The Midbrain Precommand Nucleus of the Mormyrid Electromotor Network

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The functional role of the midbrain precommand nucleus (PCN) of the electromotor system was explored in the weakly electric mormyrid fish Gnathonemus petersii, using extracellular recording of field potentials, single unit activity, and microstimulation in vivo.

Electromotor-related field potentials in PCN are linked in a one-to-one manner and with a fixed time relationship to the electric organ discharge (EOD) command cycle, but occur later than EOD command activity in the medulla. It is suggested that PCN electromotor-related field potentials arise from two sources: (1) antidromically, by backpropagation across electrotonic synapses between PCN axons and command nucleus neurons, and (2) as corollary discharge-driven feedback arriving from the command nucleus indirectly, via multisynaptic pathways.

PCN neurons can be activated by electroreceptive input, but this does not necessarily activate the whole motor command chain. Microstimulation of PCN modulates the endogenous pattern of electromotor command in a way that can mimic the structure of certain stereotyped behavioral patterns. PCN activity is regulated, and to a certain extent synchronized, by corollary discharge feedback inhibition. However, PCN does not generally function as a synchronized pacemaker driving the electromotor command chain. We propose that PCN neurons integrate information of various origins and individually relay this to the command nucleus in the medulla. Some may also have intrinsic, although normally nonsynchronized, pacemaker properties. This descending activity, integrated in the electromotor command nucleus, will play an important modulatory role in the central pattern generator decision process.

Key words: electric fish; motor command; pacemaker; corollary discharge; central pattern generator; mormyrid; premotor pathways; sensory motor integration

Rhythmic motor behaviors are part of the behavioral inventory of most vertebrates. Examples include locomotor activity, rhythmic vocalizations, electromotor behaviors of weakly electric fish, and microsaccadic eye movements. Many of these behaviors have similar physiological properties and may have developed according to common ontogenetic and phylogenetic principles (Grillner and Georgopoulos, 1996; Bass and Baker, 1997). These behaviors are repetitive, more or less stereotyped, and have a temporal pattern that is produced by brain areas referred to as central pattern generators, neural oscillators, or pacemakers (Grillner et al., 1985; Katz, 1995; Cohen et al., 1996; Grant et al., 1999). Pacemaker networks produce a basic temporal rhythm of behavior, which in turn can be modified to various degrees by premotor centers. In this paper, we investigate the physiology of the midbrain precommand nucleus (PCN) of the electromotor system in the weakly electric mormyrid fish Gnathonemus petersii, and its role in the modulation of the intrinsic rhythm of electromotor behavior.

The all-or-none, pulse-type electric organ discharge (EOD) in mormyrid fish is driven by an irregularly rhythmic central command network. Electrostimulation is used for active electrolocation (Lissmann and Machin, 1958; von der Emde, 1999) and intraspecific electrocommunication (Hopkins, 1988; Kramer, 1990). EOD displays reveal a structured temporal organization (Teyssedre et al., 1987), including endogenously controlled regularization, phase-locking to external EOD signals, and sensorimotor reflexes. Distinct electromotor behaviors are associated with exploration or social interactions (Bauer and Kramer, 1974; Bell et al., 1974; Toerring and Moller, 1984; Moller et al., 1989; Serrier and Moller, 1989). Similar EOD patterns can also be elicited experimentally by artificial electric stimuli (Moller, 1970; Bauer, 1974; Serrier, 1982).

Spinal electromotoneurons driving the electric organ receive a descending command from a central pattern generator in the medulla. This consists of two adjacent midline nuclei, the command nucleus (CN), which initiates the electromotor commands, and the medullary relay nucleus (MRN), whose function is to synchronize the descending command volley (Szabo, 1957; Aljure, 1964; Bennett et al., 1967; Bell et al., 1983; Elekes et al., 1985; Elekes and Szabo, 1985; Grant et al., 1986, 1999). The endogenous rhythm of the electromotor command depends on the membrane properties of the CN neurons and on their postsynaptic integration of afferent activity (Grant et al., 1986). The afferent and efferent connections of the medullary relay and command nuclei were established by tracing using horseradish peroxidase (Bell et al., 1983). In addition to the descending electromotor command pathway, these authors identified an ascending corollary discharge pathway projecting from the command nucleus, via the bulbar command-associated nucleus (BCA) to the mesencephalic command-associated nucleus (MCA). The bilateral PCN situated at the mesencephalic–diencephalic border (Bell et al., 1983) (Fig. 1A) was identified as the principal source of descending afferent projections to CN.

The aim of the present study was to identify the precommand nucleus electrophysiologically and to explore its functional role in electromotor command generation.

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In some experiments, the single-barrel electrode was replaced with a triple-barrel electrode (diameter of the tip of each barrel, −2 μm) once the location and depth of the PCN were ascertained. The three barrels contained, respectively, L-glutamate (Sigma; 0.1 m in water, pH 8.0), Neurobiotin (Vector, Burlingame, CA; 5% in 1 M KCl) and HRP (1:10,000). Anesthesia was maintained afterward by buccal solution (MS-222; Sandoz, Basel, Switzerland or Sigma, St. Louis, MO; concentration 1:15,000) and perfused via the heart with 3 m NaCl. To label recording sites, Neurobiotin was ejected iontophoretically with pulsed positive current (10 sec on, 3 sec off; 2–5 μA for 30 min). In other experiments, smaller deposits of horseradish peroxidase (HRP; 5% in isotonic NaCl) were used to label precise sites where maximum amplitude field potentials were recorded (tip positive, 1 μA for 1 min.). Neurobiotin labeling was developed using the ABC technique (Vector), and HRP labeling was revealed using the Hanker–Yates procedure modified by Bell et al. (1981).

While tracking through the brain, electrical microstimulation was used to identify points from which the electromotor system could be driven with a low threshold. Constant current (1–10 μA) square pulses of 0.2 msec duration or longer lasting DC currents (1–5 μA), were delivered directly through the micropipette containing 3 m NaCl. L-Glutamate iontophoresis (negative currents of 0.1–2 μA), which selectively stimulates cell bodies and dendrites but not fibers of passage, was used to stimulate PCN neurons chemically and thus to explore the effect of PCN neuron activity on the electromotor activity of the fish.

Peripheral electrosensory stimuli were applied using a stainless steel dipole (6 cm between electrodes) placed parallel to the fish at a distance of 5 cm, with the negative pole toward the head (potential gradient close to fish skin: 10–100 mV/cm). Monophasic square stimulus pulses of 0.5 msec duration were triggered by the EMN volley with a delay of 100 msec. This type of stimulation is not identical to a natural EOD but is effective in eliciting EOD responses in the behavioral context (Serrier, 1982).

Electrophysiology. After each experiment, the fish was reanesthetized deeply with MS-222 (concentration, 1:15,000) and perfused via the heart with 100 mM phosphate buffer, pH 7.4. Electrode tracks and the site of HRP deposit were reconstructed in the light microscope, from 80-μm-thick cresyl violet or neutral red counterstained sections.

RESULTS

The PCN region (Fig. 1A) has not been explored electrophysiologically previously, and although it is known that PCN axons project to the electromotor CN, it was not certain that an endogenously active motor-related field potential would be recorded at this site. For this reason, in preliminary experiments, microstimulation (1–10 μA) was used to find sites in the deeper regions of the midbrain from which the electromotor pathway could be driven with a low threshold. It was then observed that small electromotor-related field potentials were recorded at points where the threshold was
for electromotor activation was lowest. These sites were labeled by deposit of HRP or Neurobiotin. Histology showed that these recording sites corresponded to the anatomical description of PCN (Grant et al., 1999). The electromotor-related field potentials, unit activity, and electroethysynaptic responses of PCN are described below.

Field potentials in PCN

The typical field potential recorded in the center of PCN is illustrated in Figure 1, B and C (middle traces); this was recorded at a depth ranging from 3550 to 5800 μm from the dorsal surface of the brain, depending on fish size and electrode angle. PCN could be reliably identified by a two-peaked negative field potential, followed by a slow positive potential that was always associated with every EMN volley. The three components of the PCN field potential varied in size in the different regions within the nucleus. The slow positive potential (Fig. 1B, middle trace, arrow) was largest in amplitude in the center of the nucleus. In the more caudal regions of PCN, from which descending axons exit in a mediodorsal direction, the first negative peak was large, and the second negative peak was less prominent; the slow positive potential was small. Toward the rostral pole of PCN, the initial negative peak and the slow positive potential were small or absent, and multunit bursting activity of the sort illustrated in Figures 8 and 9 was predominant. Neurons in this region of the nucleus are smaller (K. Grant and G. von der Emde, unpublished observations), and it is possible that they form a functional population that is different from that formed by neurons in the center and caudal region.

The first negative peak of the PCN field potential preceded \( T_n \) by a constant fixed interval (Fig. 1B). In most fish this interval was \(~2.2\) msec (Fig. 2B,C). The second negative peak of the PCN field potential varied more in amplitude and timing at any given site; in certain records it was barely visible. It occurred between 0.75 msec before and 0.55 msec after \( T_n \) (Figs. 1B,C, 2B,C). The slow positive field potential followed the negative peaks, reaching a maximum after \( ~8–11 \) msec and lasting for 20–40 msec (Figs. 1B, 2A). This positive wave was characteristic of PCN and was recorded only in the region containing PCN cell bodies; it could usually be detected on an audio-monitor before it became visible above the background noise level on the oscilloscope trace and it was of particular value in distinguishing the nucleus field potential from other EOD command-related events occurring in neighboring regions.

PCN axons project to the command nucleus in the medulla (Bell et al., 1983), and thus the field potentials were compared in the two nuclei. Recorded in the same fish, the first negative peak in CN preceded the first negative potential in PCN by \( 0.7 \) msec (Figs. 1B,C, 2B,C). The slow positive field potential followed the negative peaks, reaching a maximum after \( ~8–11 \) msec and lasting for 20–40 msec (Figs. 1B, 2A). This positive wave was characteristic of PCN and was recorded only in the region containing PCN cell bodies; it could usually be detected on an audio-monitor before it became visible above the background noise level on the oscilloscope trace and it was of particular value in distinguishing the nucleus field potential from other EOD command-related events occurring in neighboring regions.

The MCA field potential also had two prominent negative peaks (Fig. 2A, bottom trace), although its form was often more complex than that recorded in either PCN or CN (Aljure, 1964; Bell et al., 1995). Comparison showed that the first negative peak of the field potential in PCN always occurred earlier (\(~0.2\) msec) than the earliest negative peak recorded in MCA (Fig. 2B,C). Similarly, the second negative peak in PCN almost always occurred earlier than the second negative peak of the MCA field potential, although in a few cases the timing was reversed (Fig. 2C). In general, the occurrence of the second negative peak in MCA was variable, and its

![Figure 2](image-url)
timing was not strictly correlated with that of the second negative peak in PCN. Thus, the second negative peaks of the PCN and MCA field potentials were probably neither causally related nor of common origin. The latencies of the negative field potentials recorded in PCN and MCA are compared in Figure 2C. It is also interesting to note that in MCA, the two initial sharp negative peaks were followed by a slow negative field potential lasting up to 30 msec (Fig. 2A, bottom trace), in contrast to the slow positive potential observed in PCN (Fig. 2A, middle trace).

Unitary activity in PCN

Two different sorts of unitary action potential activity were recorded extracellularly in PCN: (1) tonically active units that fired spontaneously with an irregular rhythm, some increasing their frequency just before the initiation of the EMN volley, but that were always silent for 20–70 msec immediately after the EMN volley (Figs. 3–6), and (2) units that fired a burst of action potentials during the period immediately after initiation of the EMN volley but that were otherwise silent (Figs. 7-9).

Many units of the tonically active first type fired mainly before initiation of the electromotor command and only sporadically at other times (Fig. 3). Other units of this type fired more continuously (Fig. 5), pausing only at the time of the EMN volley and for a short period afterward (Fig. 5B). Figure 3 shows that these units often fired an action potential at a fixed time before $T_0$ (Fig. 3A,B, B).
superimposed traces), resembling the first negative peak of the motor-related field potential recorded at the same site (Fig. 1C). This suggests that the first negative peak of the PCN field potential is probably in fact a focal potential corresponding to the synchronous activation of a small number of units close to the electrode tip. However, when a unit potential fired within a time window of a few milliseconds immediately before the expected occurrence of the motor-related spike, the latter was either absent (Fig. 3A, traces b–d) or appeared to be delayed (Fig. 3A, traces e–g; B, traces d–h).

This phenomenon is illustrated over 200 electromotor command cycles in Figure 4 (the same unit as Fig. 3A). The upper raster (Fig. 4A) shows the timing of unit spikes relative to T₀ for successive electromotor command cycles illustrated in the natural order in which they occurred. In some cycles the fixed motor-related spike was present, and in others it failed or was delayed. In the rasters of Figure 4, A₂ and A₃, the individual sweeps have been reordered to explore this further. Figure 4A₂ shows those cycles in which an unit spike occurred exactly at the time of first negative peak of the field potential (at 2.2 ± 0.08 msec before T₀). Figure 4A₃ shows spike timing in cycles in which the fixed-latency first negative peak was either absent (sweep numbers 29–60) or did not occur (sweep numbers 0–28). Thus, the fixed timing motor-related spike only occurred if no other unit potential fired during a time window of 3.6 msec preceding the expected motor-related spike (period a–c in Fig. 4A₂, i.e., 5.8–2.2 msec before T₀). The motor-related unit spike was present but delayed if other unit spikes fell within the period a–b (Fig. 4A₃) i.e., 5.8–4.3 msec before T₀, or 3.6 to 2.1 msec preceding the expected motor-related spike, and no motor-related spike occurred if other spikes fell within the period <2.1 msec preceding the expected motor-related spike (period b–c in Fig.
showing a large IPSP whose timing corresponds with that of the slow potentials in PCN and CN illustrated in Fig. 1.

conduction time from PCN to CN was calculated as 0.7 msec, taken

PCN (EMN triple volley and averaged extracellular field potential recorded in Figure 7.


Figure 7. Unit activity during the slow positive PCN field potential. A, EMN triple volley and averaged extracellular field potential recorded in PCN (n = 15). Arrow indicates first negative electromotor-related negative peak of PCN field potential. B, Burst-type unit activity that occurs only during the period of the corollary discharge-related positive field potential. C, An intracellular record made at a distance of 100 μm from the unit in B, showing a large IPSP whose timing corresponds with that of the slow positive field potential and the burst activity recorded extracellularly.

4A3, i.e., 4.3–22 msec before T0). The distribution of all spikes firing before T0 is shown in the histogram of Figure 4B.

This failure of firing resembles collision rather than refractoriness, because interspike intervals of <2 msec were observed in the spontaneous firing of this unit (e.g., Fig. 3Aa). It suggests that the fixed-timing motor-related unit spike may not have the same origin as the irregularly timed preceding spikes. The fixed-timing motor-related spike occurs ~0.7 msec later than activity in the command nucleus (Fig. 1C), but this interval is probably not long enough to include trans-synaptic feedback from the command nucleus via the known corollary discharge pathway. We therefore suggest that the fixed-timing PCN spike may result from antidromic invasion of PCN axons terminating on CN neurons, at the moment of the synchronous firing of CN neurons that drives the descending electromotor pathway. This antidromic backpropagation may be initiated across electric synapses because the ultrastructural study by Elekes and Szabo (1985) showed that the majority of synapses contacting CN neuron somata contain gap junctions (see Discussion).

The period in which spiking was not followed by a motor-related spike is close to the occlusion period that would be expected if the motor-related spike in the PCN unit were driven by antidromic invasion from the postsynaptic command neurons, which can be calculated as the sum of the conduction time from PCN to CN, plus the refractory period of the PCN axon, plus the conduction time from CN to PCN, i.e., 0.7 + (~ 1) + 0.7 = 2.4 msec. (The conduction time from PCN to CN was calculated as 0.7 msec, taken as the difference between the first negative peaks of the field potentials in PCN and CN illustrated in Fig. 1C.)

The unit in Figure 3B shows records from another site, illustrat-
ing a second, later motor-related unit potential that corresponded to the timing of the second negative peak of the PCN field potential observed in other recordings (Figs. 1B,C, 2A,B). The timing of this second motor-related spike was variable, and it was not always present.

Figure 5 illustrates the tonic firing pattern of a similar unit that was continuously active, except immediately after initiation of the EMN volley. To understand how spontaneous unit firing in PCN might be related to the length of inter-EMN intervals, a peri-EMN raster diagram was constructed showing all spikes occurring before and after T0, over 150 EOD command cycles. The method of construction is described in Figure 5A. In each line of the raster, all spikes occurring before each EMN volley were plotted to the left of T0, the class period and all spikes occurring after each EMN volley (and before the next EMN volley) were plotted to the right of T0. The succeeding lines in the raster thus describe the unit firing before and after successive motor commands. The symmetry of the plot arises because each PCN spike is represented twice in the raster: once to the left of T0 (if it occurred in the interval preceding the current EMN volley) and once to the right of T0 (if it occurred in the interval after the current EMN volley). In this graphic representation, the right-hand envelope of the raster may be interpreted as a sequential plot of the EMN intervals.

For the unit activity illustrated, tonic firing frequency and the length of the silent period after the EMN volley were both related to the length of the inter-EMN interval (Fig. 6). When PCN unit firing frequency was high, inter-EMN intervals tended to be short, and vice versa (Fig. 5B). This behavior is quantified in Figure 6A, which shows a negative correlation between inter-EMN interval length and PCN unit firing frequency. When the electromotor command cycle length was ~500 msec, a basal unit firing frequency (0.03 kHz) appeared to have been reached.

The length of the inter-EMN interval was also correlated with the latency of the first spike after T0 and thus with the duration of the post-EMN volley pause (Fig. 5B). However, the distribution of points in Figure 6B, showing the timing of the first PCN spike after the EMN volley, suggests that there are two preferred pause lengths (~45–60 and 65–75 msec), or two preferred inter-EMN intervals, rather than a continuous interdependent variation. Similar bimodal interval distributions are frequently observed in the naturally occurring electromotor rhythms (Teyssedre et al., 1987).

The second type of unit recorded in PCN fired a burst of action potentials during the slow positive wave of the PCN field potential (Fig. 7A,B). At all other times such units were silent. Here we associate the observation that in five short intracellular recordings made in the PCN, a large, two-component IPSP was present, which also coincided with the slow positive potential seen extracellularly (Fig. 7C). It seems probable that this IPSP, which inverted with intracellular injection of chloride ions, is related to the action potential burst seen in the type 2 bursting units described here. Together these events may reflect a corollary discharge-driven inhibitory input to PCN.

In most units of this second type, the structure and timing of the burst of action potentials was rather constant, although from one unit to another the exact timing of the first spike of the burst and number of spikes per burst differed (compare the three examples in Fig. 8A). For any given unit, the timing of the first spike in each burst was remarkably constant. Later spikes showed more variability, and the last two or three spikes of longer bursts were not always present (Fig. 8A1–3, histograms) thus producing bursts of varying duration. The units illustrated in Figure 8 were recorded during EOD command firing at a relatively low endogenous rate (Fig. 5B), and under these conditions no correlation was found between inter-EMN interval and first spike timing (Fig. 8C) or between inter-EMN interval and burst duration (Fig. 5D).

For a fourth unit of this type, however, a relationship was found between burst timing, burst structure, and inter-EMN interval (Fig. 9). Figure 9A shows that the timing of the spikes within this burst was less precisely fixed than for the units illustrated in Figure 8. In addition, the distribution of inter-EMN intervals for the
period during which this record was obtained (Fig. 9B) shows that the endogenous rhythm of the electromotor command was faster than that illustrated in Figure 8B and that most inter-EMN intervals fell between 100 and 200 msec. In this case, the latency of the first spike of the burst was negatively correlated with the inter-EMN interval: when the first spike occurred earlier, the following inter-EMN interval was longer (Fig. 9Ca). This negative correlation was more highly significant for data from electromotor command cycles in which the inter-EMN interval was <200 msec: the regression line for these points is drawn in Figure 9Ca. Inter-EMN interval was less closely related to first spike timing when electromotor command cycles were >200 msec. Inter-EMN interval was also correlated with burst duration and intraburst frequency (Fig. 9Ch,Cc), and this correlation was again only significant for data obtained from cycles in which the inter-EMN interval was <200 msec.

**PCN responses to electro sensory stimulation**

An electro sensory stimulus played to the fish through electrodes placed in the water evoked an electromotor response with an EMN volley ($T_2$) latency of 14–15 msec. In PCN, the field potential that accompanied this evoked electromotor activity was identical to that which occurred in association with intrinsic spontaneous activation of the motor command (Fig. 10, compare A, B). However, the electro sensory stimulation evoked specific, additional unitary activity that preceded the first negative peak of the PCN field potential (Fig. 10B, small arrow). A single electro sensory stimulus at twice threshold strength evoked one to three spikes with a variable latency between 8 and 14 msec (Fig. 10B, C). With repetition of the sensory stimulus, the latency of the evoked electromotor response increased (Fig. 10D), and after 8–10 trials the motor response occasionally even failed, although the sensory evoked unit activity in PCN was nevertheless still present (Fig. 10C). This suggests that adaptation, or an increase in threshold for the motor response, occurs at some other site.

**Electromotor behavior evoked by stimulation in PCN**

Electromotor activity could be evoked at short latency (4–8 msec) by microstimulation in PCN. For brief depolarizing DC pulse stimuli (0.1 msec) given via the recording microelectrode, thresholds ranged from 0.4 to 1 $\mu$A in the center of the nucleus. However both threshold and latency of the motor response depended on the time elapsed between the stimulation pulse and the preceding EMN volley.

In the center of the nucleus an electromotor response to microstimulation at twice threshold intensity was obtained with a latency of 4–5 msec, provided that the delay between the preceding EMN volley and the stimulus was >50 msec (Fig. 11A, top trace, B). At shorter EMN-stimulus intervals this short latency motor response generally failed (Fig. 11A, bottom trace, B), although in some cases, depending on the stimulation site, increasing stimulus strength could reduce the apparent “refractoriness” to 20–40 msec. When stimulating in the center of PCN, no short latency response could be evoked at EMN stimulus intervals <20 msec.

Refractory periods to stimulation in PCN were shorter in the caudal region of the nucleus, possibly because here some of the efferent axons projecting to CN were being stimulated directly and the effects of corollary discharge feedback inhibition (see below) were less strong. In the example illustrated in Figure 11E–H, as the interval between the preceding EMN volley and the stimulus was decreased, a step change in the evoked EMN response latency, from ~5 msec to a more variable value between 20 and 25 msec was
frequently observed (Fig. 11E, compare top and middle traces; see plots in Fig. 11F,G). This bimodal distribution of the evoked motor response latency (Fig. 11G, black bars) produced a discharge pattern similar to the alternating short intervals of rapid electromotor behavior observed during active electrolocation and in social encounters (Moller, 1970; Bell et al., 1974; Moller et al., 1989).

Microstimulation in PCN also induced a regularization of the spontaneous motor command rhythm. After a directly evoked short latency motor response, there was a considerably higher than normal probability that the next, subsequent EMN volley would occur after an interval of 100 msec (Fig. 11B,C, h, E,F, h'). In addition, when the short latency evoked response failed because of refractoriness, the first subsequent EMN volley would occur after a rather constant latency of 70–80 msec (Fig. 11B,F,G). The spontaneous intrinsic motor command rhythm then returned to the usual irregular pattern and in both cases, the next subsequent inter-EMN interval followed the same pattern of variability as the normal spontaneous interval distribution (Fig. 11D,H). The phenomenon underlying this regularization thus lasted for 100–200 msec, although its precise mechanism is not yet known.

Stimulation in caudoventral sites, probably within the descending axon tract leaving PCN in the direction of CN, could drive the electromotor pathway at much higher rates, down to inter-EMN intervals of as little as 4–5 msec. During such rapid repetitive activation of the electromotor pathway, the rate-limiting factor appeared to be the integrity of the triple action potential volley fired by the electromotoneurons (Aljure, 1964; Bennett et al., 1967). At high firing rates, the third action potential of the EMN volley tended to drop out, and if the stimulus was repeated as a train, the EMN volley became unsynchronized and disorganized.

In addition to brief electrical stimuli, we also used longer lasting DC electrical stimuli to drive the electromotor system from within PCN. Figure 12A shows that electrode tip-positive stimulation with an intensity of 1.5 μA caused a threefold tonic increase in EMN firing frequency. The absolute magnitude of the increase in firing rate depended on stimulus intensity. In contrast, tip-negative stimulation caused marked transient increases in discharge rate at the onset and termination of the stimulus pulse, but no tonic frequency increase during the DC stimulus plateau (Fig. 12B).

Iontophoresis of L-Glutamate in PCN also caused a tonic increase in EMN firing rate whose pattern resembled that evoked by DC electrical stimulation. This effect was dose-dependent, and spontaneous additional increases in firing rate could be superimposed on the drug-induced response (Fig. 12C). The lack of any response to reversed polarity iontophoresis in the control trace of Figure 12D shows that the observed effect was caused by L-Glutamate and not by current injection.

**DISCUSSION**

Our results confirm that in the mormyrid Gnathonemus petersii, the bilateral midbrain precommand nucleus modulates the activity of the electromotor command center and mediates both sensory-based behavioral responses to environmental stimuli and endogenously initiated social display patterns. We suggest that by its position and functional role in the electromotor system, it is func-
The origin of field potentials in PCN

Motor-related field potentials in PCN were linked in a one-to-one manner, and with a fixed time relationship, to the command of the electric organ discharge but occurred later than the two-peaked negative field potential recorded in CN. Because no large precommand field potential was recorded in PCN preceding activation of the CN, it is concluded that PCN neurons do not fire in synchrony before initiation of the EOD command and that the electromotor related field potentials recorded in PCN do not represent events driving the electromotor command. We suggest instead that they reflect backpropagation of motor command activity or that they are evoked by ascending corollary discharge activity (Bell et al., 1983, 1995).

The first and second negative peaks and the following slow depolarization recorded in PCN probably have a different origin. It is possible that the first negative peak, which occurs ~2.2 msec before the EMN volley could be evoked via a collaterals branch of the corollary discharge pathway (Fig. 13A). BCA axons projecting to MCA almost certainly run through the dendritic field of PCN neurons (Niso et al., 1989) and could have been recorded in the present study, although no BCA axon terminal field has yet been described in the region of PCN (Bell et al., 1983). However, the short delay between the first negative peaks of the field potentials in CN and PCN (0.7 msec), the lack of intrinsic sporadic firing in BCA axons (Clauss, 1985), and the apparent collision illustrated in Figures 3 and 4 make the validity of this hypothesis unlikely. An input from CN, via BCA and MCA to PCN can be excluded, because the onset of field potentials in PCN occurs earlier than those in MCA.

An alternative explanation is that the first negative peak recorded in PCN is the result of backpropagation of action potentials in PCN axons terminating on CN neurons that occurs at the moment when synchronous firing of the whole CN neuron population generates the descending electromotor command signal (Fig. 13F). This hypothesis is supported by the occlusion of the fixed latency unitary potential coincident with the first negative peak of the PCN field potential, observed when spontaneous unitary spikes occur in the preceding 2–3 msec. Occlusion could be caused by collision of an orthodromic action potential descending from PCN toward CN, with an ascending, antidromic action potential evoked in the PCN axon on firing of the postsynaptic CN neuron. This reasoning is supported by the observations of Elekes and Szabo (1985), who showed that the majority of synapses contacting command neuron somata form club endings containing gap junctions. Although these authors did not use specific labeling to identify their material, PCN axons constitute the major afferent pathway to the command nucleus (Grant and von der Emde, unpublished observation) and thus it is likely that their synaptic terminals are included in this population and that there may be electric synaptic transmission between PCN axons and CN neurons. The presence of gap junctions between PCN axons and CN neurons is also suggested by dye-coupled labeling of CN neurons observed after Neurobiotin deposit in PCN (von der Emde, unpublished results).

Other explanations for the generation of the first negative peak in PCN would require a hitherto unknown anatomical connection. It should be noted that no direct anatomical projection has been described from CN to PCN (Bell et al., 1983; Grant et al., 1986) and that insufficient time would be available for trisynaptic or multisynaptic projections from CN to PCN.

The origin of the second negative peak of the PCN field potential is less clear. It cannot be a correlate of the second negative peak of the CN field potential, which corresponds to the second action potential fired by command neurons and always occurs at a fixed latency relative to the EMN volley (Grant et al., 1986). The second PCN negative peak has a variable latency and is sometimes even absent. It seems probable that this event reflects input from the corollary discharge pathway, but as yet no possible anatomical pathway has been demonstrated.

The slow positive component of the PCN field potential occurred with a fixed timing relative to the motor command signal (EMN).
and was only observed in the immediate post-command period. It is therefore probably a corollary discharge-driven phenomenon, although the exact anatomical connections involved have not yet been described. Again, it is likely that this input to PCN arrives via a collateral branch of BCA axons that pass close to PCN en route for the MCA, or that PCN receives afferent connections from other corollary discharge associated nuclei (Bell and von der Emde, 1995).

The functional role of PCN

It is argued above that the field potentials recorded in PCN do not reflect premotor activity in this nucleus. However, our results show that many neuronal elements in PCN fire sporadically or tonically over a large part of the EOD cycle, and it is probable that these are the PCN cells that project to CN. The origin of such spontaneous activity has not been explored, and we cannot conclude whether PCN units might have an intrinsic pacemaker function, in addition to integrating information afferent to the electromotor command chain. An increase in the firing frequency of tonically firing units, or the onset of sporadic firing in otherwise silent units, was frequently associated with the generation of an EMN volley. Glutamate iontophoresis also drove the EMN firing frequency, and this was probably a result of direct stimulation of PCN neurons. Similarly, electrosensory stimulation activated unit firing in PCN at the same time that it provoked an EMN volley. Thus, it is likely that

![Figure 11](image-url)
PCN conveys many excitatory inputs to CN, although PCN neurons do not generally fire as a synchronized ensemble. We suggest that descending input from PCN is integrated at the postsynaptic level in CN. When the result of this integration process is sufficient to activate CN neurons beyond their firing threshold, initiation of the electromotor command and the subsequent firing of the entire electromotor pathway follows.

As discussed above, the most probable explanation for the timing of the first negative peak of the PCN field potential is that it is the result of backpropagation from CN. The functional role of such backpropagation is not clear, but we suggest that antidromic, synchronous invasion of the whole population of PCN output cells projecting to CN would serve as a potent resetting mechanism in the descending pathway.

The slow positive field potential associated with each electromotor event is probably the result of synchronous input to PCN provided by the corollary discharge-activated bursting units. The consequence of this input may be the generation of large IPSPs in PCN neurons. Somata of PCN cells are surrounded by large synaptic terminals that show strong anti-GAD immunoreactivity (Niso et al., 1989). Corollary discharge inhibition of PCN may act as a rate-limiting, and resetting, mechanism preventing the electromotor system from being driven too fast. Modulation of the inhibitory corollary discharge feedback to PCN may, in addition, play an important role in regulating the firing of the tonically and/or intrinsically sporadically active neurons and thus in the fine control of the length of the current inter-EOD cycle. However, the results show that the corollary discharge IPSP generated in PCN neurons does not alone decide the inter-EMN interval. Instead, the electromotor command rhythm probably depends on a regulated balance of excitatory and inhibitory modulation of distributed intrinsic pacemakers.

**Comparison with electromotor pacemaker systems of other electric fish**

Previous studies in gymnotiform electric fish (Heiligenberg et al., 1981, 1996; Kawasaki et al., 1988; Kawasaki and Heiligenberg, 1990; Keller et al., 1991; Metzner 1993) and in the wave-emitting mormyriform, *Gymnarchus niloticus* (Kawasaki and Heiligenberg, 1990; Kawasaki and Yuang-Xing, 1996), have identified similar prepacemaker nuclei (PPN) of their electromotor systems. In these fish, electromotor behavior is driven by the intrinsic regular rhythmic activity of the medullary pacemaker center. Descending pathways from PPN modulate this intrinsic pacemaker rhythm. In wave-type gymnotids, in which several subdivisions of the PPN have been described, different stereotyped modulations of the highly regular
EOD emission have been attributed to activation of the individual neuronal subpopulations. It is possible that further detailed investigation of the precommand nucleus in G. petersii may reveal a similarly complex organization.

REFERENCES


Bauer R (1974) Agonistic behavior in mormyrid fish: latency-related behavior and the presence of an as-yet-undemonstrated collateral of BCA axons projecting toward the PCN. In A, it is suggested that the first negative motor-related field potential peak in PCN might be the result of antidromic invasion of PCN axons after generation of the electromotor command in CN. In both cases, nonsynchronized descending activity from PCN to CN is integrated by the electromotor command neurons in CN. VP, Ventroposterior nucleus of the torus semicircularis.

Figure 13. Possible schemes of functional connectivity to explain the genesis of electromotor-related events in PCN (see Discussion for explanation). A, it is suggested that all motor-related events are the result of activity in the corollary discharge pathway; this would depend on the presence of an as-yet-undemonstrated collateral of BCA axons projecting toward the PCN. In B, it is suggested that the first negative motor-related field potential peak in PCN might be the result of antidromic invasion of PCN axons after generation of the electromotor command in CN. In both cases, nonsynchronized descending activity from PCN to CN is integrated by the electromotor command neurons in CN. VP, Ventroposterior nucleus of the torus semicircularis.