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RESOLVING COMPETING THEORIES FOR CONTROL OF THE JAMMING AVOIDANCE RESPONSE: THE ROLE OF AMPLITUDE MODULATIONS IN ELECTRIC ORGAN DISCHARGE DECELERATIONS

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Summary

The algorithm for the control of the jamming avoidance response (JAR) of *Eigenmannia* has been the subject of debate for over two decades. Two competing theories have been proposed to explain how fish determine the correct direction to shift their pacemaker frequency during jamming. One theory emphasizes the role of time-asymmetric beat envelopes, while the other emphasizes the role of amplitude- and phase-difference computations that arise from the differences in spatial geometry of the electric fields of neighboring fish. In repeating earlier experiments, we found that the decision to raise or lower the pacemaker frequency reliably above or below its resting level depends on the latter process, and that frequency deceleration responses to amplitude modulation appear to be sufficient to explain previous experimental results on which the former theory is based. Specifically, fish of the genus *Eigenmannia* show differential deceleration responses to asymmetric beat envelopes. The deceleration responses do not require phase modulation and show a sensitivity for amplitude modulation depth and selectivity for amplitude modulation rate comparable with that of JARs that are elicited when amplitude- and phase-difference information is available.

Key words: jamming avoidance response, electric organ discharge, amplitude modulation, electroreception, *Eigenmannia*.

Introduction

The gymnotiform electric fish *Eigenmannia* emits quasi-sinusoidal discharges from its electric organ, located in the tail. These signals are used for electrolocation and electrocommunication (Heiligenberg, 1973; Hopkins, 1988). During electrolocation, fish maintain a constant, private frequency of electric organ discharge (EOD). They shift their otherwise constant frequencies of EOD when they encounter a neighbor whose EOD frequency is close to their own. The frequency shifts always occur in the direction that increases the frequency difference between a neighbor's EOD and their own (Watanabe and Takeda, 1963; Bullock et al., 1972a,b). This 'jamming avoidance response' (JAR) serves to minimize the detrimental effects of foreign EODs on the animal's electrolocation abilities (Heiligenberg, 1973). During jamming, fish are exposed to a complex signal mixture of the fish's own and a neighbor's EODs. Using information in this combined signal, fish are able to determine the correct direction to change their EOD frequency.

Two fundamentally different theories have been proposed to explain how fish might compute the sign of frequency differences (Δf). Both theories have extensive support from behavioral analyses of computational algorithms and physiological studies.

Scheich (1974, 1977a–c) and Scheich and Bullock (1974) proposed a model which involves a temporal pattern analysis of the signal mixture. This model requires harmonic components in the fish's own EOD which create particular temporal modulation patterns of both amplitude and phase in the signal mixture. Depending on whether a neighbor's frequency is higher or lower than that of a fish's own EOD, this pattern of modulation is temporally reversed, giving fish a cue as to the direction in which to shift the EOD frequency for a 'correct' JAR. This theory does not require and does not incorporate naturally occurring spatial differences in sensory signals.

Heiligenberg and his colleagues later discovered that the higher harmonic components are unnecessary for eliciting correct JARs, provided that the animal's own and its neighbor's EOD fields have different spatial geometry, as in natural conditions (Heiligenberg et al., 1978; Heiligenberg and Bastian, 1980; Heiligenberg, 1991). In this model, the temporal asymmetry in the stimulus is not required, but analyses of the

relationships between the amplitude and phase of signals in different regions of the body surface are essential. Heiligenberg concluded that the stimulus features required in Scheich's model are not necessary for the JAR on the basis of the observation that an addition of harmonic components to essential stimuli for his model did not augment the magnitude of the JAR (Heiligenberg et al., 1978). Although Heiligenberg attempted to explain the experimental results of Scheich (1977a) using his own theory of the JAR, these arguments are not conclusive (Heiligenberg and Partridge, 1981; Heiligenberg, 1991).

In the present study, we repeated the experiments of both Scheich and Heiligenberg in search of the origin of these discrepancies. In evaluating these two theories for the control of JARs, we investigated the possibility that somewhat different behaviors were actually measured in the earlier experiments. Traditionally, the magnitude of the JAR has been measured as the difference in a fish's EOD frequency for negative Δf *versus* positive Δf stimulus conditions; changes in EOD frequency with respect to the fish's resting EOD frequency (baseline), therefore, are not typically measured. In the present experiments, we measured frequency shifts elicited by the stimulus regimens described in earlier studies and related these shifts to each fish's resting EOD frequency.

We found that *Eigenmannia* reliably increased its pacemaker frequency above baseline when a jamming signal was lower in frequency and presented with different spatial geometry from that of the EOD-substitute signal, as Heiligenberg's theory predicts; fish did not, however, consistently raise their pacemaker frequency when both signals were presented with the same spatial geometry, as in Scheich's experiments, even when the stronger signal was clipped to match the natural EOD waveform. Fish lowered their pacemaker frequency below baseline when the jamming signal was higher in frequency. These frequency deceleration responses were greatest when the EOD-substitute signal (sinusoidal or clipped) and jamming signal were presented with different spatial geometry, but substantial decelerations were observed when both signals had identical spatial geometry. We also found that the magnitude of pacemaker decelerations caused by pure amplitude modulations (Scheich, 1977a) usually differs for different shapes of amplitude modulation envelopes. Switching, therefore, between stimulus conditions that result in time-asymmetric amplitude modulation patterns results in reversals in the direction of change of the pacemaker frequency. These frequency shifts differ, however, from natural JARs in that the pacemaker frequency does not reliably increase above the resting level. Moreover, we found that the phase modulation components which were present in Scheich's stimuli were not necessary for evoking these deceleration responses.

The differential frequency deceleration that can be observed for different amplitude modulation patterns may account for the fact that when EOD-substitute and jamming signals are presented with identical spatial geometry, sign-dependent shifts in the pacemaker frequency can be observed only when the former signal is slant-clipped (Scheich, 1977a). It is likely, therefore, that the competing theories of Scheich (1977a) and Heiligenberg et al. (1978) for the control of JARs actually account for qualitatively different behaviors. As in natural JARs, shifts in the EOD frequency above resting level only occur reliably when EOD-substitute and jamming signals are presented with different spatial geometry.

Materials and methods

Animals

Eigenmannia sp. (11–17 cm total length) was obtained through a local dealer. They were kept in holding tanks at 27–29 °C under a 12 h:12 h L:D photoperiod. Water resistivity was kept between 4 and 7 kΩ cm. Fish were fed live blackworms every other day.

Experimental tank

Experiments were performed in a tank (60 cm×60 cm×13 cm) circulated with aerated and filtered water (27 °C). Water resistivity was strictly regulated between 4 and 6.6 kΩ cm in all experiments. Electrical behaviors expressed as changes in pacemaker frequencies were examined in both intact and curarized fish. Intact fish were confined in a sock made of plastic mesh which was submerged in the experimental tank. Their electric organ discharges (EODs) were recorded with a pair of carbon rod electrodes placed near the fish's head and tail. Curarized fish (intramuscular injection of 3 µl of 0.1 % gallamine triethiodide) were held by sponge-lined fish clamp and suspended in water. Their gills were perfused with aerated water. The command signal from the pacemaker nucleus which normally drives EODs was recorded by a suction electrode fitted to the tail.

Stimulus

Stimuli that were delivered to curarized fish were prepared numerically in computer arrays at 16-bit precision (Tucker-Davis Technology, AP-2). Different arrays containing different stimuli were chosen by custom-designed software and read out *via* D/A converters (Tucker-Davis Technology, DA3-4) at a sampling rate of 100 kHz. Stimuli mimicking the fish's own EOD, S_1, and mimicking a neighbor's EOD, S_2, were applied with two different modes of geometry, 'identical geometry' and 'differential geometry'. For the identical geometry mode, S_1 and S_2 were numerically added in a computer array. The mixed signal was generated by a D/A converter and presented through a stimulus isolator and a pair of electrodes. One electrode was inserted in the mouth and the other electrode was placed near the tail of the fish. For the differential geometry mode, S_1 and S_2 were prepared in separate computer arrays and were generated using two channels of D/A converters and stimulus isolators. S_1 was applied between the mouth and the tail electrodes. S_2 was applied through two carbon-rod electrodes, each 10 cm from the side of the fish. The amplitude of S_1 was measured at the gill cover and set at 1–2 mV cm^{-1} unless mentioned otherwise.

To control the amplitude and phase of signals independently in different regions of the body surface, fish were placed in a 'phase chamber' (Bullock et al., 1972a; Heiligenberg and Bastian, 1980). In this mode, a curarized fish was placed in a chamber in which its head and trunk portions were electrically isolated by a partition. The head and trunk compartments were stimulated with independent signals, each of which was manipulated in amplitude and phase. The same electronic equipment as described above was used.

Intact fish were stimulated with an amplitude-modulated EOD which was simultaneously recorded from the fish. The EOD was fed into an analog multiplier (Tucker-Davis Technology, MT-3), which received a sinusoidal signal for amplitude modulation from a function generator. The output of the analog multiplier was applied to the tank using the external pair of electrodes mentioned above *via* a stimulus isolator. This stimulus produced approximately 30 % amplitude modulation of the intact EOD measured at the gill cover. The recording pair of electrodes and the stimulating pair of electrodes were arranged perpendicularly so that the stimulus did not feed back into the recording.

The frequency difference, Δf, between S_1 and S_2 is defined as $\Delta f = f_2 - f_1$, f_n being the frequency of S_n. In natural JARs, acceleration and deceleration of the pacemaker are, respectively, expected for negative Δf and positive Δf conditions.

Data acquisition and analysis

The intact EOD and pacemaker command signal were first converted into a train of digital pulses, each of which represents a zero-crossing time of the signal. These pulses were fed into a divide-by-100 counter whose output pulses were registered by a time stamper (Tucker-Davis Technology, ET-1, 100 ns resolution). Intervals of these divide-by-100 pulses were converted into frequency. Thus, 100 cycles of the signal (200–400 Hz) were averaged, and sampling was made at a few hertz.

Before and between presentations of test stimuli, the 'background' stimulus, which differed from the test stimulus only by the tested attribute (the presence of amplitude modulation, or S_2, etc.), was constantly applied. For quantitative measurement of responses, 30 s test stimuli were repeatedly applied with 30 s resting periods. Response magnitudes are expressed as the mean frequency changes over the 30 s stimulus period (in Hz).

Results

Replication of experiments by Scheich (1977a)

Curarized fish were tested with an S_1 that simulated the natural EOD waveform and a sinusoidal S_2. S_1 consisted of a triangular wave whose bottom was slant-clipped and filtered (Fig. 1A), which mimicked the signal in Fig. 6 of Scheich (1977a). S_1 and S_2 were presented with identical spatial geometry using an electrode pair. As shown in Fig. 1A, the mixture of S_1 and S_2 showed time-asymmetric envelopes which were temporally reversed when the sign of Δf was changed. All fish tested showed frequency deceleration responses regardless of the sign of Δf. Some fish showed stronger deceleration responses to negative Δf than to positive Δf, and other fish showed the opposite preference. Alternated presentation of negative and positive Δf could induce JAR-like responses (Fig. 1B). That all fish showed frequency decelerations to both signs of Δf contradicts the statement made in Scheich (page 195, 1977a) that 'the response goes above and below the resting frequency of the pacemaker'. We attempted to replicate his result by changing the stimulus parameters. Since stimulus intensity and geometry are hardly mentioned by Scheich (1977a), we tested a range of stimulus intensities (1, 2, 6, 20 and 40 mV cm^{-1}) and two different positions of stimulus electrodes. In one condition, the positive electrode for S_1 was placed in the mouth, as in all other experiments with curarized fish in this study. In the other condition, stimuli were applied through two carbon-rod electrodes each 10 cm from the side of the fish. We tested all combinations of these parameters in 10 fish. Two of the 10 fish increased their pacemaker frequency above and below the resting level according to the sign of Δf, provided that the amplitude was large (in one fish >10 mV cm^{-1}, in a single other fish >40 mV cm^{-1}). Five fish showed frequency decelerations regardless of the sign of Δf, but the magnitude of these responses diminished as the amplitude exceeded 10 mV cm^{-1}. Three fish showed frequency decelerations at normal stimulus amplitudes, as in the other fish, but often showed large (approximately 5 Hz) frequency accelerations to both signs of Δf when the amplitude exceeded 20 mV cm^{-1}. No systematic difference was found in terms of the electrode position.

Comparison of identical and differential geometry

S_1 and S_2, given with identical geometry in Fig. 1A,B, were later applied to the same preparation with differential geometry. With differential geometry, the pacemaker frequency increased above the resting level for negative Δf and decreased for positive Δf (Fig. 1C). The absolute magnitudes of the decelerations were larger than for the accelerations in all 16 fish tested.

Frequency deceleration response to amplitude modulation (curarized fish)

The stimulus given in the experiments in Fig. 1B contains modulations in both amplitude and phase. We tested whether only amplitude modulation is sufficient for the frequency-decelerating response described above. Indeed, when the amplitude of a sinusoidal S_1 was modulated sinusoidally at low frequencies (approximately 4 Hz), curarized fish consistently decreased their pacemaker frequency. The time course of the frequency deceleration was similar to that observed during JARs. The response magnitude and time course were consistent from trial to trial, although there was individual variation. Across fish, the magnitude of responses ranged from a few Hz to 6 Hz for presentation of 30 % amplitude

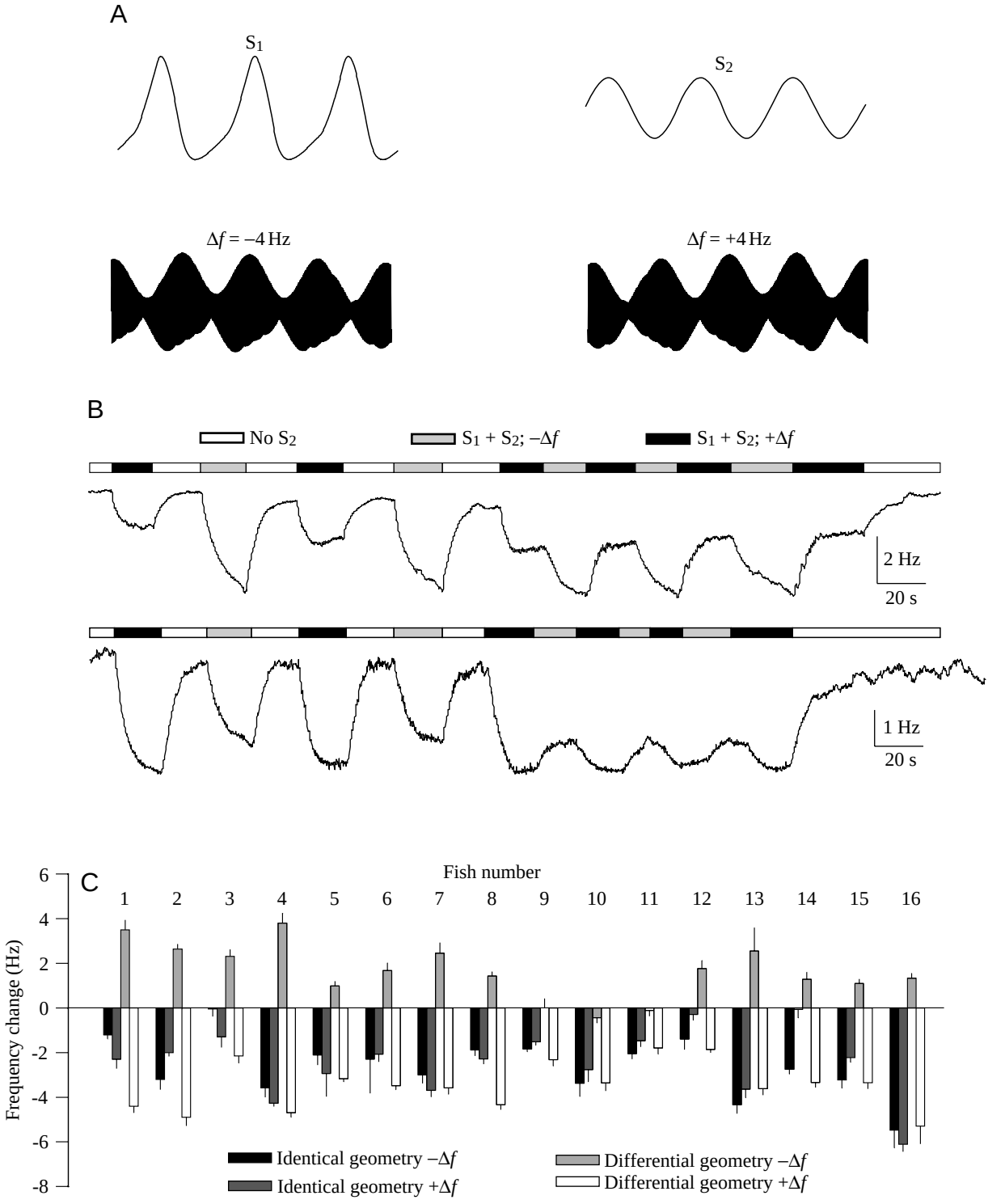

Fig. 1. (A) Top: waveform of signal S_1 (left); sinusoidal signal S_2 whose amplitude was 70 % of S_1 (right). Bottom: the sum of these two signals with their frequency differences −4 Hz (left) and +4 Hz (right). Note the temporally asymmetric envelopes. (B) Results from two representative fish which showed stronger deceleration responses to $-\Delta f$ (top) and to $+\Delta f$ (bottom). Note the jamming-avoidance-response-like responses when negative Δf and positive Δf were presented alternately (right half). (C) S_1 and S_2 shown in A were applied as identical geometry and differential geometry (see Materials and methods) to 16 randomly chosen fish. Note that frequency acceleration occurs only for negative Δf with differential geometry, and all fish showed deceleration responses to all identical geometry signals. Values are means ± S.D. Δf, frequency difference.

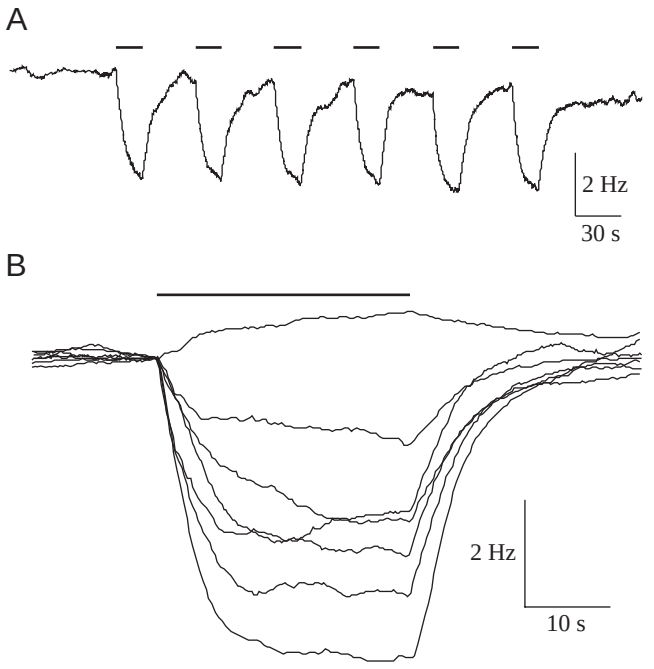

Fig. 2. Frequency deceleration responses of the pacemaker to amplitude-modulated signal S_1 (depth 30 %, modulation frequency 4 Hz) in curarized fish. (A) Amplitude-modulated S_1 was given during the horizontal bars. Unmodulated S_1 was given in all other periods. Pacemaker frequency consistently decreased by 3.5 Hz. (B) Responses to the same amplitude-modulated S_1 in seven fish, representing the range of response magnitudes. In one exceptional fish (out of more than 40 fish), we observed small but consistent frequency-increasing responses (uppermost trace).

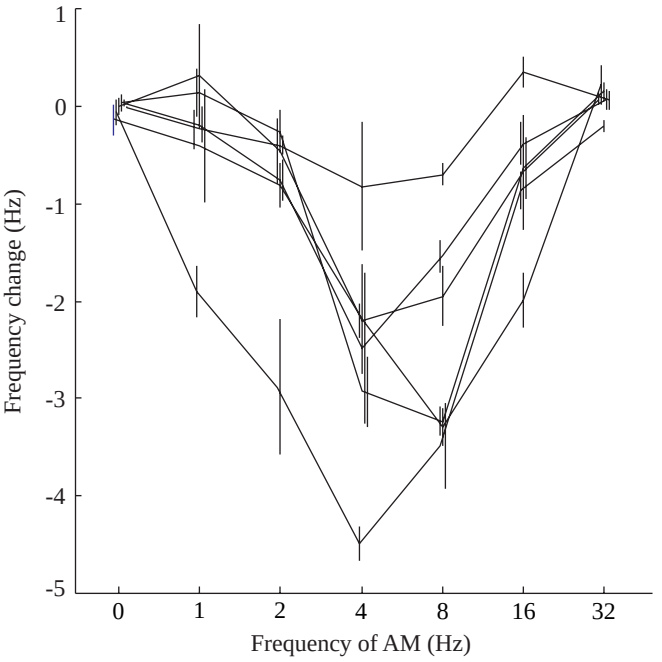

Fig. 3. Magnitude of frequency deceleration responses to 30 % sinusoidal amplitude modulation (AM) at various modulation frequencies. Different lines present data from different fish. Each point represents a mean from 4–10 trials. Error bars show standard deviations.

modulation. The direction of the response to amplitude modulation was always deceleration, with only one exceptional fish (out of more than 40 fish tested) which showed weak acceleration responses (Fig. 2). The response was greatest when the amplitude modulation frequency was approximately 4 Hz (Fig. 3) and occurred over a wide range of amplitude modulation depths (Fig. 4). An amplitude modulation depth of a few per cent was generally strong enough to induce responses. One particularly sensitive fish responded to an amplitude modulation at 0.1 %. Some individuals were insensitive, requiring an amplitude modulation of more than 10 % (Fig. 4).

Frequency deceleration response to amplitude modulation (intact fish)

In curarized preparations, the electric field that is generated by the EOD mimic (S_1) is not identical to the natural field. To assess whether the deceleration responses are restricted to the curarized preparation, we measured the EOD frequency of intact fish while the EOD was amplitude-modulated. Albeit somewhat weaker, all intact fish tested showed frequency deceleration responses when the fish's own EOD recorded in the tank was amplitude-modulated and returned to the tank (Fig. 5A). The responses of intact fish to different modulation depths and frequencies were similar to those of curarized fish. One of three fish tested showed small frequency accelerations at certain modulation parameters (Fig. 5B,C).

Temporal sensitivity of frequency deceleration responses

We used sinusoidal amplitude modulation in the experiments so far described. Other waveforms of amplitude modulation, such as triangular and square, also decelerated the pacemaker frequency below the resting level. We also compared the effects of temporally symmetrical ramps, up-ramp and down-ramp waveforms for amplitude modulation (Fig. 6A). Both up-ramp and down-ramp modulation induced frequency deceleration responses, but the response magnitude was often different for up- and down-ramps. Some fish preferred up-ramp modulation to down-ramp modulation, other fish showed the opposite preference. The differences between the effects of up- and down-ramps were usually small, but could be as large as a factor of two, as shown in Fig. 6B,C. When the preference was strong, alternated presentation of up- and down-ramp amplitude modulations resulted in JAR-like changes in the pacemaker frequency (Fig. 6B).

Effects of phase modulation

The mixture of S_1 and S_2 presented with identical geometry (Fig. 1B) exhibits modulations not only in amplitude (envelope) but also in phase. Direct measurement of the signal revealed that the zero-crossing times were modulated by approximately 200 µs. The effects of this phase modulation on

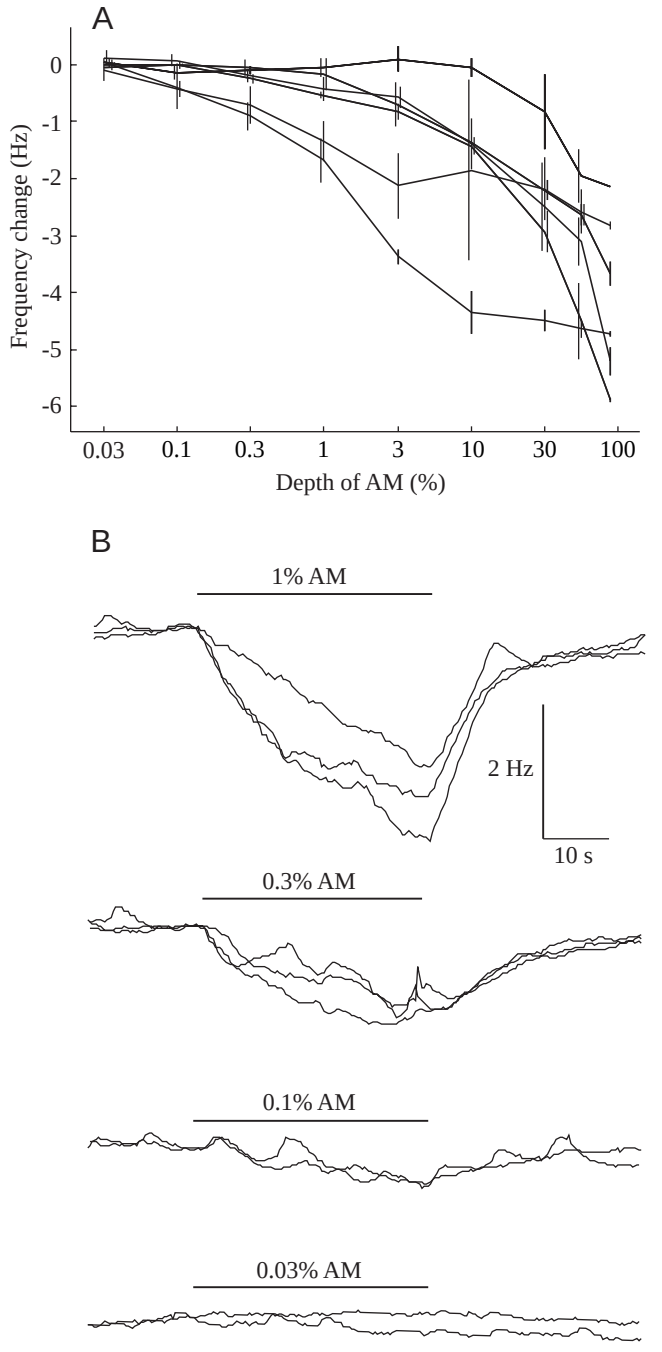

Fig. 4. (A) Magnitude of frequency deceleration responses to 4 Hz sinusoidal amplitude modulation (AM) at various modulation depths. Each line presents data from one fish. Each point represents the mean from 4–8 trials. Error bars show standard deviations. (B) Frequency traces from a particularly sensitive fish (lowest curve in A). Each trace represents a single trial (no averaging).

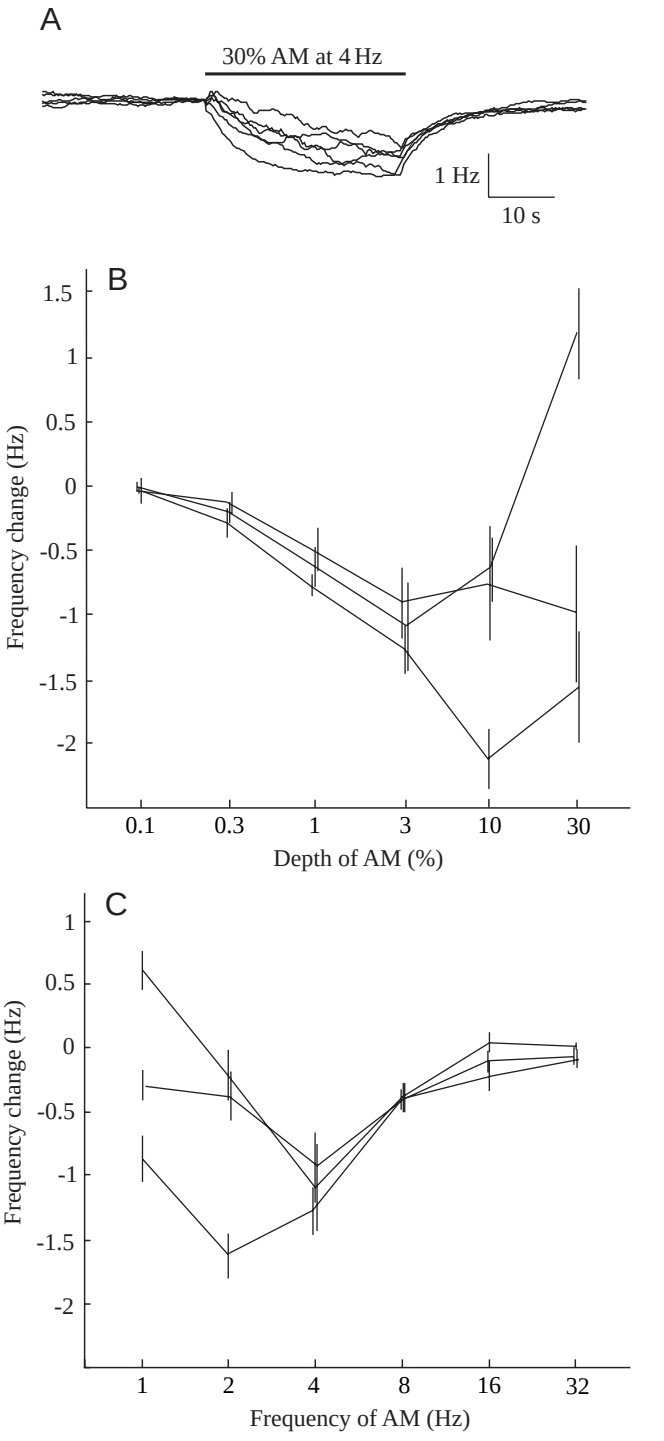

Fig. 5. (A) Six trials of 30 % amplitude modulation (AM) at 4 Hz in two intact fish showing frequency deceleration responses. (B) Magnitude of frequency deceleration responses to 4 Hz sinusoidal amplitude modulation at various modulation depths. (C) Modulation frequency tuning from three fish. Modulation depth was approximately 30 % for C. Different lines present data from different fish. Each point represents mean of 4–6 trials. Standard deviations are shown.

frequency deceleration responses were examined by using a ramp modulated sinusoidal signal whose phase was numerically manipulated. Phase modulation at the same modulation frequency as the ramp amplitude modulation frequency (4 Hz) was introduced. To examine any possible temporal interaction between amplitude and phase modulations, we tested four different phase relationships between amplitude and phase modulations (0 °, 90 °, 180 ° and

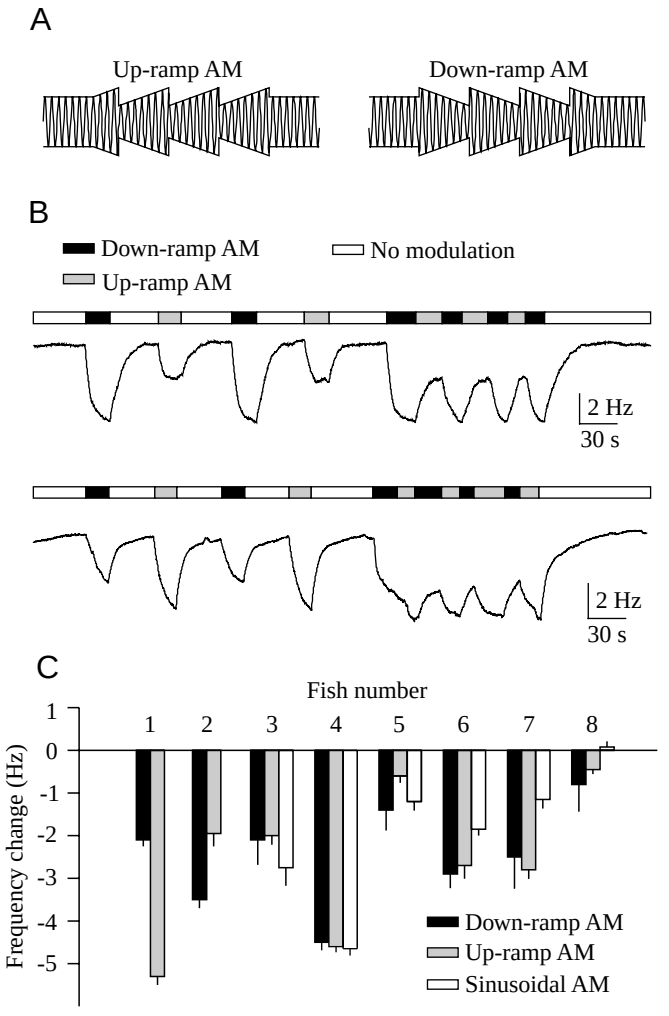

Fig. 6. (A) Envelopes of an S_1 amplitude modulated by down- and up-ramp waveforms. (B) Differential effects of down- and up-ramp amplitude modulation (AM) (30 %, 4 Hz) on frequency deceleration responses of two fish (top and bottom). The down-ramp and up-ramp were first presented intermittently to observe their individual effects (left half). When they were presented alternately, jamming-avoidance-response-like responses appeared (right half). (C) Comparison of eight individuals for up- and down-ramp modulation and for sinusoidal modulation. Each bar represents a mean value from six trials. Error bars show standard deviations. Modulation frequency was 4 Hz, depth of modulation was 30 % in all cases.

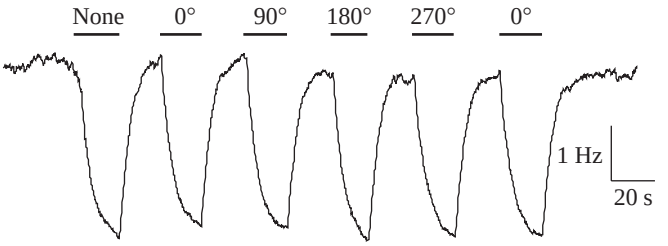

Fig. 7. Effects of phase-modulated down-ramp amplitude modulation (30 %, 4 Hz) were compared. Phase modulation (4 Hz, 200 µs peak-to-peak) was introduced in the down-ramp amplitude modulation stimulus with four different values of phase relationship (0 °, 90 °, 180 ° and 270 °) to the ramp envelope. Regardless of the presence and the relationship of phase modulation, the fish showed constant frequency deceleration responses.

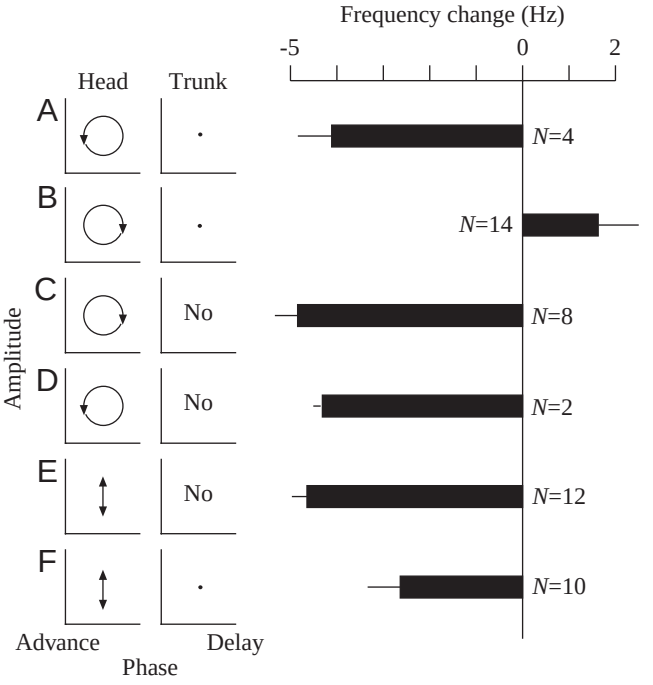

Fig. 8. The head and trunk of the fish were stimulated independently in a phase chamber. The signal in the head was modulated in amplitude and/or phase. Amplitude and phase modulation of the signals are plotted, respectively, on the ordinate and abscissa of the planes on the left. Depths of modulations were ±30 % in amplitude and ±100 µs in phase in all modulated cases. A dot in A, B and F indicates no modulation in either parameter, but the presence of an unmodulated carrier signal (a 380 Hz sinusoid). 'No' on the plane denotes the absence of a signal. Note the differential response in A and B, and equally strong frequency deceleration in C, D and E. Values are means ± S.D.

270 °). None of these signals showed any difference in causing frequency deceleration responses from the signal without phase modulation (Fig. 7).

Phase chamber experiment

Using their theory of JAR computation, Heiligenberg and Partridge (1981) attempted to explain the experimental observation of Scheich (1977a) that signals presented with identical geometry induce JAR-like responses if S_1 contains higher harmonics (for more detail, see Discussion). In their explanation, phase difference plays a crucial role. To examine the possible role of *differential* phase information in inducing the above-mentioned frequency deceleration responses to amplitude modulations, we tested fish in a phase chamber in which the amplitude and phase difference between the head and trunk areas of the body could be independently controlled (Bullock et al., 1972a; Heiligenberg and Bastian, 1980). When the amplitude and phase of the signal in the head compartment

mimicked their natural pattern of modulation for negative Δf and positive Δf, the pacemaker frequency increased and decreased from its resting level, respectively (Fig. 8A,B). As expected from their theory, this occurred only if a phase reference signal was provided in the trunk compartment. The removal of the phase reference signal from the trunk compartment, however, did not abolish shifts in the pacemaker frequency. Without a reference signal in the trunk compartment, strong frequency-decelerating responses occurred whenever the signal in the head compartment was amplitude-modulated, regardless of the absolute phase modulation that was also present in the head compartment (Fig. 8C–E). With amplitude modulation of the signal in the head compartment alone, the magnitude of these decelerations was independent of whether a reference signal (unmodulated) was also present in the tail compartment. These experiments indicate that the amplitude modulation component alone can induce strong frequency-decelerating behavior even when only part of the body surface is stimulated.

Discussion

In its JAR, *Eigenmannia* raises or lowers its EOD frequency above or below resting levels when it senses an EOD of another fish that is lower or higher than its resting frequency, respectively (Watanabe and Takeda, 1963; Bullock et al., 1972a,b). Previous work has resulted in two competing theories to explain the control of the JAR of *Eigenmannia*. These theories are based on the results of experiments in which the fish's own EOD is silenced and replaced by an artificial signal. One theory holds that the signal that substitutes for the fish's own EOD (the strongest signal, S_1) must be clipped with appropriate polarity in order to elicit correct JARs; the second (jamming) signal (S_2) can be sinusoidal (Scheich, 1977a). In this case, higher harmonics in the stimulus generate a temporally asymmetric beat pattern, and this asymmetry differs for negative *versus* positive Δf conditions (see Fig. 1). This theory is based on experimental data obtained by presenting S_1 and S_2 with the same spatial geometry. The other theory (for reviews, see Heiligenberg, 1989, 1991) postulates that the decision to change the EOD frequency in the appropriate direction results from a comparison of the amplitude and phase of signals in areas of the body surface that are differentially contaminated by the S_1 and S_2 signals. Significantly, when the two signals are presented with different geometry, this requirement is met, and fish perform correct JARs even when sinusoidal signals are used for both S_1 and S_2 (Heiligenberg et al., 1978; Heiligenberg and Bastian, 1980). Presenting the two sinusoidal signals with identical geometry, i.e. from the same pair of electrodes, results in no differential response to negative *versus* positive Δf conditions, provided that the magnitude of Δf is held constant.

Are there two mechanisms that fish can use to determine the correct direction to shift their EOD frequency or is one theory incorrect? In addressing this question, it is important to identify (1) whether sensory stimulation induces fish to change their EOD frequency above or below their resting frequency (the EOD frequency before the jamming signal has been applied), and (2) whether stimulus conditions that lead to JAR-like responses are observed when signals are in the biologically relevant range.

In attempting to replicate Scheich's experiments with a clipped S_1, we did not find increases in EOD frequency in response to negative Δf conditions when the S_1 and S_2 signals were of normal amplitude and were presented with identical spatial geometry (Fig. 1). In an earlier attempt at replicating Scheich's results, Heiligenberg (1991) reported that small JARs could be elicited when a clipped S_1 and sinusoidal S_2 were presented with identical spatial geometry, but not when both signals were sinusoids. It is not clear, however, whether the fish actually increased their EOD frequency above their resting level in response to negative Δf conditions. Further, Heiligenberg (1991) reported that 'while some individuals behaved in accordance with the original claim by shifting in the correct direction if the polarity of the EOD mimic corresponded to that of the natural EOD waveform, other individuals did exactly the opposite'. What was even more revealing was that an animal might produce the 'correct' behavior for several minutes and then slowly, after several minutes of small and unpredictable frequency fluctuations, switch to 'incorrect' behavior.' These findings are in agreement with our observations that, as the sign of Δf (clipped S_1 + sinusoidal S_2) is alternated, some fish show JAR-like fluctuations of their EOD frequency that are in the correct direction, while others change their EOD frequency in the incorrect direction (Fig. 1B,C). Significantly, however, unlike in normal JARs, these fish failed to increase their EOD frequency above the resting level. In addition, we have shown that similar types of frequency fluctuations can be produced simply by switching the symmetry of 'saw-toothed' amplitude modulation (up-ramp, down-ramp amplitude modulation). As shown in Fig. 1A, time-asymmetric amplitude envelopes result when a sinusoidal S_2 is added to a clipped S_1. Because the direction of time asymmetry of the beat envelope is opposite for negative *versus* positive Δf conditions, differential decreases in EOD frequency would be expected. It appears, therefore, that these identical-geometry stimuli, when in the normal range of amplitude, do not cause fish to shift their EOD frequency above resting level.

Scheich (1977a, p.194) states that 'the response goes above and below the resting frequency of the pacemaker'. In these experiments, frequency rises above the resting level were observed primarily in fresh preparations (T. Bullock, personal communication). Similarly, we occasionally observed frequency increases in response to identical-geometry stimuli, but only when the S_1 amplitude was well above the normal range; we did not observe a frequency rise in response to negative Δf with a normal amplitude of S_1. Both studies are in agreement, therefore, that rises in frequency above baseline cannot be reliably elicited when the EOD substitute and jamming signals are presented with identical spatial geometry. Bullock et al. (1972b, p.34) report that pure amplitude

modulation given to intact *Eigenmannia* induces an 'irregular increase and decrease of a few seconds duration', whereas our intact fish always responded with frequency deceleration. We are currently unable to determine the source of this discrepancy. This could be due to differences in conditions of curarized preparation and/or species differences of our subject fish. Scheich's original observation that differential effects of different envelope waveforms on frequency-shifting behavior (Scheich, 1977a) has been confirmed by our current study.

Heiligenberg attempted to account for the changes in EOD frequency in response to presentation of a clipped S_1 and sinusoidal S_2 by suggesting that differential phase information, which does not exist in Scheich's stimuli and is essential for the JAR, might be created within the central nervous system by the amplitude-sensitivities and the dynamics of certain 'T-type' (phase-coding) afferents (Heiligenberg and Partridge, 1981; Heiligenberg, 1991). We will call these amplitude-induced phase differences 'phantom phase differences', because they are not present in the stimulus that is received by the fish but emerge internally. In this formulation, the EOD frequency decrease in response to pure sinusoidal amplitude modulations is neglected. According to Heiligenberg's theory, sinusoidal amplitude modulations should have no effect on the EOD frequency. As can be seen in Fig. 2, this is clearly not the case. Because pure amplitude modulations elicit strong decelerations of the EOD frequency, the question arises as to whether these responses are discretely generated by activity of amplitude-coding neurons alone or are a consequence of the interaction between amplitude and phantom differential phase information in the central nervous system. We have examined the importance of the phantom phase differences on the frequency deceleration behavior using a 'phase chamber'. In this apparatus, receptive fields on the head can be stimulated separately from those caudal to the electrical partition. If amplitude-dependent modulations in the timing of T-unit firing (Heiligenberg and Partridge, 1981) were present, then phantom phase differences would be greatest when a signal was present as a phase reference in the trunk compartment. We found that strong deceleration responses to pure amplitude modulations of the signal in the head occur even without a reference signal in the trunk. Also, the presence of phase modulation within the head compartment did not contribute to the deceleration responses (Fig. 8). Adding a reference signal in the tail compartment actually attenuated the deceleration response. These findings suggest that phantom differential phase information is not responsible for the strong deceleration responses to pure amplitude modulations observed in the present study, although it may be generated and still contribute to pacemaker frequency shifts to some extent as Heiligenberg and Partridge (1981) suggested.

In the present experiments, at normal stimulus amplitudes, increases in EOD frequency above the resting level could only be elicited when S_1 and an S_2 of lower frequency (2–8 Hz is best) were presented with different spatial geometry or when the appropriate spatiotemporal relationships between signal amplitude and differential phase were provided (Heiligenberg et al., 1978; Heiligenberg and Bastian, 1980); at normal stimulus amplitudes, presentation of a clipped S_1 and a sinusoidal S_2 did not elicit increases in the EOD frequency unless they were presented with different spatial geometry. It is important in this regard to point out that categorically different behaviors that require different stimulus parameters appear previously to have been called 'JARs', as defined as the difference in EOD frequency for negative *versus* positive Δf conditions. According to this classical definition, therefore, even differential decelerations, such as those that result from the presentation of sinusoidal and clipped sine waves with identical spatial geometry, qualify as JARs. In natural jamming encounters, however, fish raise their EOD frequency above baseline in response to lower-frequency jamming signals of a neighbor. We suggest, therefore, that future measurements of frequency-shifting behaviors in *Eigenmannia* be related to each fish's resting EOD frequency. These frequency shifts should then be related to those that each fish produces in response to 'natural' jamming conditions, i.e. differential but overlapping spatial geometry of jamming and EOD-substitute signals. To be representative of natural JARs, shifts in the EOD frequency above and below baseline in response to stimuli representing negative Δf and positive Δf conditions, respectively, should be proportionately similar to those measured under 'natural' conditions; the EOD frequency must increase above resting level reliably for negative Δf conditions, as it does in natural conditions where stimuli have different spatial geometry. Decreases in EOD frequency, however, can be elicited by pure amplitude modulations, in addition to positive Δf conditions.

In conclusion, the JAR observed as a natural behavior appears to be the sum of the effects of two processes. The first component is the sensory process theorized by Heiligenberg, which requires amplitude modulation and differential phase modulation. This process differentiates the sign of Δf and creates motor effects of opposite direction. The second component is the frequency deceleration response to amplitude modulation alone, reported in this study, which was not recognized by Heiligenberg. This process does not differentiate the sign of Δf, always creating frequency deceleration responses. The relative magnitude of these decelerations depends, somewhat unpredictably, on temporal asymmetries of the amplitude envelope. What, then, might be the function of the second process since the first process seems to be sufficient for the behavioral function of the JAR? The first process may have stronger motor effects for frequency-accelerating (negative Δf) stimuli than for frequency-decelerating (positive Δf) stimuli. This idea is supported by the observation that, for most fish, the magnitude of deceleration responses is very similar for positive Δf and amplitude modulation stimuli. The frequency deceleration response to amplitude modulation components may function to 'balance' the magnitudes of excursions above and below resting levels during natural JARs. Further work is needed to test this idea.

Although these two processes could be differentially activated, some neuronal mechanisms are undoubtedly shared.

Both JARs induced by Heiligenberg's mechanism and the frequency-deceleration responses reported here occur most strongly when the modulation frequency is approximately 4 Hz. Moreover, the deceleration behavior can be measured even with amplitude modulation depths of only 0.3 % (Fig. 4B). Similarly high sensitivity to amplitude modulation is demonstrated in the JAR (Rose and Heiligenberg, 1985).

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