In rivers and lakes of tropical South America, gymnotiform fish lead a secretive, nocturnal life. During the day, they stay out of the way of visually oriented predators by hiding and moving little; they do not communicate using sound, which would be detected by predatory catfish, cichlids and piranhas. Knifefishes communicate using electric organ discharges (EODs) they sense with electroreceptor organs distributed all over their skin (for reviews, see Hagedorn, 1986; Kramer, 1990, 1994, 1996; Moller, 1995; for more detailed reviews on electric organs, see Bennett, 1971, Bass, 1986; on electroreceptor organs, Zakon, 1986, 1988). A second function of the electric system of the knifefishes is electrolocation of objects (for reviews, see Bastian, 1990, 1994), which is not dealt with here.

There are two phenotypes of EOD: pulse and wave. Pulses are brief all-or-none events of the order of 1 ms or a few milliseconds separated by much longer intervals. Fed into a loudspeaker, a buzzing sound or single clicks at very low discharge rates are heard. Wave EODs have no pauses between frequency-clamped and phase-locked to the EOD (frequency difference 0 Hz). Opening the electronic feedback loop immediately restored discrimination performance on an on/off basis, and a strong jamming avoidance response (JAR; a frequency shift away from the stimulus) accompanied every behavioural decision (to go for a food reward). The strong habituation of the JAR that occurs in response to stimuli of no behavioural consequence (the usual test situation) was not seen in the present experiments. The proposed sensory model (which is based on time-marking T electroreceptors) is supported by these experiments, and a biological function for the JAR – subserving EOD waveform discrimination is shown to be useful in a social context.

Key words: electric organ discharge, Gymnotiformes, signal waveform discrimination, amplitude spectrum, phase spectrum, frequency difference, jamming avoidance response, beat analysis, sensory mechanism, Eigenmannia.

**Summary**

The electric organ discharge (EOD) of most species of the freshwater knifefishes (Gymnotiformes) of South America is of the wave, not the pulse, type. Wave EODs are usually of constant frequency and amplitude, and show a bewildering multitude of species-characteristic waveforms. The EOD of *Eigenmannia* is sexually dimorphic in waveform and in the intensity of its higher harmonics. In a go/no go paradigm, trained food-rewarded fish discriminated between these waveforms, and naive (untrained) fish showed a significant preference. To determine whether spectral or waveform (time) cues are used by the fish, artificial stimuli of identical amplitude spectrum were synthesized that differed only in phase relationship between their harmonics, i.e. waveform, and the fish discriminated even among these stimulus waveforms (i.e. spectral cues are not required). Our sensory model predicts that, for successful waveform detection, a minimum frequency difference is required between the stimulus and the EOD. As expected, trained fish confused test stimuli of different waveform that were

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**Introduction**

In rivers and lakes of tropical South America, gymnotiform fish lead a secretive, nocturnal life. During the day, they stay out of the way of visually oriented predators by hiding and moving little; they do not communicate using sound, which would be detected by predatory catfish, cichlids and piranhas. Knifefishes communicate using electric organ discharges (EODs) they sense with electroreceptor organs distributed all over their skin (for reviews, see Hagedorn, 1986; Kramer, 1990, 1994, 1996; Moller, 1995; for more detailed reviews on electric organs, see Bennett, 1971, Bass, 1986; on electroreceptor organs, Zakon, 1986, 1988). A second function of the electric system of the knifefishes is electrolocation of objects (for reviews, see Bastian, 1990, 1994), which is not dealt with here.

There are two phenotypes of EOD: pulse and wave. Pulses are brief all-or-none events of the order of 1 ms or a few milliseconds separated by much longer intervals. Fed into a loudspeaker, a buzzing sound or single clicks at very low discharge rates are heard. Wave EODs have no pauses between them, so that they represent a continuous wave (like a sine wave) and are of a constant frequency that may be modulated when the fish is excited. Fed into a loudspeaker, wave EODs sound like a musical instrument playing a constant tone.

The African weakly electric fish, the Mormyriformes (elephantfish, snoutfish), consisting of approximately 200 freshwater species that are only distantly related to the Gymnotiformes, are all of the pulse type except for a single species that is of the wave type. This contrasts with South American freshwater electric fish (perhaps 120 species in all), most of which are of the wave type (families Sternopygidae and Apteronotidae sensu Mago-Leccia, 1994). The reason for this difference between the two major groups of electric freshwater fish that occupy, as far as we know, the same general niche in their respective continents is unknown.

An advantage of the pulse phenotype in communication is its relative immunity to cross-talk by conspecifics: the statistical probability of EOD coincidences (causing signal masking) is low because of a low duty cycle (the proportion of
the time that the signal is on to the time that it is off). Therefore, pulse fish follow a time-sharing strategy of communication, the efficiency of which is often enhanced by certain forms of phase-locking of the EOD pulse to that of another fish (‘echo’ or preferred latency responses, etc.; for a review, see Kramer, 1990). Compared with pulse fish, wave fish come off badly with respect to signal integrity because their wave EOD is always ‘on’ and, therefore, superimposed on (or masked by) that of nearby conspecifics. This is similar to what we experience when trying to communicate in a noisy crowd of people.

Therefore, we might expect wave knifefishes to be rather incompetent at electric communication (perhaps excelling in active electrolocation performance instead), but this is not borne out by the facts. The thresholds of wave fishes for frequency and intensity discrimination, a prerequisite for communication using ‘tone’ signals, are among the lowest for an octavolateral sense among all vertebrates (including the human). Within a certain frequency range, Eigenmannia, a gymniform wave fish, discriminates a change of stimulus frequency corresponding to 1% of a musical tone interval (Kramer and Kaunzinger, 1991). The absolute threshold of wave fishes for an external stimulus is similar to that of pulse fish (for a review, see Kramer, 1996).

**Wave fish analyse beats**

The underlying reason for this unexpected sensory acuity became clear as a result of a paradoxical result: surgically abolishing the EOD of the wave fish *Sternopygus macrurus* caused the electrosensory threshold of trained fish to rise steeply (by approximately 30 dB; Fleishman et al., 1992). Clearly, this fish did not profit from the weak stimulus being no longer masked by its own strong EOD that had been abolished; on the contrary, this fish experienced a serious sensitivity loss. Apparently, beating of the stimulus frequency against that of the EOD is required for the fish to detect a stimulus.

This hypothesis was confirmed by a similar sensitivity loss when intact, electrically competent *Sternopygus* (Fleishman et al., 1992) and *Eigenmannia* (Kaunzinger and Kramer, 1995) were stimulated at a frequency exactly equal to their EOD frequency or, especially for *Eigenmannia*, also one of the higher harmonics of the EOD frequency. Such stimuli were detected only at greatly increased stimulus intensities. Surgically abolishing the EOD of a wave fish or stimulating it at one of the harmonics of its EOD frequency (the fundamental included) all have in common that there is no beating – the wave EOD and the stimulus mix according to their instantaneous phase relationship (which was kept stable throughout the experiment by an electronic device in the case of *Eigenmannia*; Kaunzinger and Kramer, 1995). However, even the slightest frequency mismatch immediately restored the sensory acuity of *Eigenmannia*, and the filter slope (a spectral line-reject filter, similar to a very sharp notch response in an electronic filter) of this response is one of the steepest for a sensory filter in the animal kingdom (up to 5000 dB per octave; Kaunzinger and Kramer, 1995) and is not matched by technical filters. That is equivalent to stating that even very slow beats are detected and that the transition from the sensation ‘beat present’ to the sensation ‘beat not present’ is an abrupt one (like a switch).

Certain bats emit long echolocation calls of constant frequency that are terminated by a brief period of frequency modulation (Griffin, 1958). The calls of these CF-FM-type bats mask the faint echoes that are of slightly higher frequency because of the Doppler shift when approaching a target (for reviews, see Neuweiler, 1993; Popper and Fay, 1995). To detect the echoes in spite of the very small frequency difference from the bats’ own calls, the bats must use very sharp frequency filtering (up to 3500 dB per octave; Suga et al., 1976; Suga, 1990). Depending on how fast they approach their target, these bats lower the tone frequency of their calls such that the tone frequency of the echoes is kept at the optimum filter frequency of their highly specialised auditory system (Doppler compensation; Schnitzler, 1972).

In contrast, the sensitive T electroreceptors of wave knifefishes are only weakly V-tuned (filter slope, only 20 dB per octave even on the ‘high’ side of the ‘best’, the EOD frequency; for reviews, see Kramer, 1985; Zakon, 1988; Fleishman, 1992), and these fish use a totally different strategy of signal detection and assessment, analysis of the temporal properties of beats. The temporal properties of beats and the sensory coding, central nervous and motor responses have been studied extensively by Bullock et al. (1972a,b), Scheich and Bullock (1974), Scheich (1977a–c), Heiligenberg (1991) and others. However, exactly how these fish analyse beats has remained a matter of controversy to the present day. Our own results on how frequency and intensity are detected (Kaunzinger and Kramer, 1996) are summarised in a sensory model based on time-marking (T) electroreceptor afferences (Kramer and Kaunzinger in Kramer, 1996; for a review, see Kramer, 1999) and are not presented here.

**Wave fish detect stimulus waveform**

Wave fish can afford to avoid sharp signal filtering because of their ‘intelligent’ use of beat analysis and they enjoy considerable advantages: in spite of detecting a wide range of stimulus frequencies, their electrosensory threshold and their thresholds for frequency and intensity discrimination are exceedingly low (their electrosensory acuity is high). A cost (in the form of a sensitivity loss) of the wide working range of this system is apparent only for certain spectral lines, the EOD fundamental and its higher harmonics (a situation that, for useful echoes, does not occur in CF-FM bats because of the Doppler frequency shift). Another advantage of the system used by these fish is an undistorted stimulus waveform, because signal distortion is an unavoidable consequence of sharp filtering. Therefore, in addition to stimulus frequency and intensity, wave fish could, in theory, also assess stimulus waveform, which is the topic of the present article.

Another requirement for stimulus waveform detection is a
suitable communication channel. In contrast to sound and other mechanical forms of signal transmission, the waveform of a complex electrical signal remains unchanged with distance from the signal source (except for a gradual deterioration of the signal/noise ratio). The waveform of an electrical signal is also independent of the angle at which it is received, except for a polarity reversal that occurs with certain positional or rotational changes of the sending with respect to the receiving dipole. Therefore, the electrical communication channel does transmit potentially useful waveform information, and the electrosensory system of knifefishes may detect this information because it does not discard that information by sharp sensory filtering.

When studying the gymnotiforms of the Amazon near Manaus, I was astonished by the multitude of different waveforms of EOD in wave-type fish (Kramer et al., 1981). This diversity (Kramer, 1990, Figs 3.14–3.22) suggests that, in addition to EOD frequency, which varies from approximately 15 Hz to an exceptionally high 1800 Hz in different species, EOD waveform variation is also species-characteristic and, therefore, must be the product of selection. Mate recognition by EOD frequency, as envisaged by Hopkins and Heiligenberg (1978), seemed to be ineffective in our species-richer study area because of extensive overlap of EOD frequencies and the wide range of species-characteristic frequencies; for example, 260–650 Hz in *Eigenmannia virescens* at 27 °C (Hopkins, 1974). For so many wave species, as found near Manaus, the number of non-overlapping frequency ranges is insufficient in the available frequency space. In contrast, different species display characteristically different EOD waveforms at a similar frequency (e.g. Fig. 3.20 in Kramer, 1990), and even members of the same species, such as *Eigenmannia lineata* males and females, may differ in EOD waveform (Fig. 1) (Kramer, 1985; *Eigenmannia lineata* is considered a synonym of *E. virescens*; Planquette et al., 1996).

We tested whether *Eigenmannia* discriminates electronically generated functions, such as the sine from the sawtooth wave, of identical frequency and peak-to-peak amplitude (or, alternatively, identical root mean square amplitude in case the ‘effective’ amplitude was the relevant cue). Trained, food-rewarded fish had no difficulty with this task, nor with discriminating digitally synthesized (Kramer and Weymann, 1987) female from male EODs (Kramer and Zupanc, 1986). The play-back of female rather than male EODs proved significantly more attractive to untrained fish of both sexes that were given neither reward nor punishment (Kramer and Otto, 1988).

From these observations, we concluded that *Eigenmannia* apparently discriminates any pair of waveforms that differ sufficiently in harmonic content, as seen in an amplitude spectrum. For example, the sine wave does not possess any overtones, but the sawtooth wave is extremely rich in overtones (which are integer multiples of the fundamental frequency). A similar difference, although much less pronounced, exists for *Eigenmannia* EODs, female EODs displaying overtones of weak intensity and male EODs displaying overtones of much stronger intensity (Fig. 1). Differences in overtone intensity are the physical basis for the sensation of timbre or sound quality that enables us to recognize the various musical instruments or individual human voices.

To exclude signal discrimination by spectral analysis, or an electrosensory equivalent of timbre assessment, we used computer-generated waveforms of identical amplitude spectrum (hence, timbre) but different waveform. Two harmonically related sine waves, one octave apart in frequency and of a weaker intensity by −3 dB for the higher harmonic, as is observed in a male *Eigenmannia* EOD, were added using two different phase relationships: one in which the peaks of the two waves coincided (Fig. 2A, arbitrarily called ‘0 °’), and another with a relative delay of the higher-frequency wave by one-quarter of a cycle, or 90 ° (Fig. 2E). This yielded two characteristically different waveforms of identical timbre; an audio playback of these is indistinguishable to the human ear.

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**Fig. 1.** Waveforms (A,B) of electric organ discharges in *Eigenmannia*, with their associated Fourier amplitude spectra (C,D). The ordinates of the left-hand diagrams are arbitrary linear amplitudes (V), those of the right-hand diagrams are 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,C) Female; (B,D) male. Note the almost sinusoidal waveform of the female electric organ discharge (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).
**Eigenmannia**, however, clearly discriminated between these waveforms, again both in conditioned discrimination tests (Kramer and Otto, 1991; Kramer and Teubl, 1993) and when observing spontaneous preferences in naive fish. In the spontaneous preference tests, the more *Eigenmannia*-like 0° signal was preferred over the 90° signal that resembles an *Apteronotus albifrons* EOD fairly well (*A. albifrons* is a predator; some apteronotids prey on the tails of other knifefishes; Lundberg et al., 1996). The two artificial test waveforms need not be maximally different, as with the 0° and 90° signal pair; a much smaller waveform difference, such as that between Fig. 2A and Fig. 2C, is sufficient for discrimination (discrimination threshold, between 11° and 22° phase shift of the second relative to the first harmonic; Kramer and Teubl, 1993). Therefore, spectral cues, or differences in timbre for a sound signal, are not required for *Eigenmannia* to discriminate between two electrical stimuli of different waveform. A purely temporal sensory mechanism was called for.

The sensory mechanism of Fig. 3 is compatible with the above results in trained and naive fish, and the sensory physiology as established by Scheich, Bullock, Szabo and others (for a review, see Zakon, 1988). In this model, afferent responses from time-marking T electroreceptor organs, but not from amplitude-coding P receptors, are the relevant ones. At high sensitivity and temporal precision, afferences from T electroreceptors respond to a fish’s own EOD with one action potential per EOD cycle phase-locked to the zero-crossings of the EOD wave (it is not known whether to positive- or to negative-going zero-crossings). Any spontaneous EOD frequency shift or frequency modulation caused by an external EOD of different frequency mixing with a fish’s own EOD (thus forming a beat) is faithfully reflected by the firing rate of T receptor afferences (which is modulated back and forth in the case of beats, following the modulation of intervals between zero-crossings; Scheich, 1977a–c).

When an external EOD mixes with a fish’s own EOD, the adequate stimulus for T receptors is different for the left and right sides of the body (or head and tail region receptors, depending on the relative positions of the two fish). The external EOD adds onto a fish’s own EOD for the receptors of one body side (according to instantaneous amplitudes), whereas for the receptors on the opposite side, the superposition is subtractive because these receptors face the opposite polarity of the distant dipole generating the external EOD (that is, the external EOD is perceived as inverted, or 180° out of phase, by these receptors; see Fig. 9 in Kramer and Otto, 1991; Hopkins and Bass, 1981). Therefore, there are slight time disparities between zero-crossings of the combined signals for local T receptors on the two sides of the fish (Fig. 3). The magnitude of these disparities over time reflects the waveform of the external EOD at greatly reduced speed: one external EOD cycle (of the order of 0.0025 s) is represented by one beat cycle (of the order of 0.25 s), the duration of which the fish can control by adjusting its EOD frequency (Kramer and Otto, 1991). A suitable central nervous system circuit for detecting temporal disparities between afferent input of T receptors originating from different body regions has been discovered (Carr et al., 1986a,b; Carr, 1990) that builds on the fast electrosensory pathway of Szabo (1967).

Gymnotiform pulse fish also seem able to discriminate between the slight variations among conspecific EOD waveforms in an agonistic context (McGregor and Westby, 1992), although a conditioned discrimination test has not yet
The EOD of a wave-type electric fish

been accomplished. Hopkins and Westby (1986) suggest 'scan sampling' as a possible sensory mechanism in which regular sequences of EOD superpositions in fish with a small difference in discharge rate, separated by much longer sections without superpositions, would be the relevant cue. If confirmed, such a slow mode of signal analysis would contrast with signal analysis by trained mormyrids (Gnathonemus petersii and Pollimyrus adspersus), which discriminate between low-rate playbacks of pulse EODs (conspecific, heterospecific or synthetic) immediately and reliably, after receiving only a few (or even only one) stimulus pulses in which no such sequences of superpositions occur (Graff and Kramer, 1992; Paintner, 1998; Paintner and Kramer, 1998).

**Waveform detection depends on beat analysis**

Wave fish such as Eigenmannia apparently require beats for discriminating between different stimulus waveforms, and this hypothesis can be tested. For example, in the sensory model of Fig. 3, fish should be unable to discriminate waveforms (at least those of identical amplitude spectra) when the stimulus and EOD frequency are identical (because no beats are present at frequency identity). This was tested in six fish that, in preparation for the critical experiments, were trained to discriminate between two stimuli of identical amplitude spectrum but markedly different waveform (Fig. 2A,E). These stimuli were played back in random sequence at a constant frequency difference of 50 Hz above the fish's own EOD frequency (as determined immediately before stimulus onset). At such a large frequency difference, no jamming avoidance responses were evoked and, to avoid startle responses, a stimulus rise time of 500 ms was chosen.

Modifying the experimental arrangement of our earlier studies, the six fish (14.6–16.5 cm total length) were trained to go for a food reward 42 cm away from their daytime hiding shelter (a porous pot; Fig. 4A) when the rewarded stimulus,

![Waveform detection](image)

Fig. 3. The electric organ discharge (EOD) of a female *Eigenmannia* (400 Hz) superimposed on that of a conspecific in social context (A,B) and as probably analyzed by the T electroreceptor system (C,D). (A,C) The external EOD is that of another female; (B,D) the external EOD is that of a male (both of 30% amplitude and at 450 Hz). (A,B) Additive superposition is shown as solid lines, subtractive superposition as dotted lines (representing the adequate stimuli for local T electroreceptors on the right and left sides of the body). One full beat cycle (20 ms) is shown centred. (C,D) Time disparities (or phase differences) between the zero-crossings of curves (A,B) as a function of time. Whether positive- (●) or negative-going (○) zero-crossings (A,B) are chosen is irrelevant because the time disparities between both curves represent the waveforms of the external EODs at greatly reduced speed; the only difference being a 180° phase shift relative to the beat cycle. At the large frequency difference between mixing EODs chosen here for illustration (50 Hz), the waveform resolution is rather crude; a more realistic frequency difference of 5 Hz (i.e. a beat cycle of 200 ms) yields a tenfold better resolution (Kramer and Otto, 1991).
Fig. 4. (A) The test aquarium viewed from above (75 cm long, 40 cm wide, 45 cm deep). R, pair of recording electrodes, in line with a porous pot (P), a swimming channel formed from plastic mesh (M) and a feeder (F). T, glass tube that dispenses the food reward into the feeder. A1, A2, glass tubes that deliver ‘punishing’ air bubbles. S, pair of stimulation electrodes.

Training and testing were carried out during daylight hours only, when the fish stayed inside the shelter (P). A stimulus delivered via electrodes S evoked the fish’s ‘go’ response. In training trials, a ‘go’ response to stimulus S+ was rewarded by food at F and to S– a ‘go’ response was punished with air bubbles (at A1; A2 was only used when the fish had habituated to A1). In testing trials (as shown in B), neither S+ nor S– was followed by reinforcement. The orthogonal electrode arrangement (R, S) and the limits to the fish’s swimming path (M) made possible continuous recording of electric organ discharge (EOD) frequency even during electrical stimulation. (B) Discrimination between two stimulus waveforms in a trained Eigenmannia (test trials). Stimulus S+ is shown in Fig. 2A, stimulus S– in Fig. 2E. Trials 1–8, acquiring the association of S+ with a food reward, of S– with a light punishment, seen as dissociation of the response latency curves. Latencies greater than 60 s were truncated; both stimuli were +50 Hz re the fish’s EOD. From trial 9 onwards, two additional test stimuli were introduced into the randomized stimulus regime (S+, green; and S–, red; both stimuli were frequency-clamped and phase-locked with respect to the fish’s EOD but were otherwise identical to S+ and S–, respectively). Note that S+ and S– evoked variable response latencies, indicating confusion of waveforms by the fish, while it continued to discriminate consistently between S+ and S–. This shows that, for discrimination between waveforms, a frequency difference between the fish’s EOD and the stimulus is required (this example shows fish 4, Table 1; R. Blasius and B. Kramer, unpublished results).

S+, was presented. They were supposed to stay inside their shelter when the alternative stimulus, S–, was presented (for a period of up to 30 s). If necessary, fish were punished with a few air bubbles injected into their path when they responded in spite of an S– being played. Using a stopwatch, the progress of learning was monitored as the time interval from stimulus onset to a fish placing its mouth in the Petri dish that contained the reward. The observer was visually screened from the fish by a sheet of cloth.

When response times for S+ and S– began to separate (those for S+ shortening and those for S– lengthening), ‘test’ stimuli were included in the stimulus regimes (see below). The randomized blocks design (Cochran and Cox, 1957) of stimulus sequence for training stimulus was expanded to accommodate a test stimulus every fifth stimulation while carrying on with the training stimulus (at an inter-trial interval of at least 1 min). Test stimuli (the duration of which was a maximum of 60 s in case of no response) were followed by neither reward nor punishment; it is exclusively the responses to test stimuli that are reported in the present (and previous) papers.

Test stimuli were of four kinds, although only two stimulus waveforms, the same ones as for training stimuli, were used, and their sequence followed a randomized blocks design of their own. Two test stimuli (S+ and S–) were identical with the two training stimuli, and the difference from the fish’s own EOD frequency was also 50 Hz (30 Hz for fish 6; see Table 1). In contrast, the other two test stimuli, S+ and S– (see Table 1), consisted of presenting the same two waveforms at the fish’s own EOD frequency (A=0 Hz). To achieve maintained, precise frequency identity even when the fish changed its own EOD frequency, the stimulus was frequency-clamped and phase-locked to the fish’s EOD (at a constant phase difference of 91 °; the definition of phase is based on zero-crossings times; see Fig. 1 in Kaunzinger and Kramer, 1996). Therefore, a fish was unable to ‘escape’ from the stimulus with a frequency change, since the computerised electronic device (which was triggered cycle-by-cycle by square-wave pulses derived from the fish’s EOD) followed its EOD frequency without delay. [For a description of the basic device, see Kramer and Weymann (1987); a version expanded for phase-locking of sine-wave stimuli was used by Kaunzinger and Kramer (1996); in the present article, a version capable of using ‘arbitrary’ waveforms that are phase-locked was used. The word ‘arbitrary’ is popular with electronics companies at present advertising certain stimulus generators, although phase-locking is not offered.]

By phase-locking the stimulus to the EOD at a selectable,
The EOD of a wave-type electric fish dynamically constant phase difference, relative frequency drift or jitter that might occur when using frequency-clamping without phase-locking, and hence undefined phase relationships, were prevented (see Discussion in Kaunzinger and Kramer, 1995). Further advantages were that the sensation level (which depends on the phase difference of a stimulus of constant amplitude; Kaunzinger and Kramer, 1996) and the waveform of the mixed signal (composed of the EOD and the added stimulus) were stable throughout the experiments. Therefore, in these experiments, there were not even small parts of a beat cycle present that might give a fish cues as to the signal waveform with which it was being stimulated.

To record the EOD uncontaminated from the stimulus, the classical electrode geometry for jamming avoidance response (JAR) experiments (Watanabe and Takeda, 1963) had to be followed precisely, with the additional requirement of permitting the test fish to travel between the shelter and feeder (Fig. 4A). Therefore, the fish’s swimming path was strictly limited to a straight line connecting its shelter with the feeder and the recording electrodes, by a narrow channel made of plastic mesh (maximum diameter 9 cm, which allowed the extremely flexible fish to turn around). Stimulus intensity was 156 $\mu$V peak-to-peak cm$^{-1}$ at the fish’s head when facing the feeder inside its shelter. At that position, sensation level was 48 dB for a frequency-clamped stimulus close, but not identical, to the EOD frequency (based on thresholds determined by Kaunzinger and Kramer, 1995). For stimulation at frequency identity and 91° phase difference between EOD and stimulus,

Table 1. Conditioned discrimination between two stimulus waveforms in Eigenmannia

<table>
<thead>
<tr>
<th>Fish</th>
<th>S+</th>
<th>SIQ (s)</th>
<th>Sum of ranks</th>
<th>Mean of ranks</th>
<th>N</th>
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<td>1</td>
<td></td>
<td>4 0.5</td>
<td>278.5</td>
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<td></td>
<td>S–</td>
<td>60 0</td>
<td>1554</td>
<td>74</td>
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<td></td>
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<td></td>
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<td>42.17</td>
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<td>S–</td>
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<td></td>
<td>S+φ</td>
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<td>725.5</td>
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<td>709.5</td>
<td>37.34</td>
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<tr>
<td>6</td>
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<td>6 7.25</td>
<td>535.5</td>
<td>28.18</td>
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</tr>
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<td>S–</td>
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<td></td>
<td>S–φ</td>
<td>8 11.25</td>
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<tr>
<td></td>
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<td>831.5</td>
<td>43.76</td>
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<td>58 46.5</td>
<td>1538</td>
<td>80.95</td>
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Median response latencies (s) differ significantly between stimuli (left-hand P values, based on KW, Kruskal–Wallis ANOVA by ranks). SIQ, semi-interquartile range (s).

S+, S–, test stimuli of constant frequency (with frequency difference re EOD); S+φ, S–φ, frequency-clamped, phase-locked stimuli; S+zero, S–zero, stimuli with identical frequency re EOD, not frequency-clamped.

The fish were of Colombian origin, obtained through a direct-importing dealer. Zoologische Staatssammlung, Munich/Germany, accession nos: ZSM 28575 and ZSM 28576 (R. Blasius and B. Kramer, unpublished results).

KW, Kruskal–Wallis; N, number of tests.

For stimulus waveforms, see Fig. 2A,E.
the sensation level was only 26 dB (based on thresholds determined by Kaunzinger and Kramer, 1996).

Compared with our earlier experiments with unclamped test stimuli (Kramer and Otto, 1991; Kramer and Teubl, 1993), the present experiments were more restrictive. For example, fish had to learn to swim through a narrow channel and for a longer distance to obtain a food reward. Stimulation across the centre of the swimming channel by widely spaced electrodes, as was necessary here, yields a less natural field geometry and training situation than when a small dipole (a signal source that can be localised) close to the feeder is used.

In spite of these constraints, all six fish learned to discriminate the two test signals (of Fig. 2A,E), as shown by the consistent difference in response latencies (an example is shown in Fig. 4B). As soon as a fish discriminated (as determined for the Kruskal–Wallis statistic, Table 1), the number of fish that succeeded in performing a JAR (thereby restoring beats). This hypothesis was tested in fish 6 by introducing two more test stimuli (that is, six stimuli that were all run in parallel, following a randomised blocks design) which consisted of playing back the two signal waveforms (again of Fig. 2A,E) at a constant frequency that was identical to this fish’s EOD frequency at stimulus onset (S\textsuperscript{+}\text{zero} and S\textsuperscript{−}0). These stimuli were not frequency-clamped and, hence, were of constant frequency throughout the experiment.

As expected, fish 6 discriminated between these new, unclamped test stimuli (P < 0.01, Table 1) probably because, with the frequency clamp disabled, the fish quickly changed its EOD frequency by an amount sufficient to restore its capacity for beat analysis. This assumption is supported by the fish’s confusion of the same stimulus waveforms when phase-locked to its EOD (S\textsuperscript{+}0 and S\textsuperscript{−}0, as also observed in all other fish).

The JAR is instrumental to waveform discrimination

From the previous experiment, it follows that a fish that is stimulated at its EOD frequency must perform a JAR to discriminate between different stimulus waveforms. This hypothesis was tested in an additional five untrained Eigenmannia (11.5–15.5 cm total length; Table 2, Fig. 5A,B).

In preparation for the critical tests, fish were trained to discriminate between the same two waveforms as in the previous experiment (Fig. 2A,E). However, the frequency difference from the fish’s EOD was smaller and varied between 10 and 30 Hz, depending on the fish and at which frequency difference learning progress seemed best. Discrimination between the two test stimuli, S\textsuperscript{+} and S\textsuperscript{−}, was confirmed in each fish using the Mann–Whitney U-test (P < 0.01). In combination with previous reports (Kramer and Otto, 1991; Kramer and Teubl, 1993; Table 1), the number of fish that have successfully performed the discrimination task is now 18 out of 18 tested by four experimenters.

The critical test of the above hypothesis began (1) when S\textsuperscript{+} and S\textsuperscript{−} were replaced by S\textsuperscript{+}\text{zero} and S\textsuperscript{−}\text{zero}, respectively, and (2) when S\textsuperscript{+}0 and S\textsuperscript{−}0 were added to the regime of test stimuli (as defined for Table 1). The present test stimulus regime was identical to that used for fish 6 of Table 1, except that, for the five fish of Table 2, S\textsuperscript{+} and S\textsuperscript{−} were omitted (four test stimuli rather than six). Stimulus intensity was 165 μV peak-to-peak cm\textsuperscript{−1}, corresponding to a sensation level of 49 dB for a stimulus close, but not identical, to the EOD frequency; at a phase difference of 35°, the sensation level for frequency-clamped stimuli (S\textsuperscript{+}0 and S\textsuperscript{−}0) was 29 dB. Training stimulus duration was up to 45 s, inter-trial interval was again at least 1 min and test stimulus duration again up to 60 s. Response latency was the time from stimulus onset until the tip of the fish’s tail had left the shelter completely.

The results for the five new fish (Table 2) confirmed the result (of fish 6, Table 1) that, even when the stimuli were identical in frequency with their own EOD, all fish discriminated between the two test stimuli, S\textsuperscript{+}\text{zero} and S\textsuperscript{−}\text{zero} (P < 0.05 in each fish, Table 2). An initial frequency difference
between the stimulus and the EOD, as used for fish 1–5 in Table 1, is unnecessary and does not provide a cue for discrimination. In contrast, fish were unable to discriminate between the two phase-locked stimuli, S+φ and S−φ, when frequency identity with the EOD was maintained (P>0.05 in each fish, Table 2).

After nine full repeats of test trials, fish 5 habituated to the punishment with air bubbles that was associated with the S−zero training stimulus and no longer appeared to discriminate between stimuli, as measured by response latencies. This habituation problem had been observed in other fish before but never as persistently as in fish 5, which finally ignored the punishment, visiting the feeder in response to any stimulus. Because using a more severe form of punishment was not considered acceptable in these fragile fish, the data sampled after the ninth repeat were discarded, and it was impossible to increase the sample size beyond nine in fish 5 using the present technique. With such a small sample size, the nonparametric Kruskal–Wallis test is known to possess very little power (see, for example, Siegel and Castellan, 1988; Motulsky, 1995), increasing the probability of a ‘type II’ error (failing to reject an incorrect null hypothesis, H0), and discrimination was established at a significance level of only P<0.05 in this fish (Table 2). An F-test for fish 5 confirmed the result (that rejection of H0 is justified; F=7.408; Bonferroni’s P<0.01 for the comparison S+zero/S−zero, and P>0.05 for the comparison S+φ/S−φ; normality test of data passed with P>0.10; differences between standard deviations not significant as shown by a Bartlett’s test, P=0.1138).

On stimulation with S+zero, all fish responded by changing their EOD frequency (as measured by the frequency change DR = actual EOD frequency minus resting EOD frequency, determined immediately before stimulus onset). Except on three out of 71 occasions, these stimuli of Dφ=0 Hz evoked a frequency increase, confirming the results of earlier studies (Kramer, 1987; Kaunzinger and Kramer, 1995). The time course of the frequency change (DR, absolute values) evoked by S+zero was averaged in each fish, and individual differences were revealed. Fish 1 showed the strongest responses: 5 s after stimulus onset, its averaged DR was 7.86 Hz, whereas values of 1.16 Hz, 1.85 Hz, 1.18 Hz and 0.75 Hz were observed in fishes 2–5, respectively (Fig. 5A).

Table 2. Conditioned discrimination between two stimulus waveforms in Eigenmannia

<table>
<thead>
<tr>
<th>Fish</th>
<th>S+zero</th>
<th>S−zero</th>
<th>S+φ</th>
<th>S−φ</th>
<th>Median (s)</th>
<th>SIQ (s)</th>
<th>Sum of ranks</th>
<th>Mean of ranks</th>
<th>N</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish 1</td>
<td>10.1</td>
<td>60</td>
<td>53.2</td>
<td>32.4</td>
<td>4.65</td>
<td>8.95</td>
<td>20.45</td>
<td>24.63</td>
<td>191</td>
<td>625.5</td>
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<td>KW=23.83</td>
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<td>15</td>
<td>P&lt;0.001</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fish 2</td>
<td>23.7</td>
<td>60</td>
<td>5.5</td>
<td>8.6</td>
<td>8.65</td>
<td>2.75</td>
<td>2.85</td>
<td>2.4</td>
<td>629.5</td>
<td>1004.5</td>
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<td>17</td>
<td>P&lt;0.01</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fish 3</td>
<td>18.5</td>
<td>43.1</td>
<td>7.3</td>
<td>15.4</td>
<td>3.2</td>
<td>8.13</td>
<td>3.43</td>
<td>7.4</td>
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<td>KW=33.29</td>
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<td>15</td>
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</tr>
<tr>
<td>Fish 4</td>
<td>12.7</td>
<td>48.8</td>
<td>11</td>
<td>5.1</td>
<td>7.63</td>
<td>7.18</td>
<td>3.1</td>
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<tr>
<td>KW=37.17</td>
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<td>15</td>
<td>P&lt;0.05</td>
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<td></td>
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<tr>
<td>Fish 5</td>
<td>6.2</td>
<td>35.4</td>
<td>4.4</td>
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</table>

Median response latencies (s) differ significantly between stimuli in each fish tested (left-hand P values, based on KW, Kruskal–Wallis ANOVA by ranks).

SIQ, semi-interquartile range (s).

S+zero, S−zero, stimuli with identical frequency re EOD, not frequency-clamped; S+φ, S−φ, frequency-clamped, phase-locked stimuli.

The fish were of Guyanan origin, obtained through a direct-importing dealer. Zoologische Staatssammlung, Munich/Germany, accession nos: ZSM 28567 - 28574 (M. Orth and B. Kramer, unpublished results).

KW, Kruskal–Wallis; N, number of tests.

For stimulus waveforms, see Fig. 2A,E.
Factors contributing to the high variability of the JAR are habituation, sex and age (Kramer, 1987). In the present context, a possible further factor was of interest: is the JAR required for successful waveform discrimination, as determined by a conditioned behaviour? Therefore, the curves were aligned such that the times of decision-making (as opposed to stimulus onset) coincided and were averaged as before. The time of decision-making (to go for a food reward) was the moment when the fish left its shelter, i.e. when it showed the ‘go’ response.

Associated with the ‘go’ response, an additional frequency change superseding the one shown in Fig. 5A (where...
averaging is centred on stimulus onset) was revealed (Fig. 5B). In all fish, a steep frequency increase was observed immediately preceding the decision to ‘go’; in three fish, ‘go’ coincided with the steepest part of the frequency change. In the other two fish (fish 2 and 4) frequency continued to rise in a gentler manner once the decision to ‘go’ had been taken. The ‘go’-associated, marked frequency rise occurred within 3 s, and the highest $\Delta R$ values immediately followed ‘go’. In three fish, once they had reached the feeder, EOD frequency relaxed to frequencies below or equal to their frequency before ‘going’.

Values of $\Delta R$ around the time of ‘go’ were very large given that inter-trial intervals were usually only 1 min and that stimulus intensity was high. Short inter-trial intervals such as these, combined with the strong stimulus intensity applied here, led to marked, long-lasting habituation in ‘classical’ JAR experiments (Kramer, 1987). Habituation was not prominent in the present experiments: mean maximum $\Delta R$ values were a record 9.37 Hz in fish 1, and still remarkable in fishes 2–5 (3.87 Hz, 5.94 Hz, 2.95 Hz and 1.94 Hz, respectively). The greatest individual (non-averaged) $\Delta R$ values were 14.13 Hz in fish 1 and 11.07 Hz in fish 3.

Fish 5, which displayed the lowest $\Delta R$ values, was the one that finally habituated completely to punishment with air bubbles (see above), and the correlation does not seem accidental. The time course of the frequency change of fish 5 (very moderate $\Delta R$ at first, but a strong response at decision making) clearly demonstrates that the JAR served a sensory function in detecting stimulus waveform even in this fish. This suggests that fish 5 did discriminate between the two waveforms even after trial 9, when it stopped showing differential ‘go’ responses.

One of the functions of the frequency change behaviour of Eigenmannia – be it called JAR or ‘active beat response’ (as suggested by Kramer, 1996) – is clearly to enhance the detection of the EOD waveforms of other fish when discharge frequencies are too similar for beat analysis to be possible.

D. Weymann constructed and modified the equipment for phase-locked stimulation with ‘arbitrary’ waveforms. For their critically reading of an earlier version of the manuscript, I wish to thank S. Hanika and I. Kaunzinger. I wish to thank the editors and referees for constructive criticism. Supported by the Deutsche Forschungsgemeinschaft (grants SFB4/H1 and Kr 446/10-1 to 3).

References


