

# EOD modulations of brown ghost electric fish: JARs, chirps, rises, and dips

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## Abstract

Weakly electric “wave” fish make highly regular electric organ discharges (EODs) for precise electrolocation. Yet, they modulate the ongoing rhythmicity of their EOD during social interactions. These modulations may last from a few milliseconds to tens of minutes. In this paper we describe the different types of EOD modulations, what they may signal to recipient fish, and how they are generated on a neural level. Our main conclusions, based on a species called the brown ghost (*Apteronotus leptorhynchus*) are that fish: (1) show sexual dimorphism in the signals that they generate; (2) make different signals depending on whether they are interacting with a fish of the opposite sex or, within their own sex, to a fish of that which is dominant or subordinate to it; (3) are able to assess relative dominance from electrical cues; (4) have a type of plasticity in the pacemaker nucleus, the control center for the EOD, that occurs after stimulation of NMDA receptors that causes a long-lasting (tens of minutes to hours) change in EOD frequency; (5) that this NMDA receptor-dependent change may occur in reflexive responses, like the jamming avoidance response (JAR), as well as after certain long-lasting social signals. We propose that NMDA-receptor dependent increases in EOD frequency during the JAR adaptively shift the EOD frequency to a new value to avoid jamming by another fish and that such increases in EOD frequency during social encounters may be advantageous since social dominance seems to be positively correlated with EOD frequency in both sexes.

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## 1. Introduction

Weakly electric fish generate weak electric fields around themselves and detect those fields with specialized sensory receptor cells called electroreceptors. They detect and recognize nearby objects by the distortions these objects make in their electric fields, a process termed electrolocation. They also detect, recognize and communicate with conspecifics by means of their electric organ discharges (EODs). Weakly electric fish have evolved two strategies for emitting EODs. They emit either brief pulses at an irregular rate (“pulse” fish), or a highly regular sinusoidal discharge (“wave” fish). One advantage of utilizing a regular sinusoidal EOD is that fish can sample their environments at high frequencies, since the EOD frequencies of most wave species exceed those of pulse fish, even when they are firing at their maximum pulse rate. Further, the regularity of a sinusoidal discharge allows fish great predictive ability since

they are digitizing their environments at a highly regular rate.

Efficient electrolocation utilizing the “wave” strategy calls for a highly stable EOD frequency. The electro-motor system of the brown ghost (*Apteronotus leptorhynchus*) (Fig. 1) is the fastest firing, most precise neural oscillator known: this species may discharge in excess of 1 kHz, with sub-microsecond jitter [1]. On the other hand, if a wave fish is to use its EOD for social signaling it has a limited number of EOD parameters than it can vary, the most obvious is EOD frequency. Thus, electric wave-type fish transiently modulate EOD frequency in a variety of social circumstances for signaling purposes. Despite the extreme precision of its electromotor system that makes it a champion of electrolocation, the brown ghost also shows frequent modulations of its EOD in social circumstances or to applied electrical stimuli. These modulations range from a few milliseconds to hundreds of seconds, roughly four orders of magnitude.

This article focuses on the various types of EOD modulations made by brown ghosts, their use in social interactions, and the implications of these modulations for neural processes.

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## 2. EOD generating circuitry

As in other species of weakly electric fish, the EOD is controlled by a midline medullary nucleus called the pacemaker nucleus (PMn) (Fig. 1). About 100 electrotonically coupled pacemaker neurons in this nucleus fire synchronously at highly regular rates. These neurons determine the EOD frequency since they drive the electric organ in a 1:1 fashion. The large numbers of pacemaker neurons (species with lower EOD frequencies typically have ~50 pacemaker neurons) and their extensive electrotonic coupling aid their synchrony and precision

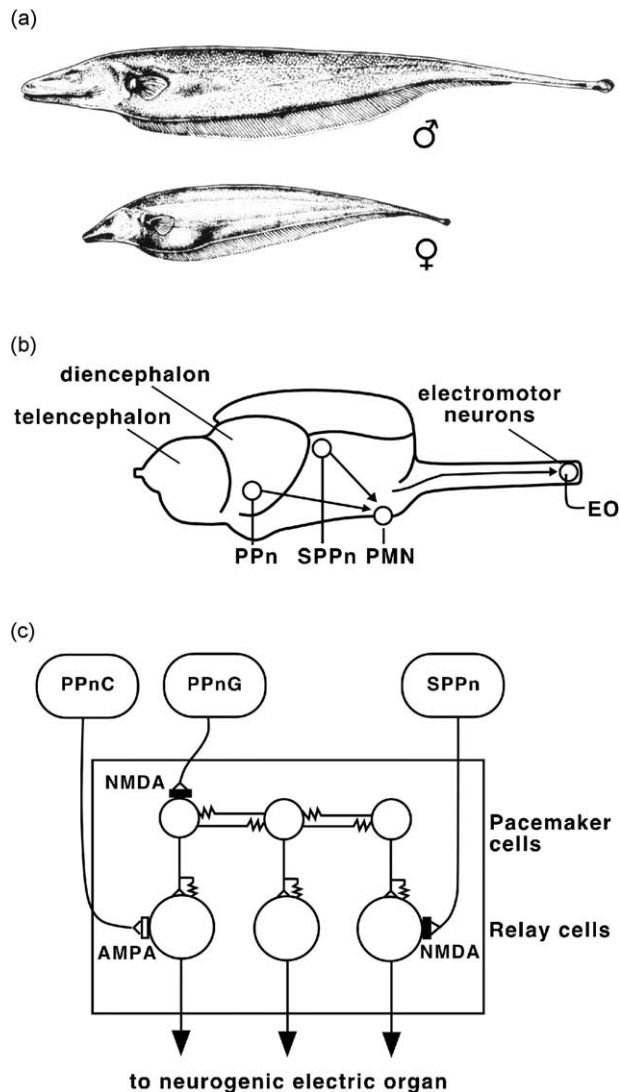


Fig. 1. Brown ghosts and their brains. (a) Male brown ghosts are larger than females (modified from Hagedorn and Heiligenberg [10]). (b) The pacemaker nucleus (PMn), which is responsible for setting the EOD frequency, is located in the ventral medulla. It receives input from two prepacemaker nuclei (PPn and SPPn) which modulate its output. (c) The apteronotid pacemaker has two major cell types, the pacemaker and relay cells (parvocells are omitted for clarity). The inputs from the PPnC, PPnG, and SPPn synapse on these two cell types with either AMPA or NMDA receptors. (modified from Juraneck and Metzner [7]).

[1,2]. The pacemaker neurons are also electrotonically coupled to a second class of neuron, the relay neuron. The 25 relay cells send their axons down the spinal cord to innervate electromotor neurons, also with electrotonic coupling. The electromotor neurons are specialized spinal motoneurons whose axons actually form the electric organ (a distinctive feature of the Apterontidae).

A third and newly described neuron type in the PMn is the parvocell [3]. These are small interneurons that label with an antibody against parvalbumin. They receive electrotonic synapses from the pacemaker and relay cells, and make chemical synapses back onto those cells. Their transmitter is unknown but is unlikely to be the inhibitory transmitters GABA or glycine since application of these on a pacemaker slice has no effect (G.T. Smith, unpublished).

The modulations of EOD frequency are mediated by inputs from two prepacemaker nuclei: the prepacemaker nucleus (PPn) in the diencephalon and the sublemniscal prepacemaker nucleus (SPPn) in the mesencephalon (Fig. 1). The PPn is subdivided into two anatomically distinct regions: the PPnG (for gradual rises) and PPnC (for chirping). These definitions are based on single unit recordings from or micro-stimulation and/or lesions of these nuclei [4,5]. The inputs from the prepacemaker nuclei make glutamatergic synapses. The SPPn synapses on relay cells via NMDA receptors, inputs from the PPnG synapse on pacemaker neurons and also utilize NMDA receptors, whereas the inputs from the PPnC activate AMPA receptors on relay cells [4,6,7]. Pharmacological evidence also suggests that type I metabotropic glutamate receptors are present in the PMn of *Apteronotus* (G.T. Smith and J. Oestreich, unpublished), as has been observed in a pulse gymnotiform fish [8], although it is not yet known which cells possess them nor which inputs access them.

In the last section in this paper, we will explain what is known about how these inputs are activated in generating various social signals.

## 3. Sex differences in EOD frequency

EOD frequency is highly sexually dimorphic in brown ghosts with males discharging from about 800–1000 Hz and females discharging from 600 to 800 Hz (at 26 °C) [9–13]. In most other gymnotiform wave fish males discharge at a lower frequency than females; this is true for the black ghost (*A. albifrons*), the only other apteronotid studied in detail [14].

EOD frequency in males is positively correlated with body size and androgen (11-ketotestosterone) level. Body size and EOD frequency are also positively correlated with aggressiveness in that males with high EOD frequencies chirp more in social encounters and successfully compete for shelters in aquaria [15,34,35].

Furthermore, when two males are placed in a group tank with a number of females, the largest male with the highest EOD frequency becomes dominant and only he mates with the females in the tank [10].

EOD frequency is not correlated, or is at best weakly positively correlated, with body size in females, at least in non-breeding individuals [13,34,36]. It is possible this is different in breeding females. However, in tanks with small groups of breeding females and two males no dominance hierarchy is established among the females and they alternately spawn with the dominant male on successive nights [10]. Females also tend to be less competitive in social interactions and to share shelters in an aquarium when they are in limited number. Nevertheless, even when sharing, females who attain shelters have higher EOD frequencies than females who are unable to compete for shelters [34]. This observation suggests that EOD frequency may be used in female–female interactions even though it is largely independent of body size.

#### 4. Types of EOD modulations

There are a number of EOD modulations that brown ghosts make in a variety of social interactions or to different types of electrical signals. These are the jamming avoidance response and the non-specific response, which are believed to be emitted reflexively to prevent jamming, and a variety of social signals such as chirps and rises.

##### 4.1. The jamming avoidance response

The electrosensory system of each fish is tuned to its own discharge frequency. If two conspecifics with nearly identical frequencies meet, their EODs will beat and this jams each other's electrosensory system [5,6]. In order to avoid jamming electric fish have evolved a jamming avoidance response (JAR) that varies depending on species. In brown ghosts, the fish with the higher EOD frequency shifts its frequency upwards, thus maximizing the difference between the two EOD frequencies and minimizing the jamming. This can be simulated in the laboratory by presenting a fish with an artificial sine wave signal 1–20 Hz below its EOD frequency [16–19]. Stimuli of 3–6 Hz below EOD are the most effective at eliciting a JAR [19]. In comparison with chirping and other social signals, the range of EOD frequencies that elicit the JAR is small. The more intense the jamming stimulus, the higher the frequency elevation achieved during the JAR. In brown ghosts, there are no sex differences in any parameter of the JAR when signals of normal amplitude are used [22], although a sex difference emerges under frequency clamp when the JAR is strongly stimulated [18]. The JAR is activated by input

from the SPPn via NMDA receptors on the relay neurons. This depolarizes the relay cells, increasing their firing frequency and elevating EOD frequency.

Unlike the wave species *Eigenmannia* in which an EOD mimic above EOD frequency causes a fish to lower its EOD, in brown ghosts, a weak stimulus a few Hz above EOD frequency often produces no response; however, a stronger stimulus may provoke a so-called non-specific response. This is a response in which a fish paradoxically raises its EOD frequency, but does so to “jump over” the intruding EOD frequency [19]. Functionally, this seems like another type of JAR in that it also minimizes jamming between two fish [20]. This response is mediated by a different pathway: input from the PPnG acting through NMDA receptors on pacemaker neurons [5].

Why does a fish need a JAR if it can swim away from the EOD of another fish? Indeed, fish in a tank will sometimes swim away from each other during the night when they are active or, if they remain in proximity, they will show a JAR. However, during the daytime they hide and, at least in the laboratory, fish are reluctant to leave a shelter then. If an artificial sine wave is played to a fish in a shelter while the lights are on, it will not leave its hiding place but, instead, makes a JAR (J. Oestreich, personal communication). This is supported by the observation that the EODs of fish sharing a shelter are typically greater than 7–11 Hz apart [34].

If a fish is exposed to a long duration jamming stimulus, it will perform a JAR for hours. During that time, the EOD generation circuit shows a form of accommodation in that the EOD frequency is gradually and adaptively reset (Fig. 2). This is evident when the jamming stimulus is terminated and the EOD is observed to be at a higher, stable frequency [21,22]. We refer to this as behavioral long-term frequency elevation (LTFE).

The magnitude and duration of the behavioral LTFE is related to the stimulus parameters of the mimic EOD. Exposure to an EOD mimic for 2 min is too brief a time to elicit an LTFE, whereas a 30 min exposure produces an LTFE comparable to a 3 h exposure. A more intense stimulus, which produces a stronger JAR, also produces a larger magnitude and longer lasting LTFE. The LTFE can last for tens of minutes or hours, depending on the duration and magnitude of the jamming stimulus and it gradually shifts downward until it returns to its baseline value [22].

LTFE can be elicited in a pacemaker slice that includes the afferent terminals from the prepacemaker nuclei. When these afferents are electrically stimulated at a low frequency, the pacemaker responds with a fictive JAR. Following cessation of a 20 min stimulation, the pacemaker firing frequency is elevated, just as in behavioral LTFE. It gradually returns to baseline after tens of minutes or hours. That an electrophysiological

LTFE mimicking behavioral LTFE in many important details can be achieved in the pacemaker slice shows that the PMn is the locus of the accommodation [22]. Furthermore, the absence of the somata of the afferent fibers in the slice, suggests that this is a postsynaptic phenomenon.

Mechanistically, it seems that LTFE can be generated by activation of NMDA receptors on either relay or pacemaker neurons (EOD mimics above a fish's EOD frequency that activate the non-specific response also result in LTFE, J. Oestreich, unpublished). On the other hand, activation of AMPA receptors does not cause LTFE. This is shown by giving a male fish an EOD mimic 40 Hz higher than its EOD frequency (see next

section), which makes it chirp but not make a JAR [22]. Under these circumstances a prolonged bout of chirping is not followed by LTFE.

Functionally, LTFE passes the “responsibility” of keeping EOD frequency raised from the synaptic input on to the postsynaptic cells. We speculate that this is adaptive for a few reasons. First, prolonged stimulation of NMDA receptors is likely to increase the calcium load of the pacemaker and relay cells, and this could be dangerous for the cells. Second, synaptic input tends to introduce more jitter, that is, to make firing rates of cells more irregular. Shifting the responsibility for the increase in firing frequency from a synaptic mechanism to a post-synaptic one ought to result in a smoother firing rate and a more stable EOD frequency.

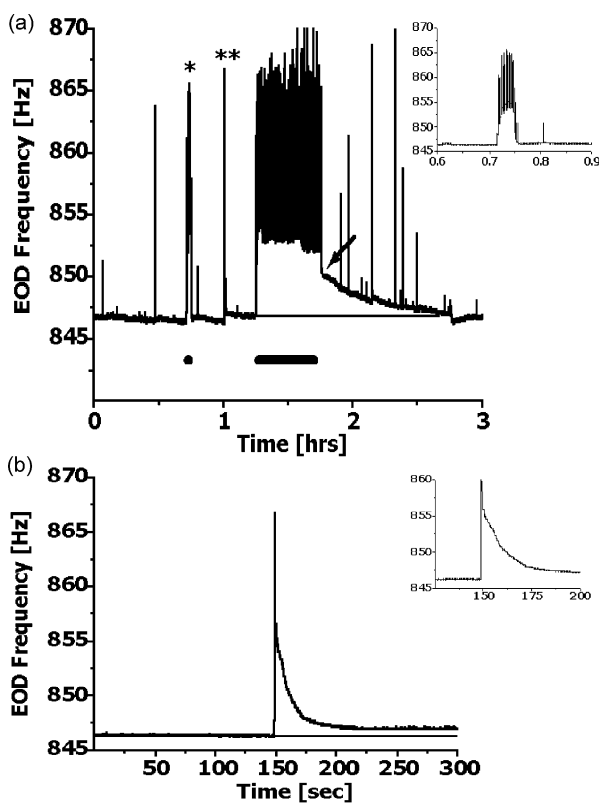


Fig. 2. A recording of EOD frequency of a male brown ghost illustrating some spontaneously generated and evoked modulations of EOD frequency. (a) The black bars beneath the records indicate when stimuli were presented to the fish. The first short black line indicates a two minute stimulation with an electrical sine wave at 843 Hz (1 mV/cm), to which the fish responds by making a JAR and chirping (single asterisk). This is better shown in the inset, which is expanded in time. The cluster of thin black lines are chirps, these are superimposed on a JAR. Note the lack of LTFE. The second black bar indicates when the same stimulus was presented for 30 min. The fish made a JAR for the total duration of the stimulus and when the stimulus was terminated, its EOD frequency was reset at a new value (at the arrow) for approximately an hour. This is an example of a long term frequency elevation (LTFE). Also in this record are a number of “yodels.” LTFEs also occur after some yodels (double asterisks). (b) The yodel demarcated by the double asterisks in (a) is expanded further in the inset. Note the rapid rise followed by a more or less exponential decay and LTFE. Other yodels are depicted in Fig. 4.

#### 4.2. Chirps

The best-studied category of social signaling is chirping. These signals are primarily made by males [19,23,24]. There are 4 types of chirps [18,24,25]: short duration chirps lasting  $\sim 20$  ms, with large frequency excursion of 200–300 Hz (type 1) or small excursions of 50 Hz (type 2) are used mostly in agonistic contexts, whereas chirps of long duration (types 3 and 4) lasting many tens to a few hundreds of milliseconds are more typically used by males to interact with and court females.

Males chirp spontaneously at low levels when alone. Not surprisingly, they chirp at much higher rates during social interactions or to EOD playbacks. Both the parameters of the stimulus that a fish is responding to as well as its own internal state influence its chirp rate. For example, males given an EOD mimic near their own EOD frequency, show a greater propensity to chirp and a higher chirp rate with increases in stimulus [2,9,26]. In staged encounters between two fish separated by a mesh barrier, males chirp at other males two to four times more frequently than they do to females [15].

Fish of both sexes tend to chirp (type 2) more at EOD mimics near their own frequencies, than to EOD mimics of distant frequencies ( $\pm 50$  Hz) whether confined to a testing chamber [18,26] or free swimming [12]. When the data from free swimming fish are analyzed more closely, it is apparent that males with low EOD frequencies, which tend to be the smallest and least mature males, chirp more at stimulus frequencies near or below their own EOD frequencies (Fig. 3). They are therefore chirping at EOD frequencies that correspond to males of similar rank, and females. They chirp much less at stimulus frequencies higher than theirs, which would correspond to the range of the larger dominant males. Conversely, males with high EOD frequencies—the larger dominant fish—chirp at EOD frequencies across the male range, but chirp less at females [12]. Interestingly, the last observations imply that fish can assess the body size of rivals by EOD frequency alone. This is an accu-

rate assessment since male EOD frequency correlates well with body size (and androgen level, see below) [15].

Very long duration chirps (type 3 or 4), are mainly made by males to females during courtship [10,27,28]. In a social interaction the role of various sensory stimuli in eliciting chirps cannot be adequately evaluated. However, electric cues alone are sufficient to elicit long-duration chirps: males will court females with whom they are interacting when they are in separate tanks connected only by wires [36]. Furthermore, long-duration chirps can even be elicited from males when they are presented with sine wave stimuli in the female, but not male, frequency range in playback experiments [12,18].

Chirp types are further differentiated by the influence of various hormones on the frequency of their occurrence. The chirp rate of males interacting through a mesh screen is correlated with their androgen (11-ketotestosterone) levels [15] and chirping is induced in female brown ghosts treated with testosterone [23,29]. Propensity to chirp to a standard test stimulus is also increased in socially-housed males compared to isolated ones, and this is correlated with increased levels of serum corticosterone in these fish [30]. This is likely to be a causal relationship since chirp rate to the same standard stimulus is higher in isolated males implanted with corticosterone [30]. On the other hand, long duration chirps in males are elicited by injection of the peptide arginine vasotocin [18], a vasopressin/oxytocin homolog known to activate courtship behaviors in a variety of amniote vertebrates.

Females chirp very rarely to playback stimuli or in group tanks. When they do, they usually make type 2 chirps, and only very occasionally make type 1 chirps [29]. There is, however, one situation in which females consistently chirp. During mating, females release a

single egg that is then fertilized by the male, and repeat this many times over a few hours. As each egg is about to be laid, the female makes a series of brief chirps (3–16 m) presumably to alert the male [10,27,28]. Then, upon laying the egg, she releases a single long duration (180–200 ms) chirp [27]. Thus, females are capable of producing all the chirp types that males make, what differs is the rate at which they make them.

#### 4.3. Rises

Previous studies had shown that gymnotiform wave fish, especially females, modulate their EOD frequency with long slow rises of EOD frequency (increases of a few to tens of Hz that last for seconds or tens of seconds) in social encounters [10,31]. Hagedorn and Heiligenberg [10] qualitatively described two types of rises: short rises (around a second in duration), and long rises (tens of seconds). This distinction was questioned by Engler et al. [25] who maintain that rises fall along a continuum and labeled them all gradual frequency rises (GFR). However, the original suggestion of distinct rise-types has been borne out by another study which noted three categories of rises: short rises, which are a new category, medium rises, which are equivalent in duration to the short rises of Hagedorn and Heiligenberg, and long rises which are equivalent to the long rises described by Hagedorn and Heiligenberg [36]. Two factors might explain the difference in results: Engler et al. used rises emitted spontaneously by lone males and analyzed rises using two variables (frequency excursion, duration), whereas Tallarovic and Zakon analyzed rises made by females that were interacting with a fish in another tank via wire electrodes, and analyzed the rises using four variables (frequency excursion, duration, rise-time and fall-time) with a computer-based clustering algorithm.

Rises have been interpreted simply as submissive signals. This is certainly an oversimplification. When presented with EOD mimic stimuli, male brown ghosts with the lowest EOD frequencies—the smallest, least mature males—make more rises (in this study, rises were not classified into sub-types) than those with higher EOD frequencies—the largest, more mature males—[12]. This is consistent with the idea that rises are submissive signals in that they are made preferentially by fish with the least dominant EOD frequencies. On the other hand, this study found that female brown ghosts with the highest EOD frequencies make more rises than females with the lowest EOD frequencies. The relationship between dominance and EOD frequency is less clear in females but there are suggestions that females with the highest EOD frequencies may also be dominant [34] (D. Lim and S. Tallarovic, unpublished). Tallarovic and Zakon corroborated that females make more rises to females with

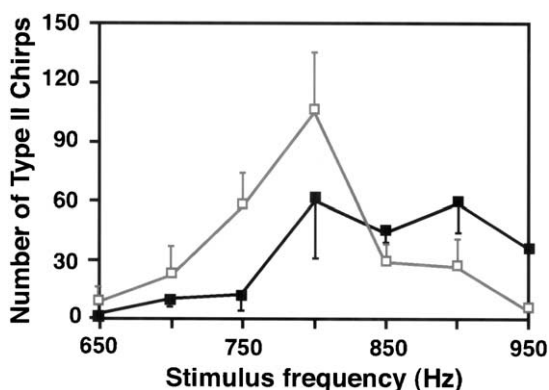


Fig. 3. Males with low EOD frequencies (open squares) chirp more at sine waves with frequencies similar to or lower than their own than they do to sine waves higher than their own EOD frequencies. Males with low EOD frequencies are likely to be subordinate males. Males with high EOD frequencies (filled squares) chirp at males across the frequency range. Males with high EOD frequencies are most likely to be dominant individuals.

lower EOD frequencies, but showed that this was mainly short rises and suggested that short rises are likely to be an aggressive signal used predominantly in female–female interactions. Interestingly, while females with lower EOD frequencies made fewer long rises to females with higher EOD frequencies, their frequency excursion was higher than the ones made by females with higher EOD frequency. Preliminary observations suggest that when two females fight, the one with the lower EOD frequency makes long rises early in the fight as if attempting to raise its EOD frequency to equal or exceed the EOD frequency of the other fish (S. Tallarovic and D. Lim, unpublished).

One other long duration EOD modulation that has been observed is a yodel [19] (Figs. 2 and 4). This is a signal with a rapid rate of rise of tens of Hz with a slow exponential decline to baseline lasting 10–15 s. Yodels are often followed by a LTFE that may last many tens of seconds, or even tens of minutes [22]. These signals were included in the category of long rises by S. Tallarovic and H. Zakon [36], although not all long rises are yodels. It is not clear what yodels signal, nor why they are followed by LTFEs. Dye [32] observed, after a fight between males, that the winner yodeled. In the same vein, yodeling occurs in fights between females over tubes (S. Tallarovic and D. Lim, unpublished). One possible explanation for LTFE following yodeling is that it is a mechanism used by fish to raise EOD fre-

quency. This has interesting implications in terms of “honesty” in signaling. Since EOD frequency is closely tied to size in males, a mechanism for raising EOD frequency such as the LTFE that occurs after yodeling may be a way that males can “cheat” and generate an EOD frequency that would appear to be more dominant than their own “ought” to be. The same might also be true for females for whom higher EOD frequencies may also signal dominant status although this may be dissociated from body size [34].

#### 4.4. Dips

One rarely observed EOD modulation is called a “dip.” This signal is a gradual lowering of EOD frequency by 10–20 Hz over tens to a few hundred milliseconds [36]. It is similar to the “frequency drop” of *Eigenmannia* although frequency drops last tens of [10]. EOD frequency decreases after an intense (type 1) chirp, presumably due to strong inactivation of Na<sup>+</sup> channels of the relay cells, but dips occur in the absence of any preceding modulation.

### 5. Neural basis of social signals

One of the interesting aspects of the brown ghost communication system is understanding how such a simple circuit as the pacemaker nucleus and its two input nuclei can generate such a diversity of signals. The time course of these signals ranges from a few milliseconds (female egg-laying chirp) over tens of milliseconds (male agonistic chirps), hundreds of milliseconds (male courtship chirps, female short rises), many seconds (medium and long rises, yodels) to many hours (jamming avoidance response).

First, given the difference in the types of signals made by males and females, we might wonder if there is a sexual dimorphism in the capacity of the PMn to make different types of signals. Although this question has yet to be specifically addressed, we believe that the PMns of both sexes are similar. While there is a strong bias for each sex to make a particular suite of signals, each sex appears to be able to produce most types of signals. For example, both males and females make rises. Although females rarely make type 1 chirps, when they do, these are identical to those of males [12,18]. While females seldom make long duration chirps, they do so when they lay eggs [27]. Furthermore, the JAR and LTFE show no sex difference in brown ghosts, at least when tested without frequency-clamp [22]. Thus, it seems that the control of sexually dimorphic discharges is more likely “upstream” in the prepacemaker nuclei.

In vitro recordings show that a single shock to the afferent bundle entering the PMn causes a postsynaptic potential that lasts about 5–10 ms, and raises instanta-

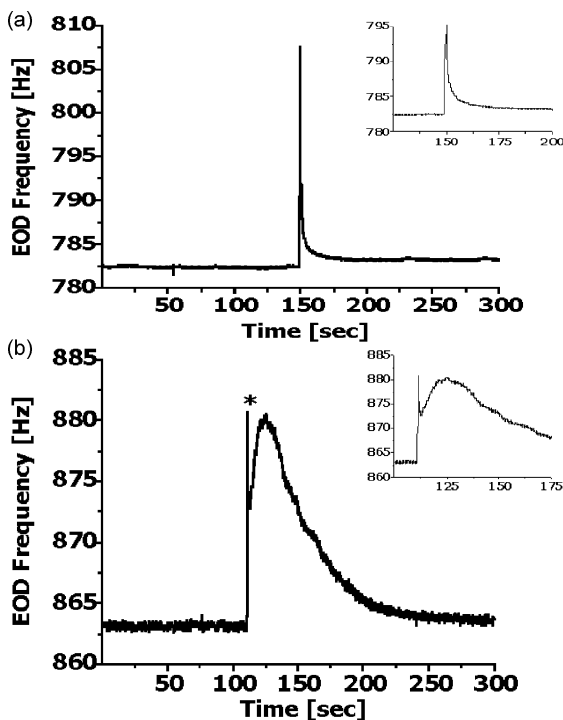


Fig. 4. Variation in long rises. (a) A “classic” rise with rapid upstroke and exponential decay and LTFE. (b) A rise showing that the rapid upstroke may be followed by a prolonged “hump.” In some long rises there is no rapid upstroke, only the “hump.” The insets for both are on a slightly expanded time scale.

neous pacemaker firing frequency by a few to tens of Hz. The strength of the postsynaptic depolarization depends on the arrival time of the presynaptic spike relative to the phase of firing of the pacemaker neurons. In other words, presynaptic spikes that arrive during the depolarizing phase of the PMn neurons have the greatest effect, whereas those that arrive immediately after the PMn has spiked, will fall into the refractory period of the PMn neurons and will have minimal effect [32]. Single unit recordings in the PPn-C of *Eigenmannia* show that each spike of a PPn-C neuron is followed by a small but discernable chirp in the EOD, the magnitude of which also depends on phase of the EOD cycle during which it occurs [33]. Thus, the effect of a single spike in a single PPn-C afferent can be thought of as generating a “quantal” chirp.

Based on these observations we suggest that the egg-laying chirp is essentially a “quantal” chirp since these are approximately the duration of a single psp (5–20 ms) [28]. Engler et al. [27] suggest that a type 2 chirp (15–28 ms) is activated by the arrival of an action potential in each of a small number of afferent fibers. It seems likely that the scatter in the arrival times of a small group of fibers might cause a summation of “quantal” chirps resulting in an increase of not only chirp frequency excursion but also duration.

Type 1 chirps are of similar or slightly longer duration (20–30 ms) than type 2 chirps, but about 4–5 times greater in frequency excursion (200–300 Hz). Engler et al. suggest that type 1 chirps result from a recruitment of a larger number of PPn-C afferents. Further, the clear separation of chirps into types 1 and 2 suggest to them that there is an intrinsic mechanism in the PPn-C for this kind of control.

Presumably, long duration (type 3 and 4) chirps are due to the activation of many fibers from the PPn-C firing bursts of action potentials: the longer the burst of presynaptic APs, the longer the duration of the chirp. In addition, Dye [32] noted that when the inputs to the pacemaker were stimulated repeatedly, they showed synaptic facilitation. Thus, a barrage of spikes in a PPn-C afferent might further increase PMn firing rate this way.

All four chirp types are likely to result from activation of AMPA receptors. Slow long duration increases in firing rate, as occur during the JAR, result from the activation of the NMDA receptors on relay or pacemaker cells. Activation of the NMDA receptor is associated with LTFE, and this happens either with a prolonged low rate of activation, as during the JAR, or a rapid intense activation as occurs during a long rise or tetanic stimulation of the prepacemaker afferents in a slice [21,22,32] (Figs. 2 and 4). However, the activation of the NMDA receptors must be either strong enough if they are activated briefly, or long enough in duration if they are activated weakly, to produce LTFE. This may

account for the lack of an obvious LTFE component in the short and medium rises.

Long rises, especially yodels, are likely due to the co-activation of chirping and JAR circuits—via AMPA and NMDA receptors—by a burst of inputs from both PPn-C and PPn-G or SPPn [2]. The AMPAergic input would likely act earlier (the axons from PPn-C are larger diameter and, therefore, presumably have a faster conduction velocity), and because AMPA receptors typically have faster activation kinetics than NMDA receptors. One indication of this is that while a “classic” yodel has a rapid rise and a smooth exponential decline, some show a sharp early peak followed by a later “bulge” or “hump” (Fig. 4). As mentioned above, yodels and other long rises are often followed by an LTFE. The fact that a long rise lasts for ten seconds or more and that it is accompanied by an increase in firing frequency of tens of Hz, suggests that the threshold for eliciting LTFE has been exceeded, as opposed to short or medium rises.

Finally, dips are an intriguing signal since we cannot explain how they are generated by our current understanding of the Apterontid pacemaker. Frequency drops in *Eigenmannia* are due to a transient decrease in the tonic excitatory input to the PMn from the SPPn. The input from either PPn-G or SPPn is not tonically active in Apterontids [5]. Since all the known inputs to it are depolarizing and none of them are active at rest, it is not obvious how the firing frequency of the PPn may be transiently lowered. It is possible that the parvo-cells are involved although at the present their neurotransmitter is unknown and it is not clear what would activate them since they receive electrotonic tonic input from the pacemaker and relay neurons. Thus, the generation of dips is a mystery.

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