ORIGINAL PAPER

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Sensory cues for the gradual frequency fall responses of the gymnotiform electric fish, *Rhamphichthys rostratus*

Abstract The sensory cues for a less known form of frequency shifting behavior, gradual frequency falls, of electric organ discharges (EODs) in a pulse-type gymnotiform electric fish, *Rhamphichthys rostratus*, were identified. We found that the gradual frequency fall occurs independently of more commonly observed momentary phase shifting behavior, and is due to perturbation of sensory feedback of the fish's own EODs by EODs of neighboring fish. The following components were identified as essential features in the signal mixture of the fish's own and the neighbor's EOD pulses: (1) the neighbor's pulses must be placed within a few millisecond of the fish's own pulses, (2) the neighbor's pulses, presented singly at low frequencies (0.2–4 Hz), were sufficient, (3) the frequency of individual pulse presentation must be below 4 Hz, (4) amplitude modulation of the sensory feedback of the fish's own pulses induced by such insertions of the neighbor's pulses must contain a high frequency component: sinusoidal amplitude modulation of the fish's own EOD feedback at these low frequencies does not induce gradual frequency falls. Differential stimulation across body surfaces, which is required for the jamming avoidance response (JAR) of wave-type gymnotiform electric fish, was not necessary for this behavior. We propose a cascade of high-pass and low-pass frequency filters within the amplitude processing pathway in the central nervous system as the mechanism of the gradual frequency fall response.

Key words Electric fish · Gymnotiform fish · Pulse-type electric fish · Jamming avoidance response · Temporal pattern

Abbreviations *EOD* electric organ discharge · *f* frequency of EOD or pacemaker command signal· *JAR* jamming avoidance response ·

S_1 stimulus mimicking fish's own EOD ·
f_1 frequency of S_1 ·
S_2 stimulus mimicking neighbor's EOD ·
f_2 frequency of S_2

Introduction

Gymnotiform electric fishes generate electric organ discharges (EODs) at a constant frequency by their electric organ in the tail. An electric field is thus established around the body, and its distortion by objects is detected by electroreceptors located over the body surfaces. The major noise source of this electrolocation system is EODs of neighboring electric fish, since the electrosensory system is stimulated by the complex mixture of the fish's own and its neighbor's EODs. When encountering neighbors' EODs, all genera of gymnotiform electric fishes but one thus far examined perform jamming avoidance responses (JARs) in which they alter the pattern of EODs to minimize the detrimental effects of signal interference between their own and their neighbors' EODs (Bullock 1982). Since the pacemaker nucleus in the medulla, which creates the rhythm of EOD and drives the electric organ, does not send corollary discharge information to the electrosensory system within the nervous system, the electrosensory system must analyze a complex signal mixture of the fish's own and the neighbor's EODs to perform an appropriate JAR (Heiligenberg 1977; Heiligenberg and Bastian 1980).

Gymnotiform electric fishes are divided into two functional groups at the level of family with regard to the form of EODs: pulse-type and wave-type (Mago-Leccia and Zaret 1978; Hopkins 1988). Pulse-type gymnotiform fishes produce short EODs with relatively longer inter-EOD intervals. In wave-type gymnotiform fishes, in contrast, the duration of each EOD is comparable to the inter-EOD interval, thus, EOD wave form is continuous. No single family of Gymnotiformes accommodates both types.

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JARs of wave- and pulse-type fishes take different forms. In wave-type species, JARs consist of gradual frequency shifts of the EOD by which a fish maximizes the frequency differences between its own and the neighbors' EODs (Watanabe and Takeda 1963; Bullock et al. 1972a, b). By increasing the frequency difference, the fish avoids the resulting low frequency beating. In contrast, JARs of all pulse-type fishes so far examined consist of rapid phase shifts of their EODs which effectively minimize the temporal coincidences of their own and their neighbors' EOD pulses (Heiligenberg 1974, 1980; Heiligenberg et al. 1978; Baker 1980). Merely making a smooth shift of EOD frequency away from that of a neighbor would, in fact, increase the rate of coincidences.

Nevertheless pulse-species occasionally perform a slow and smooth frequency shift over the course of seconds that is similar to the JAR of wave-type species. Such behavior has been observed in *Hypopygus* (Heiligenberg 1974), *Gymnotus* (Westby 1975), *Hypopomus*, *Parupygus* and *Gymnorhamphichthys* (Heiligenberg 1977), *Rhamphichthys* (Scheich et al. 1977) and *Parupygus* (Heiligenberg et al. 1978). In these studies, however, the magnitude of frequency shifts was rather small (often less than 1 Hz). In the present study, a high-frequency pulse-type species, *Rhamphichthys rostratus*, has been found to exhibit much larger gradual frequency falls (~ 10 Hz with resting EOD frequency of 50–80 Hz), in addition to the phase-shifting JARs. We have asked the following questions about this behavior: Are the sensory mechanisms of the behavior similar to those for gradual frequency shifting JARs in wave-type electric fishes? Do the frequency fall responses in *Rhamphichthys* occur under similar computational mechanisms as those of wave-type species?

Neuronal mechanisms underlying the JAR of a wave-type gymnotiform fish, *Eigenmannia*, have been extensively studied in terms of the computational algorithm, physiology and anatomy (Heiligenberg 1991). In this study, we have identified the essential sensory cues and computational features for the gradual frequency fall in *Rhamphichthys* in order to compare this type of behavior in *Rhamphichthys* with the JAR of wave-type species.

When the frequency of neighbor's EODs is slightly different from that of the fish's own discharge, the EOD pulses of the neighbor slowly shift in time through the cycle period of the fish's own EODs (Fig. 2 in Heiligenberg et al. 1978). When one displays EODs on an oscilloscope and triggers the oscilloscope with the fish's own pulses, the fish's own pulses appear stationary. The neighbor's pulses, however, travel from right to left when the neighbor's frequency is higher and left to right when the neighbor's frequency is lower. In the present study, the complex pattern of sensory stimuli created by the mixture of the two fish's EODs was experimentally simplified by replacing natural EODs with artificially created electrical pulses, which were finely controlled for the identification of essential features in the stimulus. A simple temporal pattern was found to be essential for evoking this behavior.

While the JAR of wave-type species requires a specific temporal pattern of amplitude modulation and phase modulation, the slow frequency falls of *Rhamphichthys* could be elicited with only a specific temporal pattern of amplitude modulation.

Materials and methods

Animals

Six individuals of *Rhamphichthys rostratus* (50 to 67 cm) were used. The fish were obtained from a local dealer and kept in 40 gallon tanks (26–28°C). Water resistivity in the holding and experimental tanks was kept at 5–10 k$\Omega \cdot$cm. Fish were regularly fed with live black worms. A Light-Dark regime of 12L:12D was maintained, and all experiments were performed during the fish's subjective daytime. *Rhamphichthys* stays motionless for hours during daytime and thus does not need to be restrained for behavioral experiments. EOD frequency, f, at rest ranged from 50 to 90 Hz at water temperatures of 27°C during daytime. A voucher specimen of *Rhamphichthys rostratus* has been deposited in the collection at the Smithsonian Natural History Museum (USNM337915). A drawing of a specimen and its EOD are provided in Fig. 1.

Preparation and stimulus

In *Experiments A–D* (Figs. 2–5) and *H* (Fig. 9), an intact fish was held in a loose plant (Java moss) oriented longitudinally within a tank (45 × 45 × 90 cm). A pair of carbon rod electrodes was placed at either end of the tank to record the fish's EODs, while EODs mimicking a neighbor could be presented through a second pair of electrodes

Fig. 1 *Rhamphichthys rostratus* used in this study. The EOD was recorded with a longitudinally arranged pair of electrodes (80 cm apart) with a head-positive orientation

10 cm

1 ms
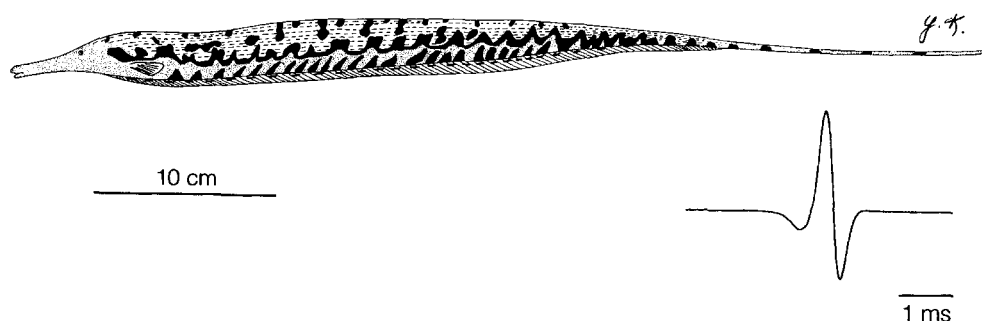
oriented perpendicularly to the first pair. The neighbor's EODs were mimicked by a train of pulses generated by a function generator. Each pulse was a single period sinusoid with 770 μs duration which corresponds to the dominant frequency component of 1.3 kHz in the EOD. This value was determined after Fourier analysis of the fish's natural EODs. Individual occurrences of these mimicking pulses, S_2, were controlled by a computer in various ways (see below). The intensity of the S_2 was typically 5 mV/cm at the center of the tank.

In *Exps. E–G* (Figs. 6–8), fish were immobilized for several hours by intramuscular injection of Flaxedil (gallamine triethiodide 1% solution, 15-20 μl) which attenuated the amplitude of the EOD greatly. Immobilized fish were held by three sponge-lined fork-shaped holders in a tank ($12 \times 45 \times 45$ cm) with a gutter-shaped extension (20 cm long) which accommodated the fish's long tail. The gills were perfused with aerated water at 100 ml/s through a glass tube inserted loosely into the mouth. The attenuated EOD shows the frequency of the fish's pacemaker command signal and is referred to here as the EOD. The attenuated EOD was recorded by a pair of wires placed alongside the tail. The attenuated EODs triggered 770 μs long, single-cycle sinusoidal pulses, S_1, which served as artificial EODs substituting for the original full sized EODs. S_1 pulses were generated by a function generator and triggered by the attenuated EOD after various delays. Identical S_1 pulses were applied through four electrodes. The positive pole of a stimulus isolator was connected to three electrodes. The first one was a silver wire inserted in the mouth cavity, the other two were pins inserted into the dorsal musculature at the anterior trunk and posterior trunk regions. The fourth electrode was placed at the tip of the tail and was connected to the negative pole of the isolator. This arrangement approximated the natural pattern of the EOD field of this elongated species.

The amplitude of natural EODs was measured before curarization in each fish at the anterior part of the body, using a fork electrode and S_1 was set to this value. The amplitude was between 15 and 25 mV/cm measured at and perpendicular to the gill cover.

In *Exp. F* (Fig. 7), S_2 was delivered in either of two ways: with differential geometry or with identical geometry. In the differential geometry condition, the S_2 was delivered in the transverse direction as discussed above. In the identical geometry condition, S_1 and S_2 were electronically mixed and delivered through the same electrodes as used for S_1.

In *Exp. H* (Fig. 9), two intact fish were housed in separate tanks ($45 \times 45 \times 90$ cm). In each tank an intact fish was held in a loose plant, and its EODs were recorded by a pair of carbon rod electrodes which were longitudinally arranged. The EOD signals from the other tank were received through a pair of carbon electrodes which were perpendicularly aligned to the first pair. The two fish thus stimulated each other when connecting electronic switches were closed. The mutual connections were made via stimulus isolators, and intervening amplifiers adjusted the stimulus intensities so that the amplitude of the neighbor's signal at the recipient fish was 5 mV/cm. The temperature of the tanks was adjusted ($\pm 3°C$) to adjust starting f.

Stimulus control

The occurrence of EODs or pacemaker command signals was registered by computers (PDP-11/73 and Gateway 2000–486 with a Tucker Davis Technology interface) with a resolution of 1 μs and was converted to frequency, f. While S_1 was always triggered by the pacemaker command signal with a delay, S_2 was controlled differently in different experiments. In *Exps. A* and *B* (Figs. 2 and 3), S_2 pulses were triggered by another function generator whose frequency, f_2, was manually controlled, with the exception of the experiment presented in Fig. 3C. In this experiment and *Exps. C–F* (Figs. 4–7), single S_2 pulses were delivered at low frequency (0.1–15 Hz) with a variable delay from the next EOD or pacemaker command. Each tick of a low frequency (0.1–15 Hz) clock evoked a process in the computer in which the next occurrence of an EOD

or pacemaker command pulse was predicted from the most recent interval, and a single S_2 pulse was delivered with a delay, which could be either positive or negative. The response magnitude was measured by presenting stimuli for 10 s. The mean frequency fall during the 10 s period was computed as integrated frequency fall over this period divided by 10 s. Frequency falls shall be expressed as negative numbers throughout the paper.

In experiments with immobilized fish, the pacemaker command signal will be referred to simply as the EOD.

Results

Experiments with intact fish

Exp. A (Fig. 2)

When intact fish were exposed to S_2 with f_2 several Hz away from f, the EOD showed two distinct types of modulations, momentary phase advances and a gradual frequency fall. In Fig. 2A and B, f_2 was manually adjusted so that S_2 pulses scanned left to right and right to left in time, respectively, a few times per second. In both conditions, momentary phase advances occurred in association with each passage of S_2 pulses over EODs. Each phase advance consisted of a sharp and brief acceleration, lasting less than 100 ms. Since the resting frequency of this fish was ca. 60–70 Hz, this involved only 6–7 EOD cycles. The magnitude of the phase advance, however, was much greater with the left to right scan of S_2 (Fig. 2A) than with the right to left scan (Fig. 2B). Figure 2C shows that the phase advances start before S_2 pulses pass the EODs and result in rapid passage of S_2 pulses over EODs. The second component of the response was the gradual frequency fall which was a slow and deep decline of EOD frequency over several seconds. The rapid phase shifting behavior could be elicited in the absence of the gradual frequency fall (not shown). Occasionally, a smooth frequency rise was also elicited by the onset of successive passages of S_2 pulses over EODs. This rise had a time constant of a few seconds (not shown) and could be observed for either left-to-right or right-to-left scan. The rise resembled novelty responses generally found in pulse-type gymnotiform fishes. In the following experiments, we attempted to identify the essential stimulus features for the gradual frequency falls. Gradual rises and momentary phase shifts were not studied further.

Exp. B (Fig. 3)

The fact that gradual frequency falls occur for either direction of S_2 scan in *Exp. A* led us to hypothesize that only a limited aspect of interaction between the EOD and the S_2 causes this behavior. To test this hypothesis, we masked a part of the scanning S_2 pulses over various parts of the fish's own EOD cycle. S_2 pulses which
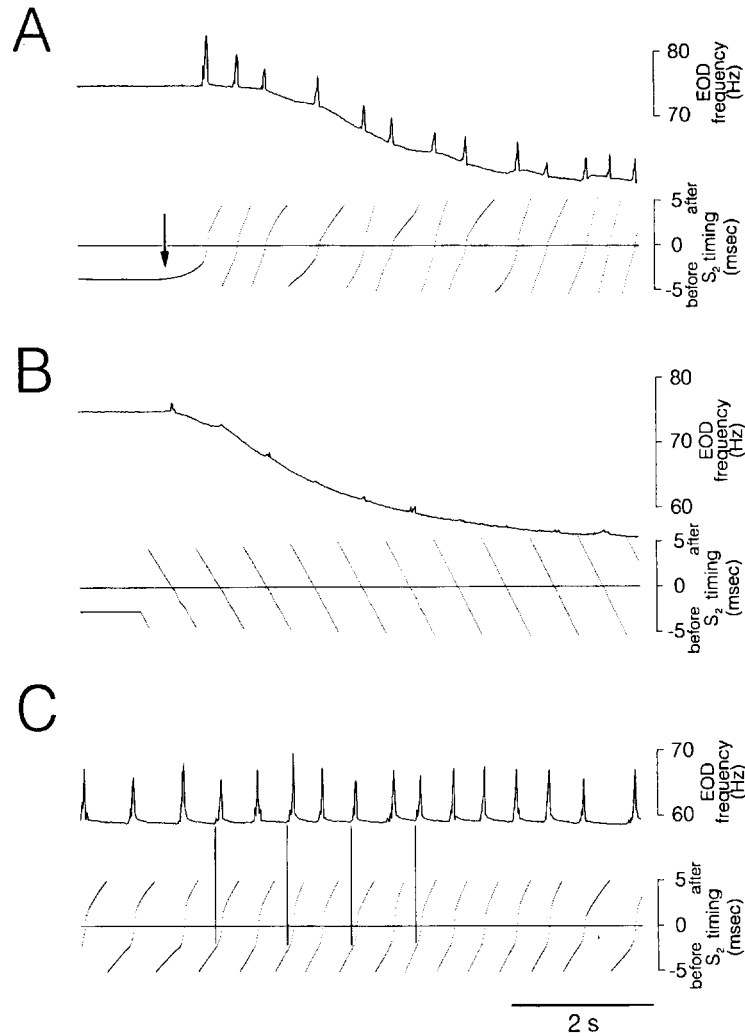
A

80
70
EOD frequency (Hz)

5 after
0
-5 before
S₂ timing (msec)

B

80
70
60
EOD frequency (Hz)

5 after
0
-5 before
S₂ timing (msec)

C

70
60
EOD frequency (Hz)

5 after
0
-5 before
S₂ timing (msec)

2 s

Fig. 2A–C *Exp. A:* Momentary phase advances and gradual frequency falls evoked in intact fish in response to S₂ pulses whose frequency was manually controlled. *Top traces:* instantaneous frequency of EOD, *bottom traces:* timing of S₂ pulses in reference to EOD timing. S₂ pulses before and after an EOD pulse are plotted below and above the zero-timing line, respectively. **A** The trace starts while S₂ timing was kept approximately 3 ms before EODs. There is no response in EOD frequency during this period. The S₂ frequency was gradually decreased at the arrow to approach EOD in the time positive direction. Momentary phase shifts, which appear as spike-like responses in the frequency trace, and a gradual fall in frequency are seen. **B** Similar experiment but S₂ pulses passed EODs in the opposite direction. While a gradual fall was still induced, the magnitude of the fast rises was much smaller. In **A** and **B**, f_2 was constantly but slowly adjusted to maintain adequate frequency difference between S₂ and EOD. **C** After prolonged (30 s) stimulation in **A**, f_2 was stabilized at approximately 2 Hz below the mean f. Thus the apparent acceleration of S₂ timing in reference to that of the EOD purely reflects the fish's behavior since f_2 was held constant throughout this trace. Vertical bars are provided to show that the phase shifting behavior before S₂ pulses coincide EODs

scan over only a short period near EOD pulses evoked gradual frequency falls that were as strong as those evoked by S₂ pulses that scanned over the entire EOD cycle (Fig. 3A and B). S₂ pulses which scan through

intermediate phases between EOD pulses and never overlap with the EOD have no effects on f (Fig. 3D). This ineffective portion of the EOD cycle extends from approximately 1 ms after an EOD to several ms before the next EOD. We further reduced the number of S₂ pulses given in one passage of S₂ through an EOD cycle, and found that single pulses applied near the EODs a few times per second were sufficient to evoke a full size gradual fall responses (Fig. 3C).

Exp. C (Fig. 4)

By varying the timing of single S₂ pulses given in Fig. 3C we could examine in more detail what portions of the S₂ scan played a role in eliciting the gradual frequency fall. Figure 4 shows that, for single S₂ pulses delivered 1.5 times per s, the strongest responses were obtained when the S₂ pulses overlapped with the EOD. S₂ pulses delivered a few ms before or after an EOD evoked weaker responses. The multiple peaks seen between -1 ms and 1 ms of S₂ timing in Fig. 4

Fig. 3A–D *Exp. B:* A sufficient stimulus component for the gradual fall. In **A**, **B** and **D**, the timing of S_2 pulses was advanced by 150 μs for every EOD pulse. The same presentation format as in Fig. 2. **A** S_2 pulses were delivered throughout EOD cycles. A strong frequency fall was induced. **B** S_2 pulses were delivered only in a restricted period between time points 4 ms before and 1 ms after EODs. **C** Single S_2 pulses were given at 1.5 Hz at 200 μs after EODs. Note frequency falls with full magnitude in **B** and **C**. **D** S_2 pulses within a time window which starts at 2.5 ms before and ends at 1 ms after the EOD pulses were omitted. Note no frequency fall. The same result as **D** was obtained when the scan direction was reversed
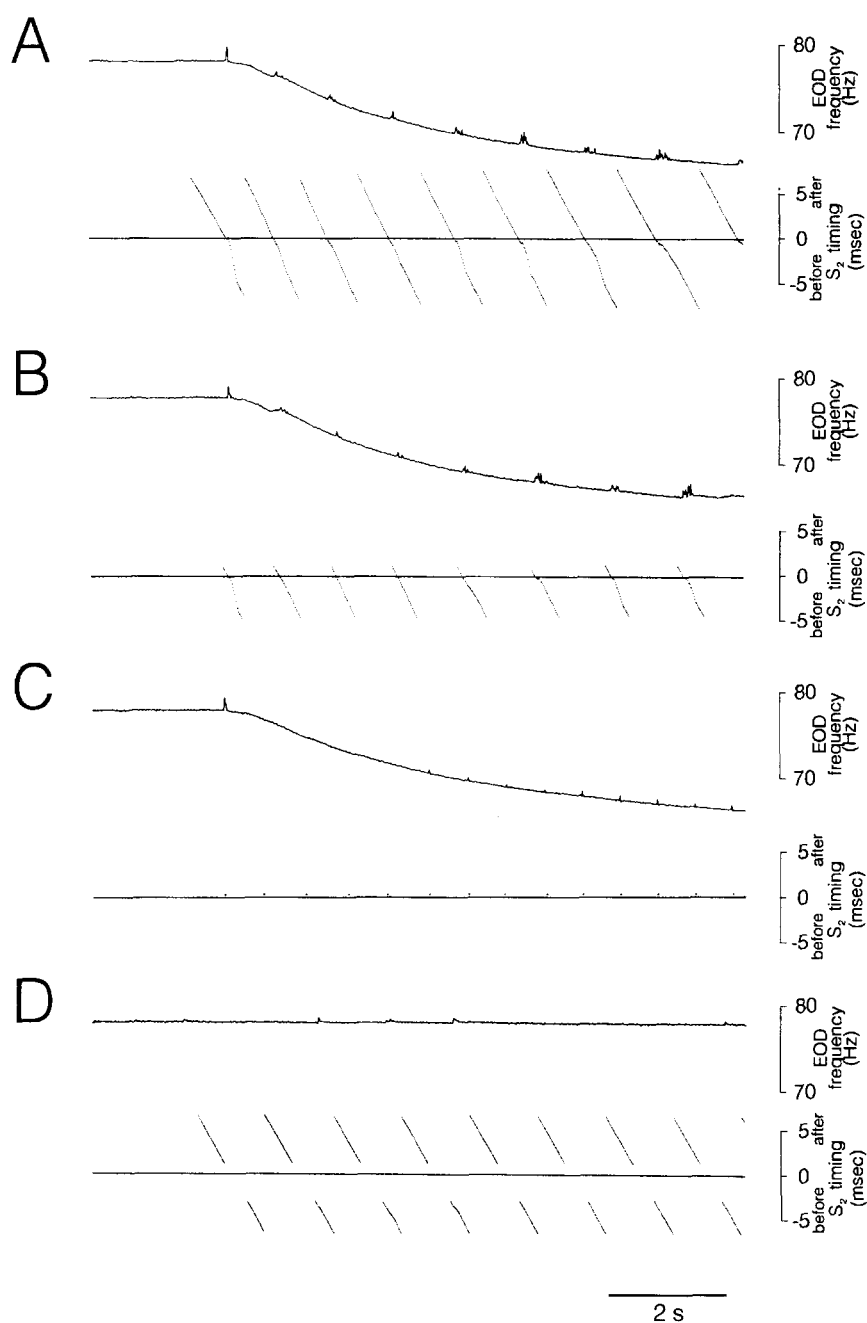
was not a general characteristic. Some delay-tuning curves had only single peak around this timing.

Exp. D (Fig. 5)

Next, we examined the effect of various frequencies of S_2 pulses. The frequency of S_2 pulse presentation in Fig. 3C was varied from 0.1 to 15 Hz and the magnitude of gradual fall was examined keeping the delay of S_2 from the EOD at zero. The tuning curve shows a sharp cut-off around 4 Hz and no responses were elicited at higher frequencies of S_2.

Experiments with immobilized fish

Exp. E (Fig. 6)

We wished to determine whether the fish performed gradual frequency falls solely on the basis of sensory afference or required reference to their internal pacemaker signals by way of a corollary discharge. To dissociate the sensory afference from the pacemaker command we sought to silence the EOD with the curare-like drug, Flaxedil, after which we could substitute an artificial EOD, S_1. As in other gymnotiform fishes, Flaxedil had no effect on the regular firing of the
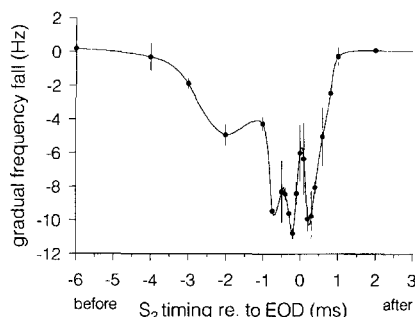
Fig. 4 *Exp. C:* Gradual frequency fall responses to single S_2 pulses presented as in Fig. 3C with various latencies. Positive latencies imply S_2 pulses applied after EOD pulses. Seven successive S_2 pulses were given at 1.5 Hz and magnitude of gradual falls during this period (see Methods) was measured. Mean and standard deviation of three to six trails are plotted for each data point
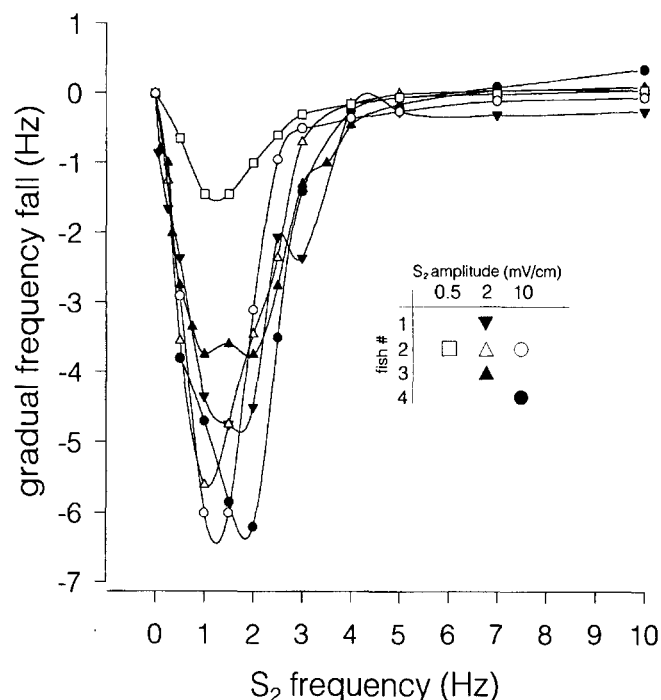

Fig. 6 *Exp. E:* Gradual frequency falls occur regardless of the temporal relation between pacemaker command signals and externally applied stimuli (S_2). S_1 pulses were applied at fixed delays (*abscissa*) with respect to the EOD. S_2 pulses were applied approximately 1.5 times per second at the same time as S_1. The resting frequencies of EOD were between 76 and 90 Hz which correspond to intervals between 11 and 13 ms. S_2 pulses coincided with S_1 pulses at zero latency. Each data point represents mean and standard deviation of several trials. Data from three fish are presented

Fig. 5 *Exp. D:* Magnitude of gradual fall to various frequencies of single S_2 pulses. S_2 pulses were delivered at a given frequency for 10 s for each trial. The magnitude of gradual fall is sharply tuned to low frequencies (< 4 Hz). Each point represents the mean of one to three trials. *Insert* shows different S_2 amplitude used

pacemaker. The discharge was strongest within a particular area of the abdomen and measured less than 0.1 mV/cm at the head area. When we attempted to replace the attenuated EODs from the main organ with S_1 pulses that were not phase locked nor frequency matched to the pacemaker signal, f showed irregular modulation. These modulations may be due to interactions between the residual EODs and S_1. Although gradual frequency falls occurred when we applied S_2 in addition to replacement S_1 pulses, the irregular EOD modulation hampered quantitative measurements. To avoid this problem, we delivered an S_1 pulse after each residual EOD pulse with a fixed delay so that EODs and S_1 pulses were interwoven. This arrangement did not alter the pattern of EODs and allowed presentation of S_1/S_2 combinations.

S_2 pulses, which coincided with S_1 in time, were intermittently applied through a transverse pair of electrodes at approximately 1.5 Hz, using a method similar to that used in Fig. 3C. The delay of S_1 pulses, and thus of the intermittent S_2 pulses, from EODs was varied throughout the EOD cycle. Gradual fall responses occurred regardless of the timing relation between the EOD and the S_1/S_2 pairs (Fig. 6).

Exp. F (Fig. 7)

In all previous experiments, S_2 was delivered through the transverse pair of electrodes (differential geometry). This arrangement of S_2 electrodes simulates the natural condition in which a neighbors' current source (electric organ) is located outside of the fish's own body, and stimulates the right and left sides of the body with opposite polarities of S_2. Therefore, when EOD or S_1 and S_2 coincide in time, the stimulus amplitude should be modulated with opposite polarities on opposite sides of the body by constructive and destructive

pacemaker as recorded by the spinal volley. While the EODs from the myogenic main electric organ in the tail were greatly attenuated, the EODs from the accessory electric organ (Bastian 1976; Caputi et al. 1994) in the abdomen, however, remained unaffected. Since the wave form and amplitude of this potential remained constant during recovery of the main organ from curarization, the accessory electric organ is probably neurogenic. The potential from this anterior electric organ measured approximately 5–10 mV/cm at and perpendicular to the abdomen, and was synchronized with the
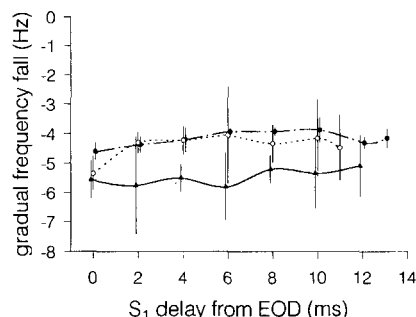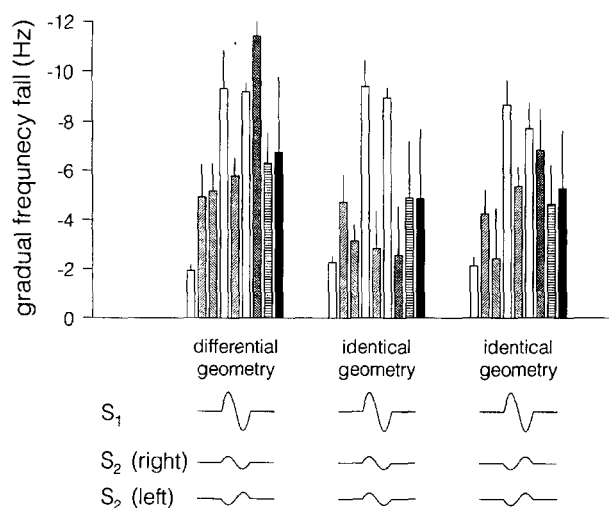
Fig. 7 *Exp. F:* Comparison of gradual frequency fall in differential and identical geometry conditions. S_1 was applied between the mouth and the trunk electrodes in all cases as in other experiments. In the differential geometry condition coincidental S_2 pulses were delivered at 1.5 Hz through an external pair of electrodes. This arrangement yielded constructive and destructive addition on the right and left sides of the body (*left column*). In identical geometry conditions, the same S_2 pulses were electrically added to (*center column*) or subtracted from (*right column*) S_1 then delivered through the same mouth-trunk electrode set. *Different keys* mark data from different fish. The *right most filled bars* represent mean of all eight cases. S_2 amplitude was 15% of S_1 amplitude in all cases

Fig. 8 *Exp. G:* Gradual frequency falls are the response to high frequency components in the amplitude modulation envelope. The S_1 pulse train in *Exp. F* was modulated in amplitude at 1.5 Hz with the various wave forms shown below. No S_2 pulses were delivered. The depth of amplitude modulation was 10%. Means and standard deviations of five sets of data from three fish (*different keys*) are plotted

addition of two pulses. In *Exp. F*, we altered the geometry of S_2 to test whether geometrical asymmetry is necessary to elicit the gradual frequency falls. When we electronically combined S_2 with S_1 thereby eliminating differential cues across the body surfaces (identical geometry, see Methods). The gradual frequency fall still occurred (Fig. 7). In the identical geometry condition, coincidental S_2 pulses momentarily increased the amplitude of the stimulus by constructive addition of S_1 and S_2 in all body surfaces. To test whether decrements in amplitude also induce gradual fall, we inverted the sign of S_2 before it was added to S_1. Gradual falls occurred under this condition as well (Fig. 7).

Exp. G (Fig. 8)

The experiments so far suggest that the primary cue for the gradual frequency fall responses is amplitude modulation of EOD feedback at low frequencies (< 4 Hz). The individual S_2 pulses inserted at 1.5 Hz in *Exps. C–F* produced a very rapid change in stimulus amplitude every 0.67 s (1/1.5 Hz). The frequency spectrum of the amplitude envelope of the mixed signal in *Exp. F* (S_1 + S_2) contains higher harmonics as well as the fundamental frequency of the S_2 pulse insertion (1.5 Hz). The following experiments determined which of these components are essential. The amplitude of the S_1 pulse train in *Exp. F* was modulated at 1.5 Hz with
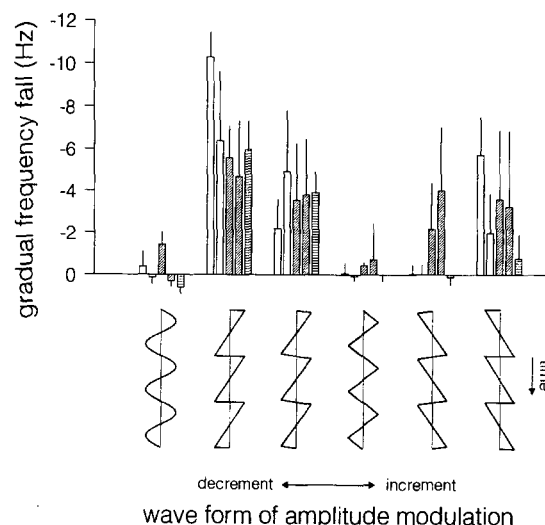
various wave forms, without using S_2 pulses. When the amplitude of the S_1 pulse train was modulated sinusoidally at 1.5 Hz, very little or no gradual fall was observed. In contrast, when the amplitude was modulated with a ramp function, which contains high frequency components, gradual frequency falls did occur (Fig. 8). When the amplitude of S_1 was modulated at 10 Hz, no gradual frequency falls were induced for any modulation wave forms, as expected from the result in *Exp. D*.

Exp. H (Fig. 9)

As predicted from *Exps. A* and *B*, when we electrically connected two fish (see Methods), both fish showed gradual frequency falls (Fig. 9A). In the figure, f of fish A started 1–1.5 Hz higher than that of fish B. When the two tanks were connected electrically, both fish showed a rise for a short period and then exhibited gradual frequency falls. In this particular case, fish A showed a larger fall response and eventually became lower in frequency. When the fish's EODs were initially 8 Hz apart, however, no gradual frequency fall ensued (Fig. 9B). This is in accordance with the fact that frequency difference higher than 4 Hz has no effect as shown in Fig. 5.

We tested if other species of pulse-type gymnotiform fishes exhibit the gradual frequency fall. Two intact individuals of *Gymnotus carapo* and *Hypopomus pinnicaudatus* (Hopkins 1991) were tested under the same experimental condition as in Fig. 3C. No gradual frequency fall was induced. We also tested several
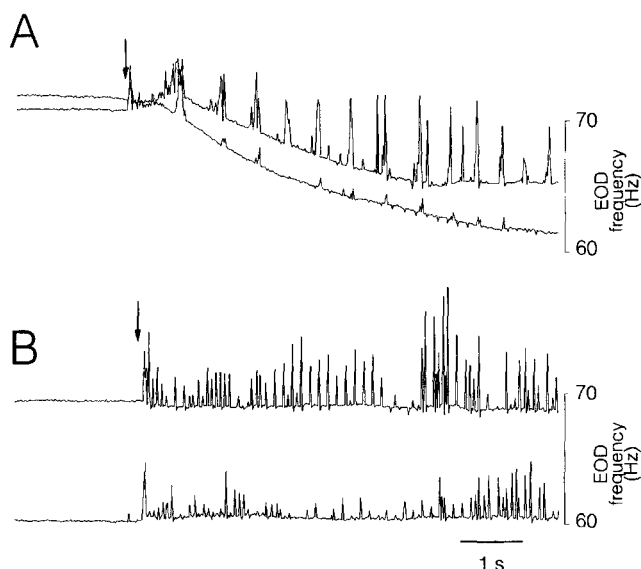
Fig. 9A–B *Exp. H:* Two intact fish were electrically connected (see Methods) at the *arrows.* **A** A gradual frequency fall occurred in both fish when initial frequency difference was 1.1 Hz. **B** No gradual frequency fall occurred when the initial frequency difference was 8 Hz

individuals of *Rhamphichthys marmoratus* (Mago-Leccia 1994) (15 cm) with the same stimulus condition. Only much smaller gradual frequency falls (< 2 Hz) could be induced in this species.

Discussion

This study identified the essential stimulus components for the gradual frequency fall responses of the high-frequency pulse-type gymnotiform fish, *Rhamphichthys rostratus. Exps. B* (Fig. 3) and *D* (Fig. 5) suggest that low frequency (< 4 Hz) amplitude modulation of sensory feedback is a necessary feature. *Exp. G* (Fig. 8), however, demonstrates that sinusoidal amplitude modulation of EOD feedback at these low frequencies does not evoke the responses. *Exp. G* also demonstrates that responses could be induced when higher harmonic components are added to the envelope of amplitude modulation by altering the wave forms used in the modulation. Therefore, we conclude that the essential stimulus feature for the gradual frequency fall is a rapid amplitude modulation of sensory feedback which occur at a low frequency (< 4 Hz). Functionally, detection of such a sensory feature could be realized by a series of frequency filters in the central nervous system. We propose that sensory feedback is initially processed by a high-pass filter which passes only high frequency components in the feedback (rapid modulations caused by singly applied pulses in *Exps. B–F*; sharp edges in modulation function in *Exp. G*), and then the signal is further processed by a low-pass filter which eliminates

responses to the high frequency components (> 4 Hz) in the envelope of amplitude modulation (Fig. 5). The higher cut-off slope of the frequency tuning curve in Fig. 5 is remarkably sharp. The functional significance of the sharp tuning has not been identified.

All known types of JARs in gymnotiform electric fishes differentiate the sign of frequency difference between the fish's own and the neighbor's EODs (Heiligenberg 1977). In the wave-type genus *Eigenmannia*, a fish will gradually raise or lower their EOD frequency in the direction that increases the frequency difference (Bullock et al. 1972a, b). In pulse-type gymnotiform fishes, a left-to-right scan of neighbor's pulses over the fish's own pulses evokes much larger phase shifting of EODs than a right-to-left scan (Heiligenberg et al. 1978; also seen in the current study: Fig. 2A and B). In contrast, the gradual frequency fall studied in this report occurs with equal magnitude in response to either scan direction. This insensivity to the scan direction was further confirmed by the experiment in Fig. 3C, in which the full response was obtained by single S_2 pulses which lacked a direction of scan. The JAR of *Eigenmannia*, which consists of gradual frequency shifting, requires a complex stimulus feature which is represented by the temporal and spatial pattern of both amplitude and phase modulation in EOD feedback. Differential stimulation of body surfaces is essential for the JAR of *Eigenmannia* (Heiligenberg and Bastian 1980). The gradual frequency fall of *Rhamphichthys*, however, occurs even when all body surfaces experience identical amplitude modulations (Figs. 7 and 8). We conclude that the JAR in wave-type gymnotiform fishes and the gradual frequency fall of *Rhamphichthys* are controlled by distinctly different sensory mechanisms.

Whether or not the gradual frequency fall is unique to *Rhamphichthys rostratus* and different from small frequency falls observed in other pulse-species is unclear. Gradual frequency falls of a fair magnitude could be elicited on most occasions by adequate stimuli. However, same individual fish had periods in which the response was refractory as well as periods in which responses could be elicited. During the refractory periods, the gradual frequency fall was either very small or absent even after repeated presentation of adequate stimuli.

Scheich et al. (1977) reported weak frequency falls in *Rhamphichthys*. The reason for the difference in response magnitude between their experiments and ours may be due to the above mentioned the fish's motivational state or different experimental parameters.

In Fig. 2A, one might argue that the gradual frequency fall is a result of momentary phase shifting (spikes in the frequency trace) which would cause periodical changes in sensory feedback of the EODs. Experiments in Fig. 2B and Fig. 3 demonstrate that this is not the case. The gradual frequency falls occur independently of the momentary phase shifts.

Exp. E (Fig. 6) demonstrated that gradual frequency falls occur as long as sensory feedback of the fish's own pulses (or S_1) is modulated by the neighbor's pulses, and that the timing of such perturbation need not be associated with the moments of pacemaker firing. This suggests that gradual frequency falls are controlled purely by a sensory feedback and that no internal association of the pacemaker command signal and S_2 pulses is necessary for this behavior. This observation is in agreement with the fact that no gymnotiform electric fish is known to possess a corollary discharge mechanism. When we applied S_2 pulses to immobilized fish without presenting a replacement S_1 signal, we observed minor gradual frequency falls only when the S_2 pulses were close in time to the EOD pulses. This is perhaps due to the interaction between the EOD from the accessory electric organ and the S_2 pulses, and may explain the one case seen in Fig. 6 (dotted line) when slightly larger gradual frequency falls were elicited when the S_1 and S_2 were placed near pacemaker firing.

Two major types of tuberous electroreceptors are known in pulse-type gymnotiform electric fishes: pulse markers and burst duration coders. Pulse markers respond with a single action potential to each pulse of sensory stimulation, and are thus suited to encode timing, or phase, information of sensory signals. Burst-duration coders give a burst of spikes for a pulse stimulus, and stimulus intensity is encoded in the number of action potentials in a burst (Hagiwara et al. 1962; Szabo and Fessard 1974). Baker (1980) recorded from both types of electroreceptors under stimulus conditions (S_2 scanning over S_1) that were similar to those in present study. He examined in the low frequency pulse species, *Hypopomus* and *Gymnotus*, and reported that while burst duration coders were affected by the scanning stimuli, pulse markers were hardly affected unless unnaturally intense S_2 was used. Burst duration coders but not pulse markers were recorded by Bastian (1976) in the primary afferent fibers in *Rhamphichthys rostratus*, although Szabo and Fessard (1974) recorded pulse-marker like field-potential responses in the magnocellular nucleus in the midbrain. When S_2 pulses were applied exactly in synchrony with S_1 pulses (as in *Exp. G*), the firing timing of pulse markers would hardly be affected. Nevertheless, gradual frequency falls occurred in response to such an arrangement of S_1 and S_2 pulses in *Exp. F*. Thus, burst duration coders are likely to be involved in encoding amplitude modulation for the gradual frequency fall.

Although functional significance of the gradual frequency fall has not been tested experimentally, one could speculate that it may serve as a jamming avoidance mechanism. As shown by *Exp. H* (Fig. 9), an electrical encounter between two fish induced a gradual frequency fall in both fish. The fish which became lower in frequency as the results of the gradual frequency fall showed much weaker phase-shifting behavior. This is

in accordance with the observation in Fig. 1A and B where the neighbor's EOD with a lower frequency (Fig. 1A) induced stronger phase-shifting responses than a neighbor with a higher EOD frequency (Fig. 2B). In Fig. 9, the fish which gained the low frequency 'position' after the gradual frequency fall, avoids pulse coincidences by neighbor's phase-shifting responses but not by his own. Although primary function of the phase-shifting behavior is thought to be jamming avoidance, the execution of the phase-shifting behavior may be in some regard costly and the two fish may be competing for the lower frequency 'position'. An attempt to measure electrolocation capability with EOD novelty responses to electrosensory stimuli (an electrical short near fish) during the gradual frequency falls and phase shifting response was unsuccessful due to a large variability in novelty responses in such conditions.

Although a frequency lowering behavior with a large magnitude and a slow time constant (tens of seconds) is not commonly observed in jamming contexts in pulse-type gymnotiform electric fishes, such behaviors are known in the communication repertoire (Hopkins 1981; Hagedorn 1988; Kawasaki 1993). *Hypopomus brevirostris* shows gradual frequency falls during courtship. These responses are known to be driven by a diencephalic prepacemaker nucleus, PPn-I, through a GABAergic pathway in the pacemaker nucleus in the medulla (Kawasaki and Heiligenberg 1989). It is likely that gradual frequency falls in *Rhamphichthys* are mediated by similar pre-motor mechanisms.

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