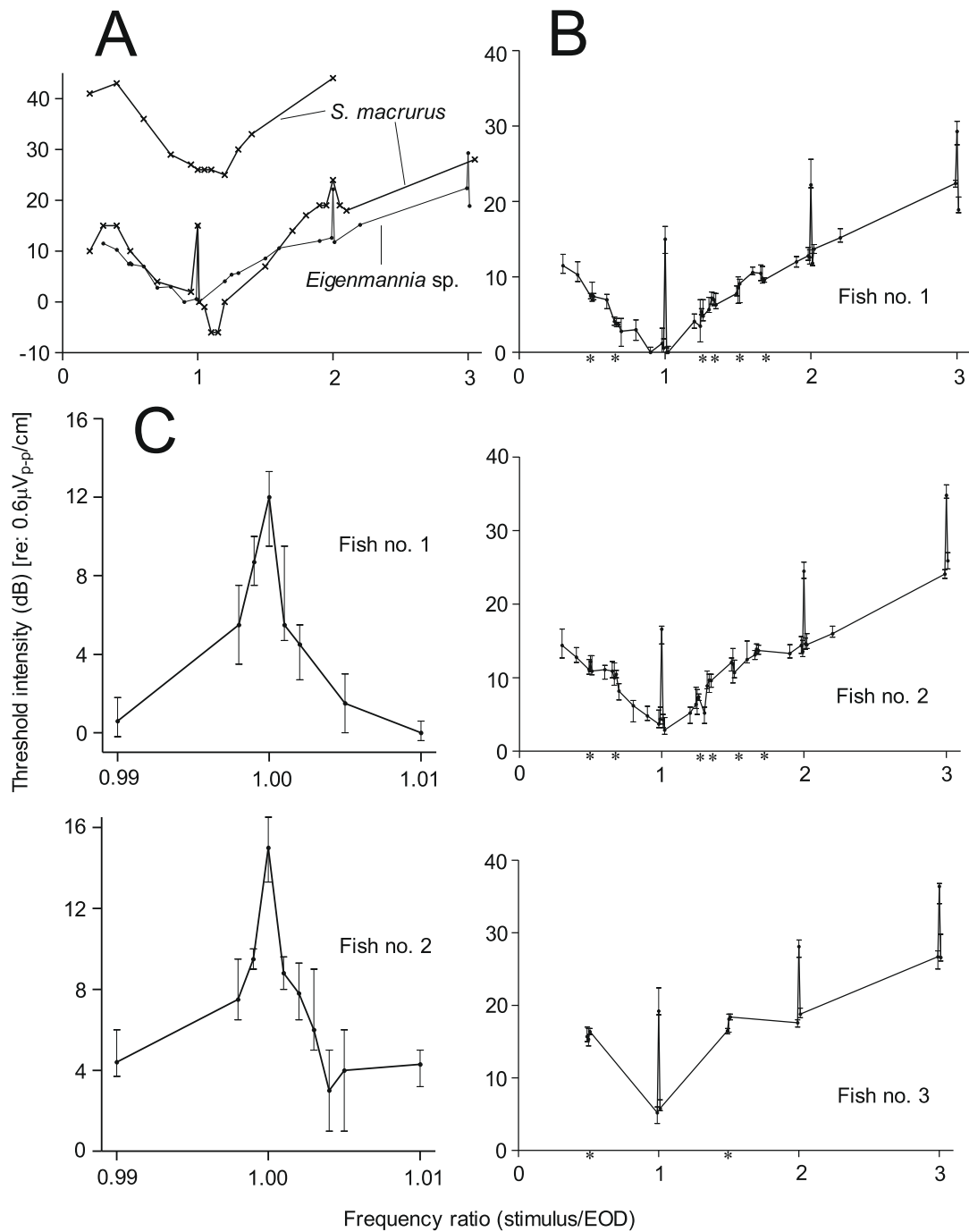
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Fig. 2 The electrosensory threshold of food-rewarded fish depends on the stimulus frequency. *Ordinates* threshold intensity of an applied stimulus at which 70% of trials resulted in a fish responding. *Abscissae* ratio of the applied stimulus frequency to the EOD frequency. All graphs refer to *Eigenmannia* sp. where the stimulus was frequency-clamped to the fish's EOD, except in (A) where also data for the related, similar fish *Sternopygus macrurus* are given and where the stimulus was *not*

frequency-clamped. (A) Good agreement of electrosensory threshold curves of *Eigenmannia* with those for *Sternopygus macrurus* (Fleishman *et al.* 1992). Uppermost curve, *S. macrurus* that had been "electrically silenced" by a brain lesion. (B) Three different *Eigenmannia* individuals are most sensitive to frequencies close to their individual EOD frequencies, however, a marked sensitivity decrease is observed when the stimulus frequency exactly equals the EOD frequency (frequency ratio = 1). This insensitivity occurs also at 2x and 3x the EOD frequency, but not for subharmonics (half or two-thirds of the EOD frequency), nor for harmonic ratios above the fundamental that represent fractions such as major third (5:4 ratio) etc., also associated with "standing wave" patterns (*asterisks*, at 1.25, 1.33, 1.50 and 1.67 times EOD frequency). 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". (C) Additional results for frequency ratios close to 1.0 show the steep threshold increase in greater detail. *Sternopygus* data in (A) from Fleishman *et al.* (1992) read off their Fig. 2B; all other data from Kaunzinger and Kramer (1995).

insensitive, and fail to discriminate between stimulus waveforms (see Section 4). By adapting its own EOD frequency to that of an external signal, for example, by performing a JAR, a fish can enhance its sensitivity and resolution for waveforms. As will be shown in the next sections, beat analysis is in three steps: (1) stimulus filtering and intensity assessment; (2) assessment of the frequency difference between a stimulus and the EOD, $\Delta f = f_{EOD} - f_{stimulus}$; (3) assessment of stimulus waveform.

2. Stimulus Filtering and Intensity Assessment

Eigenmannia's behaviourally determined electrosensory threshold-frequency curve is broadly $\vee$-shaped, with the 'best' frequency (lowest threshold) close (Knudsen, 1974), but not identical to (Kaunzinger & Kramer, 1995) a fish's individual EOD frequency (see similar results in *Sternopygus*; Fleishman *et al.*, 1992). One type of tuberous electroreceptor organ, the sensitive T receptor, shows similar tuning. This receptor behaves like a broad bandpass filter with a slope of about 20 dB on its 'high' side and even less on its 'low' side (Scheich *et al.*, 1973; Hopkins, 1976; Viancour, 1979; Fleishman, 1992). The second type of tuberous electroreceptor organ, P, is less sensitive by about 30 dB than the T type and also less clearly tuned; therefore, it is not thought to determine threshold.

Surprisingly, this only weakly tuned sensory system is capable of extracting from a stimulus the intensity of a single harmonic with high precision: that harmonic which is closest in frequency to the EOD fundamental, f_1. Efficient signal filtering became most evident when subharmonic stimuli of different waveforms were used to evoke the JAR the strength of which was the response criterion.

According to Bullock *et al.* (1972a,b) varying the waveform of a stimulus had "very little effect" on the JAR with stimuli close to EOD frequency ($\Delta 1 f$ stimuli), however, close to ½ EOD frequency ($\Delta \frac{1}{2} f$ stimuli), the sine wave was ineffective whereas the sawtooth wave still was. The assumption of Bullock *et al.* (1972a,b) that the strong second harmonic of the sawtooth was the reason for its effectiveness as a $\Delta \frac{1}{2} f$ stimulus was found correct by Kramer (1985), whereas the idea of waveform

being irrelevant in $\Delta 1f$ stimuli could not be confirmed. For example, as a $\Delta 1f$ stimulus, synthesized female EODs are more effective than male EODs (at same peak-to-peak amplitudes), however, as a $\Delta \frac{1}{2}f$ stimulus it is the male EOD with its stronger f_2 that is the much more effective stimulus. Like the sine wave, the square wave is ineffective as a $\Delta \frac{1}{2}f$ stimulus; this is because the square wave lacks all even harmonics, in contrast to the sawtooth with its complete series of higher harmonics. However, as a $\Delta \frac{1}{3}f$ stimulus the square wave is more effective than the sawtooth because the f_3 harmonic of the sawtooth is weaker than that of the square wave (equal peak-to-peak amplitudes; Kramer, 1985).

For any stimulus waveform, JAR strength was proportional to the logarithm of effective harmonic intensity (f_1 in a $\Delta 1f$ stimulus, f_2 in a $\Delta \frac{1}{2}f$ stimulus, and so on), following the Weber-Fechner Law of psychophysics. Any two stimuli of different waveform (including those of identical amplitude spectrum) evoked the same JAR strength when signal peak-to-peak amplitudes were adjusted such that the intensities of their effective harmonics were the same (Kramer, 1985).

For assessing signal intensity that determines JAR strength, fish ignore all but a single signal harmonic the intensity of which is determined at high precision. Waveform information is not gained at this step of signal analysis, on the contrary, it is rejected.

3. Assessment of the Frequency Difference

Also required for determining the strength of JAR is the frequency difference between a stimulus and the EOD, $\Delta f = f_{EOD} - f_{stimulus}$. An *Eigenmannia* will respond by a JAR when the stimulus frequency is within about ±20 Hz of its EOD frequency, or one of its higher harmonics. The most effective Δf is around ±4 Hz in a frequency-clamped stimulus (Bullock *et al.*, 1972a,b), however, in a free-running stimulus of constant frequency, the smaller the (initial) Δf the more effective the stimulus. Even $\Delta f = 0$ Hz is effective in many individuals (Kramer, 1987; Kaunzinger & Kramer, 1995, 1996).

An especially intriguing question is 'how does a fish determine the sign of Δf?' which it is obviously capable of doing judging from its JAR behaviour. The Δf range of uncertainty about which direction - frequency increase or decrease - a JAR would take varies only within ±0.3 Hz around a set point close to, but in some individuals distinctly different from, $\Delta f = 0$ Hz (up to 1 Hz; Kramer, 1987).

3.1. Previous Concepts

Scheich and colleagues worked out the sensory physiology involved, and analysed the physics of the beat signal (Scheich *et al.*, 1973; Scheich & Bullock, 1974; Scheich, 1977a-c). These authors concluded that P and T receptors follow the amplitude and phase modulations associated with beats, respectively, and that Δf is determined by detection of the asymmetries of the beat envelope that are caused by

the higher harmonics present in an *Eigenmannia*'s EOD. These asymmetries are time-asymmetric mirror images for $+$ and $-\Delta f$.

Heiligenberg *et al.* (1978), however, showed that higher harmonics are not required since when a silenced fish's EOD was replaced by a sine wave (using a so-called 'stomach electrode') it still performed correct JARs (as determined by the frequency of a pacemaker-derived signal). Heiligenberg *et al.* (1978)'s theory (e.g., review 1991) suggests that for determining the sign and magnitude of Δf, fish follow the Lissajous principle that was already favoured by Watanabe & Takeda (1963). The joint representation of beat amplitude modulation (P receptors) and beat phase modulation (T receptors) in an amplitude-phase state plane yields clockwise or counterclockwise rotating graphs, according to the sign of Δf. Neural correlates were found, and the theory became widely accepted. In Heiligenberg's view the JAR is the first vertebrate behaviour that is entirely understood, from receptors to motor output.

However, the theory never addressed several conflicting observations, theoretical and experimental. How can a behaviour be said to be fully understood when its function is still unclear (a new function is proposed in Section 4). The proposed supporting function for electrolocation performance in the presence of jamming noise from conspecific EODs is not entirely convincing given the very high intensities that are necessary to 'jam' a fish (Heiligenberg, 1977, Fig. 34; Matsubara & Heiligenberg, 1978), and probably unnatural. These high intensities contrast with the very low threshold of the JAR (around 1 µV/cm) that is identical to electrosensory threshold (stimulus intensity, 1/1000th of EOD intensity). An electrolocation function of the JAR is also difficult to reconcile with the JAR's great variability between individuals, and strong habituation. Adult males show virtually no JAR at all, and adult females usually in one direction only; only some of the juveniles behave approximately as expected under an electrolocation function hypothesis (Kramer, 1987).

The above observation that electrosensory threshold and the threshold for the JAR are the same also represents an obstacle of a more principal nature, because it is widely (if not universally) accepted that electrosensory threshold to stimuli of about EOD frequency is determined by the more sensitive T, not P, receptors (e.g., Sanchez & Zakon, 1990). According to theory, JAR threshold cannot be lower than that of the less sensitive receptor.

A further obstacle not explained by the theory is the experimental observation of juvenile individuals performing strong JARs to unclamped stimuli of $\Delta f=0$ Hz. Even when the stimulus was frequency-clamped and phase-locked to a fish's EOD, such $\Delta f=0$ Hz stimuli (square-wave) evoked JARs (Kramer, 1987). These results were confirmed and expanded using sine-wave stimuli (Kaunzinger & Kramer, 1996; see below). At $\Delta f=0$ Hz, theory predicts no JAR because the critical cues, amplitude and phase modulation in a beat signal, are absent.
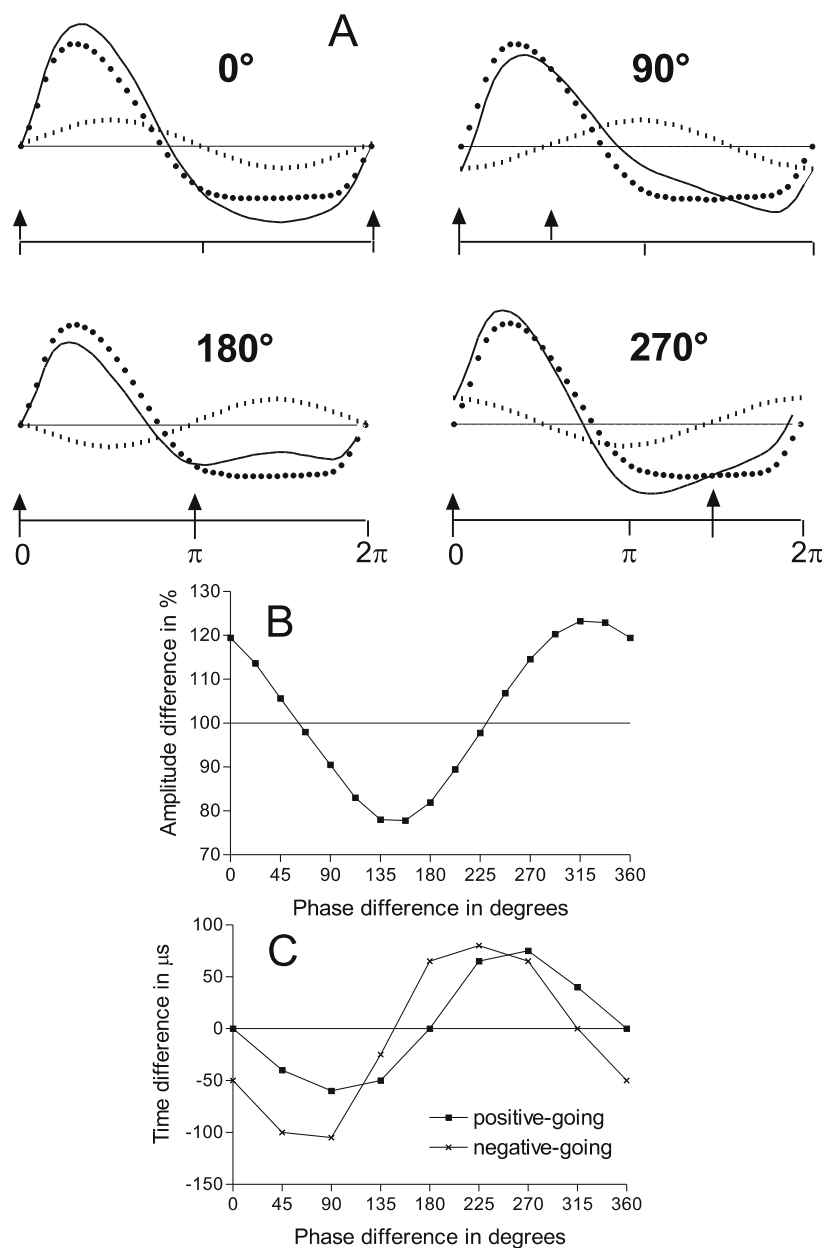
3.2 New Concept

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Fig. 3 Stimulation of *Eigenmannia* with a sine wave of exact EOD frequency (maintained by a frequency-clamp set at $\Delta f = 0$ Hz), as a function of the phase difference between EOD and stimulus (a preset phase difference was maintained dynamically constant). (A) Definition of the phase difference between EOD (*dots,* 100% amplitude, peak-to-peak) and stimulus (*vertical dashes,* 30% amplitude$_{p-p}$). 2π, one EOD cycle (or 2.5 ms in this example). *Arrows* mark the phase difference for four examples in (A), indicating the positive-going zero-crossings of both waveforms. *Line* Additive superimposition of the EOD and the stimulus (complex wave). (B) Amplitude of the complex wave in percent of EOD amplitude, as a function of the phase difference between EOD and stimulus (as detailed in (A)). (C) Time difference in μs between zero-crossings of EOD and complex wave (see (A)), as a function of the phase difference between EOD and stimulus. Curves for both positive- and negative-going zero-crossings of the complex wave are given (Kaunzinger and Kramer, 1996).

Like previous concepts, the new concept builds on our knowledge of primary receptor physiology and beat physics as established especially by Scheich, Bullock and colleagues, and is also in agreement with Hopkins (1976), Viancour (1979), Fleishman (1992), Fleishman *et al.* (1992), Sanchez & Zakon (1990), and others. The
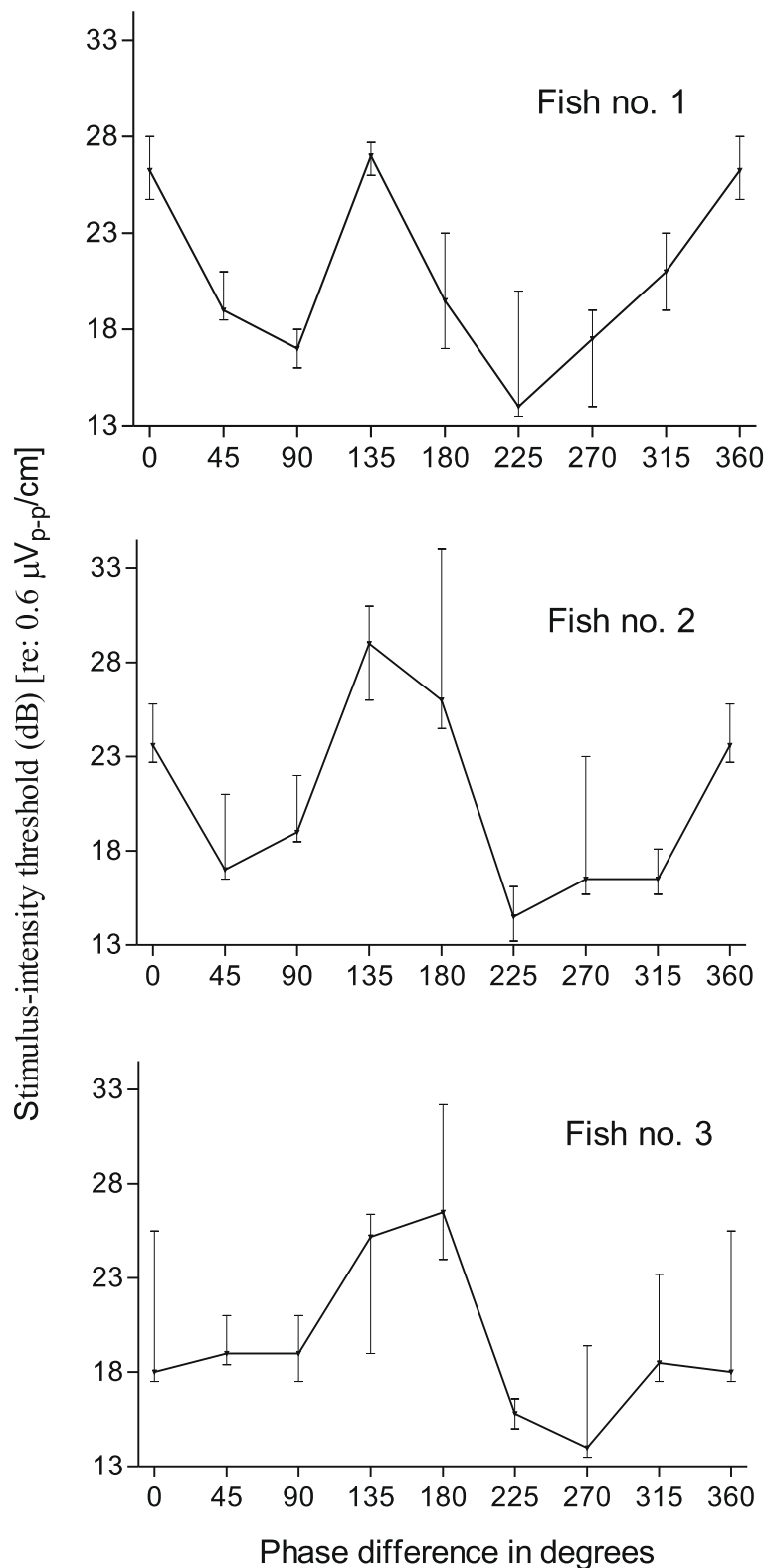
Fig. 4 Electrosensory threshold of three different *Eigenmannia* individuals to sine wave stimuli of exact EOD frequency (frequency-clamped at Δf=0 Hz), as a function of phase difference between EOD and stimulus. *Ordinate* Threshold (defined as 70% probability for a conditioned behaviour in dB [re: 0.6 μV_{p-p}/cm]; *abscissa* phase difference in degrees. "Error bars" show the range from 50% to 90% probability of the conditioned behaviour; *N*>10 for each threshold (Kaunzinger and Kramer, 1996).

new concept differs from previous concepts by suggesting that, at least in the threshold range, beat amplitude cues are not required for Δf assessment. According to the new concept time cues (phase modulation of zero-crossings) that are extracted from a beat signal are sufficient for directing the JAR.

The strongest evidence for this idea comes from work using stimuli that were frequency-clamped to *Eigenmannia*'s EOD at Δf=0 Hz, and also phase-locked at selectable phase

differences (Kaunzinger & Kramer, 1996). The superposition of a strong EOD by a weak stimulus (as seen by a local electroreceptor) is constructive or destructive, depending on the phase difference between the two signals of identical frequency. When peaks coincide the superposition is constructive, and destructive when they alternate; the peak-to-peak amplitude of the mixed signal increases or decreases accordingly. The phase difference between the two signals also affects the times of zero-crossings of the mixed signal as compared to the original EOD: when amplitude change is maximal (either constructive or destructive), there is little or no time or phase change of zero-crossings; however, when phase change is maximal, there is little or no peak-to-peak amplitude change (Fig. 3). We studied the question of whether threshold depends on the phase difference between stimulus and EOD, and, if so, whether lowest thresholds were associated with time shifts in zero-crossings or rather amplitude changes of the mixed signal.

Trained, food-rewarded fish showed significant dependence on phase difference between stimulus and their EOD. Their thresholds were significantly lower when the phase shift of zero-crossings (associated with stimulus onset) was maximal and amplitude change minimal; on the contrary, their thresholds were high when amplitude change was maximal and phase shift minimal (Fig. 4). Similar results were obtained with other fish using supra-threshold stimuli of constant intensity (also phase-locked) to evoke a JAR, and the strength of JAR significantly depended on the phase difference between stimulus and EOD. The similarity of results suggests that amplitude change is irrelevant for signal detection and evoking the JAR even well beyond threshold. Phase change of zero-crossings seems to be the relevant cue, at least in the threshold range (Kaunzinger & Kramer, 1996).

A difficulty of the new concept is ambiguity of the sign of Δf. The advantage of the previous concept was that information about beat envelope amplitude (beat phase) allows one to 'read' Δf correctly from the phase modulation of zero-crossings: when associated with a beat envelope rising in amplitude, zero-crossings lagging relative to those of the uncontaminated EOD indicate a negative sign of Δf (stimulus frequency higher); the sign of Δf is positive when zero-crossings associated with a rising beat envelope are leading (EOD frequency higher). It became widely accepted that for Δf assessment, information about phase modulation of zero-crossings is useful only when paired with beat phase information (reflected by the firing rate of P receptor afferences).

This assertion (which is equivalent to restating the Lissajous principle) is certainly inescapable for symmetrical waveforms used as 'carrier' signals, and the previous concept insisted on using a (symmetrical) sine wave rather than a fish's natural EOD. However, with a natural EOD, beats differ in envelope amplitude for opposite signs of Δf as well as in the fine detail of phase modulation of zero-crossings (Scheich, 1977a-c). Our new concept postulates that, at least in the threshold range, fish extract Δf including its sign from the asymmetries in zero-crossings phase

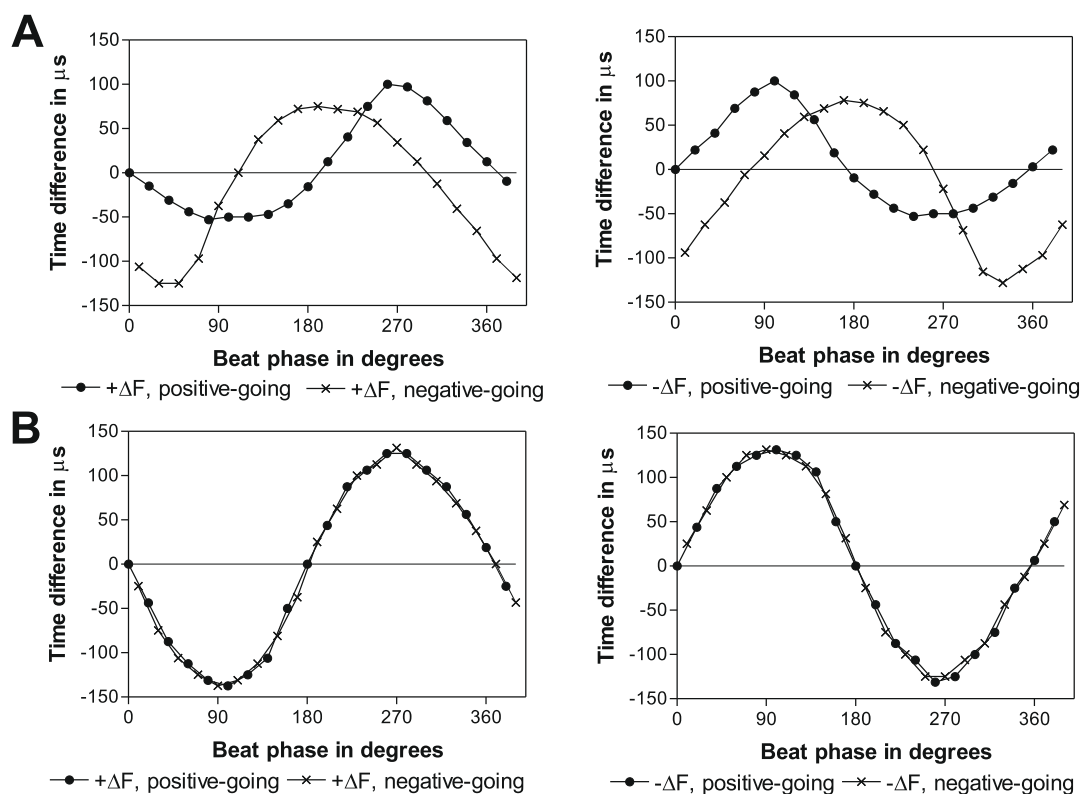modulation alone, without relying on beat amplitude (Fig. 5).

Fig. 5 (A) *Ordinates* Modulation of the zero-crossings times of an *Eigenmannia* wave-EOD by a superimposed sine wave of either plus or minus Δf=22 Hz, at 30% amplitude of the EOD (resolution of ordinates, 6.25 μs). *Abscissae* Time expressed as the phase of one beat cycle (360° corresponding to 1/22 s). *Left* Δf is positive (EOD frequency higher than stimulus frequency); *right* Δf is negative. (●) Positive-going zero-crossings; (×) negative-going zero-crossings of superimposed EOD, as reported by two populations of T electroreceptors (two populations are hypothetical). Note that the modulation patterns of zero-crossings times are characteristically different for identical Δf-values of opposite sign. For an unequivocal Δf-assessment beat-cycle phase information (abscissa) is unnecessary when, such as here, the carrier signal is a natural EOD rather than a sine wave (in this example, a male EOD was used). (B) Like (A), but both carrier and superimposed signals are sine waves; note that modulation patterns of zero-crossings are identical for $+\Delta f$ and $-\Delta f$ except for their phase within a beat cycle. To assess the sign of Δf is not possible in this case and additional information would be required (Kramer and Kaunzinger, in Kramer, 1996).

For this idea to work we obviously need (1) a natural EOD as the 'carrier' signal, although the added external signal may be of any waveform, including the sine wave, and (2), two types of T receptor, one locking onto the positive-going zero-crossings of the mixed signal, the other onto the negative-going zero-crossings (Fig. 6). Scheich (1977a-c) gave evidence for two types of T receptors reporting various
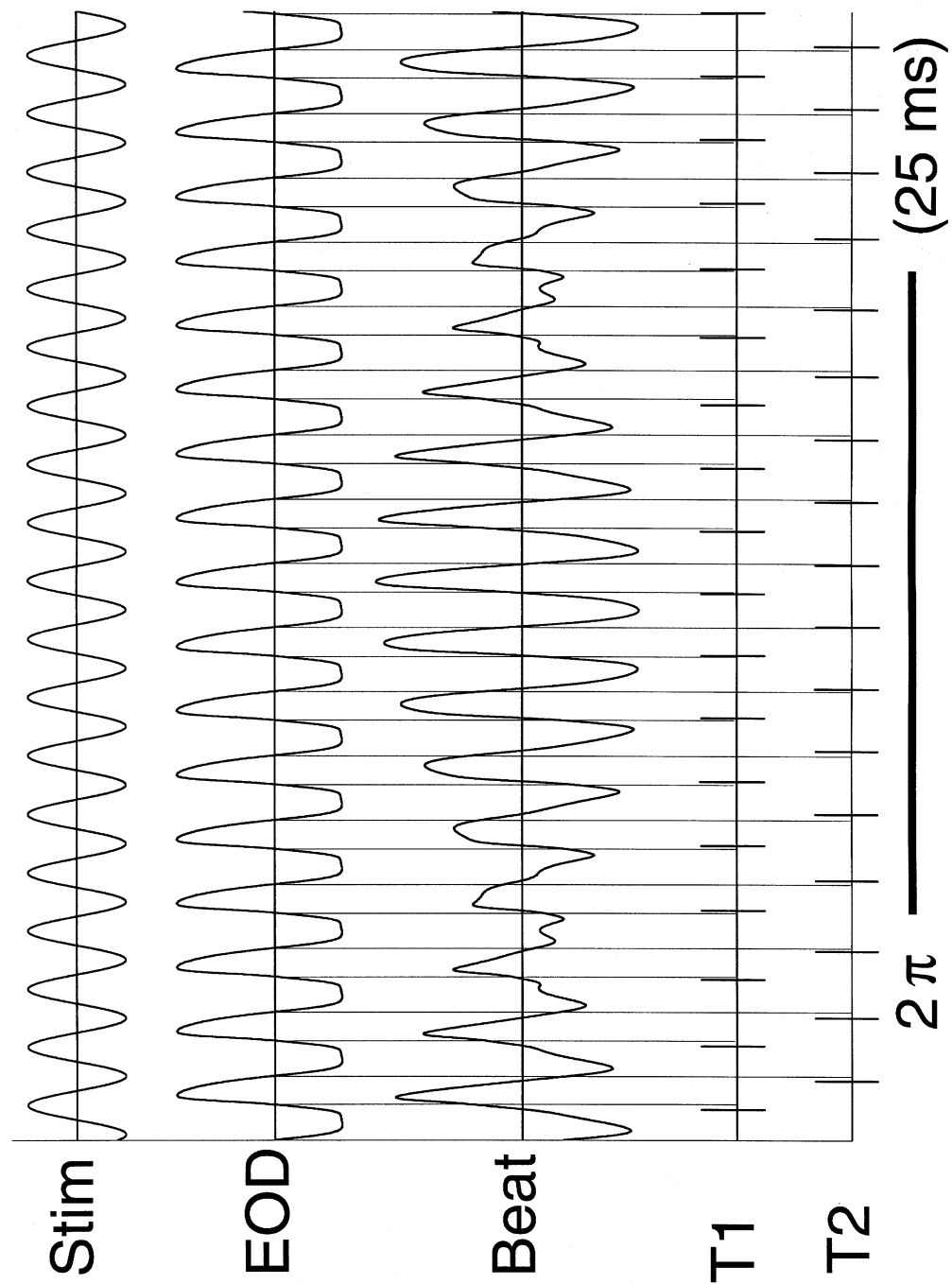
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Fig. 6 *Stim* A sine wave stimulus (of 440 Hz and 60% amplitude) and an *Eigenmannia EOD* (electric organ discharge of a female, of 400 Hz and 100% amplitude) are superimposed (*Beat*, third line). *T1* and *T2* show sensory afferences from T electroreceptors, *T1* responding to positive-going zero-crossings of the superposition signal, *T2* to negative-going ones (two types hypothetical). Note temporal disparities of action potentials with regard to zero-crossings in the original EOD (thin

vertical reference lines). When beat amplitude rises, zero-crossings are lagging, when the amplitude declines, leading with regard to the EOD. At a Δf of 40 Hz (chosen for clarity) the duration of one beat cycle, 2π, is 25 ms comprising 10 EOD cycles; no JAR would be evoked. A more realistic Δf of 4 Hz, however, usually evokes a strong JAR, and the duration of one beat cycle would be 250 ms comprising 100 EOD cycles.

properties of asymmetric beats but this should be reexamined in light of the new concept.

The new concept demonstrates that under these two assumptions, information on phase modulation within beats is sufficient for Δf assessment including its sign, and that beat amplitude information is not required. According to the best of my knowledge, this sensory model is in agreement with all known facts; however, whether fish actually use this model or rather a still different one can only be determined by further experimental research.

4. Assessment of Stimulus Waveform

From the observation that trained, food-rewarded *Eigenmannia* discriminate between male and female EODs, as well as between other waveforms, it is impossible to decide whether fish use temporal waveform cues, or rather spectral cues (Section 1.1). For an octavolateralis sensory system in vertebrates, spectral cues are the 'conventional' ones (for example, in human hearing); temporal waveform cues would represent a new sensory capacity which is, therefore, unlikely. The unlikely possibility is, however, suggested by the astounding diversity in gymnotiform EOD waveforms that must be the result of natural and/or sexual selection, referred to in Section 1.1.

In experiments testing this question, two sine waves one octave apart in frequency (for example, 400 and 800 Hz) were mixed (by additive superposition), at a variable delay of the higher-frequency wave, f_2, relative to the lower-frequency wave, f_1; f_2 was weaker by 3 dB like in an *Eigenmannia* male EOD. One complex waveform was calculated such that the peaks of the two constituent sine waves coincided, the other one with a delay of f_2 peaks relative to those of f_1 by ¼ cycle, or 90˚ (½ a cycle or 180˚ would yield a waveform identical to the former that is inverted). Discrimination by amplitude was excluded by the experimental paradigm.

Trained, food-rewarded fish discriminated between these signals of identical amplitude spectra, hence, identical 'electrosensory timbre' but different waveforms (Kramer & Otto, 1991; Kramer & Teubl, 1993). Fish discriminated also between pairs of waveforms that are much more similar to each other than those referred to above (discrimination threshold, below 22˚ phase shift of f_2 relative to f_1; Kramer & Teubl, 1993). The proposed sensory mechanism for waveform discrimination (Kramer & Otto, 1991) requires a frequency difference between stimulus and EOD; it is shown that the magnitude of the phase modulation of zero-crossings over a beat
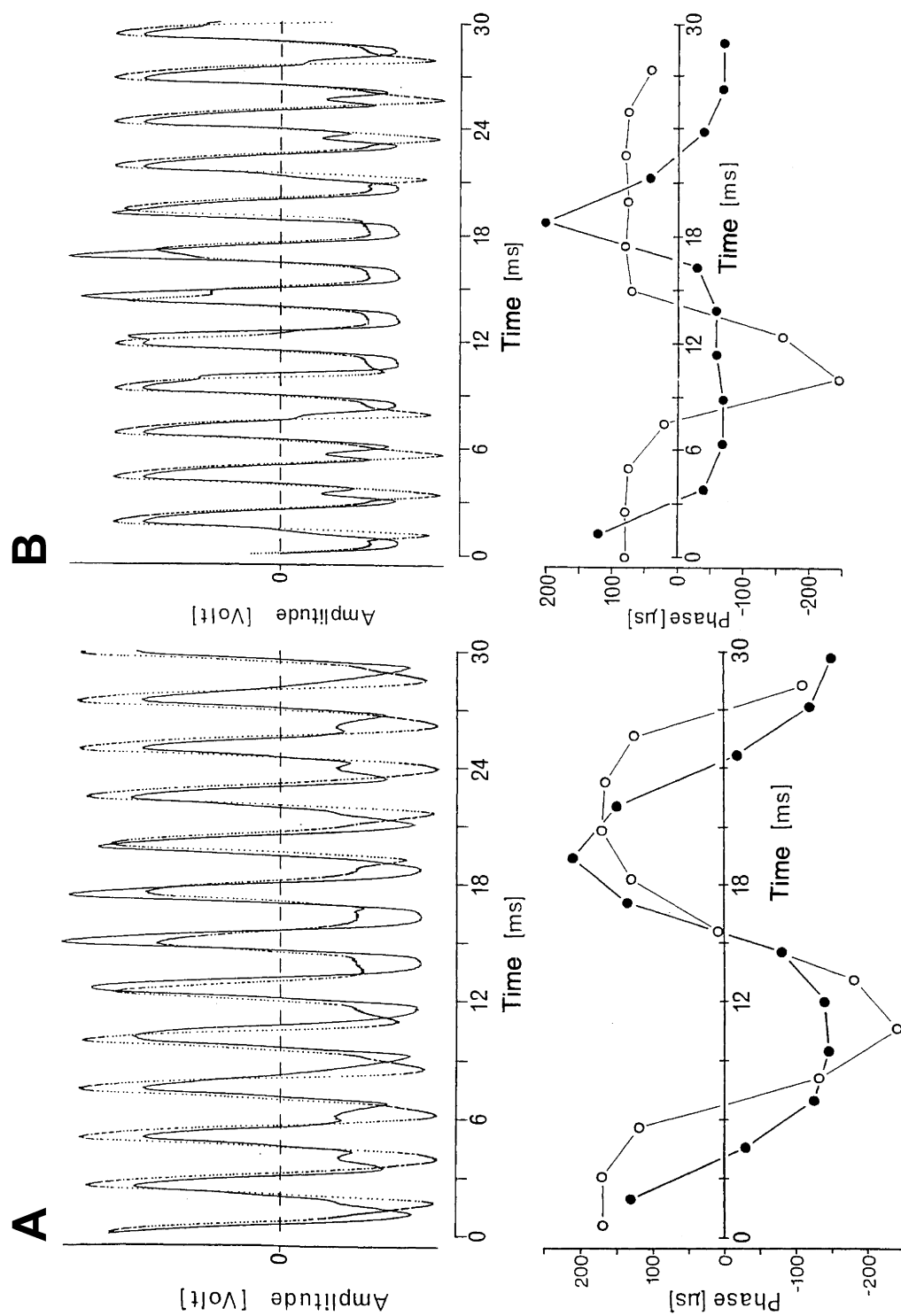
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Fig. 7

Fig. 7 A female *Eigenmannia*'s EOD (400 Hz) superimposed by that of a close-by conspecific (top panels), as probably analysed by the T electroreceptor system (lower panels). (A) The superimposing EOD is that of another female; (B) of a male (both of 30% amplitude and 450 Hz). Top panels, additive superposition shown as lines, subtractive ones dotted (representing the adequate stimuli for local T electroreceptors of right and left body sides). One full beat cycle (20 ms) is shown centred. Bottom panels, time disparities (or phase differences) between the zero-crossings of the two curves (top panels) as a function of time. Whether positive- (●) or negative-going (○) zero-crossings (top panels) are chosen is irrelevant for this calculation as the time disparities between both represent the waveforms of the superimposing EODs at greatly reduced speed; the only difference being a 180˚ phase shift relative to the beat cycle. At such a high frequency difference between superimposing EODs as chosen here for illustration (50 Hz) the waveform resolution is rather crude; a more realistic frequency difference of 5 Hz (that is, a beat cycle of 200 ms) yields a tenfold better resolution (Kramer and Otto 1991).

cycle reflects the waveform of the stimulus at greatly reduced speed (Fig. 7). For example, at 4 Hz frequency difference the 2.5 ms period of an external EOD of 400 Hz is represented by a beat cycle of 250 ms (magnification factor, ×100). Stroboscopic analysis used by the human follows the same principle by seemingly slowing down a process that is too fast for direct observation: a slight frequency difference between, e. g., an insect sensory hair vibrating in a sound field and the stroboscopic light flashes allows one to observe the full range of sensilla movement.

The hypothesis of EOD-waveform discrimination by a sensory mechanism that resembles stroboscopic analysis predicts that fish should fail to discriminate between stimulus waveforms that are phase-locked to a fish's EOD frequency (Δf=0 Hz). In an experimental design again using two stimuli of identical amplitude spectra but different waveforms, either free-running at constant frequency or else phase-locked to a fish's EOD, all six fish tested discriminated between stimuli when free-running but failed to discriminate between stimuli when phase-locked. This result was repeated in another five specimens without exception (Kramer, 1999).

Another prediction of the 'stroboscope hypothesis' is that for successful discrimination between different waveforms that are presented at a frequency identical to a fish's own EOD, fish must perform a JAR (this is impossible when stimuli are phase-locked). Results were according to expectation: each fish tested (N=5) performed a JAR first before showing by its behaviour it discriminated between the stimuli presented at constant frequency (> 70 observations in five fish).

It appears the JAR is a sensorimotor mechanism aiding social wave-fish like *Eigenmannia* in the analysis of external EOD waveforms. Powerful frequency analysis mechanisms as detailed in Sections 2 and 3 support this system by guiding the JAR such that the stroboscopic principle of signal analysis can be usefully applied, by seemingly slowing down (but not too much) a process that is too fast for direct observation.

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