

Electric signaling behavior and the mechanisms of electric organ discharge production in mormyrid fish

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Abstract

Mormyrid fish communicate and navigate using electric organ discharges (EODs). The EOD is highly stereotyped and provides information on sender identity, including species, sex, reproductive condition, and possibly relative status and individual identity. By contrast, the sequence of pulse intervals (SPI) is variable and plays more of a role in signaling behavioral states. Various types of SPI displays may be produced, including tonic patterns such as ‘random’ and ‘regularized’, and phasic patterns such as ‘bursts’ and ‘cessations’. Certain displays have been linked to specific behaviors such as aggression, submission, courtship and active exploration. In addition, interacting pairs of fish may produce stereotyped displays involving the relative timing of their EODs. The EOD waveform is controlled by the morphological and physiological properties of cells in the electric organ termed electrocytes. Differences in the innervation, morphology, size and membrane characteristics of electrocytes have been directly linked to species and sex differences in the EOD. The generation of each EOD is initiated in the medullary command nucleus (CN), which thereby determines the timing of EOD output. CN does not have any properties of a pacemaker, but rather appears to integrate descending inputs that affect the probability of EOD production. The precommand nucleus (PCN) provides a major source of excitatory input to CN and is itself inhibited by corollary discharge feedback following the production of each EOD. Changes in the activity of PCN and its inhibitory feedback neurons modify EOD output, and therefore drive the generation of SPI patterns. Current studies are addressing the mechanisms underlying the generation of these patterns and preliminary results suggest that different types of signals may be controlled by distinct components of the electromotor system. This is similar to findings in other electrogenic teleosts, suggesting that it may be a general feature in the motor control of signaling behavior.

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1. Introduction

Animal communication involves two participants, sender and receiver. As such, neuroethological studies of communication behavior generally fall into two categories: the mechanisms of signal reception and the mechanisms of signal production. The problem for the receiver is to dissect an external signal into its primary components, encode them as neuronal spike trains, and extract relevant information from these trains. Thus, in studying the mechanisms of signal reception, the experimenter presents stimuli to the animal, manipulates various components of the stimuli, and determines how each of these components are encoded by the nervous system. Good examples include the parallel processing of time and intensity information in the auditory system of barn owls for determining the

azimuth and elevation of a sound source, respectively [51], or the comparison of phase and amplitude information in the electrosensory system of gymnotiform fish for determining the frequency difference between a fish’s own electric discharge and that of its neighbor [35].

The problem for the sender is essentially the exact opposite from that of the receiver. The sender must take different types of relevant information about internal states and external conditions, represent them as descending spike trains, and combine that activity into a single, external signal that represents that information. Thus, rather than manipulate the signal and study the effects on the nervous system, the experimenter must manipulate the nervous system and study the effects on the signal. Fundamentally, this is a more difficult problem since the experimenter is not in complete control of the animal’s nervous system. Nevertheless, the problem has been addressed through: (1) observations of neuronal activity in relation to signal output; (2) extracellular and intracellular stimulation of specific brain regions to

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drive signal production; and (3) lesions of those same brain regions to induce signal production deficits.

Teleost fish from the African family Mormyridae produce weak electric organ discharges (EODs) that play an important role in both navigation [79] and communication [41]. Electroreceptors on the surface of the skin respond to distortions in the fish's own electric field caused by the local environment and also respond to the electric fields of other fish. Compared to other communication signals in vertebrates, EODs are relatively simple, consisting of a static electric field that varies in voltage over time. Thus, electric fish serve as excellent model systems for establishing general principles involved in the mechanisms of signal production and reception (see [12,35,41,45,64]). In this review, I describe electric signaling behavior in mormyrid fish, discuss the mechanisms of EOD generation, and present unanswered questions and hypotheses on the mechanisms of electric signal production.

2. Mormyrid electric signal production

2.1. The electric organ discharge (EOD)

Electric signal production in mormyrids involves two primary components, the waveform of each pulse of electricity, termed the electric organ discharge (EOD), and the timing of EOD production, represented by the sequence of pulse intervals (SPI; Fig. 1). The EOD is highly stereotyped within individuals over time [22,27]. In general, the EOD is species-specific, showing differences in polarity, number of phases, duration and overall shape across closely related species (Fig. 2A; [37,38,41]). During the breeding season, males of many species have a longer EOD than females and often have a sex-specific waveform (Fig. 2; [4,22,37,38,42,56,57,60]). In *Brienomyrus brachyistius*, EOD duration is also related to relative dominance among males [20]. High status males tend to have relatively long EODs, while low status males have EODs only slightly longer than a typical female EOD (Fig. 2B). These observations all suggest that the EOD conveys information on various aspects of the signaler's identity.

Both behavioral and physiological experiments support the role of EODs in sender recognition.

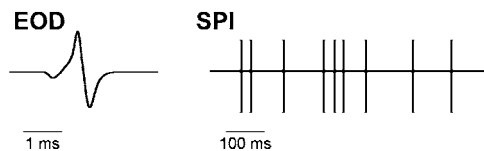


Fig. 1. The two basic components of mormyrid electric communication are the electric organ discharge (EOD) and the sequence of pulse intervals (SPI). The EOD is a stereotyped waveform that provides information on sender identity. The SPI is highly variable, and is used to generate social signals in a variety of contexts.

Conditioning experiments demonstrate that *Gnathonemus petersii* and *Pollimyrus isidori* are able to distinguish individual differences in the EOD, supporting a potential role in individual recognition [29]. In *Brienomyrus* sp. 2, males produce far more courtship responses to natural and 180° phase-shifted (reversed polarity) female EODs than to EODs phase-shifted to various intermediates [39,42]. In addition, strong courtship responses are elicited by square pulses similar in duration to female EODs, but not different duration square pulses [42]. These results suggest that temporal characteristics of the EOD are responsible for sex and species recognition. These temporal features are first coded

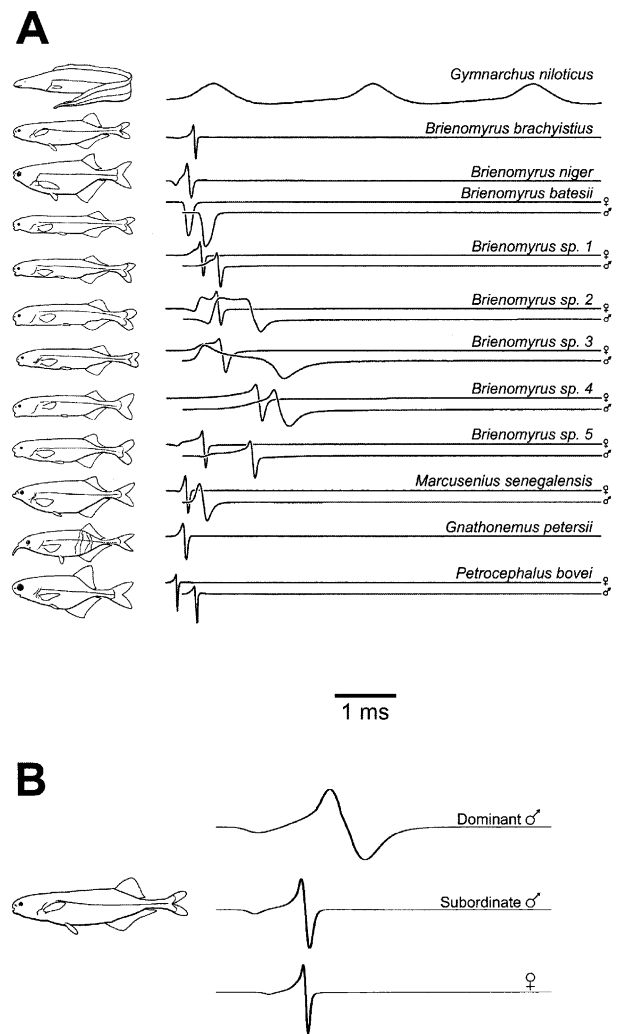


Fig. 2. (A) Species diversity and sex differences in EOD waveform in mormyrids (from [1] with permission). Several species of *Brienomyrus* are not yet described and are tentatively referred to by numbers. Note the variation in polarity, duration, number of phases, and overall shape across species and, in some cases, between the sexes. (B) Sex and dominance-related differences in EOD waveform in *Brienomyrus brachyistius*. All three individuals are members of the same community tank. The dominant male has an extremely elongated EOD, while that of the subordinate is only slightly longer than the female's EOD (data from [20]).

by knollenorgan electroreceptors, which respond with a phase-locked spike to positive voltage changes, the timing of which varies with different EOD waveforms [42]. Recent studies on the central knollenorgan pathway have identified a potential mechanism by which these spike times may be compared to determine the temporal characteristics of EODs [82].

2.2. The sequence of pulse intervals (SPI)

The sequence of pulse intervals (SPI) refers to the temporal pattern of inter-EOD intervals (Fig. 1), and is generally represented as a plot of EOD interval vs. time (Fig. 3A). In contrast to the EOD, the SPI is highly variable from moment to moment, ranging from tens to

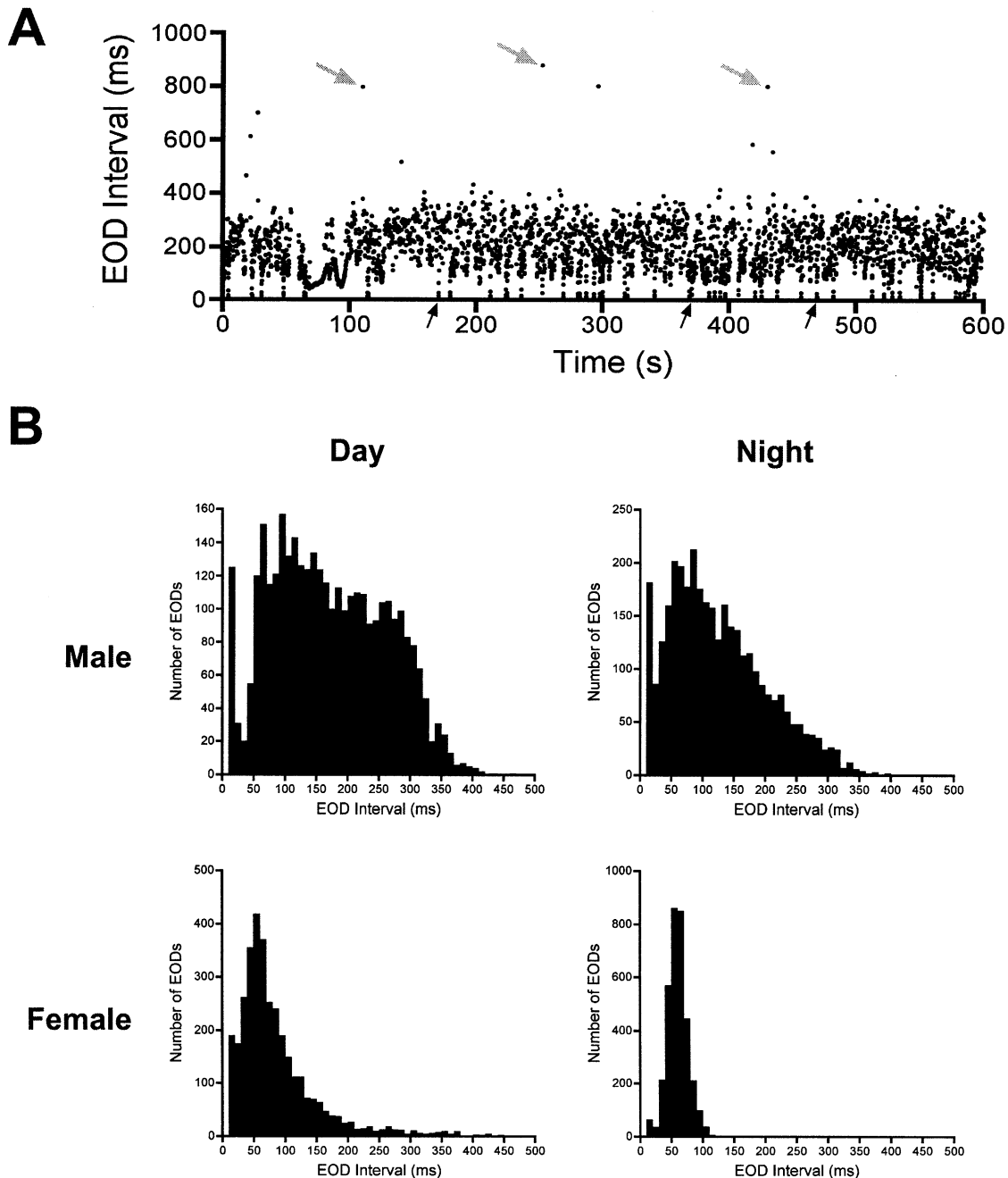


Fig. 3. (A) Sequence of pulse intervals (SPI) plot from a 10 min recording of an isolated male *Brienomyrus brachyistius* during the day. Note the wide variation in EOD interval, as well as occasional bursts (small arrows) and cessations (large arrows) that deviate from the overall resting pattern. (B) EOD interval distributions from 10-min recordings from an isolated male and female, each recorded once during the day and once at night. Note the differences in overall distribution between the sexes and from day to night, as well as the peaks at very short intervals reflecting EOD bursts.

several hundreds of milliseconds (Fig. 3). This constant variation suggests that the SPI is less important for sender recognition, and plays a greater role in signaling the behavioral state of the sender. Various SPI patterns have been linked with aggressive behavior [8,10,52,54,55,58,73], threat signaling [53], submissive behavior [10,55,65,67,73], group cohesion [66,68,69,74], detection of another fish [8,9,54,58,65,70], and courtship [17,21,42].

Limited data from playback experiments indicate that the SPI does have an important role in electric communication. In *G. petersii*, for instance, playbacks of SPIs from aggressive fish elicit more attacks on the playback electrode than playbacks of SPIs from resting fish [55]. In addition, playback of SPIs from fish in three different conditions (slow swimming, investigative swimming, and feeding) lead to differences in approach responses, and fish approach electrodes playing natural SPI patterns more often than scrambled SPI patterns [77].

Despite the variability of the SPI, there are species, sex, and individual differences in the overall distribution of EOD intervals (Fig. 3B; [17,23,40,59,67]), and there is evidence that these differences play a role in species and sex recognition for certain species [59]. However, in *Brienomyrus* sp. 2, males court playbacks of conspecific female EODs whether they are played back at a conspecific SPI, a heterospecific SPI, or a scrambled SPI, indicating that in this species, the SPI plays little, if any role in sender recognition [39,40,42]. In general, the SPI may be used for species recognition in species that have very short EODs, where temporal differences may be too small for discrimination. The SPI may also play a role in species and sex recognition in species with no differences in the EOD between the sexes or among sympatric species.

3. Electric signaling using sequence of pulse interval patterns

3.1. SPI patterns in mormyrid fish

Studying the role of SPIs in mormyrid communication has proven difficult due to the technical problem of isolating the EODs produced by different fish. Recordings from isolated fish are relatively easy, but prevent the association between signaling behavior and social context. Playback experiments add the element of electrical stimulation, but do not allow for an interaction between freely behaving animals. Several methods, each with their own advantages and disadvantages, have been used to overcome these problems, such as tethering one fish with a fine wire to isolate its EOD [10], recording interactions between two species with widely different EODs [52], electrical silencing of one fish through

surgery [40], and recording electrical activity on videotape for later visual analysis [73]. Despite the difficulties, numerous authors have described a wide range of SPI patterns in various species that have been linked to specific behavioral contexts and appear to play an important role in communication (Fig. 4; reviewed by Hopkins [40]). Following the nomenclature of Hopkins [40], these patterns can be categorized as involving one fish, in which case they can be tonic or phasic, or as involving an interaction between the outputs of two fish.

3.2. Tonic SPI patterns in single fish

While at rest, mormyrids typically produce a tonic SPI pattern with relatively long intervals, on the order of hundreds of milliseconds, and with a high degree of irregularity, with coefficients of variation ranging from 15 to over 100% [54,58,65,67,75,78]. Such an SPI pattern is referred to as 'random' (Fig. 4). Because mormyrids are nocturnal, the random pattern predominates during the day, leading to EOD interval distributions with high variability and relatively long SPIs (Fig. 3B; [65]).

This random pattern may be replaced by a highly regular tonic SPI pattern, in which coefficients of variation decrease to less than 15% [65,75]. This SPI pattern is termed 'regularized' and is usually, but not always associated with decreases in the mean EOD interval (Fig. 4). Regularized SPI patterns at relatively low mean EOD intervals occur while fish are actively probing their environment, and are probably related to active electrolocation in which the fish increases its sampling rate and regularity [78]. In addition, regularized SPI patterns occur upon detection of a conspecific or during playback of electric signals [8,9,54,58,65,67,73]. In general, the regularity increases, and the mean EOD interval decreases with increasing stimulus intensity and frequency [65]. These regularized SPI patterns may be related to signaling behavior, or to active electrolocation as the fish investigates the stimulus. As mormyrids become more active at night, the regularized pattern becomes more frequent, causing the EOD interval distribution to shift to lower SPIs with less variation (Fig. 3B; [65]).

A communication function for regularized SPI patterns has been suggested for *P. isidori* [17,23]. Normally, females produce a random SPI pattern, but on spawning nights, females produce regularized patterns with mean EOD intervals centered around 100 ms throughout courtship and spawning. When the female enters the male's territory, the male begins producing a similar regularized pattern as well. While this is not definitive evidence that these regularized patterns are involved in signaling behavior, the correlation between spawning and regularized SPI patterns suggest that they may be involved in the courtship process.

A third type of tonic SPI pattern has been described for *G. petersii*, and is termed ‘pulse pairs’, consisting of alternating long and short intervals (Fig. 4). Both the long and short intervals tend to be fairly stereotyped, ranging from 15 to 16 ms and 8 to 9 ms, respectively [10,58]. Pulse pairs are produced during overt agonistic interactions, indicating that they serve as an aggressive signal [8,10,52,58].

3.3. Phasic SPI patterns in single fish

Phasic SPI patterns also play an important role in electric communication for mormyrids. There are two basic types of phasic SPI signals, ‘cessations’ and ‘bursts’ (Figs. 3A and 4). Cessations consist of a sudden interruption in the SPI that may last from tens of milliseconds to several seconds, or even minutes in duration [10,58,65,67,70]. Cessations have generally been

grouped into short and long cessations depending on whether they are less than or greater than a certain value, respectively, which ranges from 1 to 5 s depending on the author and the species studied (Fig. 4). Short cessations are generally produced by aggressive fish during chasing, overt attack or while engaged in an anti-parallel display, and often follow or alternate with bursts [10,58].

By contrast, long cessations are produced by submissive fish upon detecting a dominant conspecific and can last up to several minutes [10,65,67,70,73]. In *B. niger*, this ‘social silence’ response can be elicited by the controlled movement of two fish into each other’s active space [70]. Under these conditions, cessation duration is negatively correlated with body size, indicating that larger, more dominant fish produce shorter cessations than smaller, more submissive fish. These long cessa-

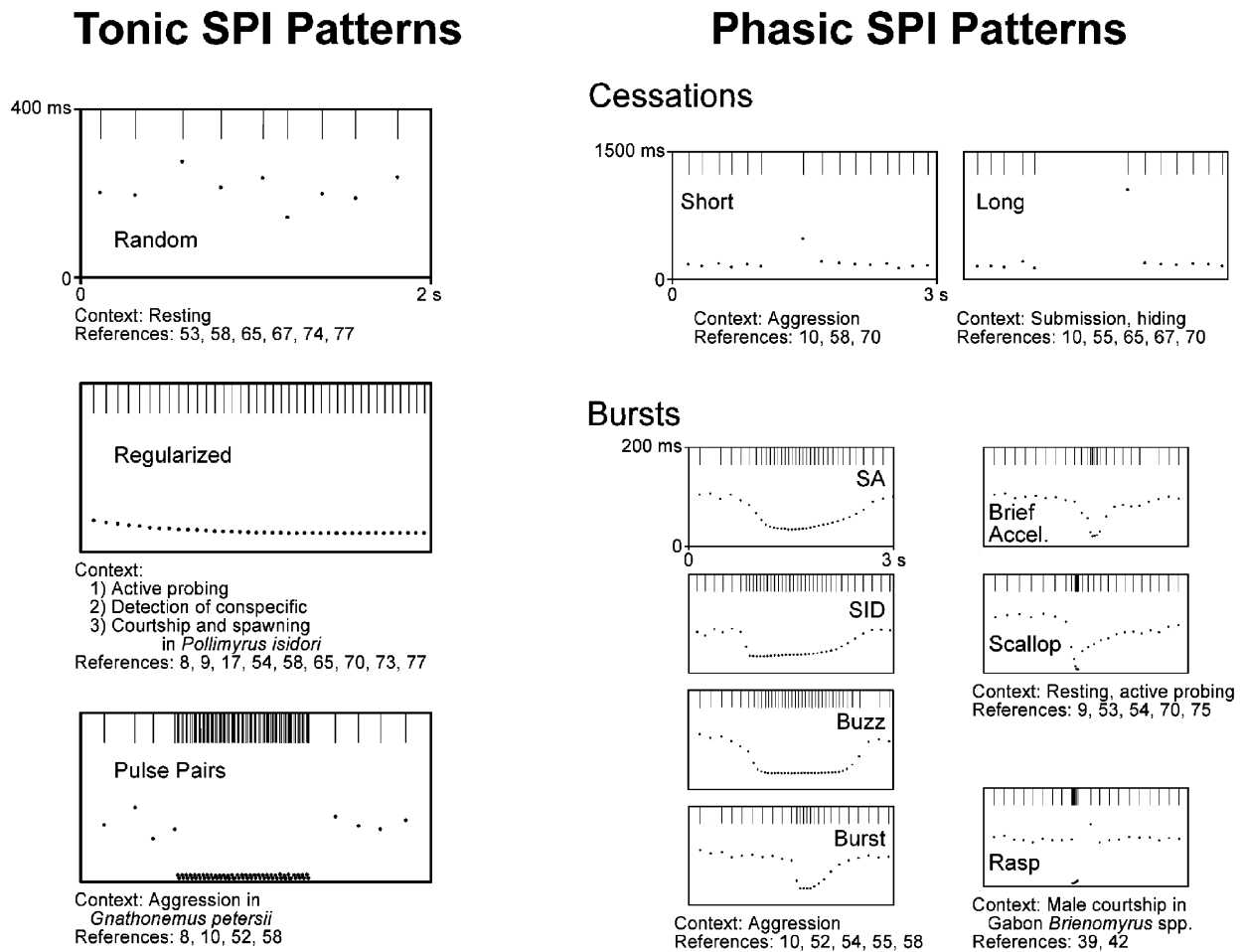


Fig. 4. Summary of SPI patterns produced by single fish as described for various species of mormyrids, including the general context in which they are produced and the references in which they are described (modified from [40]). Each pattern is represented by a plot of EOD interval vs. time with a comb plot in the upper part of the graph showing the timing of EOD activity. Stereotyped SPI patterns can be classified according to whether they are tonic (ongoing activity) or phasic (transient activity), and if phasic, as cessations or bursts. For each category, the abscissa and ordinate for each plot are identical. SA, smooth acceleration; SID, sudden increase followed by a decrease in frequency.

tions may serve as a submissive appeasement signal, or simply as a means of hiding from a potential aggressor.

Bursts have been described by many authors for several species, and have been given a variety of names, such as brief acceleration, burst activity, step-like decrease in interval, sudden increase followed by a decrease in frequency (SID), smooth acceleration, rate acceleration, gradual decrease in interval, burst, buzz, scallop and rasp (Fig. 4; [9,38–40,42,52–55,58]). In general, bursts involve a transient decrease in the SPI to values of 5–40 ms, often resulting in distinct peaks in the overall EOD interval histogram at short intervals (Fig. 3B). The onset and offset of these bursts can vary from sudden to gradual. Many of these bursts (smooth acceleration, burst, buzz, SID) have been linked to agonistic behavior, occurring during anti-parallel displays, chasing, attacking, and intense aggression [10,40,52,53,55,58,73], and as mentioned above, may alternate with short cessations. As seen in Fig. 4, these ‘aggressive bursts’ are qualitatively similar to each other. Due to a lack of quantitative analysis, it is difficult to know whether these different signals reflect discrete categories, continuous variation in one type of burst, or both. In addition, these signals are quite similar to the tonic regularization pattern, and may simply reflect relatively brief regularizations. It would be interesting to know whether these are quantitatively distinct signals or different variants of the same signal. Such variance may be directly linked to the intensity or duration of the associated behavior.

Two other types of bursts, the brief acceleration and scallop frequently occur in isolated fish, while either resting or probing their environment [9,69,75], suggesting that they may be related to active electrolocation by providing the fish with a briefly high sampling rate. Both types of bursts have shorter durations and shorter minimum EOD intervals than the aggressive bursts (Fig. 4). Because they are produced in a different context from the aggressive bursts, this difference in form may relate to a difference in function. In *B. niger*, individual scallops show a significant degree of stereotypy within individuals over a period of several days [75], suggesting that scallops may play a role in communication. The significance of these individual differences to receivers has not been tested, however.

Stereotyped bursts called rasps consist of sudden onset, intense bursts to intervals as short as 6–7 ms that are produced by males of various species in the Gabon-clade *Brienomyrus* spp. during the breeding season (Fig. 4; [38–40,42]). Rasp production increases dramatically upon the approach of a female or in response to playback of a female conspecific EOD or similar duration square pulse, suggesting that they are used as a courtship signal [39,42]. Rasps probably serve to attract females, and variation in certain characteristics of rasps may be used by females to assess male quality.

Addressing these possibilities will require quantification of rasp characteristics and playback experiments analyzing female choice.

3.4. SPI interactions between two fish

In addition to signals produced by one fish, various authors have also described specific SPI patterns resulting from the relative timing of the EODs of two fish. For instance, during agonistic interactions, fish will often produce EODs that are phase-locked at 180° out of phase with each other [10,52]. In addition, the echo response, or preferred latency response (PLR), refers to the tendency to produce an EOD at a latency of 12–20 ms after the EOD of another fish [33,52,55,62,72]. The PLR is produced by dominant fish during agonistic encounters or in response to electrical stimulation [34,52,55,62,72]. In *G. petersii*, typical latencies for the PLR range from 12 to 14 ms, while in *Mormyrus rume*, a species with a longer EOD duration, typical latencies range from 20 to 22 ms [34]. This may be a result of variance in EOD duration, or it may be directly related to communication and play a role in species recognition [72]. In contrast to the PLR, the preferred latency avoidance (PLA) refers to a tendency to avoid producing EODs at latencies of 12–20 ms after another fish’s EOD [62]. In *P. isidori*, males tend to produce the PLR in response to electric stimulation, while females tend to produce the PLA [62], suggesting that they play different roles in communication.

3.5. Remaining questions

While the studies referenced in Fig. 4 clearly show that mormyrids produce various SPI patterns in certain contexts and that they likely have communicative significance, they have generally suffered from a lack of quantitative analysis. A rigorous, quantitative description of SPI signals is absolutely essential to address important questions, such as: How much variation in signal characteristics exists within and among individuals, between sexes and between species? Are there discrete boundaries within this variation that allow us to categorize the signals or is there continuous variation in one basic signal type? How often are the signals produced in various social contexts? Do the types of signals or their characteristics change with changing context? What are the functions of these signals? What kinds and amounts of information do they contain? How do these signals affect the behavior of other fish? What are the mechanisms for signal generation and what are the mechanisms underlying their encoding? Only with quantitative approaches to these questions can we begin to address these issues of general significance to animal communication.

4. The mormyrid electromotor system

4.1. Control of EOD waveform: the electric organ

The mormyrid electric organ is located in the caudal peduncle and is composed of two rows of electrocytes on each side of the midline (Fig. 5; [13]). The synchronous activation of the electrocytes via spinal electromotor neurons (EMN) leads to a summation of their electrical potentials and the generation of a single EOD [14,16]. Therefore, the species, sex, and individual differences in EOD waveform are due to differences in the morphological and physiological properties of the electrocytes.

Mormyrid electrocytes are thin, multinucleated discs with a series of stalks that arise from the posterior or anterior face and fuse to form a large trunk that receives innervation from the electromotor nerve [2,3]. The stalks actively propagate action potentials to the electrocytes, where both the anterior and posterior face are also active [14]. Apart from this common theme, there is wide diversity in the morphology of the stalk system across species [1,2,41]. The stalks may be classified according to the site of innervation (anterior vs. posterior) and as penetrating, doubly penetrating or non-penetrating, based on whether they reverse course and penetrate the electrocyte face once or twice before fusing [1,2,14,41]. Based on these differences, seven distinct types of electrocyte stalk systems have been recognized [1,2]. These different morphologies correlate exactly with species differences in EOD polarity and number of phases. Specifically, the site of innervation determines

the polarity of the first phase, and the number of penetrations determines the total number of phases [1,2,41].

In addition to differences in polarity and number of phases, the EOD may also vary in the shape and duration of each phase across species, between the sexes, and among individuals [3,4,20,27,37,38,42]. Across species, the degree of surface invagination on the anterior face of electrocytes is directly related to the species-typical EOD duration; species with long EODs have a high degree of invagination, while those with short EODs have less proliferation of the anterior face [7]. In *Brienomyrus* sp. 2 and sp. 3, males have longer EODs than females, and this difference is associated with larger stalks, thicker electrocytes, and a greater invagination of the anterior face in males [7,26]. These morphological features can be induced by the administration of androgens to females, juveniles, and non-reproductive males [7,26], which also leads to EOD elongation and a male-typical waveform [4,5,26,36,61]. Presumably, the degree of invagination is related to the capacitance of the anterior face, which, in turn, is related to the time constant of activation, thereby affecting electrical excitability. In support of this explanation, administration of androgens to *B. brachyistius* leads to a 2–3-fold increase in the action potential duration of single electrocytes [6]. While the morphophysiological correlates of individual differences in the EOD have not been explored, dominance-related differences in EOD duration are directly correlated with plasma levels of androgens [20], suggesting that mechanisms similar to those driving sex differences may be involved.

4.2. Control of EOD production: The medullary electromotor network

While the EOD waveform is determined by the morphophysiological properties of the electrocytes, EOD timing is determined by activity in the medullary electromotor network (Fig. 5). The command to produce each EOD is initiated in the command nucleus (CN), a group of about 15–20 cells, located medially, on the ventral edge of the medulla [11,30]. CN projects to the medullary relay nucleus (MRN), which is located immediately dorsal to CN, both directly and indirectly via the bulbar command-associated nucleus (BCA; [11]). BCA also projects rostrally, serving as the origin of the corollary discharge signal that provides electrosensory regions with a reference for electromotor output [11]. MRN sends the signal down the spinal cord to electromotor neurons (EMN), which drive the electric organ (EO) to produce a single, stereotyped EOD, the waveform of which is controlled by the morphology and physiology of electrocytes in EO. The output of CN, and therefore the SPI, is influenced by two identified inputs to the electromotor system, the pre-command nucleus (PCN) and the ventroposterior nucleus (VP), as well as other potential sources. *c1–c3*, lobes 1–3 of the corpus of the cerebellum; *ELL*, electrosensory lateral line lobe; *Hyp*, hypothalamus; *Tel*, telencephalon; *Val*, valvula of the cerebellum.

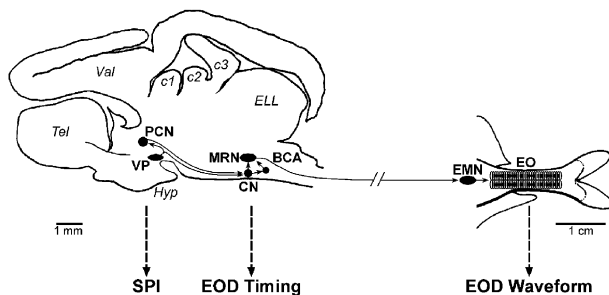


Fig. 5. Neuroanatomy of the mormyrid electromotor system (modified from [32]). Each EOD is initiated in the command nucleus (CN), which thereby determines EOD timing. This signal is sent to the medullary relay nucleus (MRN), both directly, and indirectly through the bulbar command-associated nucleus (BCA). MRN sends the signal down the spinal cord to electromotor neurons (EMN), which drive the electric organ (EO) to produce a single, stereotyped EOD, the waveform of which is controlled by the morphology and physiology of electrocytes in EO. The output of CN, and therefore the SPI, is influenced by two identified inputs to the electromotor system, the pre-command nucleus (PCN) and the ventroposterior nucleus (VP), as well as other potential sources. *c1–c3*, lobes 1–3 of the corpus of the cerebellum; *ELL*, electrosensory lateral line lobe; *Hyp*, hypothalamus; *Tel*, telencephalon; *Val*, valvula of the cerebellum.

[15,16,24,25,30]. This coupling results in a tightly linked network that rigidly preserves timing information and ensures the synchronous activation of the electrocytes, thereby maintaining a stereotyped EOD [15,16,30].

Electromotor neurons fire a phase-locked, synchronous triple action potential starting about 5 ms before EOD generation [11,16,30]. When a fish is curarized during a physiology experiment, its EOD is silenced, and the EMN activity can be recorded as an external field potential by placing an electrode next to the tail. This potential is used to monitor EOD output, with the timing of the first spike in the triple volley representing the reference time (T_0 ; Figs. 6–8). Field potentials recorded within CN and MRN show phase-locked activity in relation to EMN output, with a double negative potential beginning 3–3.5 ms before T_0 (Fig. 6; [11,80]). The CN potential starts around 200–300 μ s

before the MRN potential, indicating that the MRN potential is driven by input from CN. Intracellular recordings from CN and MRN neurons support this conclusion [30]. Both types of neurons fire double action potentials in a strict 1:1 relationship with EMN output, the timing of which agrees well with that of the double field potentials (Fig. 7A). While these times may vary across individuals, the output of CN and MRN neurons is highly phase-locked with EMN output within individuals [30].

The mechanism of generation of the double action potential in CN and MRN is unclear. It was originally suggested that the double spike in MRN resulted from the intrinsic properties of MRN neurons [16], but stimulation of the spinal cord leading to antidromic activation of MRN results in only a single action potential, which is sometimes later followed by a double action potential [30]. Furthermore, later studies demonstrated that MRN receives input via a direct and indirect pathway from CN [11]. It may be that the first spike results from the direct input, while the second spike results from the indirect input [32]. The second spike in CN may then be due to antidromic activation across the gap junctions between CN and MRN, which accounts for the second spike in CN following the second spike in MRN (Fig. 7A). Refractoriness may be responsible for ending the cycle at two spikes. Stimulation of the spinal cord does activate CN neurons, indicating that activity can be propagated in either direction through these gap junctions [30].

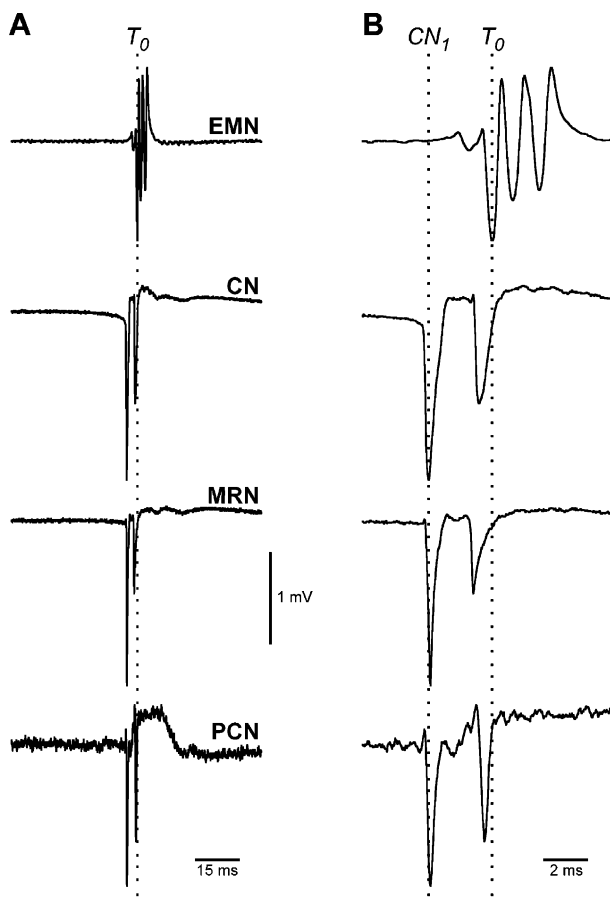


Fig. 6. Field potentials of electromotor nuclei recorded in a single individual *Brienomyrus brachyistius*, at long (A) and short (B) time scales. The electromotor neurons (EMN) fire about 5 ms before EOD generation in freely behaving animals, and the timing of the first peak in its field potential is used as the reference time (T_0). Both the command nucleus (CN) and medullary relay nucleus (MRN) have a double negative peak, starting around 3 ms before T_0 . The timing of the first peak in CN is referred to as CN_1 . The precommand nucleus (PCN) has a double negative peak that follows activity in CN, as well as a prolonged positive peak following T_0 .

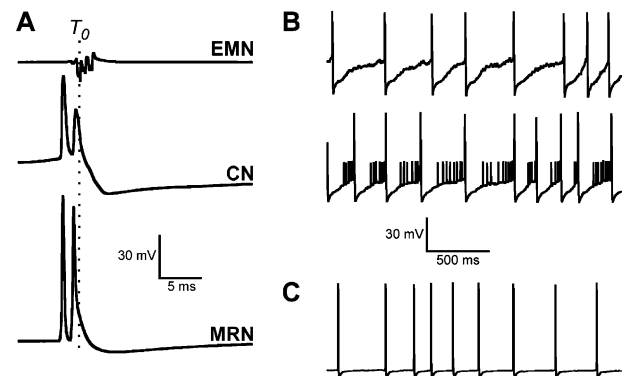


Fig. 7. Intracellular activity of command neurons (CN) and medullary relay neurons (MRN) in relation to electromotor neuron (EMN) activity in *Brienomyrus brachyistius*. (A) Both CN and MRN neurons fire a stereotyped double action potential starting about 3 ms before the time of the first EMN spike (T_0), in a 1:1 relationship with EMN output. Within individuals, the first action potential in CN precedes that in MRN by 200–300 μ s, while the second action potential in CN follows the second action potential in MRN. (B) Ongoing activity in two different CN neurons. The neuron in the upper trace shows fluctuations in the baseline voltage indicating postsynaptic activity, and the neuron in the lower trace shows spikelettes, small all-or-none events that likely reflect the descending activity of precommand neurons (PCN). (C) Ongoing activity in an MRN neuron. Note the flat baseline from which the double action potential arises, suggesting that MRN does not receive any input but the command signal from CN.

Continuous records from CN and MRN neurons reveal important differences between the two (Fig. 7B, C). CN neurons show spontaneous fluctuations in the membrane potential, indicating the occurrence of post-synaptic activity, while MRN neurons have a flat baseline voltage from which the double action potential arises [16,30]. In addition, CN neurons often have spikelets, small all-or-none events that occur before the double action potential but are absent for several milliseconds after (Fig. 7B; [30]). These may represent post-synaptic manifestations of descending activity to CN passing through electrical synapses (see below; [32]). These results suggest that CN plays an important role in integrating descending input to the electromotor system, while MRN simply relays CN output to the spinal cord, which is consistent with the roles of a command and relay nucleus, respectively, and agrees with the timing of activity in the two nuclei. There is no evidence that CN has any characteristics of a pacemaker as the depolarizing period leading up to the double action potential is highly variable (Fig. 7B), and synaptic isolation of CN by transecting the brain at the rhombencephalic-

mesencephalic border completely eliminates electromotor output [76].

The anatomy of the primary electromotor system also suggests that CN is the ultimate site of EOD initiation. Injections of HRP into CN leads to retrograde labeling in the ventroposterior nucleus of the torus (VP) and a group of cells at the mesencephalic-diencephalic border, termed the precommand nucleus (PCN; Fig. 5; [11]). However, injections into MRN only retrogradely label cells in CN and BCA, supporting the physiology that indicates MRN is dedicated to relaying CN output rather than integrating descending input [11]. In addition, intracellular labeling of CN and MRN cells demonstrate that MRN dendrites are restricted to within the nucleus, while CN dendrites extend several hundred microns into the adjacent reticular formation, where they likely receive additional synaptic inputs [30].

Immunohistochemical studies in *G. petersii* have revealed serotonergic projections to both CN and MRN, with the projection to MRN being especially dense [31,63]. These fibers likely originate from cells within the nearby raphe nuclei. The significance of these

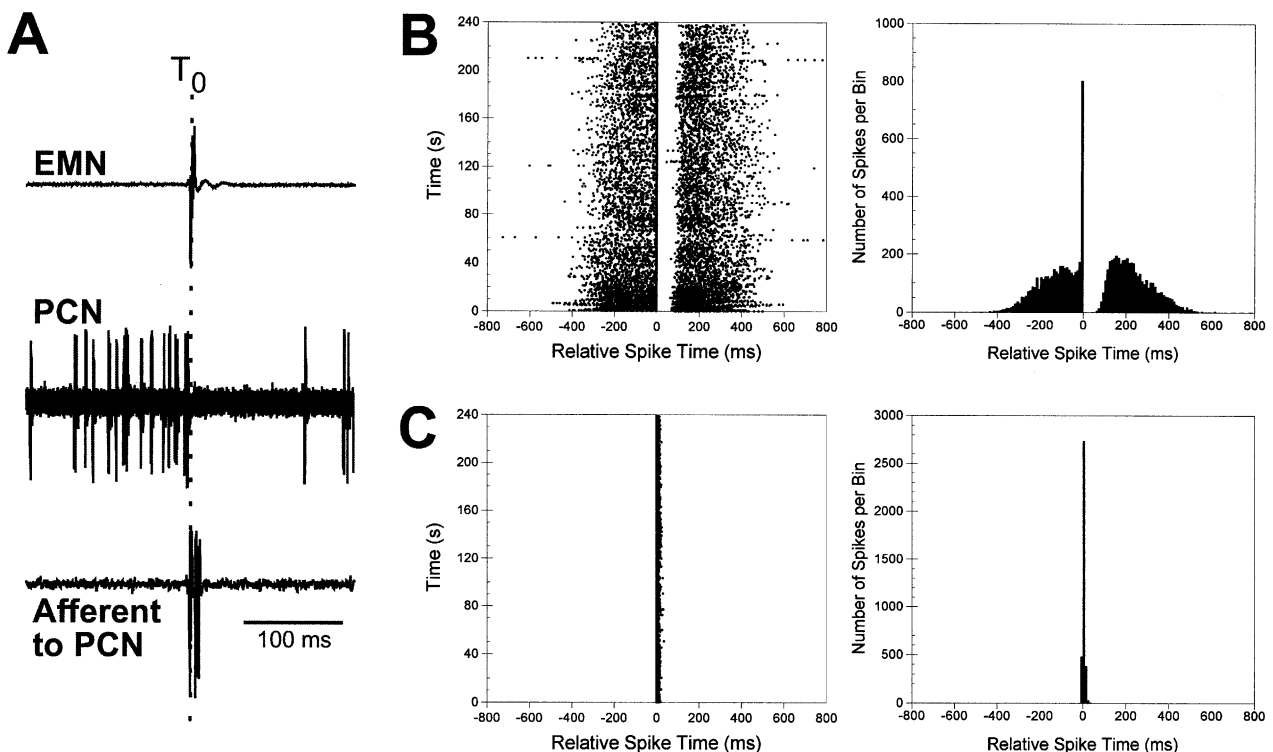


Fig. 8. Extracellular activity of single units in the precommand nucleus (PCN) in *Brienomyrus brachyistius*. (A) PCN units fire spontaneously before T_0 , fire a phase-locked spike about 2.5–3 ms before T_0 , then fall silent for tens to hundreds of milliseconds after T_0 . Afferents to PCN fire bursts of action potentials starting near T_0 , indicating that they are driven by the corollary discharge pathway and inhibit PCN units. (B) A raster and histogram from a four-minute recording from a PCN unit in relation to EMN output. The ordinate represents the time of occurrence of T_0 , while the abscissa represents the time of PCN firing relative to T_0 . Negative times reflect PCN spikes that occur before T_0 , while positive times reflect PCN spikes that occur after T_0 . Note the spontaneous firing pattern, except for the phase-locked spike at around -2.5 ms that leads to a prominent peak in the histogram, and the pronounced inhibition for ~ 70 ms after T_0 . (C) A raster and histogram from a 4 min recording from an afferent to PCN in relation to EMN output. Plots have the same format as in (B). Note the absence of activity throughout the EOD cycle except for a burst of activity centered around T_0 , indicating that these units drive the inhibition of PCN after the generation of each EOD.

projections is not known and the fact that there is a strong projection to MRN is especially intriguing since the evidence presented above strongly suggests that MRN is solely dedicated to relaying the output from CN. It may be that this input is involved in modulating the degree of electrical coupling within MRN or modifying the responses of MRN to CN activation. It is also possible that this input serves to regulate overall electromotor excitability by modifying the resting potential of CN/MRN. The large size of MRN neurons and the strong electrotonic coupling between CN and MRN suggest that the MRN resting potential would directly influence the CN resting potential. These hypotheses are, however, purely speculative at this point since the role of serotonin in modifying electromotor output has not been studied physiologically.

4.3. Control of SPI patterns: descending inputs to the medullary electromotor network

Based on retrograde injections of HRP, CN appears to receive input from two primary sources, the ventroposterior (VP) and precommand nuclei (Fig. 5; PCN; [11]). Recent results using anterograde transport of biotinylated compounds support this conclusion (Carlson, in preparation). In addition, extracellular recordings from PCN strongly support its role as an afferent input to CN (see below; [80]).

After injections of HRP into CN, 10–15 large multipolar neurons are bilaterally labeled at the ventromedial edge of VP [11]. These neurons have many large dendrites that extend several hundred microns in all directions. Their axons head ventromedial through the toropraeeminential tract to enter the ventral commissure, where they trifurcate, sending one branch to CN, and one branch each to the contralateral and ipsilateral PCN (Carlson [83]). No recordings have been made from VP neurons, so their potential role in regulating electromotor output remains to be explored.

The primary descending input to CN is from PCN, a bilateral nucleus located between the mesencephalic-diencephalic border and the dorsal thalamus that is strongly labeled after HRP injections into CN [11]. The caudal region of PCN contains medium-sized multipolar neurons that send thick dendrites to arch laterally toward the toro-praeeminential tract, while the rostral region has smaller neurons with fine, varicose dendrites that are limited to the nucleus. This rostral group of neurons extends into the dorsal thalamus (Carlson [83]).

Field potentials recorded in PCN have three characteristic features, two negative peaks, one which occurs 2–3 ms before T_0 , and one which occurs from 1 ms before to 1 ms after T_0 , as well as a weaker, prolonged positive potential following T_0 (Fig. 6; [80]). No portion of the field potential occurs before CN activation, sug-

gesting that PCN units do not fire in synchrony to drive CN output. Thus, CN must be influenced by asynchronous input across PCN units. Extracellular recordings from single PCN units support this conclusion [80]. In the period before CN activation, PCN units fire spontaneously at an irregular rhythm (Fig. 8A, B). During this period, the average firing rate of PCN units is positively correlated with EOD rate [80], indicating that the PCN to CN synapse is excitatory. Both electrical stimulation and glutamate iontophoresis in PCN drive large increases in EOD rate [80], further demonstrating that PCN provides excitatory input to CN. Apparently, the ongoing activity in PCN leads to a gradual depolarization of CN neurons until they reach threshold (Fig. 7B). The firing rates of PCN neurons thereby determine the rate of depolarization and therefore the EOD rate. This view is supported by a recent study demonstrating that resting EOD output can be replicated by modeling CN as integrating input from several units with PCN-like properties [28]. The causes of variation in PCN firing rates are unknown, but may be due to intrinsic properties of PCN neurons as well as modulatory inputs from other sources.

The first negative peak in the PCN field potential appears to result from synchronous activity across PCN units since they tend to fire a phase-locked action potential at the same time (Fig. 8B; [80]). This peak comes immediately after the first peak of the CN field potential (Fig. 6B), indicating that it is an effect of activity in CN, rather than a cause. One possibility is that this phase-locked spike results from corollary discharge feedback to PCN. However, the timing of the first peak in the field potential comes before the timing of activity in the mesencephalic command-associated nucleus (MCA; [80]), a major relay center for the corollary discharge, suggesting that this phase-locked activity cannot be due to corollary discharge feedback.

An alternative explanation is that PCN forms electrical synapses on CN, and when CN neurons are activated in synchrony, this leads to antidromic activation of the PCN axons. In support of this hypothesis, the phase-locked spike is occluded by an action potential occurring within 2–3 ms before the normal firing time of the phase-locked spike, which is longer than the refractory period of PCN units, but agrees well with the predicted time for a descending action potential to block an antidromic action potential [80]. While a detailed study of PCN terminals in CN has not been done, ultrastructural studies of CN demonstrate that the majority of synapses in CN are large club endings with gap junctions, and PCN forms the major input to CN, suggesting that the PCN to CN synapse is electrical [24]. In addition, intracellular records from CN neurons often contain spikelettes (described above) whose timing relative to EOD output is very similar to that of PCN units (Fig. 7B; [32]). This suggests that these small, all-or-none

events represent action potentials from PCN axons that passively propagate across the gap junctions into CN somata.

Another prominent feature of single-unit activity in PCN units is a period of silence starting immediately after the phase-locked spike and lasting tens to hundreds of milliseconds in duration (Fig. 8A, B; [80]). The timing of this silent period suggests that it results from negative feedback via the corollary discharge pathway, in which the generation of each EOD leads to inhibition of PCN units. A second type of unit recorded in PCN is completely silent throughout each EOD cycle, except for firing a burst of action potentials that start near T_0 (Fig. 8A, C; [80]). This timing exactly coincides with the onset of the silent period in PCN units, indicating that they are inhibitory afferents to PCN that are driven by the corollary discharge pathway. Preliminary results using immunohistochemistry have indicated that PCN neurons are surrounded by terminals containing glutamic acid decarboxylase (GAD), an enzyme involved in synthesizing GABA [71], supporting the presence of an inhibitory input to PCN. The bursts in these units vary in onset time, duration, and intraburst frequency both within and between units. For EOD intervals less than 200 ms, as EOD rate increases, the latency to the first spike in the burst increases, burst duration decreases, and intraburst frequency decreases [80]. In addition, the latency to the first spike in PCN units following inhibition is negatively correlated with EOD rate [80]. These results suggest that part of the variation in PCN activity is due to modifications in the strength of negative feedback by the inhibitory units. It is unknown at this point where these inhibitory units originate, but they must receive input from the corollary discharge pathway.

The stereotyped firing pattern and timing of these inhibitory units makes them likely responsible for the second negative peak in the PCN field potential, as well as the slow, positive potential (Fig. 6). The initial, sharp negativity likely results from a synchronous burst onset across several units, while the slow, weak positive potential probably results from asynchronous activity across many units. Individual units frequently have different burst onset times [80], which suggests that the wide variation in the timing of the second negative peak and its frequent absence may be due to variation in the unit activity near the recording electrode. The second negative peak is not likely caused by the second action potential in CN, because its timing is far more variable in PCN than CN, and the second peak in CN is never absent [80].

Electrosensory stimulation has a strong effect on EOD output, and PCN appears to play a central role in mediating this response. During physiological recordings, playing electrosensory stimuli to a fish leads to an electromotor response at latencies of 14–15 ms, which is preceded by a train of 1–3 spikes in PCN at a latency of

8–14 ms, but no change in the PCN field potential [80]. Thus, electrosensory stimuli do not synchronize PCN units, but increase their overall excitability, which drives increased electromotor output. PCN must ultimately receive input from electrosensory regions, though the pathways by which this information may reach PCN at such short latencies is unclear. Interestingly, with repeated presentation of a stimulus, the electromotor response fails after 8–10 repetitions, while the response of PCN units remains, suggesting some kind of plasticity in the electromotor system [80].

4.4. Summary of EOD generation

The generation of each EOD is initiated in the command nucleus (CN), which relays this signal through the medullary relay nucleus (MRN), which, in turn, projects down the spinal cord to activate electromotor neurons (EMN) that drive the electrocytes of the electric organ in synchrony to produce a single stereotyped EOD [11,14,15,30,32]. CN does not act as a pacemaker, but rather integrates asynchronous, descending input from neurons in the precommand nucleus (PCN; [28,80]), as well as other potential sources. The activity level of PCN units can vary widely, leading to variation in CN depolarization, and therefore EOD output patterns. Part of the variation in PCN activity appears to be caused by a modifiable negative feedback pathway that is driven by corollary discharge input [80]. This results in an immediate inhibition of PCN activity for tens to hundreds of milliseconds after each EOD. The negative feedback pathway and PCN are likely modulated by other, unidentified inputs as well. PCN is excited by electrosensory stimulation, indicating that sensory input converges through unknown pathways on PCN to drive electromotor responses to external stimuli [80].

5. Conclusions

5.1. Mechanisms of stereotyped SPI signal generation

Now that we have a basic understanding of electric signaling behavior in mormyrid fish and the mechanisms of EOD production, we can ask: What are the mechanisms underlying the generation of specific SPI patterns? While this question has not been directly addressed, several hypotheses come to mind. For instance, PCN serves as the main descending, excitatory input to CN making it a likely candidate for driving burst production. Furthermore, the rostral and caudal subregions of PCN may differ in their physiology and synaptology and each drive distinct, stereotyped bursts. Preliminary experiments indicate that the two subregions have different patterns of activity in *B. brachyistius*, suggesting that they may have different effects

on electromotor output [18]. In addition, preliminary results suggest that extracellular stimulation in the rostral part of PCN leads to different types of EOD bursts than those caused by stimulation in the caudal region [19]. The negative feedback pathway is a likely candidate for driving cessation production. Prolonged bursts of inhibitory action potentials may completely silence PCN, thereby shutting down excitatory input to CN and preventing EOD production. Preliminary recordings indicate that the burst duration of these units is indeed increased during cessation production in *B. brachyistius* [18].

The activity in these regions is most likely controlled by inputs from higher-order regions that regulate signal production based on internal and external conditions. Extracellular injections of HRP into the tectum mesencephali lead to anterograde and retrograde labeling in VP [81]. Because the tectum mesencephali is a multimodal sensori-motor processing region, this may be at least one of the main pathways by which sensory information reaches the electromotor network.

Additional experiments are needed to more fully address these issues. As already mentioned, SPI patterns have not been quantified, making it impossible to rigorously define the behaviors. However, such definitions are necessary if we wish to understand how different components of the electromotor system may drive the generation of these behaviors. Second, much about the basic physiology of descending inputs to CN remains to be studied. For example, how do VP units contribute to EOD output? What are the differences between the two subregions of PCN (rostral vs. caudal), and what do the differences mean for their relative influence on EOD output? What are the patterns of activity in PCN and VP in relation to the generation of specific SPI patterns? Can such patterns be induced by microstimulation in these regions? What are the inputs to PCN and how do they modulate PCN activity? What is the source of the inhibitory afferents to PCN that are driven by the corollary discharge pathway? Are there other inputs to these units that affect their excitability? By what path(s) does sensory information reach the electromotor network and how does it modify PCN activity?

5.2. Comparisons with other weakly electric fish

Gymnotiform fish from South America also produce EODs for communication and navigation. They are generally considered to have independently evolved electromotor systems and can therefore provide for important comparative information on electric signal generation. They can be divided into wave species, which produce a continuous, quasi-sinusoidal EOD, and pulse species, which produce discrete pulses separated in time. Unlike the pulse-type mormyrids, however, their SPI patterns are highly regular at rest. The

EOD is controlled by a medullary pacemaker nucleus (Pn), that contains both pacemaker and relay cells, and is located similarly to the mormyrid CN/MRN. There are two primary descending inputs to Pn, the centroposterior/prepacemaker nucleus (CP/PPn) located in the dorsal thalamus and the sublemniscal prepacemaker nucleus (SPPn) located in the mesencephalon [64]. In the wave-type species *Eigenmannia virescens*, CP/PPn drives EOD accelerations, with CP driving sudden, intense bursts, and PPn driving slow, sustained bursts. The SPPn tonically excites the pacemaker, and when inhibited, drives EOD decelerations. The SPPn and CP neurons have glutamatergic inputs to relay cells, though SPPn neurons activate NMDA receptors, and CP neurons activate AMPA receptors [43]. The input from PPn activates both AMPA and NMDA receptors on pacemaker cells [43].

In the wave-type gymnotiform *Sternopygus macrurus*, the PPn drives gradual frequency rises via NMDA synapses on pacemaker cells, while the SPPn drives EOD interruptions via NMDA synapses on relay cells [49]. In the pulse-type fish *Hypopomus* spp., CP/PPn is divided into three subregions, PPnC, PPnG, and PPnI. PPnC drives sudden, intense frequency increases via AMPA receptors on relay cells, PPnG drives gradual, sustained frequency increases via NMDA and AMPA receptors on pacemaker cells, and PPnI drives decelerations via GABA receptors on pacemaker cells [47,48,50]. The SPPn in *Hypopomus* spp. synapses on relay cells and drives interruptions via NMDA receptors [50].

The sole non-mormyrid species of the order Mormyriiformes, *Gymnarchus niloticus*, produces a wave-type EOD (Fig. 2). As in mormyrids, there are distinct relay (MRN) and pacemaker (PN) nuclei, and a lateral relay nucleus in a position similar to BCA that receives input from PN and projects to MRN [44]. PN/MRN receives input from two sources, a bilateral nucleus at the mesencephalic–diencephalic border, in a location similar to PCN, and a bilateral medullary nucleus [44,46]. Stimulation of the mesencephalic–diencephalic nucleus generates interruptions by inhibiting MRN, while modulation in the activity of the medullary nucleus drives EOD accelerations and decelerations [46].

Thus, in the species that have been examined, different stereotyped electrical behaviors are controlled by distinct descending inputs to the medullary electromotor system. Furthermore, at least in gymnotiforms, these distinct behaviors appear to be the result of both spatial and pharmacological differences in the synaptic organization of their inputs to the electromotor network. This suggests that the different inputs to the mormyrid CN may likewise drive distinct behaviors, and studies are currently addressing this issue [18,19]. If the same organizational scheme applies to mormyrids, then it would suggest that common selective pressures have operated on the mechanisms of electric signal

generation in unrelated taxa. Such selective pressures would likely apply to other systems as well, such as the vocal motor pathways of fish, amphibians, birds, and mammals. Thus, general principles established using weakly electric fish as model systems may prove useful in guiding future studies on more complex communication systems, where the link between behavioral output and neuronal activity may be more difficult to establish.

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