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Motor control of the jamming avoidance response of *Apteronotus leptorhynchus*: evolutionary changes of a behavior and its neuronal substrates

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Abstract The two closely related gymnotiform fishes, *Apteronotus* and *Eigenmannia*, share many similar communication and electrolocation behaviors that require modulation of the frequency of their electric organ discharges. The premotor linkages between their electrosensory system and their medullary pacemaker nucleus, which controls the repetition rate of their electric organ discharges, appear to function differently, however. In the context of the jamming avoidance response, *Eigenmannia* can raise or lower its electric organ discharge frequency from its resting level. A normally quiescent input from the diencephalic prepacemaker nucleus can be recruited to raise the electric organ discharge frequency above the resting level. Another normally active input, from the sublemniscal prepacemaker nucleus, can be inhibited to lower the electric organ discharge frequency below the resting level (Metzner 1993). In contrast, during a jamming avoidance response, *Apteronotus* cannot lower its electric organ discharge frequency below the resting level. The sublemniscal prepacemaker is normally completely inhibited and release of this inhibition allows the electric organ discharge frequency to rise during the jamming avoidance response. Further inhibition of this nucleus cannot lower the electric organ discharge frequency below the resting level. Lesions of the diencephalic prepacemaker do not affect performance of the jamming avoidance response. Thus, in *Apteronotus*, the sublemniscal prepacemaker alone controls the change of the electric organ discharge frequency during the jamming avoidance response.

Key words Electric fish · Pacemaker nucleus · Central pattern generator · Glutamate receptors · Social communication

Abbreviations *aCSF* artificial cerebrospinal fluid · *APV* D(-)2-amino-5-phosphonovaleric acid (NMDA receptor blocker) · *CNQX* 6-cyano-7-nitroquinoxaline-2,3-dione (non-NMDA receptor blocker) · *CPP* 3-(2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid (NMDA receptor blocker) · *Df* frequency difference between jamming signal and EOD · *EOD* electric organ discharge · f_{EOD} frequency of fish's own EOD · f_{JAM} frequency of jamming stimulus · *GABA* γ-amino-*n*-butyric acid · *JAR* jamming avoidance response · *NMDA* *N*-methyl-D-aspartate · *NSR* non-selective response

Introduction

The jamming avoidance response (JAR) of *Eigenmannia* has long served as a useful model system for the study of sensory information processing and the neuronal extraction of key stimulus features necessary to evoke a behavioral response [see Heiligenberg (1991) for review]. *Eigenmannia* produces electric organ discharges (EODs) at a regular rate, or resting frequency (f_{EOD}), that in the context of the JAR, can be shifted either up or down away from the frequency of an imposed jamming stimulus (f_{JAM}). Performance of the

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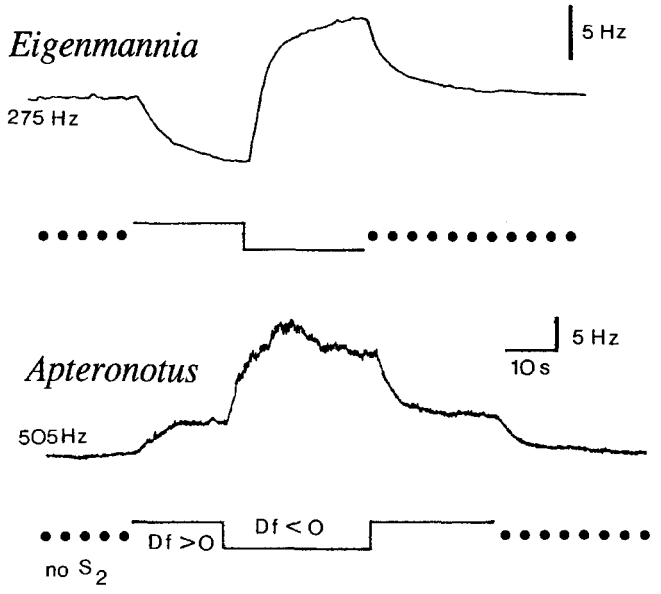


Fig. 1 Jamming avoidance response in *Eigenmannia* and *Apteronotus*. For each fish the top trace represents the EOD frequency of the curarized fish, sampled as the mean across 30 successive EOD cycles. The starting frequency for each trace, representing the resting frequency of the unstimulated fish, is listed to the left. Lower traces indicate the presence of a jamming stimulus (S2) of approximately one-third of the uncurarized fish's EOD amplitude presented transversely to the fish's body axis and frequency-clamped 4 Hz above (+ Df) and then below (-Df) the EOD frequency. *Eigenmannia* responds to the onset of the + Df jamming stimulus by lowering its frequency below the resting level. When the sign of Df is reversed, the fish raises its EOD frequency to a point above the original resting frequency. After offset of the S2, the EOD frequency relaxes back towards the original resting level. *Eigenmannia*'s JAR is usually approximately symmetrical about its resting frequency. In contrast, *Apteronotus*' JAR is asymmetrical. The fish gives an initial small rise, known as a 'non-selective response,' at the onset of the + Df jamming stimulus. After the switch to -Df jamming, a larger rise follows. In the context of the JAR, *Apteronotus*' EOD frequency never falls below its resting value

JAR critically depends upon analysis of jamming-induced modulations in the fish's own electric field to provide information about the sign and magnitude of the frequency difference ($Df = f_{JAM} - f_{EOD}$). Electrosensory cues that control the JAR are extracted in various laminae of the dorsal torus semicircularis (Heiligenberg 1991). The closely related genus, *Apteronotus* (Alves-Gomes et al. 1995) displays similar JARs with the chief difference that *Apteronotus* cannot lower its EOD frequency below its resting level (Fig. 1; Bullock et al. 1972). Since *Apteronotus* discriminates the sign of Df by the same computational rules as does *Eigenmannia* (Heiligenberg 1991), we assumed that the difference in the performance of its JAR is not attributable to differences in this sensory processing, but rather is due to differences in the premotor elements controlling the JAR that lie downstream from the midbrain torus. The present report examines similarities and differences between these two genera in the underlying premotor circuitry and its dynamic properties as one chapter in the evolution of the neuronal substrate controlling the JAR and related social behaviors.

The current understanding of the premotor circuitry controlling the JAR in *Eigenmannia* is summarized in Fig. 2A (Rose et al. 1988; Kawasaki et al. 1988; Keller et al. 1990; Metzner 1993). Each EOD of *Eigenmannia* is driven by an intrinsically rhythmic pacemaker nucleus (Pn) located in the fish's medulla. The pacemaker normally fires at a constant rate, termed the resting frequency. The resting frequency can be modulated by excitatory input to the pacemaker from both the mesencephalic sublemniscal prepacemaker (SPPn) and the diencephalic prepacemaker nucleus (PPn).

When confronted with a jamming signal of slightly higher frequency, the pacemaker frequency can be lowered below its resting discharge rate and when presented with a lower frequency jamming signal, the pacemaker frequency is raised. The torus issues instructions for raising or lowering the frequency of the pacemaker nucleus via the nucleus electrosensorius, nE (Keller and Heiligenberg 1989). Activation of the subnucleus of the nE called the nE↑ raises the pacemaker frequency through excitation of the PPn. Activation of a different subnucleus of the nE, called the nE↓, lowers the pacemaker frequency through inhibition of the tonically active SPPn (Metzner 1993). Thus, the JAR of *Eigenmannia* relies on two largely independent pathways from the nucleus electrosensorius to the Pn to control EOD frequency rises (nE↑-PPn-Pn) and falls (nE↓-SPPn-Pn), respectively.

In the present report, we show striking differences between the two genera in the functional significance of diencephalic and mesencephalic inputs to the pacemaker nucleus although all of the anatomical substrates appear to be present. In particular, whereas the motor pathway in *Eigenmannia* operates with equal participation of both the diencephalic and mesencephalic pacemaker afferents in controlling the rising and falling phases of the JAR, the motor pathway in *Apteronotus* is heavily biased to the mesencephalic sublemniscal prepacemaker nucleus (SPPn), which is reflected in its asymmetrical JAR.

Modulations of the pacemaker frequency, and hence of EODs, are also generated for communication and result from a set of higher-level inputs to the pacemaker that partially overlaps those controlling the JAR. Thus, as in many other vertebrate and invertebrate motor control systems (Pearson 1993), a single central pattern generator can provide a large repertoire of behaviorally relevant patterned output, which may, in turn, provide the necessary raw material allowing evolutionary change (Arbas et al. 1991).

Materials and methods

We performed experiments on more than 100 *Apteronotus leptorhynchus* ("brown ghosts"; body lengths between 12 and 18 cm) over

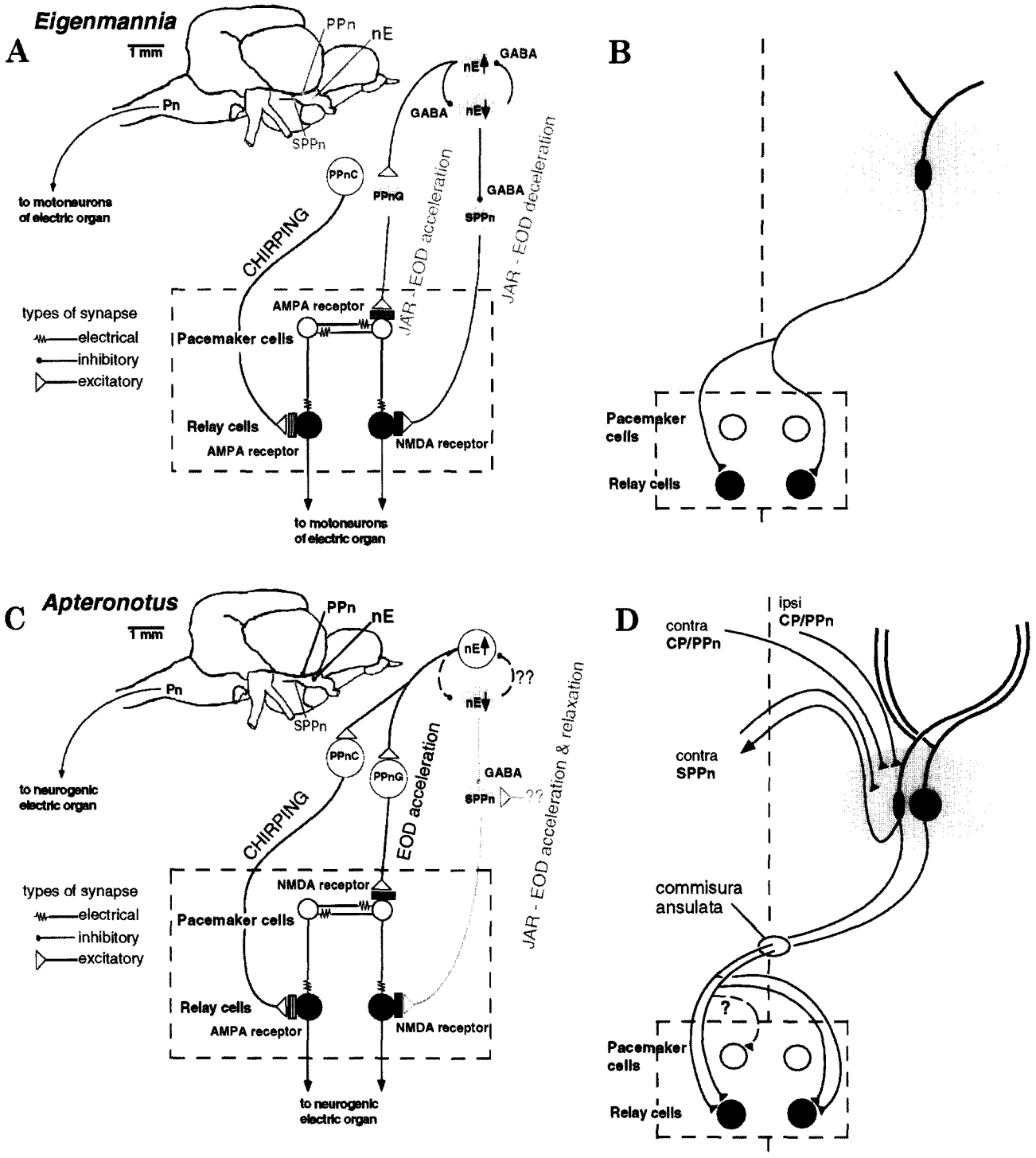


Fig. 2A–D Schematic representations of pre-electromotor circuitry in (**A**) *Eigenmannia* (after Metzner 1993) and (**C**) *Apteronotus*. Brain nuclei involved in control of the JAR are shown in *gray*. Performance of the JAR by *Eigenmannia* requires the use of both the nE↑-PPnG-Pn and nE↓-SPPn-Pn pathways for control of EOD frequency rises and falls, respectively. The *Apteronotus* JAR requires only the nE↓-SPPn-Pn pathway. Schematic representations of the SPPn of (**B**) *Eigenmannia* and (**D**) *Apteronotus* summarize the known anatomical differences between the two genera. The *Apteronotus* SPPn is more highly developed, having two cell types, more extensive dendrites, bilateral input from the CP/PPn, reciprocal connections with the contralateral SPPn, and a possible weak projection onto pacemaker cells of the Pn. Components of the pacemaker nucleus are enclosed within a dashed rectangle

the course of 4 years. Whereas *Eigenmannia* maintains a rather stable EOD frequency and generates consistent JARs over the course of hours of experimentation, the electrical behavior of *Apteronotus* is less predictable when presented with external electrical signals. The EOD frequency of an individual may fluctuate gradually by tens of Hz over the course of an hour, and the JAR often weakens if jamming signals are presented continuously. For this reason and to explore possible individual differences we always repeated critical paradigms in several animals. For convenience, we refer to our experimental subject as "*Apteronotus*" but do not know whether the results presented below are directly applicable to other members of the genus.

The physiological methods employed in these studies were identical to those used in earlier experiments (Dye et al. 1989; Kawasaki and Heiligenberg 1989, 1990; Metzner 1993). Briefly, we immobilized fish by intramuscular injection of gallamine triethiodide (1–2 µl of a solution of 20 mg·ml^{-1} of H_2O). Since the EOD in *Apteronotus* is neurogenic [review: Bass (1986)], it is not attenuated by curare-like drugs. We tested the fish's JAR by presenting a sinusoidal jamming signal and maintaining, by means of on-line computer control (Heiligenberg et al. 1978b), the frequency difference, Df, between the jamming signal and the fish's EOD alternately at + 4 or -4 Hz; Df values of this magnitude are most effective in eliciting JARs (Bullock et al. 1972).

We explored diencephalic and mesencephalic nuclei that provide inputs to the medullary pacemaker nucleus by local iontophoretic application of L-glutamate while monitoring the ensuing modulations of the pacemaker rhythm by recording the fish's EOD. The EOD signal was amplified (Grass P-15) and fed to a PDP11-70 computer for on-line frequency control and analysis or recorded to magnetic tape (HP 3964A) for later analysis. For recording of EOD-interruptions (Fig. 13), the signal was DC-amplified, recorded on tape (bandwidth of 5000 Hz) and (off-line) A/D converted at 44.1 kHz. Frequency spectra were calculated using the MATLAB "specgram" routine, with 1024 point FFTs using a Hanning window advanced by 256 points for each successive FFT.

To gain access to these diencephalic and mesencephalic structures, we removed the frontal bone overlying the rostral optic tectum and cerebellum (approximately 2 mm^2) under local topical anesthesia (2% lidocaine). In some cases, a larger hole (ca. 2×4 mm) was required to expose both tecta to allow bilateral penetrations. On occasion, rostral portions of the corpus cerebelli were removed to allow easier access. No obvious change in behavioral response resulted from this procedure. The medullary pacemaker nucleus was reached by making a small midline-incision in the skull above the caudal corpus cerebelli. Stimulating and iontophoresing electrodes were triple-barrel glass micropipettes with each barrel having an inner tip diameter of < 10 µm. By filling one barrel with L-glutamate for local stimulation, we could accurately localize the premotor brain structures of interest. The center of the pacemaker nucleus could be localized by detecting its field potential, which is phase-locked to the EOD, through an electrode barrel filled with 3 mol·l^{-1} NaCl. Electrolytic lesions were obtained by passage of a high-frequency current (Birtcher Hyfrecator, Model 733) through an indium-filled barrel whose tip was plated with platinum. Lesions were also used to histologically verify the site of the injection of pharmacological substances. Other electrode barrels could be filled with various substances, e.g., glutamate-receptor blockers for pharmacological testing. By employing two sets of electrodes simultaneously, with each set inserted at a specific location of the brain, we were able to explore the mutual functional dependence of such locations.

We applied pharmacological substances either iontophoretically or by pressure at the concentrations listed in Table 1. The artificial cerebrospinal fluid (aCSF) consisted of (mmol·l^{-1}): 124 NaCl, 2 KCl, 1.25 KH_2PO_4, 1.1 $MgSO_4$, 1.1 $CaCl_2$, 16 $NaHCO_3$ (L. Maler, pers. comm.; see Dye 1988). In most cases, more than one drug was tested in one fish, often using one drug as a control for the action of the other. Each drug was tested in several individuals.

We traced neuronal connections by extracellular, iontophoretic injections of neurobiotin (Vector Laboratories, 2% solution in 1 mol·l^{-1} KCl). Commonly, we used a multi-barrel electrode set with inner tip diameters of approximately 5 µm, one barrel filled with L-glutamate or 3 mol·l^{-1} NaCl for the physiological identification of the injection target, and another barrel filled with the neurobiotin solution. We filled the tip of this barrel by introducing a small volume of the neurobiotin solution, held between the tips of a fine forceps, into the wide end of the barrel and allowing the solution to travel along the micro filament into the tip of the capillary. This procedure ensured that no solution would invade the outside space between the barrels and thus contaminate areas along the track of the penetration. We then backfilled the electrode with 1 mol·l^{-1} KCl, leaving a small bubble between the neurobiotin solution in the tip and the KCl solution above. Electrode impedances were between 10 and 20 MΩ. While searching for the injection target, we commonly applied a negative backing current (approx. 1 µA) to the neurobiotin solution to avoid spillage. After localization of the target, we applied a positive injection current (1–2 µA) over a period of 10–60 min, depending upon the size of the

Table 1 Pharmacological agents tested

Substance	Carrier	Application	Supplier
L-glutamic acid	0.1 mol·l^{-1} in H_2O pH 8.0 (with NaOH)	− 0.2 to − 1 µA	Sigma
APV: D,L-2-amino-5-phosphonovaleric acid	50 or 500 µM in aCSF	pressure	Research Biochemicals Inc.
Bicuculline methiodide	20 mmol·l^{-1} in 165 mmol·l^{-1} NaCl; pH 3.2	+ 50 to + 100 nA	Sigma
CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione	25, 500 or 1000 µmol·l^{-1} in aCSF	pressure	Research Biochemicals Inc.
CPP: 3-(2-Carboxy piperazine-4-yl)-propyl-1-phosphonic acid	1 or 10 mmol·l^{-1} in aCSF	pressure	Research Biochemicals Inc
GABA: γ-amino-butyric acid	0.5 mol·l^{-1} in H_2O pH 3.5 (with HCl)	+ 50 to + 100 nA	Sigma

target to be labeled. Following the injection, we waited several minutes and then slowly retracted the electrode while applying negative backing current. Fish were allowed to survive between 3 and 24 h. They were then euthanized with MS-222 (tricaine-methane sulfonate) and perfused transcardially with saline followed by a solution of 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 mol·l^{-1} phosphate buffer (pH 7.4). Brains were postfixed overnight.

Brain sections were processed according to a protocol modified from procedures developed by Horikawa and Armstrong (1988), Huang et al. (1992), Lapper and Bolam (1991), and Vaney (1991). The following solutions were used: PBS (0.02 mol·l^{-1} PO_4 buffer, pH 7.2–7.4, and 0.9% NaCl), PBS-T (0.1% Triton X-100 in PBS), and TB (0.1 mol·l^{-1} TRIS buffer pH 7.2). Vibratome sections were cut at 50 µm thickness into PBS. They were then 'pre-bleached' by soaking for 10 min in 0.5% H_2O_2 in PBS to inhibit red blood cell staining in connection with the peroxidase reaction to be performed later.

Using a Vectastain ABC Elite kit (Vector Laboratories), an ABC solution was prepared (4 drops A + 4 drops B in 12 ml PBS-T) and stored for 30 min. Following bleaching, sections were washed 3 times for 10 min in PBS and incubated in the ABC solution in small, covered dishes at 4°C for 24 h. Sections were then washed 3 times for 10 min in PBS and once for 10 min in TB, then processed by the DAB (3,3'-diamino benzidine tetrahydrochloride) procedure: they were presoaked in a solution of 50 ml TB, 0.0008% nickel ammonium sulfate, and 0.1% DAB (2 × 25 mg tablets, Sigma). After 15 min, 30 µl of 3% H_2O_2 were added to this solution (final concentration = 0.0018%) and the sections incubated for 5–15 min, depending on the strength of the background label. Sections were then washed at least 3 times for 10 min in TB, mounted on subbed slides, counterstained with neutral red, and analyzed by light-microscopy.

Table 2 Anatomical abbreviations

cANS	commissura ansulata
C	cerebello-medullary cistern
CC	crista cerebellaris
Ccb	corpus cerebelli
CE	central n. of inferior lobe
CP	central posterior n.
cT	tectal commissure
cTS,d	commissure of the torus – dorsal subdivision
Dfl	n. diffusus lateralis
Dpn	dorsal posterior thalamic n.
Dtn	dorsal tegmental n.
Egp	eminentia granularis, pars posterior
eTS	efferents of the torus semicircularis
FR	fasciculus retroflexus
G	glomerular n.
Hc,v	hypothalamus, caudal and ventral subdivisions
LL	lateral lemniscus
LMRA	lateral mesencephalic reticular area
MLF	medial longitudinal fasciculus
MRF	mesencephalic reticular formation
nE↑,↓	n. electrosensorius – rise and fall subdivisions
nM	n. medialis
nMLF	n. of MLF
nI	n. isthmi
nRLI	n. recessus lateralis inferior
nsX	sensory vagal n.
PC	posterior commissure
PeG	periglomerular n.
Pgl,m	preglomerular n. – lateral and medial subdivisions
PPnC,G	prepacemaker n. – chirp and gradual subdivisions
Pn	pacemaker n.
R	red n.
Rd	raphé n. – dorsal subdivision
RF	reticular formation
RMT	rostral mesencephalic tegmental n.
SPPn	sublemniscal prepacemaker n.
SE	n. subelectrosensorius
TA	n. tuberis anterior
TeO	tectum opticum
TL	torus longitudinalis
TPP	periventricular n. of posterior tuberculum
Tsd,v	torus semicircularis – dorsal and ventral subdivisions
Tsl	torus longitudinalis
tSp-Cbl	spino-cerebellar tract
tTb	tectobulbar tract
V	ventricle
VCbl	lateral valvula cerebelli

Results

In the following sections we present the results of anatomical and physiological studies on the electromotor control circuit in *Apteronotus* (Fig. 2C, D) and compare them with the equivalent data for *Eigenmannia* (Fig. 2A, B). Figure 2C serves as a guide to the experimental detail of the following sections, while Fig. 2D emphasizes the electromotor links of the SPPn of *Apteronotus*, to be compared with those of *Eigenmannia* in Fig. 2B. First, we will describe the anatomical organization of the inputs to the pacemaker nucleus, followed by physiological evidence for the functional roles of these inputs in both the JAR and courtship behaviors.

The diencephalic and mesencephalic nuclei that mediate pacemaker modulations are small (<200 µm in diameter) and thus require a combination of physiological and stereotaxic methods to be localized for accurate placement of lesions or injections. By using a glass capillary with a tip diameter of a few µm filled with a solution of L-glutamic acid and by applying negative iontophoretic currents, one can stimulate neurons that project either directly or indirectly to the medullary pacemaker nucleus without stimulating fibers of passage. Most importantly, one can identify the nature of such nuclei from the kind of EOD modulations that are caused by their stimulation, and after one particular nucleus has been localized, others can readily be found by moving the electrode to specific locations relative to the first nucleus. We make use of this technique throughout the following experiments to locate our electrodes within the brain regions of interest.

Anatomy

Discrete extracellular injections of neurobiotin into the pacemaker nucleus of *Apteronotus* retrogradely label somata in two bilateral cell clusters, the diencephalic PPn and the mesencephalic SPPn (Fig. 3). The PPn of *Apteronotus* comprises two subnuclei (Kawasaki et al. 1988), the PPnG and PPnC. The PPnG appears as a ventro-lateral extension of the central posterior

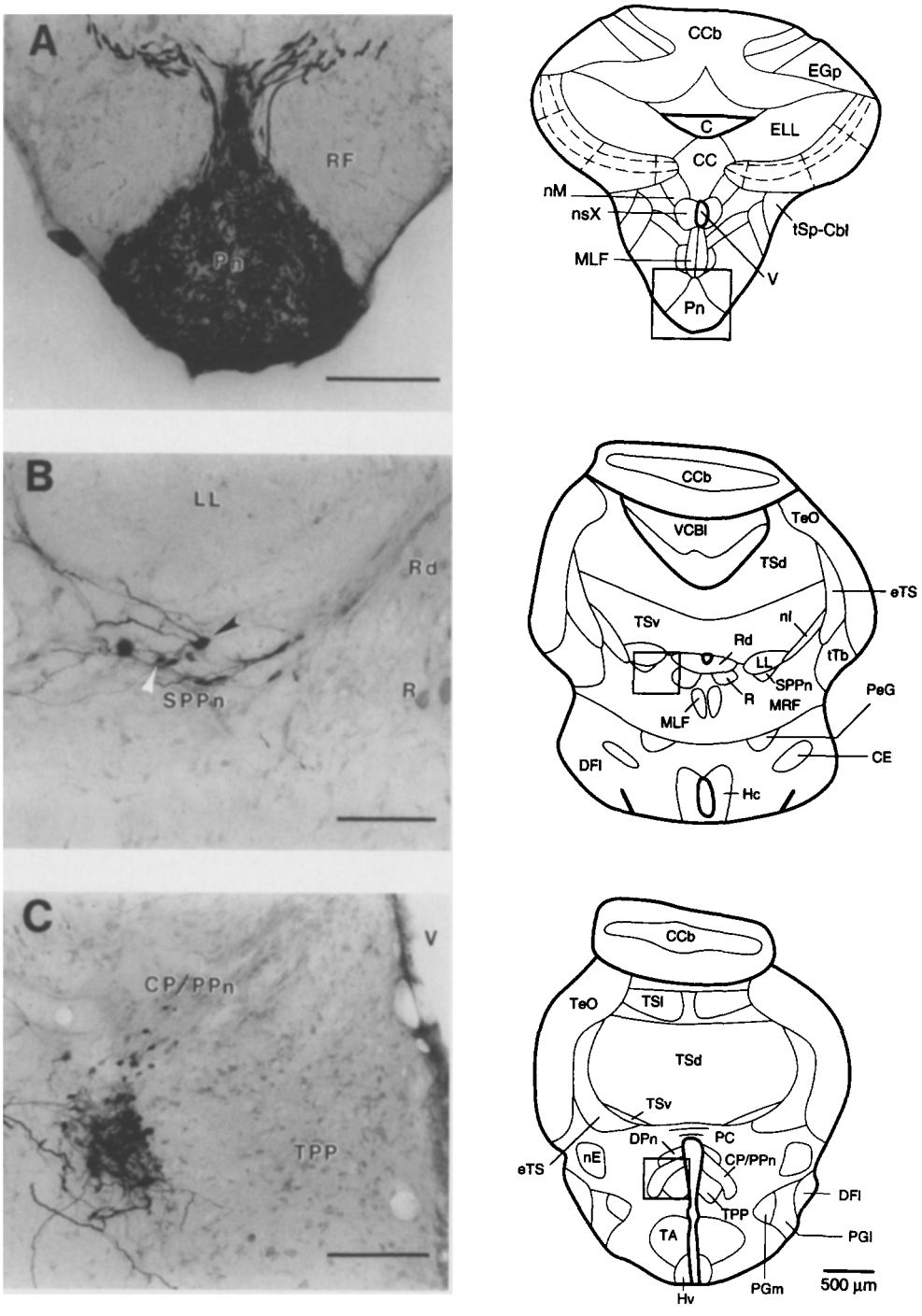


Fig. 3A–C Injection of neurobiotin into the medullary pacemaker nucleus. Photomicrographs of injection site and retrogradely labeled cell bodies with corresponding areas drawn on the right: **A**) Pacemaker nucleus in the medulla of *Apteronotus*. Large (4.3 µm diameter) relay cell axons exit the nucleus dorsally along the midline, and proceed bilaterally down the spinal cord to innervate electromotor neurons, which in *Apteronotus* constitute the electric organ; **B**) retrogradely labeled SPPn neurons with lateral extending dendrites (*white arrowhead* small cell; *black arrowhead* large cell); **C**) retrogradely labeled PPn neurons with extensive dendritic plexi (Kawasaki et al. 1988). Drawings correspond to levels − 6, + 15 and + 19 for **A**, **B** and **C**, respectively, of the brain atlas for *Apteronotus* (Maler et al., 1991). *Scale bar* for photomicrographs = 200 µm in **A**, 100 µm in **B** and **C**

nucleus of the thalamus [CP; see also Zupanc and Zupanc (1992); Zupanc and Heiligenberg (1992)], containing small round cells. Slightly caudal and ventral to the PPnG lies a collection of large multipolar cells with broadly spreading dendrites, the PPnC. We restrict the further description of pacemaker afferents to those arising from the SPPn, since a more detailed anatomical examination of the PPn in *Apteronotus* has been published previously (Kawasaki et al. 1988).

The SPPn is a bilateral cluster of cells located immediately ventral to the lateral lemniscus (Fig. 3) at the transverse level of the red nucleus, and is similar to that found in other gymnotiform genera (Keller et al. 1991; Kennedy and Heiligenberg 1992; Metzner 1993). The rostro-caudal extent of retrogradely labeled somata in the SPPn is only 150 µm, comprising approximately 15 cells/side. We can recognize two distinct cell types in approximately equal proportions: larger, round cells

(ca. 18 μm in diameter) and smaller spindle-shaped cells (15 μm × 4 μm). These give rise to two sizes of axons that exit the SPPn ventro-medially (ca. 3 and 1 μm in diameter, measured with oil immersion optics). Most, but not all, axons appear to decussate through the commissura ansulata. We cannot segregate by size the axons that do or do not cross the midline and the functional significance of these crossed fibers is unclear.

Both classes of SPPn cells give rise to dendrites that expand into several different regions. A broad lateral field extends rostrally to the caudal pole of the nE↑. A latero-dorsal field courses medial of the nucleus isthmi towards the paralemniscal nucleus. More medially, there are fibers that sweep dorso-medially in a tight bundle and run between the ventral torus and the dorsal raphe. Some of these fibers then extend rostrally to the periventricular pretectal nucleus, which is known to project to the torus semicircularis (Carr et al. 1981; Carr and Maler 1986; Keller et al. 1990). Other fibers in this medial bundle are probably commissural axons which project to the contralateral SPPn and perhaps axons from the CP/PPn.

Following a focal nE↑ injection a single large SPPn neuron and its axon were fortuitously labeled, probably by uptake of neurobiotin by its dendrites (Fig. 4). This cell's axon crosses the midline through the commissura ansulata and bifurcates before entering the pacemaker nucleus, with one collateral remaining contralateral to its cell body of origin and the other recrossing the midline to innervate the other half of the midline pacemaker nucleus. By comparing the axonal trajectory of this single SPPn cell as well as unilateral SPPn injections versus unilateral PPn injections of neurobiotin, it is clear that the SPPn axons occupy an only partially overlapping trajectory with those arising from cell bodies in the diencephalic PPn. At the mesencephalic/rhombencephalic junction, the PPn axons course caudally in a more ventral and lateral position right along the base of the brain, approximately 400 μm from the midline, whereas the SPPn axons are situated more medial and approximately 100 μm more dorsal to the PPn axons. Moreover, whereas the PPn axons are exclusively uncrossed until the level of the pacemaker nucleus, most SPPn axons cross through the commissura ansulata. The final innervation of the pacemaker nucleus from both the PPn and the SPPn is distributed throughout the entire nucleus, although there is a segregation of various inputs onto pacemaker or relay cell types as in other gymnotiform genera [review: by Metzner and Viete (1996)].

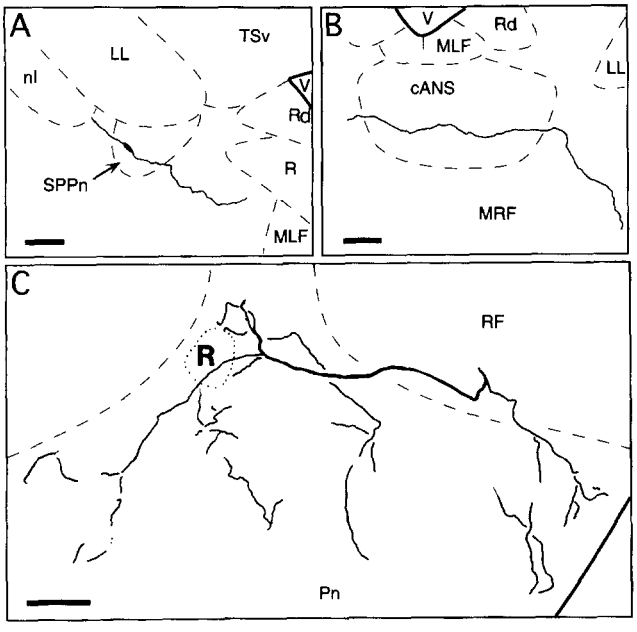


Fig. 4A–C Camera lucida reconstruction of a single SPPn cell and its axon following an injection of neurobiotin into the nE↑: **A** a single large SPPn cell sends an axon which turns medial (reconstructed over 350 μm from transverse sections) and **B** decussates caudal to its cell body through the commissura ansulata. The axon then courses caudally and bifurcates prior to entering the pacemaker nucleus. (Axon reconstructed over 500 μm.); **C** reconstruction of the axonal arbor in the pacemaker nucleus. The axon enters the nucleus contralateral to the cell body and bifurcates to innervate both sides of the midline nucleus. (Axon reconstructed over the entire pacemaker nucleus)

The SPPn and PPn both appear to be connected with many other diencephalic and telencephalic nuclei (Wong 1995; C. Wong, C. Keller and W. Metzner unpublished observations). In the present study we present only those connections as they relate directly to the JAR circuit and in which connectivity could be confirmed by reciprocally placed injections. Unilateral iontophoretic injections of neurobiotin centered on, but not necessarily confined to the SPPn (Fig. 5A) label cells and terminals in the contralateral SPPn (Fig. 5B). Cells projecting to the contralateral SPPn appear similar to the smaller spindle cells that are retrogradely labeled following pacemaker nucleus injections. It is possible that this reciprocal connection may serve to coordinate activity between both SPPn's. In other gymnotiform genera, the SPPn projects exclusively to the relay cells of the pacemaker nucleus [*Sternopygus:* Keller et al. (1991); *Eigenmannia:* Metzner (1993); *Hypopomus:* Kennedy and Heiligenberg (1994)]. In *Apteronotus*, however, although the innervation was primarily onto relay cells (Fig. 5C), an occasional pacemaker cell was also seen to be innervated .

Previous studies in *Eigenmannia* suggested that the PPn and the SPPn comprise independent pathways for control of the pacemaker nucleus (Metzner 1993). In *Apteronotus* there may be more interaction between PPn and SPPn. Unilateral injections of neurobiotin in SPPn frequently retrogradely labeled the PPnG and the medially contiguous CP bilaterally though mostly ipsilaterally (Fig. 6A). Zupanc and Zupanc (1992) and Zupanc and Heiligenberg (1992) have argued for the

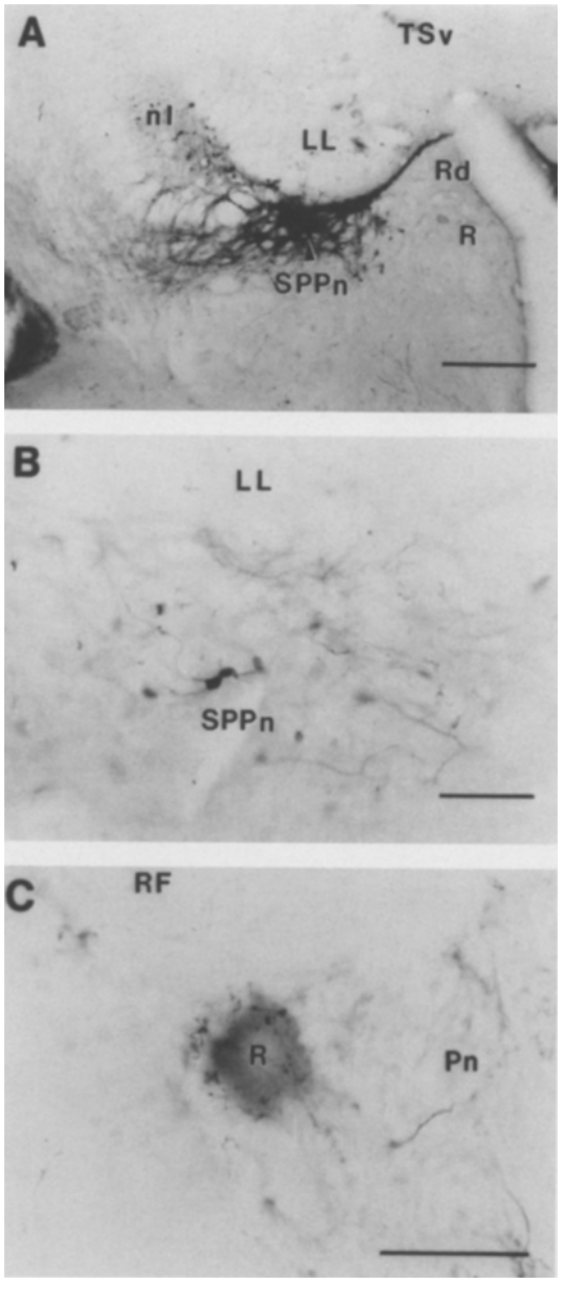


Fig. 5A–C Injection of neurobiotin into the SPPn: **A** low power photomicrograph of injection site. A tight bundle of axons consisting of some commissural fibers (*arrow*) courses dorso-medially between the dorsal raphe and the ventral torus semicircularis. *Scale bar* 200 μm; **B** Contralateral side to A with retrogradely labeled cell bodies and terminal fields heavily labeled. The commissural cells are smaller and more spindle shaped. Scale bar 50 μm; **C** anterogradely labeled terminals in the pacemaker nucleus following SPPn injection. Note clustering of putative synaptic terminals around the relay cell. *Scale bar* = 50 μm

developmental continuity of the CP and medial PPn (PPnG) and thus, although we label this area CP/PPn in our figures, we shorten this designation to PPnG throughout the text unless specifically referring to the putative connection described here. These retrogradely labeled cell bodies are round and relatively small (4 μm × 7 μm). Corroborating this new connection, injections into CP/PPn produced extensive bilateral label in the area where SPPn cells are located. These putative CP/PPn axons course medio-dorsal before turning and heading caudal from PPn, along the nucleus of the medial longitudinal fasciculus. At the rostral pole of the dorsal raphe this axon bundle splits off and courses bilaterally between the lateral lemniscus and the lateral edge of the dorsal raphe prior to innervating both SPPn's. Currently, we have no physiological evidence for this connection as lesions of the SPPn do *not* affect stimulation of PPnG. This connection may represent a unique feature of *Apteronotus*, since a similar connection in *Eigenmannia* has not been identified (C. Wong unpublished observations).

The nE of *Eigenmannia* is known to contain subnuclei that either respond to specific communication signals or cause, upon stimulation, specific frequency shifts of the pacemaker (Keller and Heiligenberg 1989; Keller et al. 1990; Heiligenberg et al. 1991). Preliminary comparative studies by Keller (1989) in *Apteronotus* had suggested a functional organization of the nE similar to that in *Eigenmannia*. A relatively large cluster of cells within the nE is retrogradely labeled by injections of neurobiotin centered within the PPn (Fig. 7A). This cluster is located approximately 150 μm dorsal and 800 μm lateral to the area of maximum response within the PPnC and occupies a position similar to the nE↑ in *Eigenmannia*. In contrast, neurobiotin injection into the SPPn retrogradely labels a different group of cells within the complex of the nE (Fig. 7C). This cell group is labeled primarily ipsilaterally and located slightly rostral, ventral and medial to the cell group retrogradely labeled after PPn injections. Injections of neurobiotin to this area of the nE labels terminals in the SPPn, thus confirming this connection. Based upon topography and connectivity, these two subdivisions of the nE that connect to the PPn and SPPn, respectively, appear very similar to the nE↑ and nE↓ of *Eigenmannia*. Further physiological evidence, detailed below, confirms this designation. We therefore refer to these two subregions of the nucleus electrosensorius in *Apteronotus* as the nE↑ and nE↓.

In summary, the premotor elements for controlling the JAR of *Eigenmannia* appear to be present and generally similar in connectivity and topography in *Apteronotus*. We do note some differences, however, in the organization of the SPPn of *Apteronotus* (compare Figs. 2B and 2D): first, two cell types are present whereas only one cell type is found in the SPPn of *Eigenmannia*. Second, there is a reciprocal connection between the left and right SPPn in *Apteronotus*. Finally, the SPPn receives an afferent projection from the CP/PPn in *Apteronotus*.

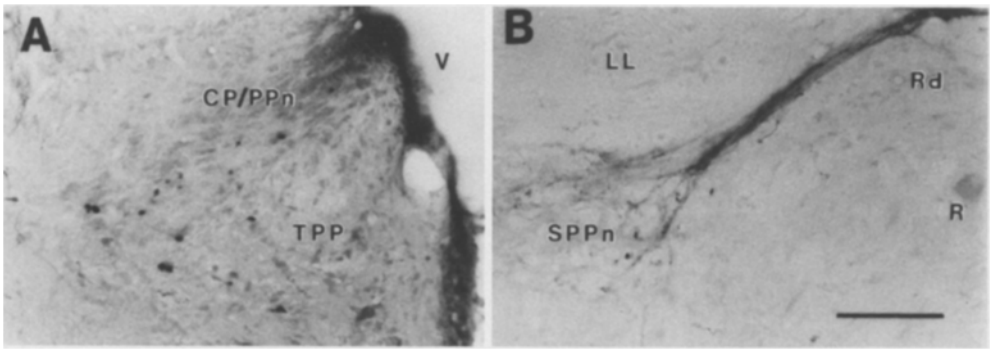


Fig. 6A, B Putative connection between the CP/PPn and the SPPn. **A** retrogradely labeled cells in CP/PPn following injection of neurobiotin into the SPPn. Same case as in Fig. 4; **B** anterogradely labeled fibers and putative terminals in the ipsilateral SPPn following an injection of neurobiotin into CP/PPn. Extensive label is also present in the contralateral SPPn (not shown). *Scale bars* = 100 μm

Physiology

The nE↑-PPnG-Pn pathway

In *Eigenmannia* the nE↑-PPnG-Pn pathway serves to raise the EOD frequency during jamming with negative Dfs (Kawasaki et al. 1988; Keller and Heiligenberg 1989; Metzner 1993). Stimulation of either the nE↑ or the PPnG with L-glutamate causes gradual EOD frequency rises. Bilateral lesion of either nucleus results in the fish's inability to raise its EOD frequency above the resting level. Below, we describe a number of experiments designed to ascertain whether the anatomically similar pathway in *Apteronotus* fulfills the same functional role for the JAR.

The nE↑ of *Apteronotus* (Fig. 8) is located 700–800 μm lateral, 50–150 μm caudal, and approximately 200 μm dorsal to the center of the PPnC. As in *Eigenmannia*, iontophoretic stimulation of the nE↑ with L-glutamate causes a brisk rise of the pacemaker frequency (Fig. 8A, top trace) which, in contrast to the case in *Eigenmannia*, is sometimes superimposed with chirp-like transients (Fig. 8B, top trace). The effects of stimulation can only be elicited in an area of no more than 100–150 μm diameter, centered slightly caudal to the somata of the nE↑.

The nE↑ projects to the PPnG but not to the SPPn. As this would suggest, the effects of nE↑ stimulation are eliminated after an electrolytic lesion of the ipsilateral PPnG (Fig. 8B) but are immune to bilateral electrolytic lesions of the SPPn (Fig. 8A).

Earlier stimulation and lesion experiments in *Eigenmannia* (Keller and Heiligenberg 1989) had shown that the nE↑ is necessary for mediating the rise of the fish's pacemaker frequency above its resting value within the context of the JAR. These findings, and the observation that stimulation of these subnuclei in *Apteronotus* caused similar frequency shifts as in *Eigenmannia*, suggested that lesions of the nE↑ would impair the JAR in *Apteronotus* in a similar manner. To our surprise, however, electrolytic lesions of the nE↑ did not affect the JAR (Fig. 9). Instead, over a few minutes following the lesion of this area, the JAR often increased in size and then returned to its pre-lesion level as if the lesion current had only excited nearby pathways involved in the JAR. Therefore, we conclude that the JAR of *Apteronotus*, contrary to that of *Eigenmannia*, does not require the nE↑.

In *Eigenmannia* stimulation of the nE↑ raises the pacemaker frequency through activation of the PPnG (Fig. 2A; Metzner 1993). Kawasaki and colleagues (1988) have shown that in *Apteronotus*, as in *Eigenmannia*, stimulation of the PPnG causes a gradual rise in EOD frequency, whereas stimulation of the PPnC causes chirp-like EOD modulations similar to ones seen during courtship and aggression. Thus, we expected the PPnG to mediate EOD frequency rises during the JAR in *Apteronotus* as well.

In *Apteronotus*, however, even complete, bilateral electrolytic lesions of the PPn that also include areas adjacent to the PPn, leave the JAR unaffected (Fig. 10). This further supports the conclusion that the nE↑, which is linked to the pacemaker by way of the PPnG, is not necessary for performance of a complete JAR. The lesions shown in Fig. 10 also included much, if not all, of the PPnC thus accounting for the loss of chirping seen in the bottom trace. Small portions of the CP were spared, but fibers connecting these areas with the SPPn pass through the lesioned area and thus did not survive the lesion.

These results lead us to conclude that the nE↑-PPnG-Pn pathway is not necessary for performance of the JAR in *Apteronotus*. We present evidence below, however, that shows this pathway to be active during jamming, but with a selectivity for the sign of Df that is opposite to that of the JAR.

The nE↓-SPPn-Pn pathway

In *Eigenmannia* the nE↓-SPPn-Pn pathway mediates EOD frequency falls during jamming with positive Dfs

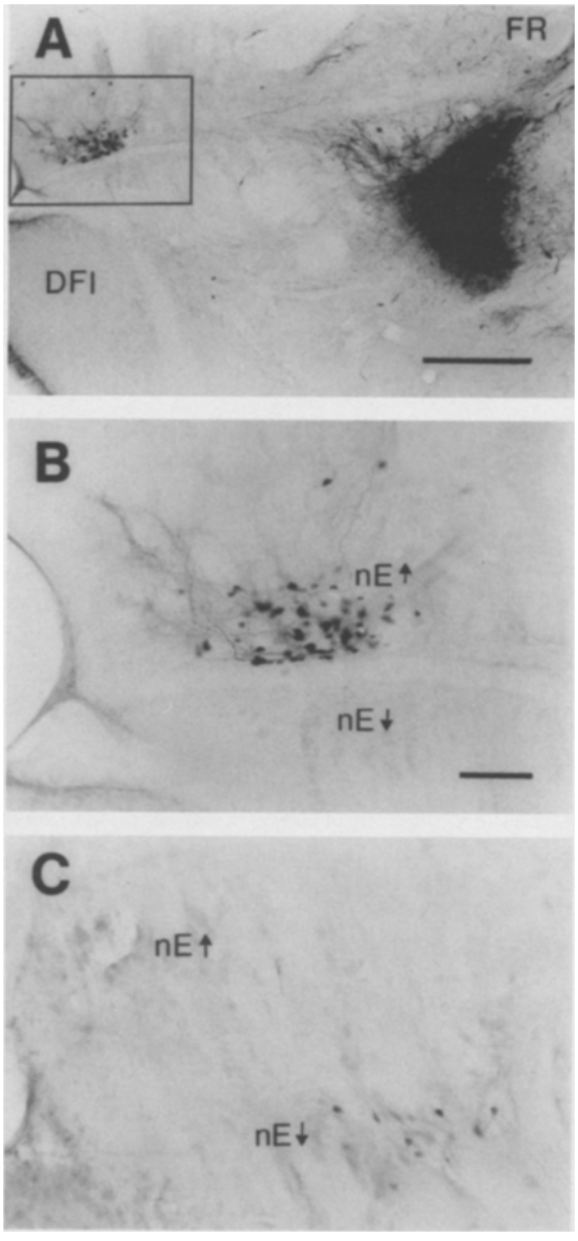


Fig. 7A–C Retrogradely labeled cells in the nucleus electrosensorius. **A** low power photomicrograph of retrogradely labeled cells in the nE↑ following an injection of neurobiotin focused on CP/PPn. *Scale bar* 250 μm; **B** high power micrograph of **A** showing the cells of the nE↑. *Scale bar* 50 μm; **C** high power micrograph of retrogradely labeled cells in the nE↓ following injection of neurobiotin into the SPPn. Approximately same rostro-caudal level as **A** and **B**. Same magnification as in **B**. Corresponds to level + 18 in Maler et al. (1991)

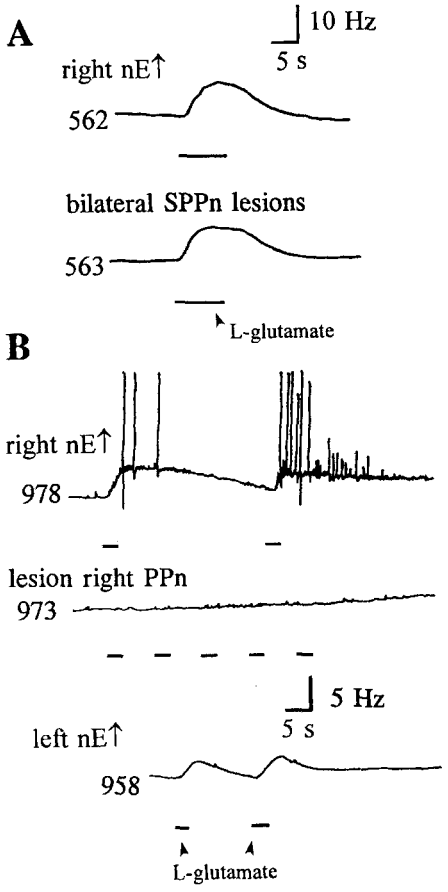


Fig. 8A B nE↑ stimulations with L-glutamate. **A** bilateral lesion of the SPPn does not alter the effects of nE↑ stimulation. L-glutamate was applied to the right nE↑ by localized iontophoresis (*bars* beneath each frequency trace indicate 10-ms periods of iontophoresis). *Top trace*: EOD frequency rise elicited with both SPPn intact. *Lower trace*: response elicited after bilateral electrolytic SPPn lesion; **B** in a different fish, lesion of the ipsilateral PPn eliminated the response to nE↑ stimulation. L-glutamate was first applied to the right nE↑ (*top trace*, 3 ms applications) eliciting gradual frequency rises as well as chirps (seen as transient deflections of the frequency trace). After electrolytically lesioning the right (ipsilateral) PPn, stimulation of the right nE↑ with L-glutamate was no longer effective. L-glutamate application to the left nE↑ (contralateral, *bottom trace*) still evoked gradual frequency rises. Presentation follows that of Fig. 1

(Keller and Heiligenberg 1989; Metzner 1993). The SPPn provides tonic excitation to the pacemaker, resulting in a resting EOD frequency that can be lowered by suppression of SPPn activity. Activation of the nE↓ accomplishes this via GABA-mediated inhibition of the SPPn. The nE↓ of *Eigenmannia* can readily be located by iontophoresis of L-glutamate, whereas the SPPn can be localized by iontophoresis of GABA. Either application results in a prompt lowering of the fish's EOD frequency.

Although iontophoretic stimulation of the *Apteronotus* nE↓ with L-glutamate causes a small drop of the pacemaker frequency, these responses are not very robust. Much stronger and far more reliable depressions of the pacemaker frequency can be obtained by electrical stimulation of the rostral portion of the nE complex, which is heavily invaded by dendrites of the nE↓, or by stimulation of the toral efferent fiber tract that lies between the torus and tectum and supplies input to the nE complex among other targets. While this electrical stimulation might act by way of different (as yet undescribed) pathways, we tentatively ascribe

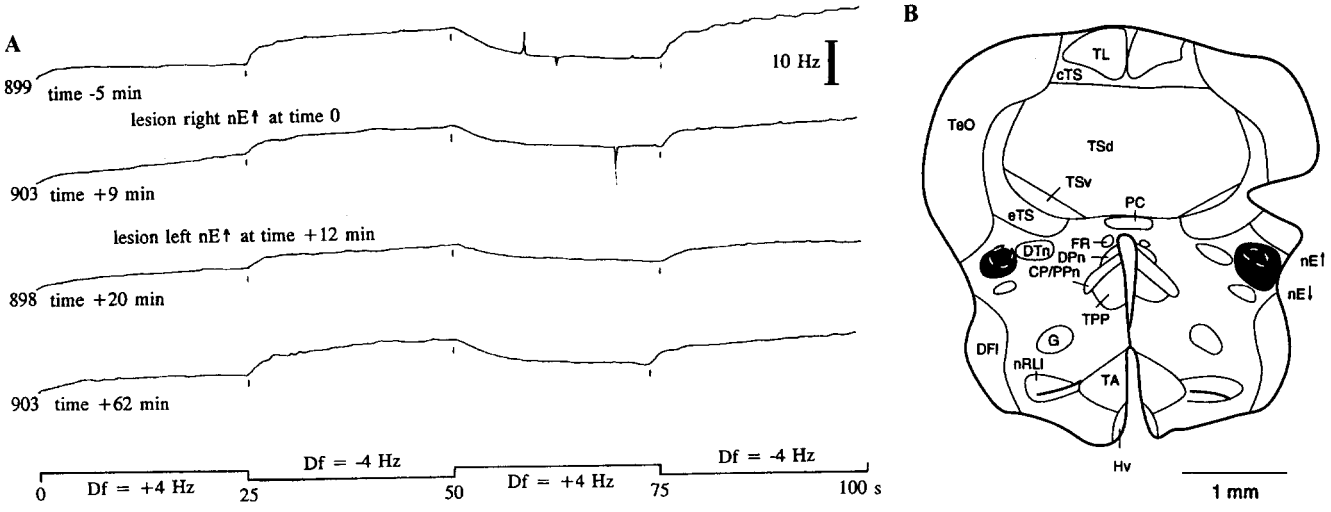
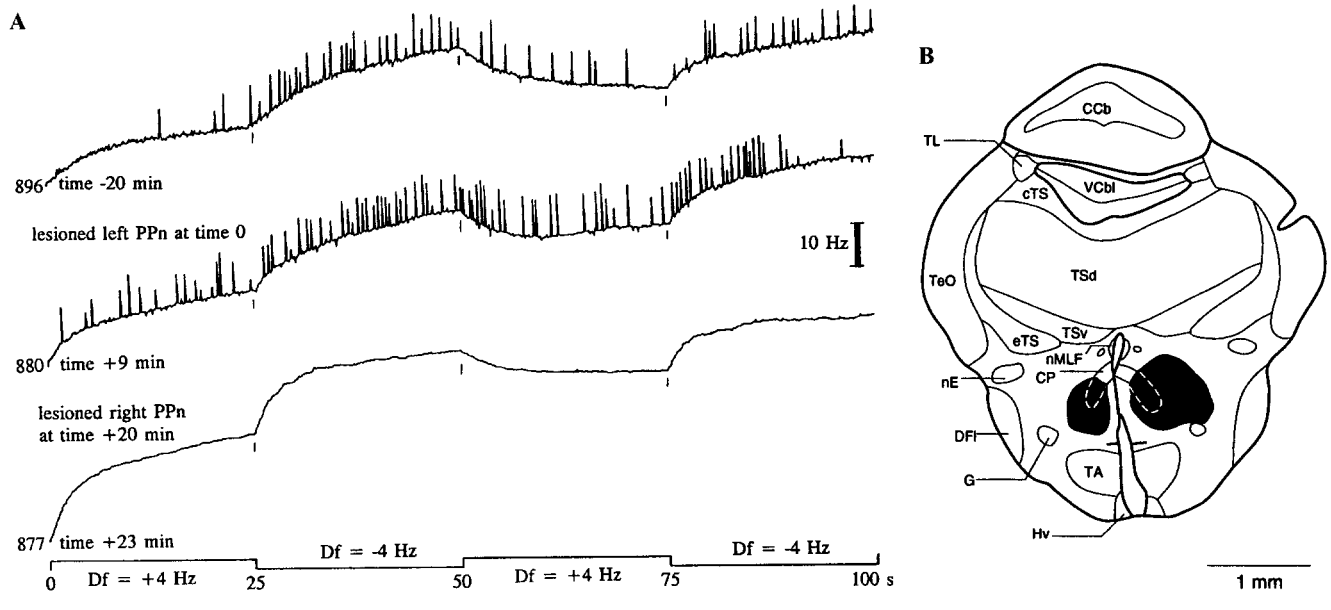


Fig. 9A, B Bilateral lesions of the nE↑: **A** the JAR and a relatively weak non-selective response both remain intact after bilateral electrolytic lesioning of the nE↑. The sign of the frequency difference, Df, was switched every 25 s as indicated by the *tick marks* underneath each trace. At the beginning of each trace the time relative to the first lesion and the initial EOD frequency (Hz) are given. The intact fish (with an indium electrode already positioned in the right nE↑, *top trace*) responds to the onset of the stimulus with a frequency rise. A switch to a negative Df then causes a further rise in frequency, while a switch to a positive Df initiates a return towards the baseline frequency. Neither these Df sign-dependent changes in EOD frequency, nor the non-selective response are greatly affected by lesioning the nE↑ bilaterally (*lower traces*). Transient upward or downward deflections of the frequency trace reflect 'chirps' elicited by the presence of the jamming stimulus. Presentation follows that of Fig. 1; **B** Camera lucida drawing of a transverse section of the brain showing the lesioned areas (*black*) within the nE↑ (*dashed*). Corresponds to levels + 18 to + 19 in Maler et al. (1991)

these responses to activation of the nE↓. We therefore utilized the electrical stimulation of the rostral nE rather than chemical stimulation of the nE↓ itself in all physiological studies described below.

Fig. 10A, B Bilateral lesions of the PPn do not affect performance of the JAR. **A** neither the JAR, nor the non-selective response are reduced by electrolytically lesioning the PPn bilaterally (*bottom trace*). This fish chirped in response to the jamming stimulus, but chirps were eliminated by the lesions, which included most or all of the portion of the PPn that drives chirping behavior (PPnC). Presentation follows that of Fig. 1; **B** camera lucida drawing of a transverse section of the brain showing the areas of the PPn that were lesioned. Approximately equivalent to levels + 17 to + 18 of Maler et al. (1991)



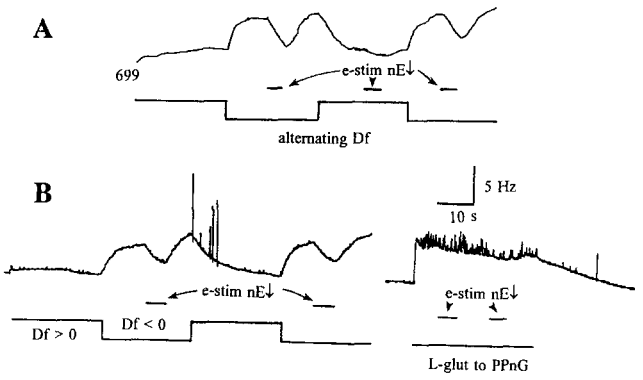


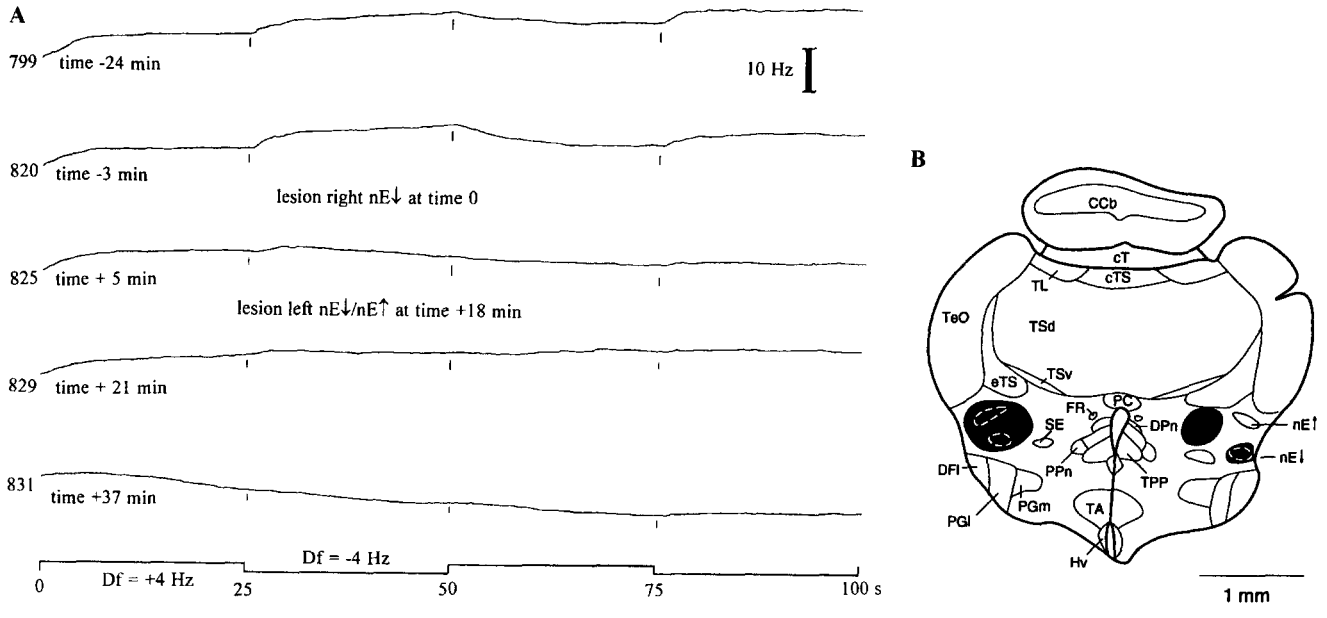
Fig. 11 **A** Electrical stimulation of the nE↓ lowers the EOD frequency after it has been raised in response to a jamming stimulus. With -Df stimuli, electrical stimulation of the nE↓ elicits relatively large frequency falls. With + Df stimuli, when the EOD is raised only slightly above the resting level, nE↓ electrical stimulation results in only a minimal frequency drop. nE↓ electrical stimulation is ineffective when no jamming stimulus is present (not shown). **B** An electrode containing L-glutamate was placed into the fish's right PPnG, while an indium electrode was located within the right nE↓ for electrical stimulation. Electrical stimulation of the nE↓ elicited the usual frequency falls when the EOD had been raised by jamming with -Dfs (*left traces*), but was ineffective when the EOD was raised to about the same level by L-glutamate stimulation of the PPnG. Presentation follows the conventions of Fig. 1

In contrast to stimulation of the nE↓ in *Eigenmannia*, which lowers the fish's pacemaker frequency below its resting level, electrical stimulation of the putative nE↓ in *Apteronotus* only lowers the fish's pacemaker frequency after it has been raised sufficiently above its resting level, i.e., the pacemaker frequency can never be driven below its resting level (Fig. 11A; Keller 1989). This conforms to the observation that the JAR of *Apteronotus* also fails to drive the fish's pacemaker frequency below its resting level in response to positive Dfs (Fig. 1; Bullock et al. 1972). We conclude from these observations that the SPPn of *Apteronotus*, in contrast to that of *Eigenmannia*, provides little, if any, tonic excitation to the pacemaker nucleus.

In the absence of a jamming stimulus, the SPPn will normally remain quiescent and thus electrical stimulation of the nE↓ is necessarily ineffective. Direct stimulation of the PPn raises the pacemaker frequency independently of the nE↓-SPPn-Pn pathway and should not be affected by nE↓ stimulation. Figure 11B demonstrates that raising the pacemaker frequency by L-glutamate stimulation of the PPn is indeed unaffected by electrical stimulation of the putative nE↓.

In *Eigenmannia* bilateral lesion of the nE↓ eliminates the fish's ability to lower its discharge frequency below the resting level (Keller and Heiligenberg 1989). The fish is still able to perform a limited JAR, however, by raising and lowering its frequency within a range above the resting level if the nE↑-PPnG-Pn pathway is left intact. In contrast to these results, bilateral lesion of the nE↓ in *Apteronotus* totally eliminates the JAR (Fig. 12). However, because accurate placement of the lesioning electrode was difficult using electrical stimulation of the nE↓, we were only successful in selectively and bilaterally lesioning the nE↓ in two fish. In each of these

Fig. 12A, B Bilateral electrolytic lesions of the nE↓. eliminate the JAR: **A** the resting EOD frequency was quite variable over the course of this experiment, but observations from another fish showed that electrolytic elimination of the nE↓ need not be accompanied by a rise in pacemaker frequency. Presentation follows the conventions of Fig. 1; **B** camera lucida drawing of a transverse section of the brain showing the areas of the nE that were lesioned. Approximately equivalent to level + 18 in Maler et al. (1991)



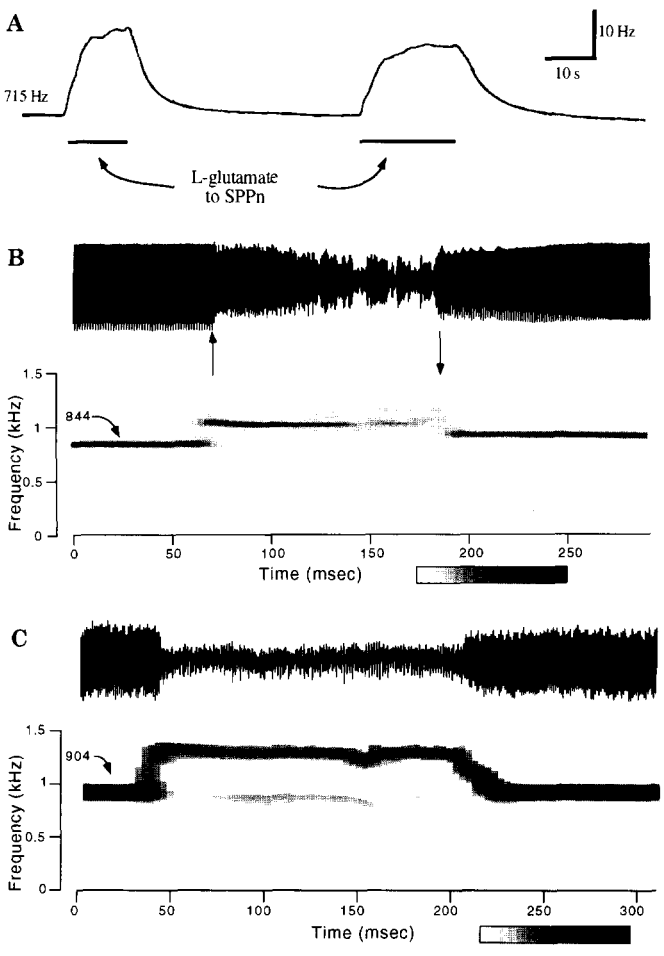


Fig. 13A–C SPPn stimulations and EOD interruptions. **A** L-glutamate stimulation of the SPPn causes a brisk rise in EOD frequency. Presentation follows the conventions of Fig. 1; **B** *upper trace* (voltage versus time): EOD-interruption elicited by electrical stimulation of the SPPn (stimulus was present for the period indicated by the *upward-* and *downward-pointing arrows*). During the interruption, the regular EOD pattern is replaced by a weaker and highly irregular signal caused by the desynchronized activity of the spinal electromotor neurons. *Lower trace*: frequency spectrogram covering the same time interval (see Materials and Methods). Frequency is represented on the vertical axis and linear amplitude is coded by the *gray scale*. The normal EOD has a frequency peak at 844 Hz that is lost during the EOD-interruption; **C** EOD-interruption of a courting male *Apteronotus*. Voltage-versus-time trace recorded by a pair of differential electrodes placed within the male's territory. Small variations in the peaks of the regular EOD pattern are due to insufficient sampling and movement by the fish. Presentation follows that of part B

fish, portions of the nE↑ were also lesioned unilaterally, but since we have already shown that bilateral nE↑ lesions do not affect the JAR, we interpret these results as being due solely to the bilateral loss of the nE↓. Additionally, in several control fish, the JAR was unaffected when we lesioned larger areas of the nE complex within a few hundred micrometers of, but sparing, the nE↓. Because the entire JAR was eliminated by lesioning the nE↓ bilaterally, we conclude that both the rising and falling phases of the *Apteronotus* JAR are mediated by the nE↓.

The SPPn of *Apteronotus* can be localized by iontophoresis of L-glutamate approximately 700–800 μm caudal and 300–400 μm dorsal to the center of the PPnC. Stimulation of the SPPn with L-glutamate causes a brisk rise of the EOD frequency of up to about 20 Hz (Fig. 13A). With weak electrical stimulation, and rarely by iontophoresis of L-glutamate, this acceleration may lead to a prolonged interruption of the EOD, leaving a weak, broad-band remnant signal (Fig. 13B). It is possible that L-glutamate stimulation may be ineffective in synchronously activating the widely spreading dendrites of the *Apteronotus* SPPn. EOD-interruptions can only be elicited by electrical stimulation within about 100–150 μm of the anatomical center of the SPPn. Thus, although the site of activation is less certain than for responses that can be reliably elicited by stimulation with L-glutamate, it seems reasonable to conclude that the observed EOD-interruptions are indeed due to SPPn activation. Additionally, in two related genera [*Sternopygus:* Keller et al. 1991; and *Hypopomus:* Kawasaki and Heiligenberg (1989)] L-glutamate stimulation of the SPPn results in relatively small frequency rises that are usually coupled with an EOD-interruption similar to those shown in Fig. 13 (see Discussion). These responses to stimulation of the SPPn contrast with findings from *Eigenmannia*, in which EOD modulations can only be obtained by inhibition of the SPPn by, for example, GABA iontophoresis. EOD-interruptions similar to those elicited by putative SPPn stimulation in *Apteronotus* are sometimes produced during intensive courtship and spawning (Fig. 13C; Hopkins 1974; Hagedorn and Heiligenberg 1985).

As in *Eigenmannia* the iontophoretic application of GABA to the SPPn can mimic the effects of stimulating the nE↓ (Fig. 14). In *Apteronotus*, however, GABA application results in a lowering of the EOD frequency, but only if the EOD frequency has been raised above the resting level by, for example, the presence of a jamming stimulus. The role of GABA in the inhibition of the SPPn by the nE↓ can also be demonstrated by applying bicuculline to the SPPn. The application of bicuculline, which blocks GABA-A type receptors, has two, possibly related, effects (Fig. 15): It suppresses the inhibition normally caused by stimulation of the nE↓, while also enhancing the frequency rise occurring in response to jamming with negative Dfs. We attribute the frequency falls seen in response to jamming with positive Dfs to activation of the nE↓ in a manner similar to the activation produced by our electrical stimulations of the putative nE↓. Thus, the SPPn appears to be under a tonic GABA-ergic inhibition that may play a large role in determining the change in pacemaker frequency during jamming with either sign of Df.

The SPPn of *Apteronotus* thus appears to be normally quiescent, but to be activated during jamming. Such

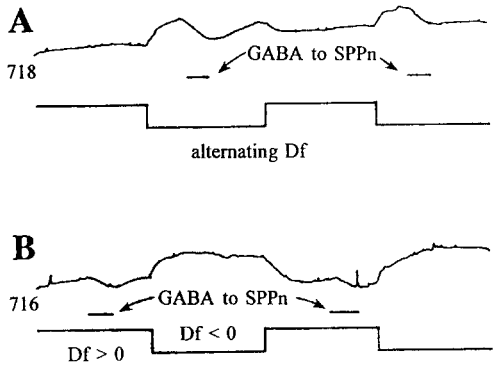


Fig. 14A, B Application of the inhibitory neurotransmitter GABA to the SPPn elicits EOD-frequency falls similar to those seen with stimulation of the nE↓: **A** GABA application during jamming with + Dfs leads to large falls in frequency; **B** GABA application during jamming with negative Dfs elicits smaller falls in frequency. The magnitude of this frequency fall is limited by the extent of the frequency rise above the resting level during the non-selective response. Conventions follow those of Fig. 1

activation could result from excitatory input to the SPPn during jamming with either sign of Df, and/or from a relaxation of the GABA-ergic inhibition, discussed above, that normally suppresses an intrinsically high level of SPPn activity. The source or sources of possible excitatory input is not yet known, but one candidate is the CP/PPn to SPPn projection described above. To explore this possibility, we stimulated the PPnG with L-glutamate to cause a smooth rise of the pacemaker frequency, and assessed this effect before and after lesions of the SPPn. Since bilateral lesions of the SPPn did not alter the effect of PPnG stimulations upon the pacemaker frequency, and since bilateral PPn lesions do not affect performance of the JAR (Fig. 10), we conclude that the CP/PPn to SPPn projection is not necessary for performance of the JAR.

Following bicuculline application to the SPPn, EOD frequency falls formerly elicited by electrical stimulation of the nE↓ were replaced by large, rapid frequency rises (Fig. 15, traces at + 9, + 11, + 28 min). These rises may be due to recruitment of passing fibers or of SPPn dendrites that lay close to the nE↓ by our use of electrical stimulation. They may, alternatively, indicate a heretofore unknown excitatory pathway from the nE complex to the SPPn, but we have no further evidence for the existence of such a pathway. Rises such as these may also occur spontaneously (Fig. 15, trace at + 9 min; arrowheads) and are similar to those elicited by direct SPPn stimulation. Similar frequency rises were termed 'yodels' by Dye (1987) and were observed to occur following the offset of long periods of jamming. Thus, GABA-ergic inhibition of the SPPn may also suppress SPPn excitations that cause yodels.

In *Eigenmannia* bilateral lesioning of the SPPn lowers the fish's resting EOD frequency and eliminates frequency falls below this new level (Metzner 1993). The fish is still able to perform a limited JAR, much as

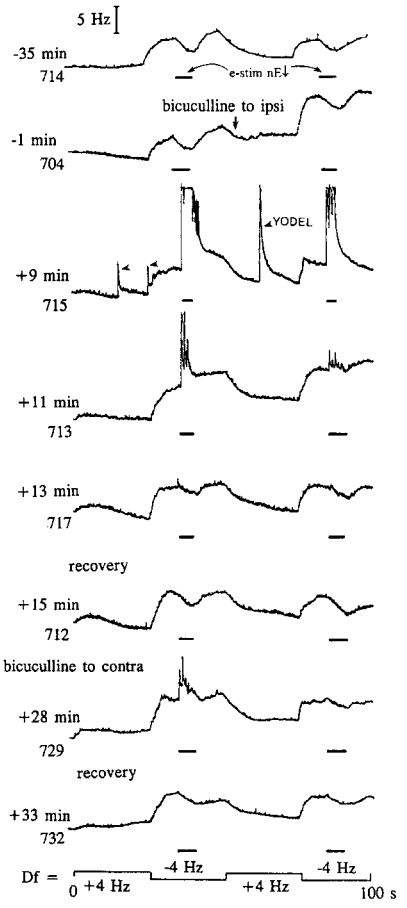


Fig. 15 Application of bicuculline to the SPPn suppresses its inhibition by the nE↓. Eight JAR records show the effects of bicuculline applied first to the ipsilateral SPPn (from the arrow at time 0, for 3 min), and then to the contralateral SPPn (from time + 25 for 2 min). *Bars* under each trace indicate electrical, unilateral stimulation of the nE↓, which normally causes a drop in EOD frequency, if presented during jamming with a negative Df (traces at times − 35, − 1, + 13, + 15, + 33 min). With bicuculline application, however, stimulation of the nE↓ no longer lowers the EOD frequency but instead causes strong rises (traces at times + 9, + 11, + 28 min). Moreover, similar rises (also known as 'yodels', *arrowheads* in trace at time + 9 min) occur even without nE↓ stimulation. Conventions follow those of Fig. 1

following nE↓ lesions, that consists of frequency rises and relaxations back to the new resting level. In contrast, bilateral electrolytic lesions of the SPPn of *Apteronotus* eliminate the entire JAR instantly and permanently (Fig. 16). The resting EOD frequency of *Apteronotus* is highly variable during the course of our experiments, but despite this variability, and in contrast to the results from *Eigenmannia*, there is no (consistent) EOD frequency drop following these lesions. This suggests that, in contrast to the SPPn of *Eigenmannia*, the SPPn of *Apteronotus* provides little or no tonic excitation to the pacemaker.

The SPPn is so named because it lies directly beneath the lateral lemniscus, which is the pathway for all ascending electrosensory input to the torus

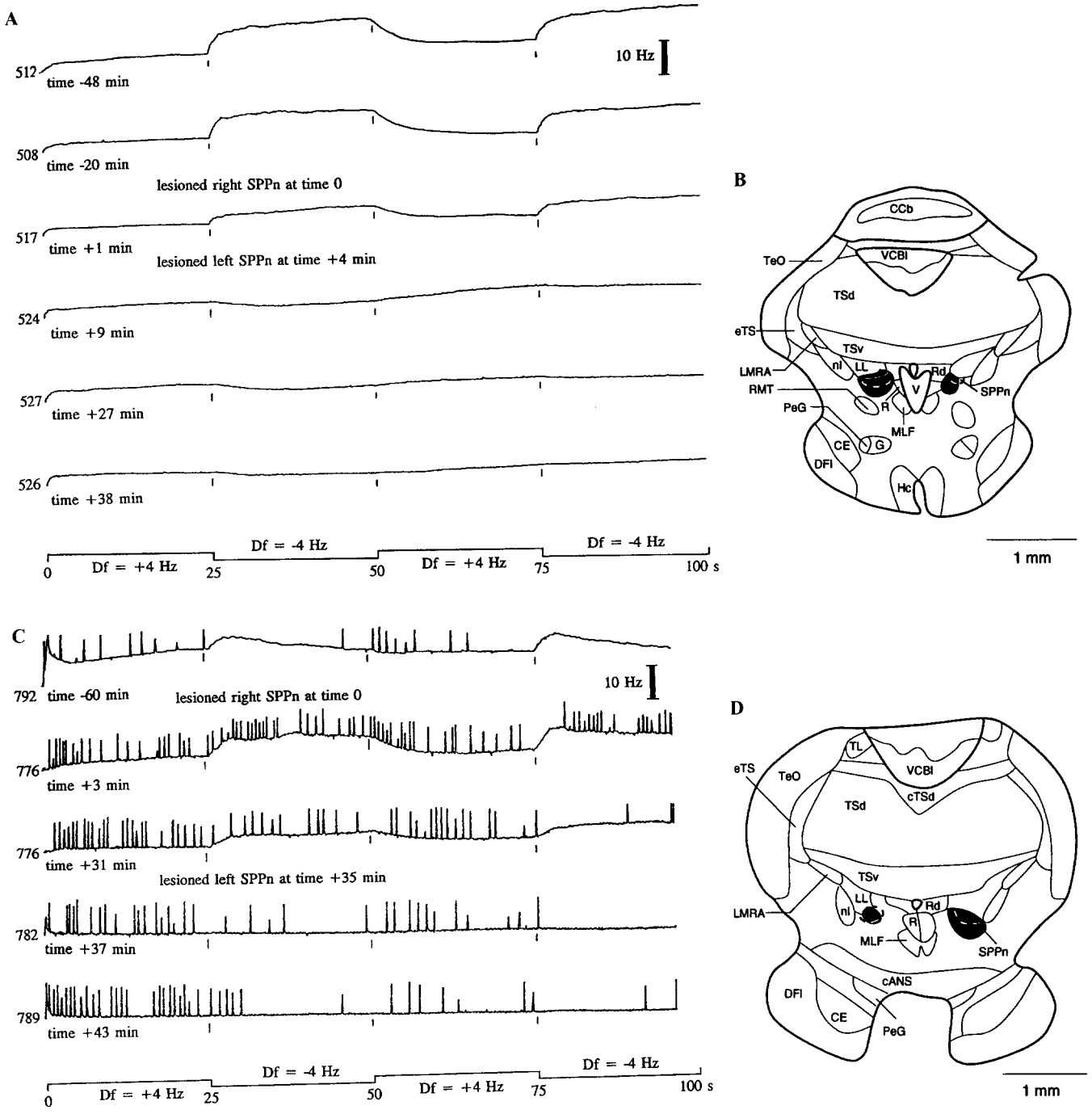


Fig. 16A–D Bilateral electrolytic lesions of the SPPn eliminate the JAR completely. **A** the JAR decreases by approximately 50% after the electrolytic lesion of one SPPn and disappears completely after the lesion of the contralateral SPPn. The remaining non-selective response shows a very slight inverse "sign selectivity" to the normal JAR (*bottom three traces*). Presentation follows that of Fig. 1; **B** camera lucida drawing of a transverse section of the brain showing the lesioned areas of the SPPn (equivalent to level + 13 of Maler et al. 1991); **C** the JAR is abolished in a second fish after bilateral SPPn lesion. Chirping behavior continues, however, and is strongest during jamming with a positive Df. Thus, lesioning of the SPPn does not seem to affect either the fish's control of chirping, nor its ability to distinguish the sign of Df; **D** camera lucida drawing showing the lesioned areas of the PPn (equivalent to level + 13 in Maler et al. 1991)

semicircularis. This close juxtaposition raises the possibility that electrolytically lesioning the SPPn may have damaged sensory input necessary for performance of the JAR. Several pieces of evidence suggest that the sensory apparatus remained intact following these lesions: 1) Histological inspection revealed that the lateral lemniscus remained intact; 2) We were still able to record phase-modulated activity within lamina VI of the torus. Such activity requires phase-locked input arriving from various parts of the body surface via the lateral lemniscus (Heiligenberg 1991); 3) Some fish (e.g., Fig. 16C) chirp in response to jamming signals and

often do so preferentially for either positive or for negative Dfs (Dye 1987, Zupanc and Maler 1993). The response shown in Fig. 16C shows that even following SPPn lesions that eliminated the JAR, the fish chirps more readily in response to positive Dfs, thus demonstrating a retained ability to discriminate the sign of Df.

Since even complete bilateral lesions of the PPn do not affect the JAR of *Apteronotus* (Fig. 10), it would appear that the *Apteronotus* JAR depends entirely upon the nE↓-SPPn-Pn pathway. This is in strong contrast to the situation in *Eigenmannia*, where both, PPn and SPPn participate in the control of this behavior (Fig. 2A), the PPn required for raising the pacemaker frequency and the SPPn required for lowering it.

The nE↑ provides pacemaker excitation for the NSR

While we conclude that the nE↑-PPn-Pn pathway does not participate in the performance of the JAR in *Apteronotus*, several pieces of evidence demonstrate that this pathway does indeed provide excitatory input to the pacemaker during jamming. This is most evident when, at the onset of a jamming stimulus, *Apteronotus* normally shows a rise of its EOD frequency that persists at some level through the duration of the jamming stimulus (Fig. 1). The frequency rise occurs regardless of whether the jamming stimulus begins with a positive or negative Df and hence has been termed a 'non-selective response', NSR (Dye 1987). The NSR varies tremendously in amplitude and time-course both between individual fish and over the course of an experiment. A weak form of this behavior that requires the nE-complex has been observed in *Eigenmannia* (Heiligenberg et al. 1991). The much stronger effect in *Apteronotus* appears to use both the SPPn-Pn and PPn-Pn pathways, since we noticed in our lesion experiments that it survives the loss of either the SPPn, or the PPn and only vanishes after bilateral loss of both nuclei.

Excitatory input to the pacemaker from the nE↑ appears to be at least partially suppressed during jamming. As was shown in Fig. 8, glutamate stimulation of the nE↑ causes both a gradual EOD-frequency rise and chirping. However, if one first elevates the pacemaker frequency by presentation of a jamming signal, a subsequent stimulation of the nE↑ with L-glutamate will produce little if any additional increment of the pacemaker frequency. The induced chirp-response remains intact, however. Removal of the jamming signal results, over tens of seconds, in a gradual return of the response to nE↑ stimulation. The efficacy of suppression may or may not be equivalent with either sign of Df, and may vary over the course of an experimental session. This suppression must occur at the level of the nE↑ and not the PPnG because similar experiments using stimulation of the PPnG instead of the nE↑, do not show similar suppression of the PPnG-mediated frequency rise.

Stronger suppression of the nE↑ during negative Df-jamming might account for the several cases in which we noticed that after bilateral lesion of the SPPn (when the only input to the pacemaker came via the nE↑-PPn-Pn pathway), the remaining frequency modulation was opposite to the normal JAR. In other words, the EOD frequency was raised in response to jamming with positive Dfs (f_{EOD} moved towards f_{JAM}) and relaxed back towards the original resting frequency (again, towards f_{JAM}) during negative Dfs.

Glutamate receptor pharmacology in the pacemaker nucleus.

In *Eigenmannia* NMDA-type glutamate receptors within the pacemaker nucleus mediate SPPn excitation of the pacemaker during the JAR (Metzner 1993). In contrast, pacemaker excitation via the PPnG during the JAR or via the PPnC during chirping are each mediated by non-NMDA-type glutamate receptors (Dye et al. 1989; Metzner 1993). Dye et al. (1989) reported similar findings for *Apteronotus* based on *in vitro* experiments, but the interpretation of this data was left unclear by the rudimentary knowledge of the premotor pathways controlling the JAR in *Apteronotus*. We were able to confirm and expand upon these earlier results by applying to the pacemaker nucleus specific blockers for different glutamate receptors while the fish either executed a JAR or chirped voluntarily, or while we stimulated premotor brain structures. Results of these experiments are summarized below.

In accord with the earlier results, chirps but not the JAR could be blocked by application of CNQX, indicating that pacemaker excitation from the PPnC is mediated by non-NMDA-type glutamate receptors. In contrast, the antagonist to NMDA-type glutamate receptors, APV, had no effect on the production of chirps but completely blocked the JAR. Once the SPPn's role in the JAR had been elucidated, these results could be interpreted to suggest that the nE↓-SPPn-Pn pathway for the JAR made use of NMDA-type glutamate receptors in the pacemaker. To test this, we electrically stimulated the SPPn to elicit consistent frequency rises that had both a fast and a slow component (data not shown). The slow component, which resembled the response to glutamate stimulation, was always attenuated by CPP (another NMDA-type blocker), sometimes by APV, but never by CNQX. The origin of the fast component is not clear, but it was blocked by CNQX and not by CPP or APV.

Given the findings that the nE↑-PPnG-Pn pathway is not necessary for the JAR of *Apteronotus*, the receptor-type used by this pathway remained to be tested. For these experiments we electrically stimulated either the PPnG or the nE↑. with identical results, hence we present results only for stimulations of the PPnG (Fig. 17). Electrical stimulation resulted in frequency

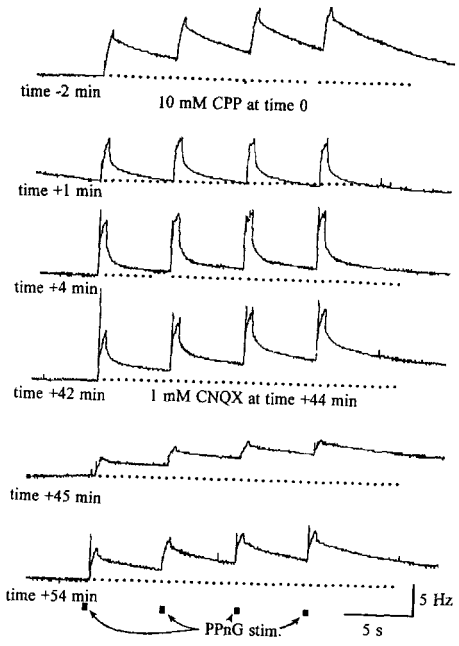


Fig. 17 Application of glutamate-receptor blockers to the pacemaker nucleus while electrically stimulating the PPnG. A series of four brief electrical stimulations of the PPnG normally elicited rapid and brief frequency rises coupled with a slower increase in frequency that summates through the series (trace − 2 min). Administration of CPP, which blocks NMDA-type glutamate receptors, reversibly blocked the slow, summating frequency rise with little effect on the fast component (traces at + 1, + 4, + 42 min). In contrast, application of CNQX, which blocks non-NMDA type glutamate receptors, attenuated only the fast component (traces at + 45, + 54 min.). Similar records were obtained with electrical stimulation of the nE↑ instead. Conventions follow those of Fig. 1

rises that had both fast and slow components. These components might be attributable to activation of the nE↑-PPnC-Pn and nE↑-PPnG-Pn pathways, respectively. The rapid component was attenuated by CNQX (Fig. 17, traces at + 45, + 54 min), while the slower, sustained component was reduced by APV in some individuals and by CPP in all individuals (Fig. 17, traces at + 1, + 4 min). The same APV solutions that failed to show a significant effect in some individuals of *Apteronotus*, however, never failed to block the NMDA-mediated effect of the SPPn upon the pacemaker in *Eigenmannia*. The lack of effect for a particular solution of APV in a given individual of *Apteronotus*, therefore, was not due to batch variability but rather to probable differences in the nature or accessibility of NMDA-type receptors across individuals of this species. NMDA-receptor-like immunoreactivity has recently been localized within the pacemaker nucleus of both *Eigenmannia* and *Apteronotus* (Spiro et al. 1994; Bottai et al. 1995).

Discussion

The electromotor system of gymnotiform fishes comprises an intrinsically rhythmic medullary pacemaker nucleus, its modulatory inputs from diencephalic and mesencephalic sources, and the spinal motor neurons and/or electrocytes of the electric organ. A wide variety of species-specific electromotor behaviors are driven by the pacemaker nucleus and hence are also controlled by its modulatory inputs. These inputs are strictly defined by both topographic and connectional criteria (i.e., they project to the pacemaker nucleus) and are likely homologous since nuclei consisting of similar cell types that project to the pacemaker have been found in all gymnotiforms examined to date. Thus, to begin to identify what might be common design features for modulatory inputs to the pacemaker, we consider the similarities and differences among these circuits within the evolutionary context of the gymnotiforms and also within a broader comparative context of vertebrate and invertebrate motor control.

Gymnotiform phylogeny and the JAR

A recent phylogenetic hypothesis proposed for the gymnotiforms (Alves-Gomes et al. 1995) suggests that the families Eigenmanniidae (consisting of the genera *Eigenmannia*, *Rhabdolichops*, etc.) and Apteronotidae (*Apteronotus*, *Adontosternarchus*, etc.) are sister groups. Preliminary investigations suggest that, similar to *Eigenmannia*, *Rhabdolichops* can lower its pacemaker frequency below a resting level during the JAR (Heiligenberg et al. 1986). In contrast, none of the apteronotids tested thus far [*Apteronotus leptorhynchus* and *Apteronotus albifrons*: Bullock et al. 1972 and *Adontosternarchus devenanzii*: C. Keller, unpublished observations] can lower their EOD frequency below the resting level during a JAR, and thus their JARs display asymmetries similar to that shown for *A. leptorhynchus* in Fig. 1. These observations suggest that the ability to lower the EOD frequency below the resting level during a JAR may be a distinguishing feature between the Apteronotidae and Eigenmanniidae. Pulse-type fish of the families Rhamphichthyidae and Hypopomidae comprise the next phylogenetic outgroup. These fish also perform JARs, but their JARs are very different from and probably convergently evolved with those of *Eigenmannia* and *Apteronotus* (Heiligenberg et al. 1978a; Baker 1981, Kawasaki et al. 1996). The most distantly related outgroup to all gymnotiforms is the family Sternopygidae (*Sternopygus*). The phylogeny proposed by Alves-Gomes et al. (1995) suggests that the Sternopygidae evolved a wave-type discharge convergently with the Eigenmanniidae and Apteronotidae. Since *Sternopygus* does not perform a JAR (Bullock et al. 1972, 1975; Matsubara and Heiligenberg 1978) and the JARs of pulse species are not homologous to those of the wave species, we are currently unable to resolve whether the JAR of *Eigenmannia* or that of *Apteronotus* is the primitive condition of the behavior. An unrelated African electric

fish, *Gymnarchus*, has convergently evolved a wave-type EOD (Bullock et al. 1983) as well as a JAR which uses a similar computational algorithm to that of *Eigenmannia* and *Apteronotus* (Bullock et al. 1975; Kawasaki 1993).

Reshaping the electromotor pathway

A comparison of the pathways controlling the pacemaker nucleus in *Eigenmannia* and *Apteronotus* reveals a similar basic blueprint as well as a few distinct anatomical and functional differences (compare Fig. 2A, B with 2C, D). Both genera are able to produce very similar forms of 'chirps', i.e., brief and rapid rises of their EOD frequency accompanied by a reduction of their EOD amplitude. In both genera, chirps are caused by activation of the PPnC and involve an excitation of the relay cells via non-NMDA-type glutamate receptors. In addition, both genera can gradually raise the frequency of their pacemaker through activation of the PPnG which excites pacemaker cells directly, but whereas this gradual excitation appears to be mediated by non-NMDA-type glutamate receptors in *Eigenmannia* (Metzner 1993), NMDA-type glutamate receptors are involved in *Apteronotus* (Fig. 17).

Significant differences are present in both the behavior and the neuronal control of the JAR despite the presence of much the same neuronal circuitry in the two groups. The pacemaker nucleus of *Eigenmannia* receives a tonic excitatory drive from the SPPn that is necessary to keep the pacemaker at an elevated resting frequency. This tonic input can be inhibited (by activation of the nE↓) to lower the pacemaker frequency or supplemented by excitatory input from the nE↑-PPnG-Pn to further raise the pacemaker frequency (Metzner 1993). In *Apteronotus* this degree of flexibility is absent. The nE↑-PPnG-Pn pathway is not necessary for *Apteronotus* to perform a JAR. Further, there appears to be little or no tonic excitatory input to the pacemaker and hence its frequency cannot be lowered below the resting level. Combined inhibitory and excitatory control of the SPPn is sufficient to sculpt both the rising and falling phases of the *Apteronotus* JAR.

The SPPn of *Eigenmannia* appears to fulfill only the single functional role of the JAR, whereas in *Apteronotus* the SPPn also plays a second role. Excitation of the *Apteronotus* SPPn causes a swift rise in EOD frequency and in extreme cases leads to a sustained desynchronization of the electric organ similar to naturally occurring EOD-interruptions seen during courtship (Fig. 13). A similar form of desynchronization, caused by the SPPn and NMDA-receptor mediated excitation of the relay cells, is observed in the genera *Sternopygus* (Keller et al. 1991) and *Hypopomus* (Kawasaki and Heiligenberg 1989, 1990) but has never been observed in *Eigenmannia*. Furthermore, there are two retrogradely labeled cell types within the SPPn of *Apteronotus* but only one cell-type has been found in the SPPn of *Eigenmannia*. Thus, contrary to the unitary function of *Eigenmannia*'s SPPn, the SPPn of *Apteronotus* appears to comprise two functionally distinct subunits which might be mapped onto the two cell types.

Both genera show a GABA-ergic inhibition of the SPPn via the nE↓. The normal quiescence of the *Apteronotus* SPPn may be due to tonic, and perhaps complete, inhibition originating from the nE↓. Under such a scenario, SPPn-caused frequency rises would result from a release of nE↓ inhibition onto the SPPn. This suggestion is consistent with all of our observations except that lesion of the nE↓ in *Apteronotus* (Fig. 12) does not result in a rise of pacemaker frequency as would be expected by a release from tonic inhibition. Thus, we cannot rule out an additional excitatory input to the SPPn that might also be eliminated by these lesions. Tonic and complete suppression of the SPPn is analogous to control of the mesencephalic locomotory region of cats (Mori 1987) and to control of the visual saccade-generating circuitry in the superior colliculus of monkeys (Hikosaka and Wurtz 1985). Each pathway is active only after having been released from a tonic inhibition originating within the substantia nigra.

In *Eigenmannia* reciprocal inhibition between the nE↑ and nE↓ adjusts the balance of the push-pull arrangement of antagonistically acting electromotor pathways (nE↑-PPnG-Pn versus nE↓-SPPn-Pn) used for control of the pacemaker frequency during the JAR. A similar inhibition between the nE↑ and nE↓ also may occur in *Apteronotus* but is not necessary for control of the JAR. However, biasing of this reciprocal inhibition in favor of either the nE↑ or nE↓ would likely affect the sign-selectivity of jamming-induced chirping and/or the non-selective response both of which use the nE↓-PPnG-Pn pathway.

One pacemaker but many behaviors

Closely related behaviors or different aspects of a single behavior are often controlled by a common central pattern generator through modulatory inputs (Harris-Warrick and Marder 1991). Multiple descending inputs can be addressed to their own unique, or partially overlapping, targets within the central pattern generator and may initiate, modulate or even stop a given behavior. What rules might there be for the evolution of these descending pathways?

In the pacemaker nucleus of gymnotiforms, the generation of distinctive behaviors can be achieved by a variety of mechanisms such as the anatomical segregation of inputs onto pacemaker and relay cells (Kennedy and Heiligenberg 1994) and/or the segregation of inputs onto receptor-types having distinct kinetic properties. In both *Eigenmannia* and *Apteronotus*

the PPnC and PPnG provide anatomically juxtaposed but functionally distinct inputs to the pacemaker nucleus for rapid and gradual pacemaker excitations, respectively (Kawasaki et al. 1988). The rapid EOD-modulations seen during chirping utilize non-NMDA type glutamate receptors which in other vertebrate systems (Daw et al. 1993) show relatively fast kinetics. In contrast to chirps, which are mediated through exclusive input to relay cells, smooth rises are generated by PPnG projections onto pacemaker cells using non-NMDA-type receptors in *Eigenmannia* (Metzner 1993) and NMDA-type glutamate receptors in *Apteronotus*. The significance of this difference in PPnG pharmacology between taxa is unclear at this time. The kinetic differences between glutamate receptor types underlie differences in behaviors of other systems as well. For example, fast or slow rates of fictive swimming can be elicited from the isolated lamprey spinal cord by activation of either non-NMDA or NMDA receptors, respectively (Brodin and Grillner 1985). Excitation of NMDA and non-NMDA receptor-types appear to have synergistic excitatory effects on the respiratory rates of mammals (Feldman and Smith 1989).

In some systems, elements of the central pattern generator can be effectively uncoupled or re-coupled into a new configuration, thus allowing production of new motor patterns (Getting and Dekin 1985; Harris-Warrick and Marder 1991). Similar uncoupling occurs during SPPn-generated EOD interruptions in *Sternopygus* (Keller et al. 1991), *Hypopomus* (Kawasaki and Heiligenberg 1989; Spiro 1994) and presumably *Apteronotus*. In each case, relay cells are strongly depolarized, effectively blocking transmission of the ongoing pacemaker cell activity and causing an interruption of the EOD.

Changing transmitters might also allow evolutionarily novel behaviors. The pulse-type fish, *Hypopomus*, has an extremely broad repertoire of frequency modulations (Kawasaki and Heiligenberg 1989, 1990), including gradual decreases in frequency that may include a complete cessation of pacemaking activity. These frequency decreases are mediated, at least in part, via GABA-ergic inhibition of the pacemaker originating from a subdivision of the PPn, designated the PPnI. This subdivision has not been found in wave-type gymnotiforms (Kawasaki and Heiligenberg 1989, 1990; Wong and Heiligenberg 1993; Kennedy and Heiligenberg 1994) and there appears to be no direct GABA-ergic inhibition of the pacemaker in these fish (G. Kennedy, personal communication). Thus, the evolutionary history of the PPnI is still unknown. In other systems GABA-ergic inhibition may play either a direct and/or a modulatory role in rhythm generation, e.g. lamprey locomotion (Grillner and Matsushima 1991), mammalian respiration (Feldman and Smith 1989) and vertebrate scratch reflex (Stein 1989). Diversifying to new transmitters may, at least in some systems, be a difficult evolutionary step; for example, despite extensive evolutionary modification of synaptic connectivity and morphology in arthropod visual ganglia, the photoreceptor transmitter histamine appears to be conserved (Stavenga and Hardie 1989).

Thus, evolutionary diversification of electromotor behaviors seems to have occurred not so much from structural changes within the pacemaker nucleus as from changes in extrinsic modulatory circuits and their connections to the pacemaker. This theme has also been described in other systems. Neurons located within the stomatogastric ganglion of lobsters provide rhythmic output for control of gut motility. Control of this rhythmic output has been extensively studied and found to vary greatly under a variety of neuromodulatory influences (Harris-Warrick and Marder 1991). In other decapods, such as shrimps, very similar pattern-generating circuitry produces vastly different motor patterns under the influence of quite different modulatory inputs (Meyrand and Moulins 1988). In frogs and toads there has also been considerable evolution of the inputs that modulate activity of the central pattern generator controlling feeding (Nishikawa et al. 1992). Some more derived species have protrusible tongues, and only these species require hypoglossal feedback to coordinate tongue protrusion with mouth opening (Nishikawa and Gans 1992).

Constraints on the electromotor system

Several authors have suggested that the constraints of multifunctionality on pattern-generating circuitry might limit their being subject to evolutionary modification (Nishikawa et al. 1992). An unstated corollary of this idea is that minor modifications in the central pattern generator itself might necessitate changes in descending control structures producing profound behavioral consequences. Such constraining modifications may go unrecognized if the descending pathways are more amenable to experimental manipulation than is the central pattern generator itself. Thus, it is important to consider what is already known about differences in pacemaker function of *Apteronotus* and *Eigenmannia*.

All gymnotiforms possess a single midline pacemaker nucleus comprised of up to several hundred pacemaker and relay cells. The relative number, spatial association (segregation) and connectivity of relay and pacemaker cells differs between groups (Dye and Meyer 1986). Relay and pacemaker cells are spatially most separate in pulse-type genera, moderately so in *Sternopygus*, less so in *Eigenmannia* and most interspersed in the Apteronotids. In *Apteronotus*, pacemaker cells are strongly connected by electrical synapses both to other pacemaker cells and often to relay cells. Relay cells appear to be only weakly interconnected but are indirectly electrically coupled via the pacemaker cells. In *Eigenmannia*, pacemaker cells are richly

interconnected by mixed chemical and electrical synapses (Elekes and Szabo 1981), relay cells appear to also be interconnected with mixed synapses and electrical coupling between relay and pacemaker cells is probably weaker than in *Apteronotus* (Szabo and Enger 1964). A new class of small cells having elongate processes was recently found within the *Apteronotus* pacemaker (Turner and Moroz 1995). To date, no physiological properties of these cells are known, nor is it clear whether or not they are neural in character.

A need for synchronous control of the EOD may have been the dominant selective pressure leading to the unpaired midline arrangement of the pacemaker nucleus and to the high levels of electrical coupling between both equivalent and successive elements of the system (Bennett 1968). The demands for synchrony and regularity probably become more stringent at higher discharge frequencies and thus the high frequency Apteronotids are the only ones requiring electrical transmission at every stage. The demands of high EOD repetition rates probably also underlie the evolutionary change to neurogenic electric organs in adult Apteronotids from the myogenic organs of other gymnotiforms and juvenile Apteronotids [see Bass (1986) for review].

Since all Apteronotids tested show asymmetrical JARs and cannot lower their EOD frequency below the resting level, it is possible that adaptations allowing synchrony at such high frequencies preclude the presence of a tonically active input. The repetition rate of EODs in *Apteronotus* is remarkably constant over short intervals (Bullock 1970; Moortgat and Keller 1995) and this may be important for the fish's discrimination of small phase differences. While a tonic excitatory input to the pacemaker would provide the flexibility to lower the pacemaker frequency, spontaneous activity of these inputs may also cause unwanted jitter in the EOD repetition rate. Adaptations that allow the pacemaker to fire at such high repetition rates and with such low jitter may make the pacemaker more susceptible to such fluctuations in input. High frequency alone does not preclude such an input, however, as is shown by its inferred presence in *Rhabdolichops*, a member of the Eigenmanniidae that discharges at 700–800 Hz. As mentioned above, *Rhabdolichops* can lower its pacemaker frequency below the resting level (Heiligenberg et al. 1986) and thus is likely to possess a tonic excitatory input to its pacemaker as does *Eigenmannia*. Further study of the *Rhabdolichops* electromotor pathways may further illuminate these speculations.

Raw material for evolutionary change

Central pattern generators that retain the flexibility to subserve multiple behavioral functions provide ample raw material for evolutionary change (Arbas et al. 1991). Centrally generated patterns can be modified to control functionally similar behaviors or even behaviors that are quite different, perhaps even using newly appropriated neuromusculature. Thus, locomotory movements in lampreys, fish and tetrapods may all share many elements of an ancestral central pattern generator despite substantially different muscles and movements (Fetcho and Faber 1988; Fetcho 1992). Similarly, in two closely related species of sand crabs, homologous motor neurons and tail musculature have been extensively modified to control very different forms of swimming (Paul 1981a, b). We suggest that alterations in tonic activity levels underlie the very different roles of the nE↓-SPPn-Pn pathway during the JAR. Other rather subtle changes in activity levels, connectivity, receptor expression and the like may provide other possible substrates upon which nature may act to form new behaviors. For example, many *Apteronotus* chirp in response to jamming stimuli, and do so preferentially for one or the other sign of Df (Dye 1987, Zupanc and Maler 1993). Such "sign-selective" chirping appears to be mediated by the nE↑-PPnC-Pn pathway in *Apteronotus* (Fig 7, Fig 15C). *Eigenmannia* does not normally chirp in response to jamming stimuli and L-glutamate stimulation of the nE↑ does not cause chirping. Recently, however, sign-selective chirping was observed in several individual *Eigenmannia* (J. Kozlowski, F. Gabbiani, pers. comm.) suggesting that the neural substrate is indeed present but may normally be only very weakly expressed or may be actively suppressed.

Similarly, an "inverse sign-selectivity" to the nE↑-PPnG-Pn based non-selective response may reflect circuitry underlying behaviors that are only transiently expressed in developing *Eigenmannia* (Hagedorn et al. 1988; Viete and Heiligenberg 1991). Some young fish (1.2–1.6 cm) show first only a frequency rise when presented with jamming stimuli, and the JAR develops soon thereafter. A few fish transiently express an "inverse" JAR and in some the JAR and frequency rise combined appear remarkably similar in form to the JAR of *Apteronotus* shown in Fig. 1 (Hagedorn and Heiligenberg 1988, their Fig. 4). A JAR having the normal adult form (but of reduced magnitude), coupled with little or no additional frequency rise is attained when the fish are about 1.8 cm in length. How these developmental shifts in behavior relate to changes in underlying premotor circuitry or its evolutionary history is not yet known.

Our tracing of anatomical connections has revealed a number of weak connections of yet unknown functional significance. For example, we found a relatively weak projection from the CP/PPn to the SPPn of *Apteronotus* (Fig. 6). All of our physiological tests, however, support the notion that the PPn and SPPn represent functionally separate inputs to the pacemaker. There may be many more weak connections of this kind; too weak perhaps to reveal a striking

physiological function, but available for eventual amplification and the establishment of significant functions in the course of evolution. Weak connections of this kind could also be remnants of connections that were stronger in the past and fulfilled a function now lost or taken over by other structures. The evolution of neural circuits is probably not dominated by any single process but rather is a series of selective losses and gains of both cell groups and connections (Striedter 1992).

Analogous to differences in patterns of connectivity in the electromotor control circuits, we also found differences in receptor expression by the cells of the pacemaker nucleus for these two genera. By varying the relative expression or location of receptor types over the course of evolution the temporal dynamics of specific pacemaker modulations could be easily altered. Pending examination of other gymnotiforms, our findings indicate that relatively small changes in the strength of connections between nuclei as well as in the anatomical, physiological and pharmacological properties of individual neurons can lead to extensive diversification of behaviors in closely related genera.

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