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NEURAL COMMAND OF ELECTROMOTOR OUTPUT IN MORMYRIDS

KIRSTY GRANT[1,*], GERHARD VON DER EMDE[2], LEONEL GOMEZ SENA[3] AND CLAUDIA MOHR[2]

[1]*Institut Alfred Fessard, CNRS, 91198 Gif sur Yvette, France*, [2]*Institut für Zoologie, Universität Bonn, Germany* and [3]*Department of Biomathematics, University of the Republique, Montevideo, Uruguay*

*e-mail: grant@iaf.cnrs-gif.fr

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Summary

The electric discharge of mormyrid fish has an irregular pattern controlled by the electromotor command nucleus in the medulla. Anatomical studies suggest that much of the descending information integrated by the command nucleus comes from the diencephalic precommand nucleus. But field potentials related to the motor command occur later in the precommand nucleus than in the command nucleus, suggesting that they are a corollary rather than a cause of electromotor command initiation. Recorded extracellularly, certain precommand nucleus units fire spontaneously between electromotor commands but pause briefly following each command; others units fire a burst of spikes only during the post-command pause. The firing frequency of the former is correlated with the duration of the interval between successive electromotor commands when the fish is discharging at more than approximately 5 Hz. The post-command pause in spontaneous firing is due to corollary-discharge-mediated feedback inhibition, probably generated by the activity of the bursting units that fire only during this period. Precommand nucleus neurons are activated by electrosensory input, and stimulation of the precommand nucleus modulates the endogenous pattern of electromotor command. We propose that the irregular rhythm of the motor command depends largely on the integration of descending information of various origins, conveyed *via* the precommand nucleus to the command nucleus, and that this process is regulated by corollary discharge feedback inhibition to the precommand nucleus.

Key words: electric fish, motor command, electric organ discharge, precommand nucleus, mormyrid, *Gnathonemus petersii*.

Introduction

The electric organ discharge (EOD) of mormyrid fish is a species-specific, bi- or triphasic pulse of constant form and amplitude emitted with an irregular rhythm in which the interval between consecutive EOD pulses varies instantaneously and continuously. This irregular pattern of electro-emission is quite different from the steady, pacemaker-like discharge pattern of pulse- or wave-emitting gymnotid electric fish. How does the mormyrid electromotor central pattern generator produce this irregular rhythm and how is it modulated intrinsically and in response to external stimuli? Here, we review our current understanding of the anatomy and cellular physiology of the electromotor command pathways in mormyrid fish, which is based mainly on studies of *Gnathonemus petersii*.

Mormyrid patterns of electro-emission

The mormyrid electromotor repertoire includes modulations of the irregular EOD rhythm over a wide range: the interval between two successive EOD pulses can vary by tens or hundreds of milliseconds, and the frequency of the discharge pattern can switch instantaneously from more than 100 Hz to less than 1 Hz, or even to complete silence for brief periods (for reviews, see Kramer, 1990; Moller, 1995). In a search for basal rhythmicity as an intrinsic property of the mormyrid electromotor command, Teyssedre and Boudinot (1987) maintained *Gnathonemus petersii* in a quiet and constant sensory environment and used a curarizing agent to block the electric organ discharge, to abolish reafferent electrosensory feedback and to avoid skeletal movement. Under these basal conditions, the intervals between successive EOD command signals (recorded using an external electrode placed against the electric organ; see Fig. 1B) fell into two categories, one centered around 100 ms in all fish, and the other which varied from 250 to 400 ms depending on the individual. Even under quiet conditions, the serial ordering of the two categories of intervals was not random and the structure of the discharge pattern varied according to the individual. The EOD rhythm does becomes regular under narcosis with metomidate (Hypnodil, Janssen LeBrun). Under metomidate (80–100 $\mu l\,l^{-1}$ of aquarium water), the basal rate is much slower, generally less than 1 Hz, but reaches different values in different individuals (Clausse, 1986). Teyssedre and Boudinot (1987) concluded that, although there may be a subliminal tendency towards a basal rhythm in the intrinsic command circuit, the activity of the central program generator would normally be

structured by electroreceptive feedback. It is probable that additional descending pathways conveying information from other sensory modalities, or of internal origin, can also modulate the frequency of the central program generator. A marked circadian variation in mean discharge frequency has been reported (Moller et al., 1979; Cobert, 1984), and mormyrid fish are more active at night, suggesting that the central program generator is also subject to neurohormonal modulation.

Behavioral observations have described many complex more or less stereotyped patterns of electro-emission, which are apparent when fish are active and engaged in changing environmental or social situations (for reviews, see Kramer, 1990; Moller, 1995). These include brief regularizations centered on a stable frequency, structured alternating accelerations and decelerations, novelty responses, echo responses triggered by the electric activity of another fish, and a variety of more complex behaviours that occur during social encounters or in association with exploratory motor activity. These studies have established that electro-emission, accompanied by the electric sense, serves both for social interaction between individuals and for active imaging of the close environment. The rhythmic structure of the pattern of electro-emission clearly has meaning for the fish, expressed by the intrinsically modulated central electromotor command system.

The electromotor command pathway

The electric organ develops from fast striated muscle fibers in the deep lateral muscle of the caudal peduncle immediately anterior to the tail fin (Szabo, 1957a, 1960) and receives its innervation from a specific population of electromotor neurons in the caudal spinal cord. The descending control of electromotor neurons comes from the medullary relay nucleus (Szabo, 1957b; Bennett et al., 1967a) (Fig. 1A). This, in turn, is driven by input from the adjacent electromotor command nucleus, where the command signal is initiated (Bell et al., 1983; Grant et al., 1986). Together, the medullary relay nucleus and command nucleus constitute the central program generator that drives the electric organ. The timing of the extracellular field potentials that precede the electromotor neuron volley is compared in Fig. 1B. There is little evidence of intrinsic depolarizing pacemaker-type oscillatory activity in any of the neurons of the premotor pathway.

Electromotor neurons

The spinal electromotor neurons accommodate rapidly to injected current and do not seem to have any basal rhythmic oscillations of their own (Aljure, 1964). Electromotor neurons are strongly electrotonically coupled (Bennett et al., 1963) and, although individual electromotor neurons may be activated experimentally by weak stimulation of the spinal cord (Aljure, 1964), the whole population is normally activated as a single ensemble by the strong double descending volley of the medullary relay nucleus axons. Synchronous activation of the electromotor neurons is necessary to produce the discharge of the electric organ. In response to the descending medullary

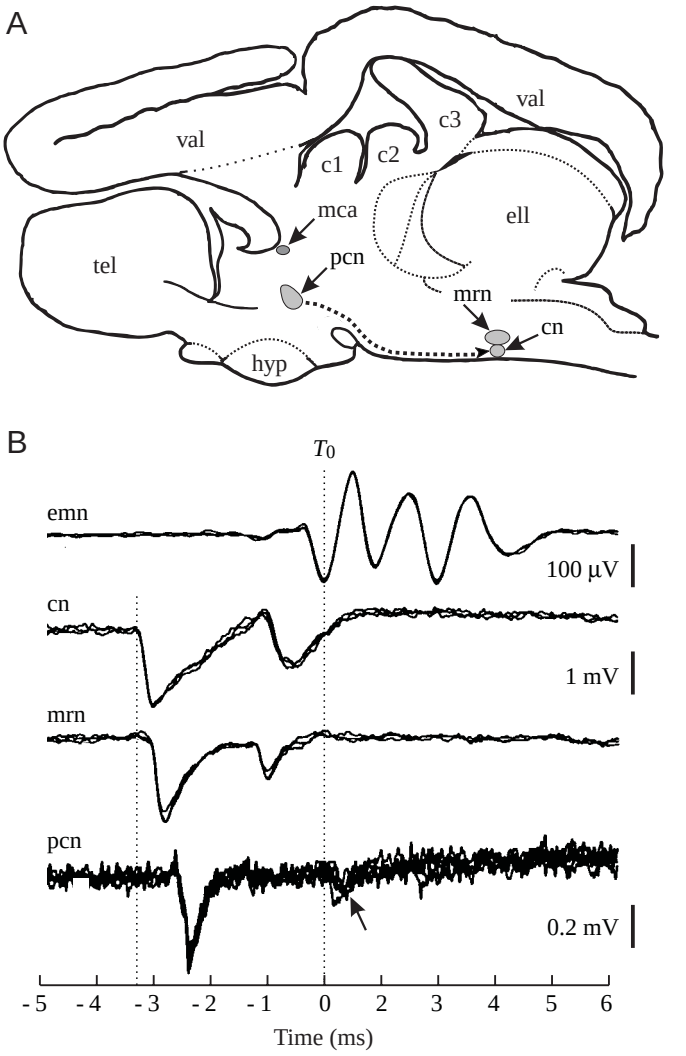

Fig. 1. (A) A schematic parasagittal section through the brain of *Gnathonemus petersii* showing the medullary relay nucleus (mrn) and the command nucleus (cn) in the rhombencephalon and the precommand nucleus (pcn) at the diencephalic/mesencephalic border. The medullary relay nucleus and command nucleus are midline nuclei, but the precommand nucleus is found bilaterally, approximately 1000 μm from the midline. Descending precommand nucleus axons (dotted line) cross the ventral commissure dorsally and then course along the ventral surface of the brain close to the midline before turning dorsally to enter the command nucleus. c1–c3, cerebellar lobes; ell, electrosensory lobe; hyp, hypothalamus; mca, mesencephalic command-associated nucleus; tel, telencephalon; val, valvula cerebelli. (B) The electromotor neuron (emn) triple volley showing the temporal reference T_0, and field potentials recorded extracellularly in the same fish from the command nucleus (cn), the medullary relay nucleus (mrn) and the precommand nucleus (pcn). Note that electromotor-related activity begins first in the command nucleus and last in the precommand nucleus. The arrow in the bottom trace indicates the second negative peak of the precommand nucleus motor-related field potential.

relay neuron volley, electromotor neurons fire a stereotyped triple action potential volley (Fig. 1B) whose precise timing has a constant relationship to the firing of the electric organ.

Medullary relay nucleus

The medullary relay nucleus, first known as the paraseptal nucleus, later as the command nucleus (Szabo, 1957b, 1961) and still later given its present name by Bennett et al. (1967a), is the only known source of direct descending input able to drive the electromotor neurons. It is situated on the midline close to the ventral surface of the medulla, ventral to the Mauthner cell axons and the fiber bundles of the medial longitudinal fasciculus. The nucleus consists of 20–30 very large neurons (soma diameter 30–40 μm) whose richly branching dendritic arborization remains mainly within the nucleus itself. Intracellular labeling with horseradish peroxidase (Grant et al., 1986) or biocytin (K. Grant and C. Mohr, unpublished observations) shows that there are very large somato-somatic appositions between neighboring medullary relay nucleus neurons, through which biocytin appears to diffuse readily. Electron microscopy has identified gap junctions at these appositions (Bennett et al., 1967a; Grant et al., 1986), providing ultrastructural confirmation of the electronic coupling demonstrated electrophysiologically (Aljure, 1964; Bennett et al., 1967a).

Because of their strong electrotonic coupling, medullary relay neurons only generate action potentials as a synchronous ensemble. This property regulates the structure and pattern of the descending motor command. Medullary relay neurons always fire a double action potential whose timing is very constant relative to the electromotor neuron triple volley. This is reflected in the two negative peaks of the extracellularly recorded field potential in Fig. 1B. The first action potential of the stereotyped pair rises sharply from the resting membrane potential with no preceding slow depolarization. No subthreshold excitatory or inhibitory postsynaptic activity is visible in the interval between action potentials. This indicates that the medullary relay nucleus is activated in a highly synchronous manner by some other structure and does not itself generate the intrinsic rhythm of the electric organ system (Aljure, 1964; Grant et al., 1986). It was originally suggested (Bennett et al., 1967a) that the generation of the second spike of the double action potential in the medullary relay neurons was due to their intrinsic membrane properties. However, more recent pathway tracing (Bell et al., 1983) has revealed a disynaptic loop from the command nucleus to the medullary relay nucleus (see below) that might also provide a mechanism for the generation of the characteristic second spike.

Medullary relay neurons receive the major part of their afferent synaptic input from the neighboring command nucleus (Bell et al., 1983). The second, disynaptic input from the command nucleus to the medullary relay nucleus comes *via* the bilateral bulbar command-associated nucleus, which itself is activated by a collateral branch of command nucleus axons (Bell et al., 1983; Clausse, 1986; Grant et al., 1986). This collateral pathway originating from the command nucleus *via* the bilateral bulbar command-associated nucleus is also the origin of the corollary signal discharge (efference copy) which feeds forward to the electrosensory processing nuclei and which serves sensorimotor coordination and an active sensory filtering mechanism (Bell et al., 1983).

Immunohistochemical studies have shown that medullary relay neurons are also surrounded by networks of serotonin-positive and noradrenaline-positive varicose processes whose morphology suggests the presence of synaptic terminals (Grant et al., 1989; Meek and Joosten, 1989; Meek et al., 1993). This has not been studied under the electron microscope, and no electrophysiological studies are yet available concerning the possible modulation of the relay neuron excitability by serotoninergic or noradrenergic input.

The command nucleus

The command nucleus, situated just ventral to medullary relay nucleus (Fig. 1A), supplies the major afferent pathway to the medullary relay nucleus and is the site of initiation of the descending electromotor command signal (Bell et al., 1983; Grant et al., 1986). A comparison of the field potentials recorded extracellularly from the medullary relay nucleus and the command nucleus (Fig. 1B) shows that activity is initiated earliest in the command nucleus, 200–300 μs before that in the medullary relay nucleus and approximately 3.4 ms before the first negative peak of the electromotor neuron triple volley which has been defined as a temporal reference (Fig. 1B; T_0).

In *Gnathonemus petersii*, the command nucleus contains 15–20 multipolar neurons whose axons form a dense terminal field around medullary relay neuron somata. Their large club-ending-type axon terminals probably make electrical synapses *via* the gap junctions present at contacts with the medullary relay neurons, but the axon terminals also contain vesicles suggestive of chemical synaptic transmission (Elekes et al., 1985; Grant et al., 1986). Collateral branches of these same axons also project bilaterally to the bilateral bulbar command-associated nucleus, as mentioned above (Bell et al., 1983; Grant et al., 1986). Command neurons have a widespread dendritic arborization which extends bilaterally several hundred micrometers into the ventral reticular formation (Grant et al., 1986). Many descending and ascending pathways pass through this region, and it is likely that some at least make contact with the command neuron dendrites.

Intracellular recordings show that command neurons always fire a stereotyped double action potential and that the nucleus always fires as an ensemble, resulting in activation of the whole medullary relay nucleus and the subsequent triple electromotor neuron volley (Fig. 2A). Individual command neurons never fire action potentials alone, and firing always results in the generation of a descending command signal to the electric organ. Large spikelets several millivolts in amplitude are also frequently observed in command neurons (Fig. 2A,B). Their occurrence is not strictly linked to activation of the double action potential or to the duration of the interval between successive command signals, and they are not present all the time in any given cell (Grant et al., 1986). Intracellular

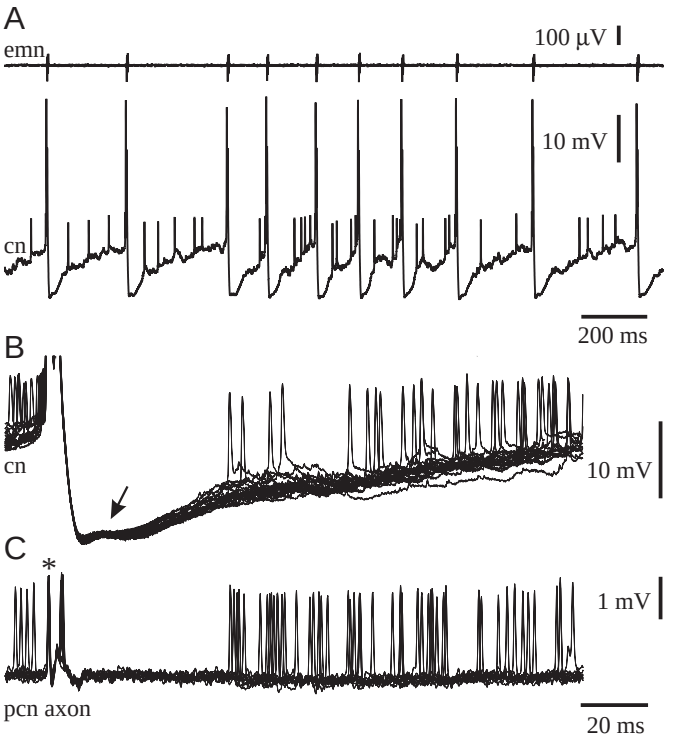

Fig. 2. Spiking activity of command neurons and a putative precommand nucleus axon recorded in the command nucleus. (A) emn, electromotor neuron command signal; cn, intracellular recording from a command neuron illustrating large double action potentials time-locked to the electromotor neuron command signal and smaller spikelets which do not activate the descending electromotor command. Note the presence of many smaller subthreshold postsynaptic potentials. (B) Superimposed intracellular recordings from a command neuron, illustrating the large afterhyperpolarization that follows the double action potential. Note the inflexion immediately after the peak of the afterhyperpolarization (arrow). During the afterhyperpolarization, spikelets and postsynaptic potentials were not observed. (C) Extracellular a.c.-coupled recording from a putative precommand nucleus axon (pcn) obtained 15 μm from the command neuron illustrated in B. This axon fired one action potential strictly time-locked to the electromotor activity (asterisk); a second motor-related spike was present in many, but not all, traces. Following the post-motor command pause, spiking began again in the putative axon at the same time that spikelets reappeared in the command neuron (see B) and significantly earlier than any full action potentials were generated in the command neuron.

recordings from command neurons also show a large number of postsynaptic potentials, reflecting a high level of synaptic input (Fig. 2A).

The integration of excitatory synaptic input is probably the most important factor contributing to the initiation of the double action potential and the generation of the descending command signal, but no single afferent input alone is sufficient to drive the command neurons. Despite the statistical tendency towards a basal frequency of approximately 10 Hz in the intrinsic rhythm of the electromotor system cited above, intracellular recordings from command neurons show no evidence of regular depolarizing oscillations of the membrane potential (Fig. 2A), such as those visible in pacemaker neurons of gymnotid fish (Bennett et al., 1967b). The duration of the large afterhyperpolarization that follows the double action potential and interrupts the integration of synaptic input for 40 ms or more is probably a major factor in deciding the length of the firing interval.

The ion channels responsible for this large afterhyperpolarization have not yet been investigated. An inflexion shortly after the beginning of recovery from the peak of the afterhyperpolarization (Fig. 2B, arrow) suggests that the afterhyperpolarization might in fact consist of two phases, perhaps due to Ca^{2+}-sensitive outward K^+ currents with different kinetics (e.g. fAHP and sAHP; see Lancaster and Nicoll, 1987). In addition, the afterhyperpolarization is sufficient in size and duration to de-inactivate any outward K^+ current of the I_A type, which would tend to reduce the probability of firing during the repolarizing phase. Another possible mechanism contributing to the later phase of the afterhyperpolarisation could be that of a recurrent shunting inhibition. In this context, the action of the narcotic metomidate, which markedly slows and regularizes the electromotor rhythm, is particularly interesting since the closely related compound etomidate is known to be a potentiator of postsynaptic γ-aminobutyric acid (GABA) receptors (Uchida et al., 1995). Metomidate slows the electromotor rhythm without changing the form of the EOD or the timing of the electromotor neuron volley. While intracellular recordings from medullary relay neurons show no change in the action potential under metomidate, the large afterhyperpolarization in command neurons is prolonged by up to several hundred milliseconds and visible postsynaptic activity is very significantly reduced (Clausse, 1986; Clausse and Grant, 1986). Despite this observation, it cannot easily be concluded that feedback inhibition contributes to the regulation of firing in command neurons. Anatomical tracing (Bell et al., 1983; K. Grant and G. von der Emde, unpublished observations) and intracellular labeling with horseradish peroxidase (Grant et al., 1986) have not identified any central, paucisynaptic inhibitory feedback pathways, and immunohistochemistry has provided no evidence of a strong GABAergic input to the soma or proximal dendritic regions of command neurons (J. P. Denizot and K. Grant, unpublished observations). Although stimulation of the spinal cord at low intensity (below the threshold for antidromic activation of the motor command pathway) did evoke a short-lasting inhibitory postsynaptic potential (IPSP) in command neurons with a latency of approximately 4 ms (Grant et al., 1986), neither the anatomical pathway involved nor the physiological relevance of this response is known. Finally, the possible direct action of metomidate on the kinetics of ion channels underlying the afterhyperpolarization has not yet been investigated.

Further experimentation will clarify our understanding of the mechanisms involved; it also remains possible that the slowing of the electromotor rhythm in response metomidate is

partly, if not wholly, due to a reduction in excitatory drive to the command neurons and is the result of potentiated inhibition of the descending excitatory pathways afferent to the command nucleus, at their source.

Afferent pathways to the electromotor command nucleus

The sources of afferent pathways to the command nucleus have been studied using retrograde labeling with horseradish peroxidase and biocytin (Bell et al., 1983; Niso et al., 1989; K. Grant, G. von der Emde and C. Mohr, unpublished observations). This has identified two principal sources of input to the center of the nucleus: the bilateral precommand nucleus situated at the diencephalic/mesencephalic border a few hundred micrometers from the midline and dorsal to the hypothalamus (Figs 1A, 3), and the ventroposterior nucleus of the torus semicircularis (not illustrated). It is also possible that command neuron dendrites in the reticular formation receive significant inputs from other sources that are not labeled when tracer substances are deposited in the center of the command nucleus. Retrograde transport following larger deposits of biocytin, centered on the command nucleus, but which also spread into the area of reticular formation containing the command neuron dendritic arborization, results in the labeling of several small groups of neurons in the hypothalamus, in the thalamus immediately dorsal and medial to the precommand nucleus, at the border of the lateral nucleus and the base of the optic tectum, and in the dorsal mesencephalic reticular formation (K. Grant and C. Mohr, unpublished observations). The command nucleus does not seem to receive the same serotonin-positive and noradrenaline-positive inputs that have been observed in the medullary relay nucleus (Grant et al., 1989; Meek and Joosten, 1989; Meek et al., 1993).

The precommand nucleus

The precommand nucleus, situated at the diencephalic/mesencephalic border (Fig. 3), does not stand out cytoarchitecturally in histological preparations unless labeled by retrograde transport of tracers from the command nucleus or in material treated immunohistochemically to reveal glutamic acid decarboxylase (GAD) labeling (Niso et al., 1989; K. Grant and J. P. Denizot, unpublished observations; see below). The most caudal region of the precommand nucleus contains medium-sized multipolar neurons (soma diameter 8–12 μm) whose most distinctive features are long, large-diameter, smooth dendrites extending laterally towards the tectum marklager. Precommand neuron axons leave the nucleus ventromedially, crossing over the ventral commissure before coursing close to the midline along the ventral surface of the brainstem to the level of the command nucleus in the caudal brainstem (Fig. 1A). Here, they turn abruptly dorsally and end in contact with command neurons; they do not appear to enter the medullary relay nucleus. The ultrastructure of the precommand axon terminal arborization has not yet been investigated in tracer-labeled material, but these axons represent the major source of input to command neuron soma.

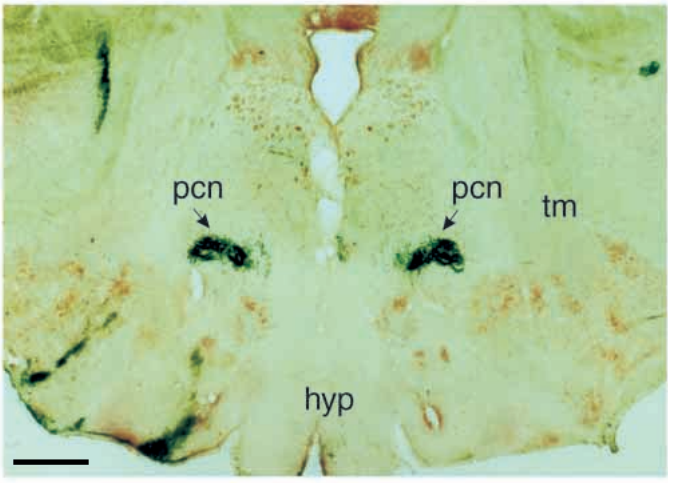

Fig. 3. A frontal section through the midbrain of *Gnathonemus petersii* showing the precommand nucleus labeled by retrograde transport of biocytin deposited in the command nucleus (cresyl violet counterstain). hyp, hypothalamus; pcn, precommand nucleus; tm, tectum marklager (toro-preeminential tract). Scale bar, 750 μm.

They probably correspond to the synaptic club endings containing vesicles and forming gap junctions in contact with the command neurons described by Elekes and Szabo (1985).

The more rostral part of the precommand nucleus contains smaller, round cell bodies and a dendritic arborization that is dense, but much finer and varicose, and extends dorsomedially towards the centroposterior nucleus of the thalamus. These cells are also descending projection neurons since they are labeled retrogradely following deposit of tracers in the command nucleus.

Anti-GABA immunohistochemistry has not revealed labeling of cell bodies in the precommand nucleus, but the nucleus stands out clearly in anti-GAD-treated material because the neuron somata are almost completely surrounded by large anti-GAD-labeled synaptic terminals (Niso et al., 1989; J. P. Denizot and K. Grant, unpublished observations). The cell bodies at the source of this GABAergic pathway have not yet been identified, but electrophysiological recordings (see below) suggest strongly that they are part of the corollary-discharge-driven feedback network. This may also be the site affected by metomidate, which slows the electromotor rhythm (described above).

The precommand nucleus can be identified electrophysiologically by the field potential illustrated in Fig. 1B, which occurs time-locked to the electromotor neuron volley and T_0 and which characterizes recordings made in the center and caudal regions of the nucleus. It may be noted immediately that the initial sharp negative potential begins almost 1 ms later than premotor activity in the command nucleus. It is therefore a corollary, rather than a causal factor, of the electromotor command. A second, smaller, negative component of the field potential is often, but not always, visible with a further delay of approximately 2.5 ms (Fig. 1B, arrow).

Several types of intrinsic neuronal activity have been

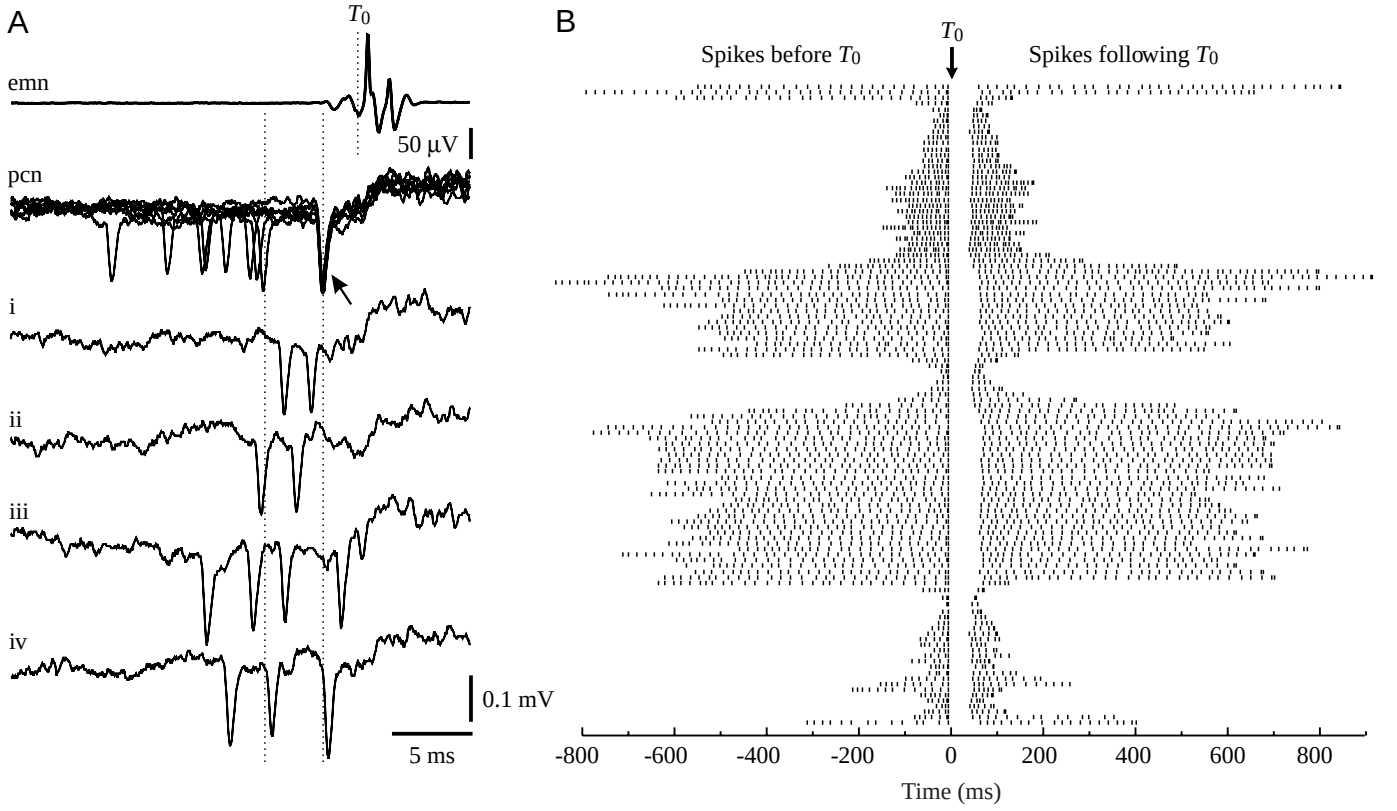

Fig. 4. Intrinsic unit activity recorded in the precommand nucleus (pcn). (A) Certain units fire sporadically with a high probability of spiking in the period 10–20 ms before T_0 (see Fig. 1). One of these spikes is very often time-locked relative to the electromotor neuron volley (emn; T_0) (pcn; superimposed traces, arrow); but note that this spike occurs later than activation of the command nucleus (see Fig. 1). If spontaneous spikes occur in the period 3–4 ms (indicated by vertical dotted lines) before the expected time-locked motor-related spike, the latter does not occur (traces i,ii) or is delayed (traces iii,iv). All traces in A were obtained from the same unit. (B) Similar precommand nucleus units fire more continuously between electromotor command signals, also with the highest probability of spiking in the 10–20 ms before T_0. These units always paused for 30–60 ms following T_0. The raster display, illustrating the responses of a single unit, shows all spikes occurring before (to the left of T_0) and after (to the right of T_0) each electromotor command signal (indicated by T_0) over 115 cycles represented in successive lines of the raster. The length of the raster line to the left of T_0 indicates the time since the previous T_0; the length of the raster line to the right of T_0 indicates the time before a new motor command (T_0) is generated. A high mean firing frequency and a short post-T_0 pause were correlated with short inter-motor command cycles.

recorded extracellularly in the precommand nucleus (Figs 4, 5). Many units fire sporadically, and the probability of firing spikes is increased during the 10–20 ms preceding the electromotor neuron volley (Fig. 4A, superimposed traces). The exact timing of these spikes is variable and, apart from the exception described below, they are not strictly time-locked to the motor activity. The exception to the very loosely structured, sporadic firing of such units is a single, motor-related, time-locked spike that occurs frequently (see superimposed traces labeled precommand nucleus in Fig. 4A, arrow), but not always (single traces in Fig. 4Ai–iv, from the same cell), at exactly the time of the first negative peak of the motor-related field potential described in Fig. 1B (bottom trace). It seems probable that local summation of the time-locked spike fired by such units is the cause of the electromotor-related field potential illustrated in Fig. 1B. This precisely timed motor-related spike in the precommand nucleus is not always present and drops out, or is delayed, if other spontaneous spikes occur within the 3–4 ms preceding the first negative peak of the field potential (Fig. 4Ai–iv). This occlusion is probably not due to refractoriness since, at other moments, the same units fire spikes separated by even shorter intervals (e.g. in Fig. 4Ai).

Other similar units fire more regularly throughout the command cycle, except during a period of 30–60 ms immediately after the generation of the electromotor command signal (Fig. 4B). The firing pattern of a neuron of this type is illustrated in the raster diagram in Fig. 4B. This raster has a complex structure in which all the spikes occurring in the command cycle before T_0 are shown to the left of T_0 and all the spikes occurring after T_0 (and before the next T_0) are shown to the right of T_0. The timings of the spikes occurring before and after each T_0 over the 115 command cycles analyzed are shown in the successive lines of the raster (Fig. 4B appears symmetrical because the spikes following T_0 in line 1 are also those occurring before T_0 in line 2, etc.). This raster shows the absence of spikes during a period of approximately 50 ms

following each T_0 (immediately to the right of T_0 in every line of the raster) and illustrates the post-command pause in firing of this otherwise intrinsically active unit. In addition, the length of the raster line to the left of each T_0 shows the time since the previous T_0, and the length of the raster line to the left of T_0 shows the time until the next T_0. From this, it can be seen that there was a loose inverse correlation between firing frequency and inter-T_0 (inter-command signal) interval length, and also that there was a tendency for the length of the post-command (T_0) pause and the duration of the previous and following inter-command intervals to be correlated.

The origin of the post-command (post-T_0) pause in the firing of the sporadically and tonically firing units described above may be explained by the activity of a third type of unit recorded in the precommand nucleus, which fired a stereotyped burst of action potentials following every command signal and which was silent at all other times (Fig. 5A–C). The timing of this burst was correlated with the silent period of tonically active cells and also with large, post-command IPSPs observed in a small number of neurons that have been recorded intracellularly (N=5) in the precommand nucleus (Fig. 5D). This suggests that these bursting units may represent the axonal firing of an inhibitory input to the precommand nucleus. Because of its timing, it is presumed that this post-command bursting activity is driven by feedback from the corollary discharge pathway, although no anatomical substrate for such a projection to the precommand nucleus has yet been described.

The size and density of the precommand nucleus axonal projection to the somatic region of the command nucleus make it likely that it constitutes a major source of afferent input to the command generator of the electromotor pathway. However, since no large premotor-related field potentials are recorded in the precommand nucleus, the intrinsic activity of precommand nucleus neurons is probably not synchronized over the whole population constituting the nucleus. Microstimulation with currents of a few microvolts (>10 μV), which probably synchronizes activation within the precommand nucleus, overrides the endogenous rhythm of the motor command and drives the electromotor pathway up to a certain following frequency (Niso et al., 1989). However, if the stimulating electrode is in the center of precommand nucleus, evoked motor responses begin to fail when the stimulus repetition rate exceeds 20 Hz (50 ms between stimulus pulses). This is probably due to the inhibitory corollary discharge feedback described above, which acts as a rate-limiting mechanism and regulates the descending stream of activity towards the command nucleus. When the stimulating electrode is placed ventrocaudally in the nucleus, in the region of the departure of the descending axons, the electromotor command can be driven much faster, presumably avoiding the inhibitory feedback to the somatic region of precommand nucleus neurons.

Electrosensory stimulation evoked a burst of spiking activity in the sporadically active units in the precommand nucleus (Niso et al., 1989). The latency of the sensory response varied

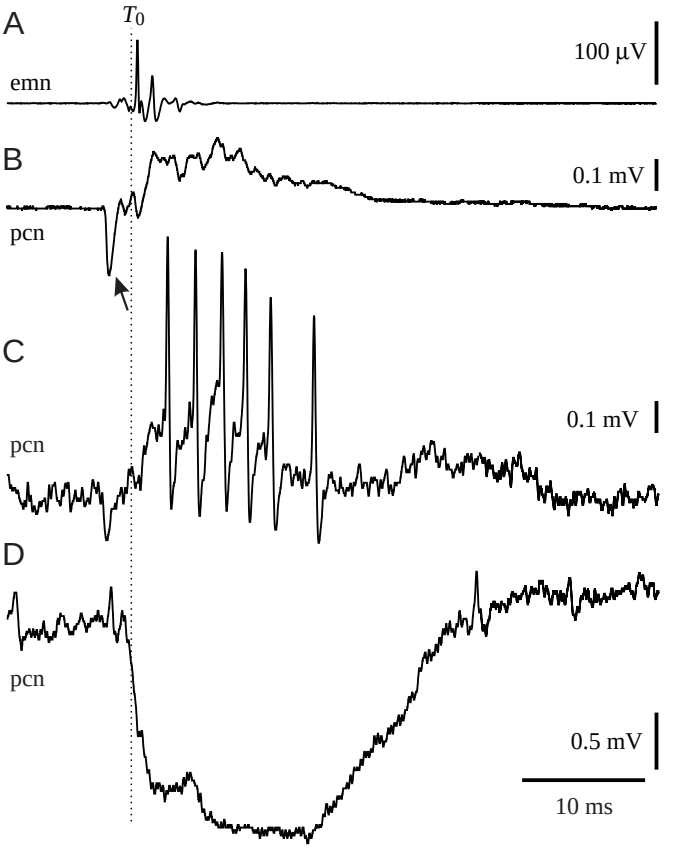

Fig. 5. Corollary-discharge-driven activity in the precommand nucleus. (A) The electromotor neuron (emn) command signal indicating T_0 (see Fig. 1) (B) Direct-current-coupled recording of the extracellular field potential in the precommand nucleus (pcn), showing a slow depolarizing wave which followed the motor-related negative population spike (arrow). (C) Extracellularly recorded burst of unit spikes occurring during the period of the depolarizing field potential seen in B. (D) Intracellular recording from a precommand nucleus neuron, illustrating a large inhibitory postsynaptic potential (IPSP) which occurred during the same period as the extracellular slow depolarizing potential (in B) and the burst of spikes (in C). Note that this cell was depolarized (–40 mV) and no full-size spontaneous action potentials were recorded, although small spikelets similar to the sporadic firing of the units in Fig. 4A were visible.

from approximately 8 to 14 ms, and when the stimulus was repeated infrequently in an isolated manner triggered 100 ms after a spontaneous motor command signal, the sensory-evoked burst of activity in the precommand nucleus was generally associated with subsequent activation of an electromotor command. However, the ability of an electrosensory stimulus to evoke motor responses adapted rapidly when the stimulus was repeated in the same time-locked manner following every spontaneously occurring electromotor command, and after 10–15 trials the probability of entraining activation of the electromotor command was reduced by 20–40 %. Despite this adaptation of the motor response, the sensory-elicited activity in the precommand nucleus was not affected, and the same burst of unit activity

continued to be evoked in the precommand nucleus in response to the sensory stimulus.

Putative precommand nucleus axonal activity recorded in the command nucleus

An as yet poorly understood class of unit activities recorded in the command nucleus shows a pattern of activity very similar to that of the intrinsic firing of units in the precommand nucleus described above (compare Fig. 2C and Fig. 4B). We suggest that these may be the axons of precommand nucleus neurons recorded at the level of their terminal arborization close to the command neurons. These units have been recorded only extracellularly in the command nucleus; they fire much faster than the stereotyped double action potentials of the command neurons, and the timing of their motor-related spikes (Fig. 2C) is less perfectly locked to the electromotor neuron volley than the stereotyped double action potentials of the command neurons. However, it is interesting to note the similarity between the firing pattern of these units and that of the spikelet-like events sometimes observed in intracellular recordings from command neurons (Fig. 2B,C). These could possibly be pre- and postsynaptic (electric) manifestations of the same events. An alternative explanation would be that the extracellular single spikes and the intracellular spikelets represent the axonal (but not somatic) activity of the command neurons themselves. This interpretation seems less likely since it would then be expected that their postsynaptic effects would be visible as subthreshold excitatory postsynaptic potentials (EPSPs) in medullary relay nucleus neurons, and this is never the case (Grant et al., 1986).

The ventroposterior nucleus of the torus semicircularis

Some (10–15) large multipolar neurons are labeled within the ventroposterior nucleus on either side of the brain following deposits of horseradish peroxidase or biocytin in the command nucleus (Bell et al., 1983; K. Grant and G. von der Emde, unpublished results). These neurons are found along the dorsolateral margin of the tectum marklager. They give rise to very long dendrites that extend through the tectum marklager and to axons that pass ventromedially between the same fiber bundles and join the ventral commissure. In the ventral commissure ventroposterior nucleus, the axons divide, sending branches to the precommand nucleus bilaterally and a descending collateral branch which projects to the command nucleus, running parallel to the descending precommand nucleus axons. No electrophysiological recordings have yet been made in this region, and the firing pattern of the ventroposterior nucleus neurons relative to that of other nuclei of the electromotor command chain is unknown.

The origin of the irregular electromotor command rhythm

The regulation of the firing of command neurons appears to be a complex process depending on both the intrinsic membrane properties of the command neurons and their integration of descending afferent input. No regular depolarizing pacemaker potentials, such as those visible in pacemaker neurons of gymnotid fish (Bennett et al., 1967b), are discernible in mormyrid electromotor command neurons. The primary rate-limiting factor regulating action potential generation is the duration of the afterhyperpolarization that follows the double action potential characteristic of these neurons. The ionic mechanisms responsible for this large afterhyperpolarization, which impose a minimum ‘refractoriness’ on the command system, will require future investigation.

Descending input to the command nucleus probably comes principally from the precommand nucleus, since these axons provide the major synaptic input to command neuron somata. It is suggested that action potential generation in the command neurons depends to a large extent on the integration of these many, convergent normally nonsynchronous synaptic inputs originating from the precommand nucleus. To understand this mechanism more fully, further experiments will also be required to identify the afferent pathways to the precommand nucleus and also to explore the role of the ventroposterior nucleus of the torus whose neurons project to both the precommand nucleus and the command nucleus, but whose activity has not yet been recorded. Reciprocal connections between the bilateral precommand nuclei are not present (K. Grant and G. von der Emde, unpublished observation), but since ventroposterior neurons project to the precommand nucleus bilaterally, as well as sending decending axons to the somatic region of the command neurons, they may also play an important role in the coordination or modulation of the two precommand nuclei and the resulting strength of the descending input to the command nucleus.

Corollary-discharge-mediated inhibition of the precommand nucleus also prevents the motor system from being driven at more than approximately 20 Hz *via* the direct descending pathway to the command nucleus. The location of the somata of the inhibitory interneurons responsible for this inhibition has not yet been identified but, because of the timing of their activation, it is likely that they receive input from the ascending axons of the bulbar command-associated nucleus.

Behavioral observations have described firing of the electromotor system at a faster rate in some circumstances, both in response to external stimuli and as an intrinsically initiated motor pattern. This would require another means for descending activation of the command nucleus, exempt from corollary discharge inhibition. It is possible either that the projection from the ventroposterior nucleus might fill this role or that other, as yet unidentified, afferent pathways are involved.

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