

Neuroanatomy of the Mormyrid Electromotor Control System

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ABSTRACT

Mormyrid fish produce a diverse range of electric signals that are under the control of a central electromotor network. The anatomical organization of this network was delineated by injecting biotinylated compounds into neurophysiologically identified nuclei. Previous work using retrograde labeling with horseradish peroxidase indicated that the medullary command nucleus (CN) receives inputs from the precommand nucleus (PCN) at the mesencephalic–diencephalic border and the ventroposterior nucleus (VP) in the torus semicircularis. This study confirms these projections and identifies the dorsal posterior nucleus (DP) in the thalamus as an additional input to CN. DP and PCN form a bilateral column of cells extending ventrolaterally and caudally from the dorsal thalamus. The primary input to DP/PCN is from VP, which is identified as having two distinct subdivisions. A small group of large, multipolar cells along the ventral edge projects to DP/PCN and to CN, whereas a dorsal group of small, ovoid cells projects to DP/PCN but not to CN. VP receives input from the tectum mesencephali and the mesencephalic command-associated nucleus (MCA). As in all vertebrates, the tectum mesencephali receives input from several sources and likely provides multimodal sensory input to the electromotor system. MCA is part of the electromotor corollary discharge pathway, and its projection to VP suggests a feedback loop. These results, combined with recent physiological studies and comparisons with other taxa, suggest that modifiable feedback to DP/PCN plays a critical role in electromotor control and that the different inputs to CN may each be responsible for generating distinct electric signals. *J. Comp. Neurol.* 454:440–455, 2002. © 2002 Wiley-Liss, Inc.

Indexing terms: electric organ discharge; electric fish; pacemaker; command; motor; communication

Mormyrid Electric fish from Africa produce weak electric organ discharges (EODs) that are used for communication and navigation (see Carlson, 2002). The waveform of each EOD pulse is constant within individuals and shows stereotypic variation among species, between the sexes, and among individuals of differing relative status (Hopkins, 1980, 1981; Bass and Hopkins, 1983; Friedman and Hopkins, 1996; Carlson et al., 2000). By contrast, the rhythmic pattern of EOD pulse production or the sequence of pulse intervals (SPI) is highly variable, with interpulse intervals varying continuously from as short as 5 msec to several seconds in duration (see Carlson, 2002). Stereotyped patterns such as bursts, cessations, and regularizations are periodically produced, and the available evidence suggests that these play roles in both communication and electrolocation (for reviews see Kramer, 1994; Carlson, 2002).

Production of each EOD pulse results from the synchronous activation of cells within the electric organ, termed electrocytes (Bennett and Grundfest, 1961). The stereo-

typed waveform of the EOD appears to be a direct consequence of the morphological and physiological properties of the electrocytes (Bennett and Grundfest, 1961; Bennett et al., 1967; Bass, 1986; Bass et al., 1986). Each EOD is initiated in the command nucleus (CN), a ventral midline nucleus located in the caudal medulla that determines the timing of each EOD pulse and therefore the SPI (see Fig. 1A; Bell et al., 1983; Grant et al., 1986, 1999; Carlson,

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2002). CN projects dorsally to the medullary relay nucleus (MRN) and caudally and laterally to the bilateral bulbar command-associated nucleus (BCA), which also projects to MRN (Bell et al., 1983). MRN projects down the spinal cord to innervate the electromotor neurons that drive the electric organ (Bennett et al., 1967). Together, CN, MRN, and BCA constitute the medullary electromotor network that drives EOD production (Bell et al., 1983).

Rather than function as a pacemaker, as does the CN of South American electric fish (see Dye and Meyer, 1986), the CN is thought to play a role in integrating descending input that influences EOD production (Grant et al., 1986, 1999; Carlson, 2002). A previous anatomical study by Bell et al. (1983) indicated two potential sources of input to CN based on retrograde labeling of horseradish peroxidase: the precommand nucleus (PCN), located at the border between the mesencephalon and the diencephalon, and a small group of cells located at the ventral edge of the ventroposterior nucleus (VP) in the torus semicircularis. These connections have not been verified by anterograde

transport, although recent physiological studies on PCN strongly support its role as an excitatory afferent to CN (von der Emde et al., 2000; Carlson, 2002).

The medullary electromotor network also generates a corollary discharge that originates in BCA, which sends an ascending projection to the paratrigeminal command-associated nucleus and the mesencephalic command-associated nucleus (MCA; Bell et al., 1983). MCA projects to the sublemniscal nucleus, which sends inputs to various electrosensory regions, either directly or indirectly, and provides a timing reference of EOD production (Bell et al., 1983, 1995; Bell and von der Emde, 1995). Extracellular recordings of single units within PCN indicate that it receives inhibitory input from the corollary discharge pathway (von der Emde et al., 2000; Carlson 2002). This input likely serves as negative feedback for electromotor output, but the pathway by which the corollary discharge reaches PCN is unknown.

The goals of the current study were to 1) provide anterograde verification of the afferent inputs to CN, 2)

Abbreviations

A	anterior nucleus of the thalamus	pc	posterior commissure
ac	anterior commissure	PCA	paratrigeminal command-associated nucleus
BCA	bulbar command-associated nucleus	pch	postchiasmatic commissure
C1	cerebellar lobe 1	PCN	precommand nucleus
cc	corpus of the cerebellum	PGc	caudal subdivision of the preglomerular nucleus
CN	command nucleus	PGd	dorsal subdivision of the preglomerular nucleus
CP	central posterior nucleus of the thalamus	PGm	medial subdivision of the preglomerular nucleus
CPN	central pretectal nucleus	PGv	ventral subdivision of the preglomerular nucleus
Dd	dorsal division of the dorsal telencephalon	Pn	pacemaker nucleus
Dm	medial division of the dorsal telencephalon	PPa	anterior periventricular preoptic area
DP	dorsal posterior nucleus of the thalamus	PPd	dorsal periventricular nucleus
De	central division of the dorsal telencephalon	PPn	prepacemaker nucleus
DLZ	dorsolateral zone of the electrosensory lateral line lobe	PPp	posterior periventricular preoptic area
DT	dorsal tegmental nucleus	PPv	ventral periventricular nucleus
dV	descending trigeminal tract	RD	rostradorsal tegmental nucleus
EGa	eminetia granularis pars anterior	RT	rostral tegmental nucleus
EGp	eminetia granularis pars posterior	sch	suprachiasmatic commissure
ELa	extero-lateral nucleus of the torus semicircularis, pars anterior	slem	sublemniscal nucleus
ELL	electrosensory lateral line lobe	SPI	sequence of pulse intervals
ELp	extero-lateral nucleus of the torus semicircularis, pars posterior	SPPn	sublemniscal prepacemaker nucleus
EMN	electromotor neurons	SRF	superficial reticular formation
EOD	electric organ discharge	stt	sub-toro-praeeminential tract neurons
FR	fasciculus retroflexus	Ta	anterior tuberal nucleus
H	habenula	tc	tecto-cerebellar tract
Hc	caudal hypothalamus	tel	telencephalon
Hd	dorsal hypothalamus	TL	torus longitudinalis
HI	lateral hypothalamus	TM	tectum mesencephali
Hyp	hypothalamus	Tp	posterior tuberal nucleus
IL	inferior lobe of the hypothalamus	TPp	periventricular nucleus of the posterior tuberculum
JL	juxtalobar nucleus	TS	torus semicircularis
L	lateral nucleus of the torus semicircularis	tt	toro-praeeminential tract
LC	locus coeruleus	v	ventricle
LCC	caudal cerebellar lobe	Val	valvula of the cerebellum
ll	lateral lemniscus	vc	ventral commissure
MCA	mesencephalic command-associated nucleus	VIIIm	facial motor nucleus
MD	mediodorsal nucleus of the torus semicircularis	VIIIs	facial sensory nucleus
MLF	medial longitudinal fasciculus	VIIIId	descending vestibular nucleus
MON	medial octavolateral nucleus	Vs	ventral telencephalon, supra commissural nucleus
MRN	medullary relay nucleus	VL	ventrolateral nucleus of the thalamus
MV	medioventral nucleus of the torus semicircularis	VLZ	ventrolateral zone of the electrosensory lateral line lobe
MZ	medial zone of the electrosensory lateral line lobe	VM	ventromedial nucleus of the thalamus
nE	nucleus electrosensorius	VP	ventroposterior nucleus of the torus semicircularis
nELL	nucleus of the electrosensory lateral line lobe	VPd	ventroposterior nucleus of the torus semicircularis, dorsal subdivision
nI	nucleus isthmus	VPv	ventroposterior nucleus of the torus semicircularis, ventral subdivision
nLR	nucleus of the lateral recess	Xm	vagal motor nucleus
nP	nucleus praeeminentialis	Xs	vagal sensory nucleus
OB	olfactory bulb		

analyze in detail the projection patterns and connectivity of the descending inputs to the medullary electromotor network, 3) describe the pathway by which corollary discharge feedback reaches PCN, and 4) determine potential sources of input to the electromotor system that may modulate EOD output. This knowledge is necessary for studying the mechanisms of EOD production and the generation of SPI patterns. Because of the relative simplicity and stereotypy of electrical communication, an appreciation of the substrates and mechanisms involved in electrical signaling should prove relevant in developing general concepts of signal production mechanisms. Portions of these results have appeared in abstract form (Carlson and Hopkins, 2000, 2001).

MATERIALS AND METHODS

Experimental subjects

In total 42 individual *Brienomyrus brachyistius* were used in these experiments, ranging in size from 7.0 to 47.0 g and from 7.9 to 19.3 cm in total length. They were part of a laboratory stock composed of a mix of imported and laboratory-bred fish, held in 400 liter community tanks, at a temperature of 23–29°C, conductivity of 150–300 $\mu\text{s}/\text{cm}$, and with a 12:12 hour light:dark cycle. All procedures were in accordance with the guidelines established by the National Institutes of Health and were approved by the Cornell University Institutional Animal Care and Use Committee.

Surgery and tracer injection

Animals were initially anesthetized in a solution of 500 mg/liter tricaine methanesulfonate (MS-222; Sigma Chemical Co., St. Louis, MO) and then respiration under a solution of 160 mg/liter MS-222 for the duration of the surgery. A flap of skin was removed from the head, and the underlying tissue was scraped away to expose the dorsal surface of the skull. Lidocaine (100–200 μl of a 2% solution; Radix Laboratories, Inc., Eau Claire, WI) was applied to each edge of the incision as a local anesthetic and was periodically reapplied throughout the experiments to maintain local anesthesia. A metal post was affixed to the skull using superglue to hold the skull in place. A dental drill was used to weaken four edges enclosing a rectangle in the skull, and the enclosed portion of the skull was removed with the underlying meninges to expose the dorsal surface of the brain (Fig. 1A). A reference electrode was placed in the dorsal musculature that originates from the caudal portion of the skull. The fish were then immobilized with an intramuscular injection of flaxedil (galamine triethiodide; 100–300 μl of a 3 mg/ml solution; Sigma Chemical Co.), and the respiration was switched to fresh water to bring the fish out of general anesthesia for physiological recording.

Glass-walled electrodes were pulled using a Sutter Flaming Brown Micropipette Puller (model P-87) and broken to a tip diameter of 5–10 μm . The electrodes were filled with solutions of 5% neurobiotin (Vector Laboratories, Burlingame, CA), biocytin (Sigma Chemical Co.), or biotin dextran-amine (BDA; 10 kDa; Molecular Probes, Eugene, OR) in 1 M NaCl, giving a tip resistance of 1–3 M Ω . Previous studies have demonstrated that biotin and neurobiotin, but not BDA, may be transported transynaptically (Huang et al., 1992; Bass et al., 1994; Carlson and

Bass, 2000). Thus, BDA is a useful tracer for verifying direct connections between nuclei. For some of the injection sites, only neurobiotin was used. However, direct connections were verified in these cases by tracing fiber pathways and comparing results with those from other injection sites. Each electrode was placed in a microelectrode drive (Inchworm 6000; Burleigh Instruments, Inc., Fishers, NY) that was held by a micromanipulator (model 462-XY-M; Newport Co., Fountain Valley, CA). The signals from the electrode and reference were sent to a differential AC amplifier (model 1700; A-M Systems, Inc., Everett, WA) and amplified $\times 10,000$. To monitor electromotor output, a silver wire was placed against the caudal peduncle, with a reference several centimeters away. Although flaxedil silences the electric organ, the electromotor neurons in the spinal cord that drive the electric organ still fire, and their activity can be seen as a three-spike field potential, the first spike of which was used as a timing reference for electromotor output (T_0 ; Bennett et al., 1967). Electromotor regions were generally localized using anatomical landmarks on the dorsal surface of the brain (Fig. 1A) and then more specifically localized by recording field potentials that showed a 1:1 relationship with electromotor neuron output (Fig. 1B). Field potentials and electromotor neuron output were digitized at 100 kHz using an A-D board (model AD2; Tucker-Davis Technologies, Gainesville, FL) and stored on a PC. There were a total of 42 injections, 14 in the command nucleus (CN; three biocytin, five neurobiotin, six BDA), six in the pre-command nucleus (PCN; all neurobiotin), four in the dorsal posterior thalamic nucleus (DP; three neurobiotin, one BDA), 13 in the ventroposterior toral nucleus (VP; all neurobiotin), three in the mesencephalic command-associated nucleus (MCA; all neurobiotin), one in the sublemniscal nucleus (BDA), and one in the tectum mesencephali (neurobiotin). There were four different injection sites spread throughout the tectum mesencephali to ensure that most afferent and efferent fibers were labeled.

After we located the electromotor regions, tracer was iontophoresed by injecting a DC current of +3.0 μA , for 3–15 minutes. The electrode was then slowly withdrawn from the brain, and the hole in the skull was sealed using cellophane paper and superglue. After survival times of 2–18 hours, the fish were placed back under general anesthesia (160 mg/liter MS-222) and then perfused transcardially with Hickman's ringer solution (6.48 g/liter NaCl, 0.15 g/liter KCl, 0.29 g/liter CaCl_2 , 0.12 g/liter MgSO_4 , 0.084 g/liter NaHCO_3 , 0.06 g/liter NaH_2PO_4), followed by ice-cold 4% paraformaldehyde/1% glutaraldehyde in 0.1 M phosphate buffer (PB; pH 7.2) for fixation. The brains were removed and postfixed overnight and then transferred to 0.1 M PB for storage.

Histological preparation

Brains were transferred to a solution of 30% sucrose in 0.1 M PB on the night prior to sectioning. Transverse sections were cut on a freezing microtome at 50 μm and processed as follows: 1) 30 min incubation in 0.4% Triton-X in phosphate-buffered saline (PBS; pH 7.2); 2) 3 hour incubation in an avidin-biotinylated horseradish peroxidase (HRP) complex (Elite Kit; Vector Laboratories); 3) two 10 minute rinses in 0.1 M PB; 4) 1–2 minute incubation in 0.05% diaminobenzidine (Sigma Chemical Co.), 0.01% hydrogen peroxide dissolved in 0.1 M PB (visualized for reaction product); and 5) two rinses in 0.1 M PB.

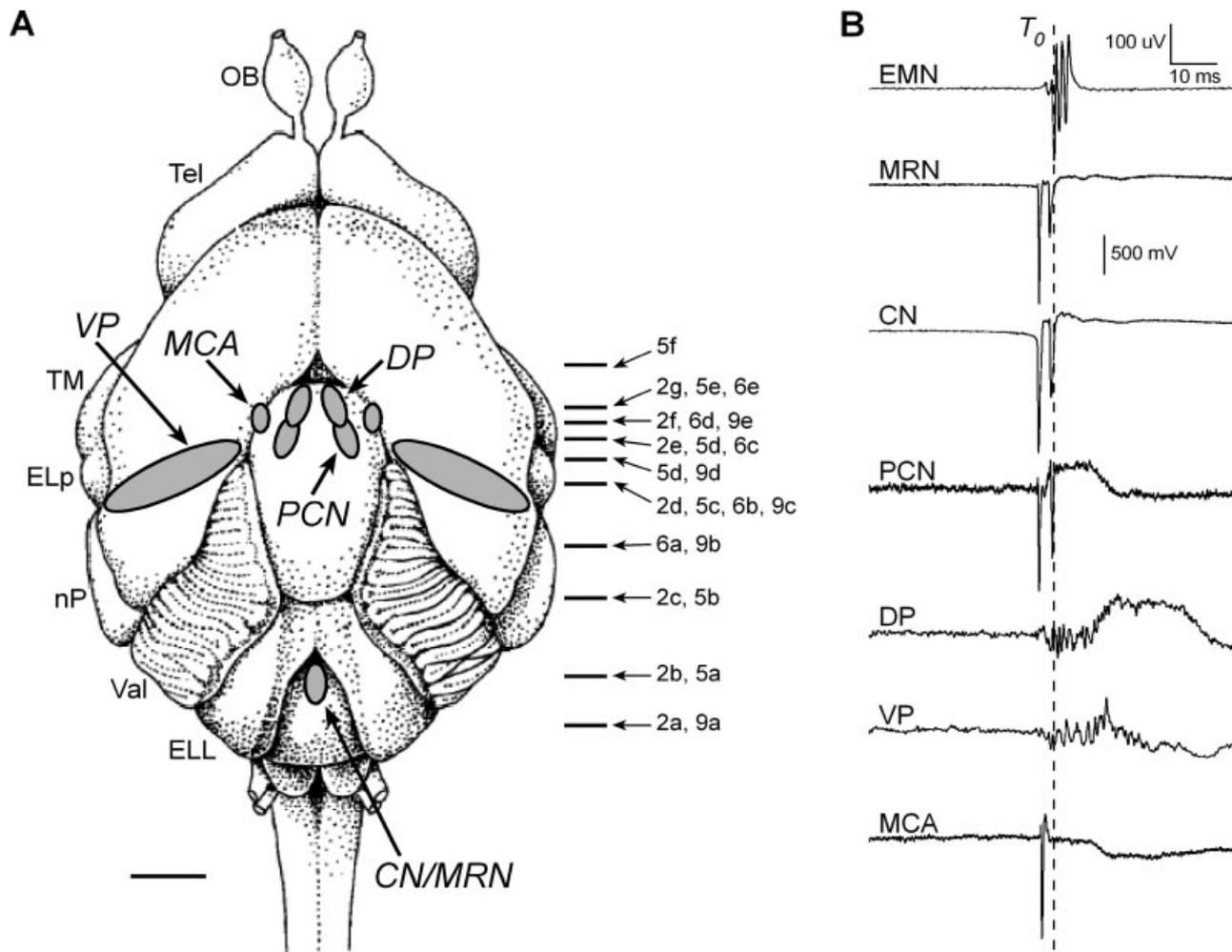


Fig. 1. **A:** A dorsal view of the brain of *Brienomyrus brachyistius* indicating the approximate locations of the main injection sites in the electromotor system, several distinctive landmarks, and the approximate rostrocaudal locations of sections shown in Figures 2, 5, 6, and 9. Scale bar = 1 mm. **B:** Extracellular recordings of electromotor-

related field potentials. Each trace represents an average of 20 recordings. The dashed line indicates the timing of the first spike (T_0) in the three-spike volley of the spinal electromotor neurons (EMN), which in freely behaving animals precedes each EOD by ~ 5 msec.

Sections were then stored in PB until mounting on chromalum-subbed slides. Slides were counterstained with cresyl violet, dehydrated in a graded alcohol series, and coverslipped with Permount (Sigma Chemical Co.).

Camera lucida line drawings were made using an Olympus BH-2 microscope with a drawing tube attachment. Images were scanned and assembled into figures in Photoshop 5.5 (Adobe Systems, Inc., San Jose, CA) and Freehand 9.0.2 (Macromedia, Inc., San Francisco, CA). Photomicrographs were taken using a Leitz DMR microscope with a Leica Wild MPS48 camera attachment. Photomicrographic images were adjusted for brightness and contrast and assembled into figures using Photoshop 5.5.

RESULTS

Electromotor-related field potentials

Extracellular recordings from the various electromotor nuclei revealed characteristic electromotor-related field

potentials (Fig. 1B). The electromotor neurons in the spinal cord always produced a three-spike potential (Bennett et al., 1967). The timing of the peak in the first spike was marked as T_0 . Field potentials recorded in CN, MRN, PCN, and MCA and described below were qualitatively identical to those already described for *Gnathonemus petersii* (Bell et al., 1983, 1995; von der Emde et al., 2000). Both CN and MRN had a double negative potential preceding T_0 by 3–3.5 msec, whereas PCN typically had a double negative potential starting 2–3 msec before T_0 , followed by a slow positive potential that lasted for 10–20 msec after T_0 . The second negative potential in PCN was not always present and varied considerably in amplitude between fish and recording sites. DP did not have any potentials occurring before T_0 but typically had oscillatory activity starting around T_0 , followed by a long, slow positive potential lasting from 15 to 50 msec. Recordings from VP revealed oscillatory activity starting shortly before T_0 and lasting for 10–20 msec. The field potential in MCA

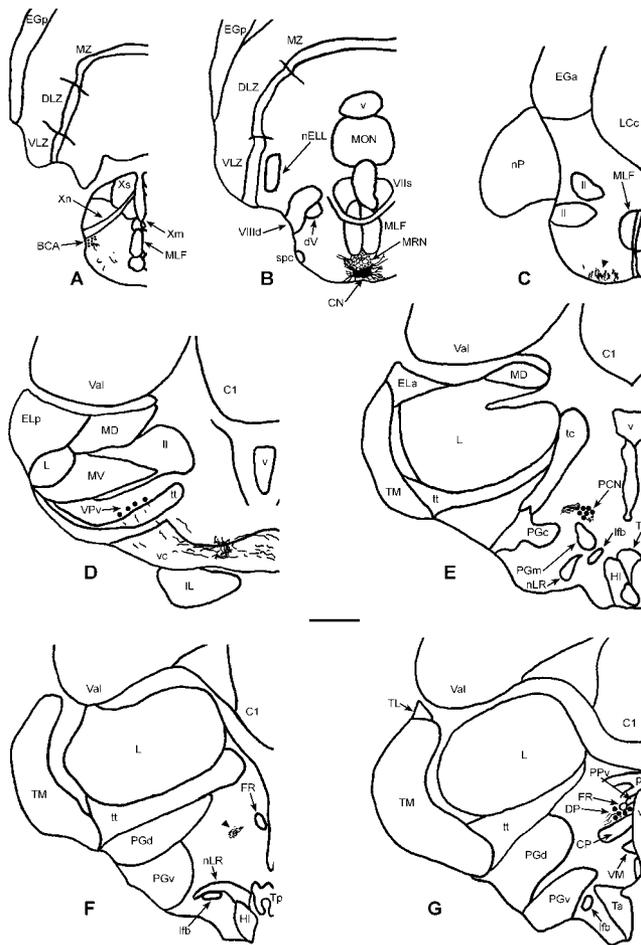


Fig. 2. **A–G:** Camera lucida line drawings of transverse sections in a caudal to rostral sequence through the brain following an injection of neurobiotin into CN. Large dots represent filled cells, small dots represent terminals, and thin lines represent fibers. The large open circles in MRN in B represent the boundaries of individual counterstained MRN cells. The injection site is shown in B as a large black mass. The arrowhead in C demarks the location of descending fibers projecting to CN, whereas that in F indicates the location of a cell-sparse region between PCN and DP, where only labeled fibers are visible. Scale bar = 500 μ m.

had a strong, sharp negative potential occurring around 2–2.5 msec before T_0 , which was sometimes followed by a second negative potential (not shown) near T_0 . These general characteristics of the field potentials were highly consistent among fish and could be used to locate the nuclei reliably for injections.

Injections into CN

CN and MRN are located on the ventral midline of the medulla, at the level of the facial motor and sensory nuclei, with MRN located immediately dorsal to CN and ventral to the medial longitudinal fasciculus (Figs. 2B, 3A; Bell et al., 1983). The majority of injections into CN were restricted to within the nucleus (Fig. 3A; $n = 9$), though in a few cases the injection sites spread dorsally into MRN and laterally into the adjacent reticular formation ($n = 5$). Irrespective of the extent of the injection or type of tracer,

retrogradely filled cells were located within PCN (Figs. 2E, 3B–D), DP (Figs. 2G, 3D,E), and the ventral edge of VP (VPv; Figs. 2D, 3F,G). Labeled cells were also seen to be interspersed throughout the reticular formation (not shown), though these possibly were due to labeling of nearby fibers, insofar as the reticular formation is located immediately lateral to CN.

PCN consisted of a bilateral group of cells starting caudally at the mesencephalic–diencephalic border and extending dorsomedially in the rostral direction toward the dorsal thalamus (Figs. 2E, 3B–D). At caudal levels, PCN was located immediately dorsal to the medial preglomerular nucleus, with relatively large multipolar cell bodies and thick dendrites arching laterally (Figs. 2E, 3B,C). More rostrally, the cell bodies became smaller and ovoid and the dendrites thinner and restricted to within the nucleus (Fig. 3D). Labeled DP neurons were located immediately rostral to PCN within the dorsal thalamus (Figs. 2G, 3D,E). The two groups of cells were distinguished by a distinct cell-sparse region that was approximately 50–100 μ m in width (Figs. 2F, 3D). Labeled soma in DP were smaller than those in PCN, forming a column of cells extending in a dorsomedial to ventrolateral direction. At rostral levels, they formed a dense cluster along the ventricle, immediately ventral to the fasciculus retroflexus and dorsal to the central posterior nucleus (Figs. 2G, 3E). The central posterior nucleus was clearly visible as a series of bands of somata extending ventrolaterally from the ventricle (Fig. 3E). The dendrites of DP neurons were thin and restricted to within the nucleus (Figs. 3D,E). In general, DP/PCN can be considered as a single complex starting caudally as relatively large cells with thick, extrinsic dendrites and changing rostrally into small, tightly clustered cells with thin, intrinsic dendrites. The axons from DP/PCN formed a dense band that extended ipsilaterally toward the ventral commissure (Fig. 2D) and then headed caudally along the ventral edge of the brain towards CN (Fig. 2C).

Only 5–15 labeled cells were seen bilaterally in VP after injections into CN, and they were always located at the ventral edge (VPv), immediately dorsolateral to the toro-praeeminential tract (Figs. 2D, 3F,G). They were typically large and multipolar, with several dendrites emanating in multiple directions (Fig. 3F,G). Their axons crossed the toro-praeeminential tract to enter the ventral commissure, where they headed medially to join the PCN and DP axons projecting caudally to CN (Fig. 2C,D). Some fibers could also be seen crossing the midline within the ventral commissure.

In cases in which the injection site was restricted to CN, labeled fibers with dense terminals could be seen immediately dorsal within MRN (Fig. 2B) and bilateral within BCA (Fig. 2A), which was located just ventral to the tenth sensory nerve at the level of the vagal motor and sensory nuclei (as described by Bell et al., 1983). With larger injections that spread into MRN, cells within BCA were also densely labeled, and large, thick, bilateral MRN axons were seen to head caudally toward the spinal cord within the bulbospinal command tract. These results confirm and extend the findings of Bell et al. (1983).

Injections into PCN and DP

Most injection sites in PCN and DP were restricted to just one of these nuclei ($n = 5$ in PCN, Fig. 4A; $n = 3$ in DP, Fig. 4B), whereas one injection in each nucleus was

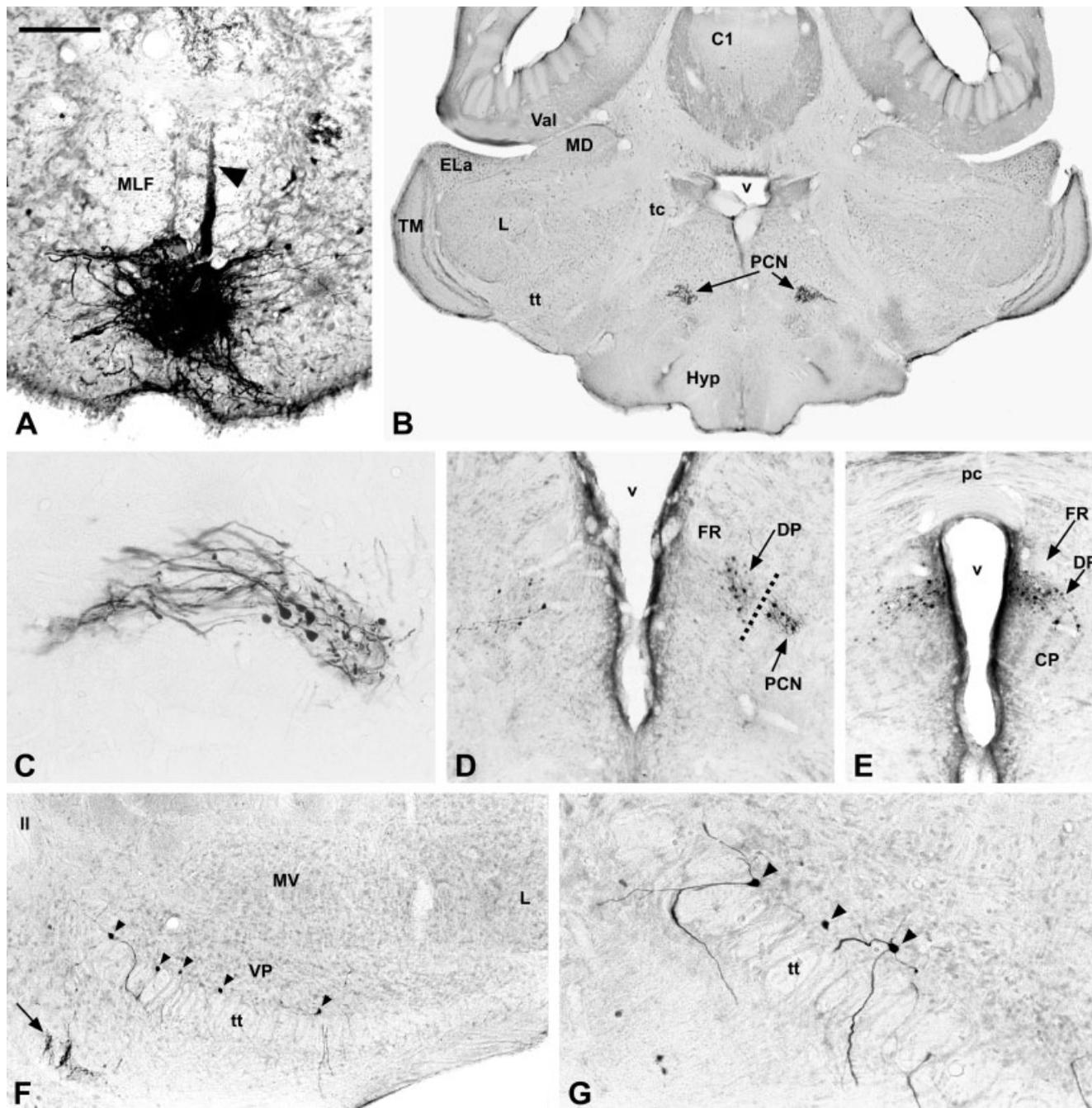


Fig. 3. Photomicrographs of label after neurobiotin injections into CN. **A:** A single injection site restricted within CN, which appears as a large black mass just below MRN. Damage from the electrode tract can be seen through the MLF (arrowhead). **B:** Bilateral labeling of PCN at the mesencephalic–diencephalic border. **C:** Close-up view of PCN. Note how the relatively large dendrites extend laterally from the cell bodies. **D:** A distinct cell-sparse region usually clearly separated PCN and DP (dotted line). **E:** Relatively small, ovoid cells located more rostrally in DP. **F:** Large, multipolar cells in VPv (arrowheads). Labeled dendrites from PCN cells can also be seen (arrow). **G:** Close-up of three VPv cells (arrowheads). Scale bar = 250 μm for A, 450 μm for B, 70 μm for C, 180 μm for D, 150 μm for E,G, 300 μm for F.

relatively large and entered adjacent areas. Each injection in PCN led to retrograde labeling of cells within DP, but not dense label or neuronal damage typical of an injection site, whereas injections in DP did not label any cells in PCN. Thus, injection sites in the two regions were limited

to either the rostral (DP) or the caudal (PCN) groups of cells within the DP/PCN complex. After injections into either nucleus, labeled axons headed ventrally to the ventral commissure before turning caudally along the base of the brainstem (Fig. 5B,C). At the level of CN/MRN, they

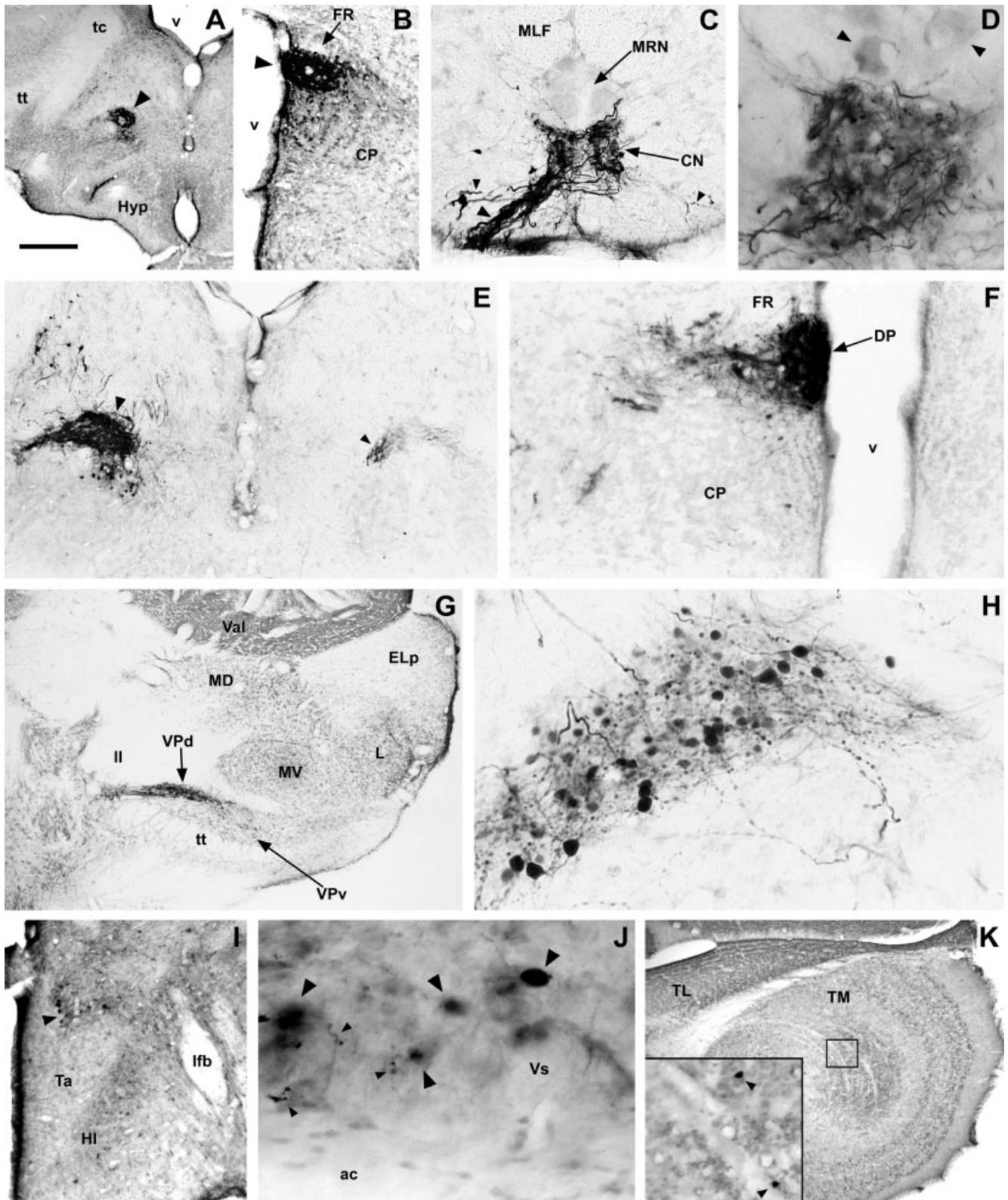


Fig. 4. Photomicrographs of label after neurobiotin injections into PCN and DP. **A:** Injection site restricted within PCN (arrowhead). **B:** Injection site restricted within DP (arrowhead). **C:** Anterograde fibers and terminals within CN following a PCN injection. Note the two groups of fibers, a large ipsilateral bundle located ventromedial (large arrowhead) and a smaller bilateral bundle located dorsolateral (small arrowheads). The large ipsilateral group of fibers is from DP/PCN afferents, whereas the small bilateral group is from VPv afferents. Note that the fibers are restricted to CN and do not terminate in MRN. **D:** Anterograde fibers and terminals within CN following a DP injection. The fibers terminate on CN cells but not MRN cells (arrowheads). **E:** Fibers and terminals in the contralateral PCN (small arrowhead) after a neurobiotin injection into PCN (large arrowhead).

F: Dense retrograde label in the ipsilateral DP following a PCN injection. **G:** After PCN and DP injections, a thick band of retrogradely labeled cells is seen in VPd, with a few scattered cells in VPv. **H:** A closer view of VPd reveals a dense plexus of cells, fibers, and terminals. **I:** Anterogradely labeled cells in the anterior tuberal nucleus (arrowhead) following a PCN injection. **J:** Anterogradely labeled terminals (small arrowheads) and retrogradely labeled cells (large arrowheads) in the supra commissural nucleus of the ventral telenkephalon after a DP injection. **K:** Label in TM following a DP injection. **Inset** shows an expanded view of the area enclosed by the box in K, where two labeled cells are visible (arrowheads). Scale bar = 260 μm for A, 100 μm for B,I, 80 μm for C, 35 μm for D, 200 μm for E,K, 50 μm for F, inset, 250 μm for G, 40 μm for H, 10 μm for J.

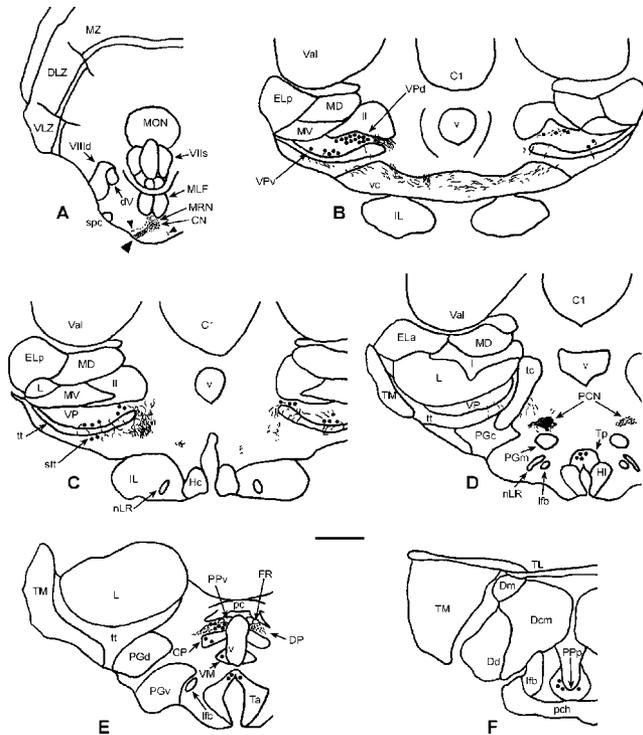


Fig. 5. **A–F:** Camera lucida line drawings of transverse sections in a caudal to rostral sequence through the brain following an injection of neurobiotin into PCN. Large dots represent filled cells, small dots represent terminals, and thin lines represent fibers. The large open circles in MRN in A represent the boundaries of individual counterstained MRN cells. The injection site is shown in D as a large black mass. In A, the small arrowheads mark the location of bilaterally labeled VPv fibers, whereas the large arrowhead marks the location of ipsilaterally labeled PCN fibers. Scale bar = 500 μm .

then abruptly turned dorsomedially to innervate CN bilaterally, forming a dense terminal field within CN, but not MRN (Figs. 4C,D, 5A). This verifies that DP and PCN both independently project directly to CN. A smaller group of bilateral fibers that also terminated in CN was located just dorsolateral to the primary bundle of ipsilateral fibers (Figs. 4C, 5A). These bilateral fibers most likely originate from retrogradely labeled VPv neurons, insofar as these were bilaterally labeled after PCN and DP injections (see below).

PCN injections led to dense labeling of cells within the ipsilateral DP and VP, with less intense labeling of contralateral VP cells and no labeling of cells in the contralateral DP or PCN (Figs. 4F–H, 5B–E). Labeled cells in DP were generally much more numerous and darkly stained than cells labeled through CN injections, forming an intense dark band just dorsal to the centroposterior nucleus and ventral to the fasciculus retroflexus (Fig. 4F). Unlike the case with CN injections, labeled cells in VP extended throughout the entire nucleus (Figs. 4G,H, 5B,C). These cells included the large, multipolar neurons located along the ventral edge that were labeled after CN injections (VPv) plus a dense band of smaller, ovoid cells located more dorsally in a tightly packed plexus of fibers and terminals (VPd; Fig. 4H). This dense labeling of terminals within VPd likely is due to intrinsic connections among

VPd neurons, insofar as injections into VPd do not result in retrograde labeling of PCN or DP neurons (see below). Whereas VPv axons exited the nucleus by crossing the toro-praeeminential tract, the majority of axons from VPd exited from the dorsomedial edge of the nucleus, between the lateral lemniscus and the toro-praeeminential tract (Figs. 4G, 5B,C). The axons from VPd generally headed directly towards the ipsilateral PCN, although some collaterals projected to the ventral commissure and others to a small group of cells located just ventral to the toro-praeeminential tract that were also retrogradely labeled ipsilaterally after PCN injections (Fig. 5C). Those that entered the ventral commissure joined the axons from VPv and headed contralaterally along the ventral commissure to terminate in the contralateral PCN and DP (Figs. 4E, 5B–E). No labeled cells were seen in the contralateral PCN and DP, confirming that the contralateral label in PCN and DP was due not to reciprocal connections but to a common afferent input (VP).

With relatively large injections into PCN, additional cells were often labeled within other thalamic nuclei, including the central posterior, ventromedial, and anterior nuclei, though these cells were not labeled with smaller injections, suggesting that this was due solely to the spread of label. In both small and large injections, retrogradely labeled cells were found in the dorsal hypothalamus (not shown), anterior and posterior tuberal nuclei (Figs. 4I, 5D,E), and anterior and posterior preoptic areas (Fig. 5F). However, these connections were not verified anterogradely.

Injections into DP resulted in a pattern of retrograde labeling nearly identical to that with injections into PCN, with densely labeled cells found ipsilaterally in VPd and VPv and fewer cells stained contralaterally. The small group of cells located just ventral to the toro-praeeminential tract that were labeled after PCN injections were also labeled after DP injections. No labeled cells were found in the ipsilateral PCN, although there was a dense terminal field surrounding the PCN dendrites and cell bodies. As with PCN injections, weak terminal fields were found in the contralateral PCN and DP, but no labeled cells. Large injections led to retrograde labeling throughout the thalamus, but smaller injections led to very minor labeling in other thalamic nuclei, suggesting again that this was due simply to the spread of label. Unlike the case with PCN injections, label was also found within the telencephalon and tectum mesencephali. A small, diffuse group of fibers passed through the forebrain bundle that led to both retrograde and anterograde label in the ventral telencephalon, just dorsal to the anterior commissure within the supra commissural nucleus (Fig. 4J). Very small numbers of fibers, terminals, and cells were labeled within the deeper layers of the tectum mesencephali (Fig. 4K).

Injections into VP and the tectum mesencephali

Among 13 injection sites into the area of VP, six were limited to VPd (Figs. 6B, 7A), two were centered on but not limited to VPv, and five were located just ventral to the toro-praeeminential tract. Injections into all three areas led to anterograde label in the ipsilateral PCN and DP, with weaker label contralaterally. The terminal fields resulting from VPd injections were especially dense, forming a dark outline of PCN and DP (Figs. 6C–E, 7B,C). Injec-



Fig. 6. **A–E**: Camera lucida line drawings of transverse sections in a caudal to rostral sequence through the brain following an injection of neurobiotin into VPd. Large dots represent filled cells, small dots represent terminals, and thin lines represent fibers. The injection site is shown in B as a large black mass. Scale bar = 500 μ m.

tions into VPv, but not the other two sites, led to weakly labeled ipsilateral axons projecting caudally to terminate in CN (Fig. 7D). The location of these axons as they entered CN was the same as the position of the small bilateral fiber bundles terminating in CN after PCN injections (Fig. 4C), verifying that the smaller group represents VPv axons and the larger, more ventromedial group PCN axons.

Injections centered ventrally to the toro-praeeminential tract led to retrograde and anterograde label in VPd, and injections into VPd led to retrograde and anterograde labeling in this region (Fig. 6C), suggesting reciprocal connections between these two regions. Each injection into VPd and VPv resulted in labeling throughout both subregions of VP. However, because the two subregions are so close to each other, it is unclear whether this was caused by actual connections between VPd and VPv or the injection sites simply spreading into the adjacent subregion.

Injections into all three areas led to strong anterograde and retrograde label within the ipsilateral tectum mesencephali (Figs. 6C–E, 7G). After VPd injections, small numbers of fibers and terminals were observed in the contralateral VPd (Fig. 6B), but no labeled cells were observed, suggesting that this label resulted from retrograde labeling of a bilateral projection to VPd.

Injections into VPd led to retrograde labeling of several large, darkly stained cells in the ipsilateral MCA, located immediately dorsal to the tectocerebellar tract (Figs. 6D, 7E,F). These injections also resulted in a small terminal field within the ipsilateral sublemniscal nucleus (Fig. 6A), a known target of MCA axons (Bell et al., 1995). These labeled terminals most likely were due to retrogradely labeled MCA axon collaterals rather than a projection from VPd, in that injections into the sublemniscal nucleus

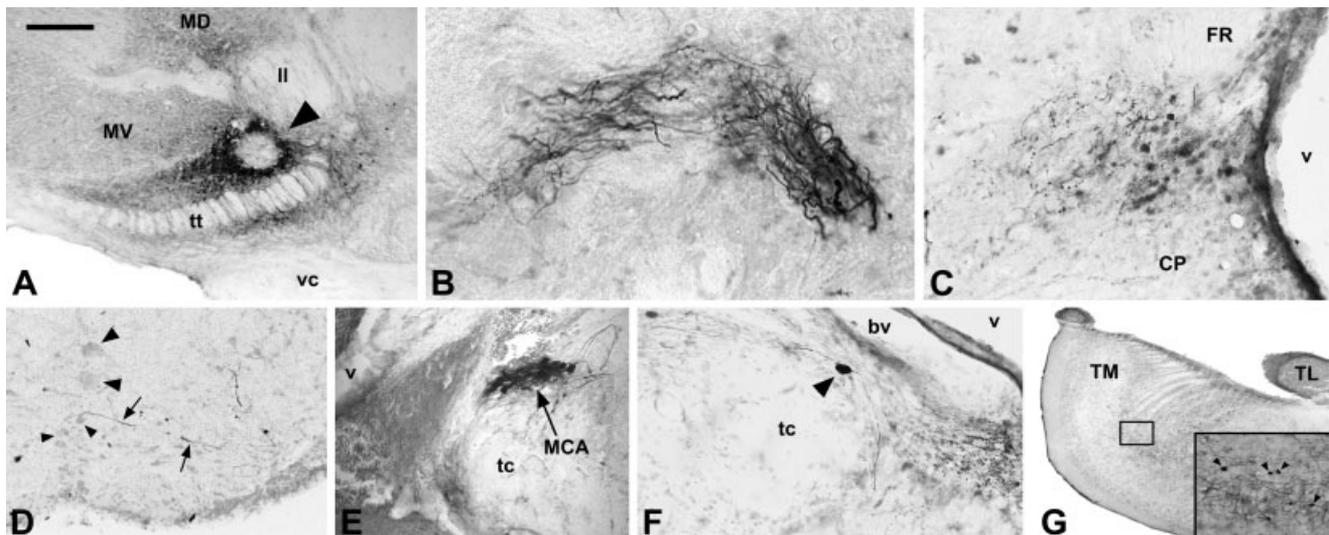


Fig. 7. Photomicrographs of label after neurobiotin injections into VP. **A**: A single injection site restricted within VPd (arrowhead). **B**: Anterograde label in the ipsilateral PCN following a VPd injection. **C**: Anterograde label in the ipsilateral DP following a VPd injection. **D**: Anterogradely labeled fibers projecting to CN following a VPv injection. A single fiber (arrows) can be seen to head dorsomedially toward CN. Individual counterstained neurons are visible in CN (small arrowheads) and MRN (large arrowheads). **E**: Retrogradely

labeled cells in MCA following a VPd injection. **F**: Close-up of a single retrogradely labeled MCA neuron (arrowhead) following a VPd injection. **G**: Label in TM following an injection in VPd. **Inset** shows an expanded view of the area enclosed by the box in G, where a dense network of fibers and terminals are visible, as well as labeled cells (arrowheads). Scale bar = 250 μ m for A, 65 μ m for B,C, 100 μ m for D, inset, 130 μ m for E, 50 μ m for F, 400 μ m for G.

did not retrogradely label cells in VP (see below). Retrograde labeling in MCA was not observed after injections into VPv or in the area ventral to the toro-praeeminential tract.

After VPd injections, retrogradely labeled cells were found within the medial octavolateral nucleus. Labeled cells, fibers, and terminals were also found at the ventral edge of the dorsal preglomerular nucleus, and small numbers of fibers and terminals were found in the ventral preglomerular nucleus (Fig. 6E). Retrogradely labeled cell bodies were also found in the anterior tuberal nucleus and dorsal hypothalamus (Fig. 6E) as well as the valvula (not shown). In addition, a terminal field was observed just medial to MCA, ventrolateral to the ventricle (Fig. 6D). With relatively large injections into VPd, additional label was found in several toral nuclei, including the medioventral, mediadorsal, lateral, and extero-lateral nucleus pars posterior, as well as a few labeled cells in the electrosensory lateral line lobe and nucleus praeeminentialis. However, this label was not observed after smaller injections, and these nuclei have been well studied (see Bell and Szabo, 1986); none are known to project to VP, so this result likely is due to labeling fibers within the lateral lemniscus, which is located immediately dorsal to VP.

The connectivity of the mormyrid tectum mesencephali has already been characterized for *Gnathonemus petersii* (Wullimann and Northcutt, 1990), and the goal of the single tectum mesencephali injection was primarily for direct comparison with the other injections. Reciprocal connections were found with several toral nuclei, including the extero-lateral pars posterior, medioventral, and ventroposterior nuclei, whereas the mediadorsal and lateral nuclei were only retrogradely labeled (Fig. 8F). Both anterograde and retrograde label were found in DP, although the amount of label was quite small relative to that at other labeled sites (Fig. 8E). Several additional diencephalic nuclei were labeled, with reciprocal connections with the central pretectal and dorsal periventricular nuclei and retrograde label in the ventromedial and ventrolateral nuclei. Retrograde label was also found in the valvula and corpus of the cerebellum, central area of the dorsal telencephalon, locus coeruleus, nucleus isthmi, superficial reticular formation, rostradorsal tegmental nucleus, and rostral tegmental nucleus. Anterograde label was also found in the dorsal preglomerular nucleus, dorsal tegmental nucleus, nucleus isthmi, and superficial reticular formation. These results confirm the findings of Wullimann and Northcutt (1990).

Injections into MCA and the sublemniscal nucleus

Injections into MCA (Figs. 8A, 9E) led to retrograde labeling in the contralateral BCA, just ventral to the tenth sensory nerve (Fig. 9A), as previously described for *G. petersii* (Bell et al., 1983). Dense terminal fields were found ipsilateral to the MCA injection site in the sublemniscal nucleus, located just ventral to the lateral lemniscus at the level of nucleus praeeminentialis (Figs. 8B, 9B). Fibers and terminals were also found within the ipsilateral VPd but not the VPv (Figs. 8C,D, 9C,D). The axons of MCA cells entered VPd from its dorsomedial edge, between the lateral lemniscus and the toro-praeeminential tract (Figs. 8C, 9C,D). Upon entering VPd, they branched and formed several distinct terminals throughout VPd

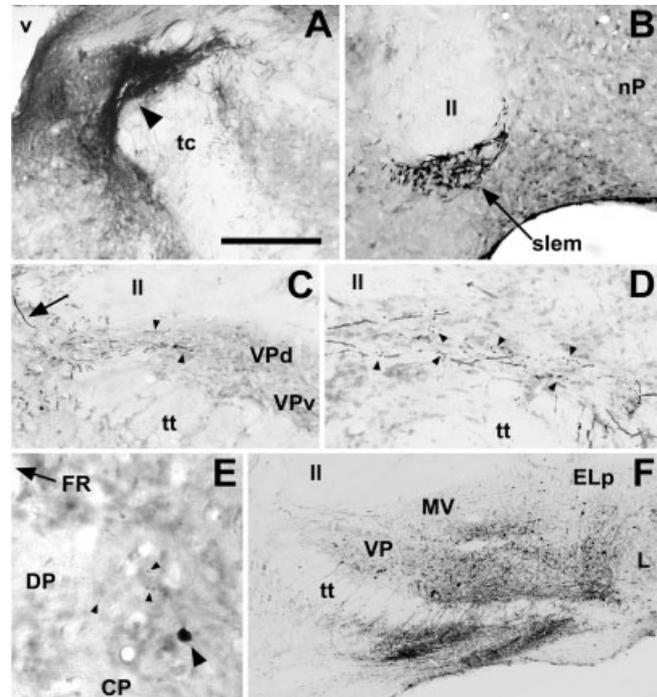


Fig. 8. Photomicrographs of label after neurobiotin injections into MCA and TM. **A:** A single injection site restricted within MCA (arrowhead). **B:** Anterograde label in the ipsilateral sublemniscal nucleus following an MCA injection. **C:** Anterograde label in the ipsilateral VPd following an MCA injection. A single large primary axon is visible just medial to the lateral lemniscus, heading ventrally (arrow), and smaller collaterals are seen entering VPd from its dorsomedial edge (arrowheads). **D:** Close-up view of VPd following an MCA injection. Numerous terminals are visible (arrowheads). **E:** Close-up of anterograde and retrograde label in DP following a TM injection. The label is fairly sparse, although distinct terminals are visible (small arrowheads), as is a single labeled cell (large arrowhead). **F:** Label in the torus semicircularis following an injection in TM. Dense retrograde and anterograde label is visible in several nuclei. Scale bar = 150 μ m for A,B, 160 μ m for C, 80 μ m for D, 10 μ m for E, 200 μ m for F.

(Fig. 8D). These fibers appeared much smaller than the large axons located along the medial and ventral edge of the lemniscus that head caudally to innervate the sublemniscal nucleus (Figs. 8C, 9C,D), suggesting that VPd receives its input from MCA via axon collaterals that branch off from these primary axons.

This is supported by the single injection into the sublemniscal nucleus (not shown), which led to anterograde fibers and terminals within the ipsilateral VPd. Because the sublemniscal nucleus is not retrogradely labeled following VP injections, this suggests that this label resulted from retrograde labeling of MCA axons. The retrograde label in MCA was qualitatively more dense than the label in MCA following VPd injections, supporting the observation that the MCA to sublemniscal projection is stronger than that from MCA to VPd. Fibers and terminal fields were found ipsilaterally within the juxtalobar nucleus and nucleus of the electrosensory lateral line lobe in the hind-brain and in nucleus praeeminentialis, as indicated by previous work in *G. petersii* (Bell et al., 1995).

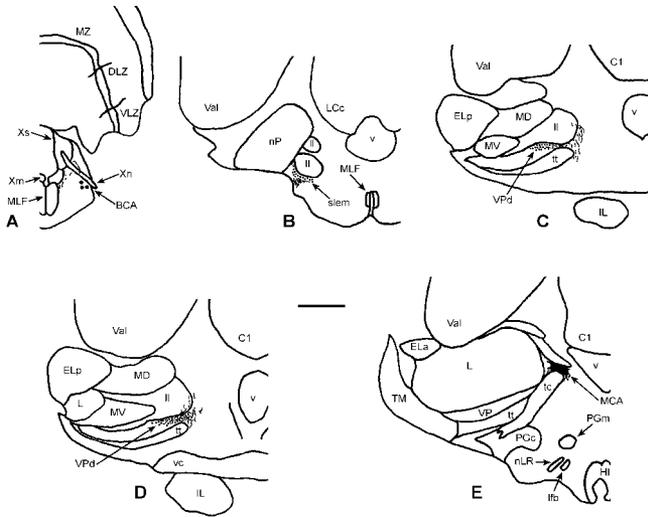


Fig. 9. **A–E:** Camera lucida line drawings of transverse sections in a caudal to rostral sequence through the brain following an injection of neurobiotin into MCA. Large dots represent filled cells, small dots represent injection terminals, and thin lines represent fibers. The injection site is shown in E as a large black mass. Scale bar = 500 μ m.

DISCUSSION

Summary of the mormyrid electromotor system

The anatomy of the central electromotor pathway in mormyrids was first studied by Bell et al. (1983); they 1) documented the connectivity of the medullary electromotor network, 2) demonstrated the existence and anatomy of a corollary discharge pathway, and 3) suggested that PCN and neurons along the ventral edge of VP provide descending input to CN based on retrograde labeling. The current study confirms these results and provides several new findings on the anatomical substrates for electromotor signaling behavior in mormyrids: 1) The medullary electromotor network receives inputs that terminate in CN from three distinct sources; in addition to verifying the inputs from PCN and VP using anterograde transport, we identified DP as an additional afferent to CN; 2) VP is identified as having two distinct subdivisions; a ventral subdivision (VPv) projects to CN and bilaterally to PCN and DP, whereas a dorsal subdivision (VPd) projects only bilaterally to PCN and DP; 3) the corollary discharge pathway projects to VPd via MCA, providing a pathway for electromotor feedback to influence descending activity; and 4) the tectum mesencephali has reciprocal connections with VPd and DP, which documents a pathway that may influence electromotor output based on sensory input, other motor commands, and internal conditions. A diagram of the mormyrid electromotor pathway summarizing the findings of these two studies is shown in Figure 10.

Comparisons with other teleosts

Gymnotiform fish from South America also produce weak electric organ discharges that are used for communication and active electrolocation. They are distantly related to the mormyrids and share no common electrogenic or electrosensory ancestors (Bullock et al., 1982, 1983).

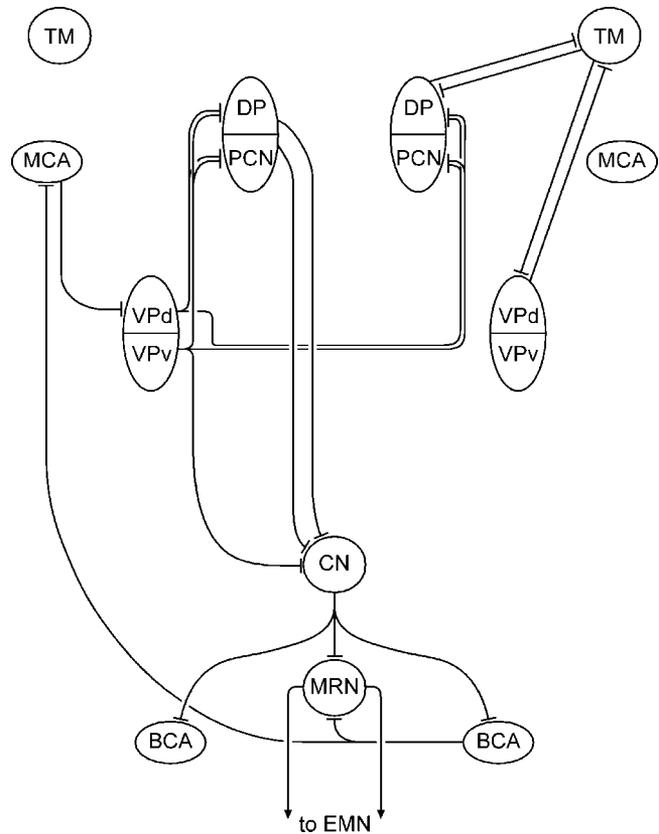


Fig. 10. Diagram summarizing the connectivity of the mormyrid electromotor system based on the current study and that of Bell et al. (1983).

They are therefore considered to have evolved their electrosensory and electromotor systems independently. Thus, studies of the central anatomy of these systems provide a rare opportunity for increasing our understanding of the evolution of vertebrate nervous systems and the behaviors they govern. Several authors have noted remarkable similarities as well as differences in their electromotor and electrosensory systems (Bass and Hopkins, 1982; Bullock et al., 1982; 1983; Dye and Meyer, 1986; Finger et al., 1986; Hopkins, 1995; Carlson, 2002). In both groups of fish, EOD production is initiated in a ventral midline nucleus in the caudal medulla, which appears to play a role as an integrative structure in mormyrids (CN) and a pacemaker in gymnotiforms (Pn; see Dye and Meyer, 1986; Grant et al., 1999; Carlson, 2002). In both cases, these neurons relay their output through extremely large relay neurons either located within the same nucleus (high frequency gymnotiforms) or separated from the command/pacemaker cells (low-frequency gymnotiforms and mormyrids), which then project down the spinal cord to innervate the electromotor neurons that drive the electric organ. Both systems are characterized by large axons, large cell bodies, and extensive electrical coupling both within and between nuclei (Bennett et al., 1963, 1967; Elekes and Szabo, 1982, 1985a, b; Elekes et al., 1985; Grant et al., 1986), as is characteristic of neural pathways in which preservation of timing information is paramount (Carr, 1993; Carr et al., 2001).

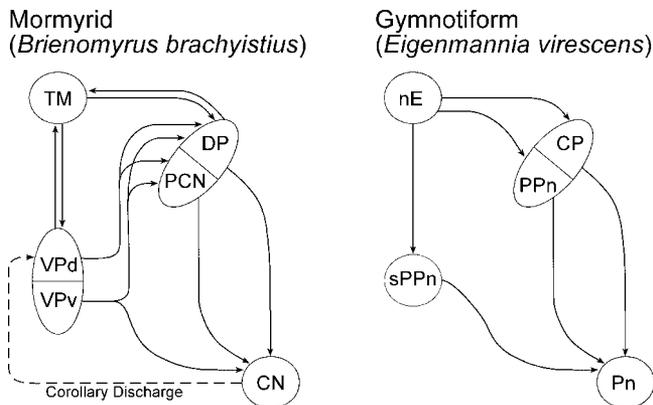


Fig. 11. Simplified flow diagrams comparing the independently evolved electromotor systems of mormyrids, based on *Brienomyrus brachyistius*, and gymnotiforms, based on *Eigenmannia virescens*.

The results of this study point toward additional convergences in the electromotor systems of these fish (Fig. 11). In gymnotiforms, the primary descending input to Pn is the central posterior-prepacemaker complex (CP/PPn), which includes a group of small, tightly packed, ovoid cells within the central posterior nucleus (CP) of the dorsal thalamus that extend slightly caudally in a ventrolateral direction to form the prepacemaker nucleus (PPn), a group of larger, multipolar cells with extrinsic dendrites (Kawasaki et al., 1988). This general organization is similar to the organization of the mormyrid DP/PCN, in which the rostral cells are small and ovoid and located within a dorsal thalamic nucleus, whereas the caudal cells are larger, are multipolar, and form a ventrolateral extension from the thalamus, with large, extrinsic dendrites (PCN). These similarities may be directly linked to functional convergence in the descending control of electromotor output.

Together, DP and CP make up the caudal portion of the dorsal thalamus in teleosts, which rostrally gives way to the anterior thalamic nucleus (for review see Meek and Nieuwenhuys, 1998). Whereas specifics vary across species, DP is generally defined as a cluster of cells located just ventral to the fasciculus retroflexus, whereas CP consists of a heterogeneous population of cells located ventral to DP, forming an obliquely oriented sheet that extends ventrolaterally from the ventricle (Echteler and Saidel, 1981; Echteler, 1984; Murakami et al., 1986; Striedter, 1990, 1991; Bass et al., 2000). CP is generally considered an auditory nucleus that receives its primary input from the acousticlateral torus semicircularis and, depending on the species, projects to the preglomerular nucleus, tectum mesencephali, anterior tubular nucleus, and/or telencephalon (Echteler, 1984; Murakami et al., 1986; Striedter, 1990, 1991; Kozloski and Crawford, 1998; Bass et al., 2000). By contrast, DP is considered a visual relay center that receives its primary input from the tectum mesencephali and projects to the telencephalon (Echteler and Saidel, 1981; Striedter, 1990; Wullimann and Northcutt, 1990).

In the present study, these two nuclei were clearly distinguishable based on morphological criteria, and the delineation between DP and CP is consistent with that of

Wullimann and Northcutt (1990) for the mormyrid *G. petersii*. Based on these criteria, the pattern of labeling indicates that DP, but not CP, provides descending input to CN in mormyrids. In addition, this region was shown to have connections with the tectum mesencephali and potentially the telencephalon but not with the auditory torus semicircularis. This pattern is consistent with a DP rather than a CP projection to CN. Considering the general differences in the connectivity and function of CP and DP in sensory processing among teleosts, it is quite interesting that they have been linked to the electromotor system of gymnotiforms and mormyrids, respectively. This difference likely reflects the different evolutionary histories of the two groups and may be related to functional differences in the control of electric signaling behavior. Comparative studies of the connectivity and physiological properties of the dorsal thalamic nuclei in teleosts may provide functional hypotheses for this difference and how it relates to the behavior and ecology of the two groups of fish.

In both mormyrids and gymnotiforms, the medullary electromotor network receives input from an additional mesencephalic source, VPv and the sublemniscal prepacemaker nucleus (SPPn), respectively (Fig. 11; Keller et al., 1991). Unlike the DP/PCN and CP/PPn comparison, however, these two nuclei are anatomically quite different, since VPv is a small group of large cells located within the torus semicircularis, whereas SPPn is a group of small cells located just ventral to the lateral lemniscus. In addition, VPv also projects to DP/PCN, and such a connection has not been documented for SPPn and CP/PPn, although in one species there is a prominent projection from CP/PPn to SPPn (Heiligenberg et al., 1996).

In mormyrids, the tectum mesencephali appears to play an important role as a sensorimotor interface for the electromotor system, insofar as it has reciprocal connections with both VPd and DP and receives input from several different sources (Figs. 10, 11). By contrast, in the gymnotiform species that have been studied, the tectum mesencephali does not project directly to the electromotor network. Instead, the diencephalic nucleus electrosensorius (nE) plays a critical role as a sensorimotor interface; it receives input from several regions of the torus semicircularis and projects to CP, PPn, and SPPn (Fig. 11; Carr et al., 1981; Keller et al., 1990). However, the detailed connectivity of the gymnotiform descending electromotor system has been studied only in select wave-type species, and there is little information available about sensory inputs to the electromotor system in pulse-type gymnotiform species. In the absence of this important comparative information, it is unclear whether this difference is related simply to disparate evolutionary histories or also to functional differences in electromotor control between pulse- and wave-type electric fish.

Inputs to the mormyrid electromotor system

The tectum mesencephali as a sensorimotor interface. The teleostean tectum mesencephali is considered to be a primary sensorimotor integration region (see Meek and Nieuwenhuys, 1998). In general, it receives sensory input from multiple sensory regions throughout the brain as well as motor feedback information from the rostral spinal cord and brainstem. Tectal efferents terminate in several areas, forming reciprocal connections with many of these inputs, and give rise to projections to midbrain

tegmental areas, a tectospinal and tectocerebellar pathway, and a prominent tectobulbar pathway that terminates throughout the reticular formation. The results of several studies point toward the tectum mesencephali as involved in integrating wide ranging information about sensory inputs and the effects of motor output to initiate and coordinate directed movements (for review see Meek and Nieuwenhuys, 1998). The single injection into the tectum mesencephali in the current study confirms the results of Wulliman and Northcutt (1990) in *G. petersii* and furthers the sensorimotor function of the tectum mesencephali to the likely coordination of electromotor output in mormyrids.

Several sensory nuclei in the torus semicircularis project to the tectum mesencephali in mormyrids, including medialis ventralis, medialis dorsalis, extero-lateralis pars posterior, and lateralis (Wulliman and Northcutt, 1990). Both medialis ventralis and extero-lateralis pars posterior are electrosensory nuclei that play a role in electrical communication, whereas lateralis is involved in electrosensory-mediated navigation (see Bell et al., 1981; Bell and Szabo, 1986). The mediodorsal nucleus is an auditory and lateral line recipient nucleus (Bell, 1981; Haugedé-Carré, 1983; Kozloski and Crawford, 1998). Several visual areas also project to the tectum mesencephali, including the retina, the central pretectal nucleus, and the dorsal periventricular thalamic nucleus (Lazar et al., 1984; Meek et al., 1986a, b; Wullimann and Northcutt, 1990). In addition, the tectum mesencephali receives input from the valvula of the cerebellum, which receives input from many different sensory regions (Russell and Bell, 1978; Haugedé-Carré, 1979; Bell, 1981; Finger et al., 1981; Szabo, 1983), and from the central area of the dorsal telencephalon, which receives input from the ventral preglomerular nucleus and has units that respond to a variety of different sensory modalities (Wullimann and Northcutt, 1990; Prechtl et al., 1998; von der Emde and Prechtl, 1999). Thus, the available anatomical evidence suggests that sensory inputs to the mormyrid electromotor system may largely be mediated by the tectum mesencephali.

Possible additional sensory inputs. Although the anatomical results suggest that the tectum mesencephali plays a primary role as a sensorimotor interface for electromotor behavior in mormyrids, other regions may be involved in relaying sensory information to the electromotor system as well. After VPd injections, retrogradely filled cells were found in the medial octavolateral nucleus, a lateral line recipient region in the hindbrain. This connection was not verified anterogradely, but injections of horseradish peroxidase into the medial octavolateral nucleus in *G. petersii* lead to primary anterograde label in medialis dorsalis with collaterals terminating in the ventral nucleus praeeminentialis as well as in VP (Bell, 1981). It is therefore likely that lateral line information reaches the electromotor pathway through this relatively direct route.

After VPd injections, retrogradely filled cells were also found in the dorsal preglomerular nucleus, which in mormyrids receives input from the telencephalon (Wulliman and Northcutt, 1990) and the electrosensory torus semicircularis (Finger et al., 1981). This therefore represents an additional pathway by which sensory input may reach the electromotor system. This connection was not verified anterogradely, but pin application of the fluorescent tracer DiI to the ventral preglomerular nucleus is

reported to lead to both anterograde and retrograde label in VP (von der Emde and Prechtl, 1999). In the current study, no labeled cells were observed within the ventral preglomerular nucleus after VP injections (though some anterograde label was present), but labeled cells in the dorsal preglomerular nucleus were located immediately dorsal to the ventral preglomerular nucleus. Thus, this finding by von der Emde and Prechtl (1999) might have resulted from the injection site in the ventral preglomerular nucleus encroaching slightly on the overlying dorsal preglomerular nucleus, which would be consistent with the results in the current study.

Injections into DP, PCN, and VPd all resulted in labeling of cells within the anterior tuberal nucleus of the hypothalamus. In teleosts, the anterior tuberal nucleus is generally thought to be involved in acousticolateral processing, because it receives inputs from auditory and mechanoreceptive regions of the torus semicircularis and diencephalon as well as the dorsal telencephalon and preoptic region (see Meek and Nieuwenhuys, 1998). The anterior tuberal nucleus may therefore represent an additional sensory input to the mormyrid electromotor system.

Sensory inputs and electromotor behavior. The connections between the tectum mesencephali and descending electromotor nuclei provide a potential pathway for a rich variety of sensory information to influence electromotor output. Inputs from several different modalities can lead to modulations in electromotor output for the purposes of both communication and active electrolocation (for review see Carlson, 2002), and it is likely that the tectal input to the electromotor system is responsible for orchestrating many of these responses. However, certain electrosensory-mediated electromotor behaviors involve extremely-short-latency responses that are not likely mediated by this pathway. For example, a single electrical stimulus pulse can result in an "echo" or "preferred latency response," in which the receiver produces an EOD at latencies as short as 10–14 msec (Russell et al., 1974). Electrical stimulation can also induce a "preferred latency avoidance," in which EOD latencies of 10–20 msec are avoided (Lücker and Kramer, 1981).

The delay from CN activation to EOD production is approximately 8 msec (3 msec delay to electromotor neuron activation, followed by 5 msec delay to EOD production; Bennett et al., 1967). This leaves only 2–6 msec from sensory input to CN activation for these responses, suggesting that they must be mediated through a pathway other than the tectum mesencephali or other potential sensory inputs identified in this study. Furthermore, the available evidence suggests that the echo response is mediated by the mormyromast electroreceptor pathway (Russell et al., 1974). Minimum latencies for mormyromast action potentials are approximately 2 msec (Bell, 1990), which would seem to suggest that mormyromasts must provide direct input to CN, although such a projection has not been observed in this or any other study (see Bell and Russell, 1978; Bell et al., 1983). The anatomical pathway for these short-latency responses, therefore, remains a mystery. It is worth noting that PCN units do respond to electrosensory stimulation, and this generally results in an electromotor response, but the latency for PCN responses is about 8–14 msec, which is too long to explain the echo response (von der Emde et al., 2000).

Possible links with reproductive/endocrine centers. After DP injections, both anterograde and retrograde label were found in a restricted area within the telencephalon, the supracommisural nucleus of the ventral telencephalon. This was not verified anterogradely, although an earlier study demonstrated retrograde labeling in DP after large telencephalic pin injections of horseradish peroxidase (Wullimann and Northcutt, 1990). The supracommisural nucleus is generally associated with sexual function; it receives inputs from the olfactory bulb, ventral and dorsal areas of the ventral telencephalon, and ventromedial thalamic nucleus and projects to the olfactory bulb, dorsal telencephalon, habenula, hypothalamus, and midbrain tegmentum (see Meek and Nieuwenhuys, 1998). If the connection between the supracommisural nucleus and DP is real, this represents a pathway by which reproductive behavior may be linked to electromotor signaling.

Additional areas that were consistently retrogradely labeled by injections into PCN and DP were the anterior and posterior preoptic areas and the dorsal hypothalamus. The dorsal hypothalamus was also retrogradely labeled following VPd injections. Although these projections were not confirmed by anterograde labeling, they represent additional pathways by which electromotor output may be modulated by socially relevant stimuli and/or influenced by reproductive condition, insofar as these regions are known to play an integral role in the neuroendocrine regulation of reproductive and social behavior across vertebrates (see Bass and Grober, 2001). In the gymnotiform electromotor system, CP/PPn has direct or indirect reciprocal connections with several hypothalamic and preoptic areas (Wong, 1997a, b; Correa and Zupanc, 2002). These projections appear to play an important role in social signaling, in that androgen-induced changes in communication behavior correlate with increased substance P-like immunoreactivity in CP/PPn (Dulka et al., 1995), and electrical stimulation of the preoptic area evokes EOD interruptions (Wong, 2000), which play a role in agonistic and sexual interactions (Hopkins, 1974; Hagedorn and Heiligenberg, 1985).

Implications for the mechanisms of electric signaling

The physiology of the medullary electromotor network in mormyrids has been well studied (Bennett et al., 1967; Bell et al., 1983; Grant et al., 1986, 1999; Carlson, 2002), and a recent study on the physiology of single units within PCN demonstrated some of the descending mechanisms involved in electromotor generation (von der Emde et al., 2000). The initiation of each EOD begins in CN, which fires in a 1:1 manner with EOD output. Intracellular recordings from CN neurons indicate that they do not display any pacemaker properties but, instead, appear to integrate descending input that influences EOD production (see Grant et al., 1999; Carlson, 2002). Indeed, transecting the brain between the mesencephalon and the rhombencephalon completely eliminates EOD production (Szabo, 1961).

One type of unit recorded within PCN shows spontaneous patterns of ongoing activity, with a slightly increased probability of firing slightly before CN activation (von der Emde et al., 2000). In addition, these units tend to fire a phase-locked spike that occurs immediately after CN activation at the same time as the first negative spike in the PCN field potential (see Fig. 1B). This is followed by a

silent period immediately after EOD generation. The authors formed three conclusions based on these observations: 1) PCN provides excitatory input to CN, 2) electrical synapses between PCN axons and CN result in antidromic activity in PCN following CN activation, and 3) PCN is inhibited by corollary discharge feedback after the generation of each EOD. A second type of unit recorded within PCN is completely silent except for firing a burst of action potentials starting around the time of EOD generation (von der Emde et al., 2000), which exactly corresponds to the onset of the silent period in PCN units. Thus, the authors concluded that these units are inhibitory afferents to PCN that are driven by the corollary discharge pathway and provide negative feedback for electromotor output.

The results of the current study provide anatomical support for this conclusion and delineate a likely pathway, in that the corollary discharge pathway feeds into PCN via the projection from MCA to VPd (Fig. 10). This suggests that the inhibitory afferents to PCN originate in VPd. This is supported by field potentials recorded within VPd, which showed oscillatory activity typical of multiunit bursting centered around the time of EOD generation (Fig. 1B). The main input to DP is also from VPd, suggesting that it is probably under similar negative feedback control. This is also supported by field potentials recorded within DP, which show a slow positive potential starting shortly after the time of EOD generation, similar to the corollary-discharge-driven slow positive potential observed in PCN (Fig. 1B; von der Emde et al., 2000; Carlson, 2002). Studies on single-unit activity in DP, PCN, and VPd are currently addressing these hypotheses (Carlson and Hopkins, 2000). It is unclear what role VPv may serve in the mormyrid electromotor system. Anatomically, its involvement would appear relatively minor compared with the strong projections from VPd to DP/PCN and from DP/PCN to CN. However, because it projects bilaterally to DP/PCN as well as to CN, it may play a critical role in coordinating the activity patterns of the entire electromotor system.

Because VPd appears to be involved in providing negative feedback to DP/PCN, it is quite interesting that it also receives such a significant input from the tectum mesencephali. This suggests that modulations in electromotor output may be partially controlled through modification of the inhibitory feedback provided by VPd. Indeed, the burst durations of corollary-discharge-driven inhibitory units (presumed to be VPd units) show a negative relationship with instantaneous EOD rates (von der Emde et al., 2000).

A wealth of research on the physiology of the gymnotiform electromotor system has made it an excellent model system for understanding the mechanisms of signal production (for review see Metzner, 1999). A major theme that has emerged from this research is that the different inputs (CP, PPn, and SPPn) to the pacemaker nucleus (Pn) are each responsible for controlling the production of distinct modulations in EOD output through differences in the location and pharmacology of synapses within Pn (see Metzner, 1999). The general similarities between the descending electromotor systems of mormyrids and gymnotiforms raise the possibility that the different inputs to the mormyrid CN are also responsible for generating different EOD modulations. A recent study of electromotor signaling behavior in *B. brachyistius* has demonstrated that there are three distinct kinds of stereotyped EOD

bursts, termed scallops, rasps, and accelerations (Carlson and Hopkins, unpublished observations). Furthermore, evidence on the quantitative characteristics of these bursts suggests that rasp production results from a combination of scallop and acceleration production. This raises the possibility that the two main inputs to CN (DP and PCN) each drive a different kind of burst (scallop or acceleration) and together drive rasps. Studies using single-unit recording and extracellular stimulation of these areas are currently addressing this issue (see Carlson and Hopkins, 2000, 2001). Future studies on the physiology of the mormyrid electromotor system will be needed to understand better the mechanisms involved in regulating electromotor output. From a comparative perspective, this information should prove relevant to the establishment of general principles in the mechanisms of signal production.

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LITERATURE CITED

- Bass AH. 1986. Species differences in electric organs of mormyrids: substrates for species-typical electric organ discharge waveforms. *J Comp Neurol* 244:313–330.
- Bass AH, Grober MS. 2001. Social and neural modulation of sexual plasticity in teleost fish. *Brain Behav Evol* 57:293–300.
- Bass AH, Hopkins CD. 1982. Comparative aspects of brain organization of an African “wave” electric fish, *Gymnarchus niloticus*. *J Morphol* 174:313–334.
- Bass AH, Hopkins CD. 1983. Hormonal control of sexual differentiation: changes in electric organ discharge waveform. *Science* 220:971–974.
- Bass AH, Denizot JP, Marchaterre MA. 1986. Ultrastructural features and hormone-dependent sex differences of mormyrid electric organs. *J Comp Neurol* 254:511–528.
- Bass AH, Marchaterre MA, Baker R. 1994. Vocal–acoustic pathways in a teleost fish. *J Neurosci* 14:4025–4039.
- Bass AH, Bodnar DA, Marchaterre MA. 2000. Midbrain acoustic circuitry in a vocalizing fish. *J Comp Neurol* 419:515–531.
- Bell CC. 1981. Some central connections of medullary octavolateral centers in a mormyrid fish. In: Fay RR, Popper AN, Tavolga WN, editors. *Hearing and sound communication in fishes*. Berlin: Springer-Verlag. p 383–392.
- Bell CC. 1990. Mormyromast electroreceptor organs and their afferent fibers in mormyrid fish. III. Physiological differences between two morphological types of fibers. *J Neurophysiol* 63:319–332.
- Bell CC, Russell CJ. 1978. Termination of electroreceptor and mechanical lateral line afferents in the mormyrid acusticolateral area. *J Comp Neurol* 182:367–382.
- Bell CC, Szabo T. 1986. Electroreception in mormyrid fish: central anatomy. In: Bullock TH, Heiligenberg W, editors. *Electroreception*. New York: John Wiley & Sons. p 375–421.
- Bell CC, von der Emde G. 1995. Electric organ corollary discharge pathways in mormyrid fish: II. The medial juxtalobar nucleus. *J Comp Neurol* A177:463–479.
- Bell CC, Finger TE, Russell CJ. 1981. Central connections of the posterior lateral line lobe in mormyrid fish. *J Comp Neurol* 216:327–338.
- Bell CC, Libouban S, Szabo T. 1983. Pathways of the electric organ discharge command and its corollary discharges in mormyrid fish. *J Comp Neurol* 216:327–338.
- Bell CC, Dunn K, Hall C, Caputi A. 1995. Electric organ corollary discharge pathways in mormyrid fish: I. The mesencephalic command associated nucleus. *J Comp Neurol* A177:449–462.
- Bennett MVL, Grundfest H. 1961. Studies on the morphology and electrophysiology of electric organs. III. Electrophysiology of electric organs in mormyrids. In: Chagas C, del Carvahlo P, editors. *Bioelectrogenesis*. Amsterdam: Elsevier. p 113–135.
- Bennett MVL, Aljure E, Nakajima Y, Pappas GD. 1963. Electrotonic junctions between teleost spinal neurons: electrophysiology and ultrastructure. *Science* 141:262–264.
- Bennett MVL, Pappas G, Aljure E, Nakajima Y. 1967. Physiology and ultrastructure of electrotonic junctions. II. Spinal and medullary electromotor nuclei in mormyrid fish. *J Neurophysiol* 30:180–208.
- Bullock TH, Northcutt RG, Bodznick DA. 1982. Evolution of electroreception. *Trends Neurosci* 5:50–53.
- Bullock TH, Bodznick DA, Northcutt RG. 1983. The phylogenetic distribution of electroreception: evidence for convergent evolution of a primitive vertebrate sense modality. *Brain Res Rev* 6:25–46.
- Carlson BA. 2002. Electric signaling behavior and the mechanisms of electric organ discharge production in mormyrid fish. *J Physiol (Paris)* (in press).
- Carlson BA, Bass AH. 2000. Sonic/vocal motor pathways in squirrelfish (Teleostei, Holocentridae). *Brain Behav Evol* 56:14–28.
- Carlson BA, Hopkins CD. 2000. Neural mechanisms in the generation of electric signals in mormyrid fish. *Soc Neurosci Abstr* 26:1522.
- Carlson BA, Hopkins CD. 2001. Extracellular stimulation of descending electromotor nuclei in the mormyrid *Brienomyrus brachyistis* and the control of electric signaling behavior. 6th Int Cong Neuroethol, Bonn, Germany. p 221.
- Carlson BA, Hopkins CD, Thomas P. 2000. Androgen correlates of socially induced changes in the electric organ discharge waveform of a mormyrid fish. *Horm Behav* 38:177–186.
- Carr CE. 1993. Processing of temporal information in the brain. *Annu Rev Neurosci* 16:223–243.
- Carr CE, Heiligenberg WH, Sas E. 1981. Laminar organization of the afferent and efferent systems of the torus semicircularis of gymnotiform fish: morphological substrates for parallel processing in the electrosensory system. *J Comp Neurol* 203:1982.
- Carr CE, Soares D, Parameshwaran S, Perney T. 2001. Evolution and development of time coding systems. *Curr Opin Neurobiol* 11:727–733.
- Correa SAL, Zupanc GKH. 2002. Connections between the central posterior/prepacemaker nucleus and hypothalamic areas in the weakly electric fish *Apteronotus leptorhynchus*: evidence for an indirect, but not a direct link. *J Comp Neurol* 442:348–364.
- Dulka JG, Maler L, Ellis W. 1995. Androgen-induced changes in electrocommunicatory behavior are correlated with changes in substance P-like immunoreactivity in the brain of the electric fish *Apteronotus leptorhynchus*. *J Neurosci* 15:1879–1890.
- Dye JC, Meyer JH. 1986. Central control of the electric organ discharge in weakly electric fish. In: Bullock TH, Heiligenberg W, editors. *Electroreception*. New York: John Wiley & Sons. p 71–102.
- Echteler SM. 1984. Connections of the auditory midbrain in a teleost fish, *Cyprinus carpio*. *J Comp Neurol* 230:536–551.
- Echteler SM, Saidel WM. 1981. Forebrain connections in the goldfish support telencephalic homologues with land vertebrates. *Science* 212:683–685.
- Elekes K, Szabo T. 1982. Synaptic organization in the pacemaker nucleus of a medium frequency weakly electric fish, *Eigenmannia* sp. *Brain Res* 237:267–282.
- Elekes K, Szabo T. 1985a. The mormyrid brainstem 3. Ultrastructure and synaptic organization of the medullary pacemaker nucleus. *Neuroscience* 15:431–444.
- Elekes K, Szabo T. 1985b. Synaptology of the medullary command pacemaker nucleus of the weakly electric fish *Apteronotus leptorhynchus*, with particular reference to comparative aspects. *Exp Brain Res* 60:509–520.
- Elekes K, Ravaille M, Bell CC, Libouban S, Szabo T. 1985. The mormyrid brainstem 2. The medullary electromotor relay nucleus: an ultrastructural horseradish peroxidase study. *Neuroscience* 15:417–430.
- Finger TE, Bell CC, Russell CJ. 1981. Electrosensory pathways to the valvula cerebelli in mormyrid fish. *Exp Brain Res* 42:23–33.
- Finger TE, Bell CC, Carr CE. 1986. Comparisons among electroreceptive

- teleosts: why are electrosensory systems so similar? In: Bullock TH, Heiligenberg W, editors. *Electroreception*. New York: John Wiley & Sons. p 465–481.
- Friedman MA, Hopkins CD. 1996. Tracking individual mormyrid electric fish in the field using electric organ discharge waveforms. *Anim Behav* 51:391–407.
- Grant K, Bell CC, Clause S, Ravaille M. 1986. Morphology and physiology of the brainstem nuclei controlling the electric organ discharge in mormyrid fish. *J Comp Neurol* 245:514–530.
- Grant K, von der Emde G, Sena LG, Mohr C. 1999. Neural command of electromotor output in mormyrids. *J Exp Biol* 202:1399–1407.
- Hagedorn M, Heiligenberg W. 1985. Court and spark: electric signals in the courtship and mating of gymnotoid fish. *Anim Behav* 33:254–265.
- Haugedé-Carré F. 1979. The mesencephalic extero-lateral posterior nucleus of the mormyrid fish *Brienomyrus niger*: efferent connections studied by the horseradish peroxidase method. *Brain Res* 178:179–184.
- Haugedé-Carré F. 1983. The mormyrid mesencephalon. II. The medio-dorsal nucleus of the torus semicircularis: afferent and efferent connections studied with the HRP method. *Brain Res* 268:1–14.
- Heiligenberg W, Metzner W, Wong CJH, Keller CH. 1996. Motor control of the jamming avoidance response of *Apteronotus leptorhynchus*: evolutionary changes of a behavior and its neuronal substrates. *J Comp Physiol A* 179:653–674.
- Hopkins CD. 1974. Electric communication: functions in the social behavior of *Eigenmannia virescens*. *Behavior* 50:270–305.
- Hopkins CD. 1980. Evolution of electric communication channels of mormyrids. *Behav Ecol Sociobiol* 7:1–13.
- Hopkins CD. 1981. On the diversity of electric signals in a community of mormyrid electric fish in West Africa. *Am Zool* 21:211–222.
- Hopkins CD. 1995. Convergent designs for electrogenesis and electroreception. *Curr Opin Neurobiol* 5:769–77.
- Huang Q, Zhou D, DiFiglia M. 1992. Neurobiotin, a useful neuroanatomical tracer for in vivo anterograde, retrograde and transneuronal tract-tracing and for in vitro labeling of neurons. *J Neurosci Methods* 41:31–43.
- Kawasaki M, Maler L, Rose GJ, Heiligenberg W. 1988. Anatomical and functional organization of the prepacemaker nucleus in gymnotiform electric fish: the accommodation of two behaviors in one nucleus. *J Comp Neurol* 276:113–131.
- Keller CH, Maler L, Heiligenberg W. 1990. Structural and functional organization of a diencephalic sensorimotor interface in the gymnotiform fish *Eigenmannia*. *J Comp Neurol* 293:347–376.
- Keller CH, Kawasaki M, Heiligenberg W. 1991. The control of pacemaker modulations for social communication in the weakly electric fish *Sternopygus*. *J Comp Physiol A* 169:441–450.
- Kozloski J, Crawford JD. 1998. Functional neuroanatomy of auditory pathways in the sound-producing fish *Pollimyrus*. *J Comp Neurol* 401:227–252.
- Kramer B. 1994. Communication behavior and sensory mechanisms in weakly electric fishes. In: Slater PJB, Rosenblatt JS, Snowdon CT, Milinski M, editors. *Advances in the study of behavior*. San Diego: Academic Press. p 233–270.
- Lazar G, Libouban S, Szabo T. 1984. The mormyrid mesencephalon 3. Retinal projections in a weakly electric fish (*Gnathonemus petersii*). *J Comp Neurol* 230:1–12.
- Lücker H, Kramer B. 1981. Development of a sex difference in the preferred latency response in the weakly electric fish, *Pollimyrus isidori* (Cuvier et Valenciennes; Mormyridae, Teleostei). *Behav Ecol Sociobiol* 9:103–109.
- Meek J, Nieuwenhuys R. 1998. Holosteans and teleosts. In: Nieuwenhuys R, ten Donkelaar HJ, Nicholson C, editors. *The central nervous system of vertebrates*. New York: Springer. p 759–937.
- Meek J, Nieuwenhuys R, Elsevier D. 1986a. Afferent and efferent connections of cerebellar lobe C1 of the mormyrid fish *Gnathonemus petersii*: a horseradish peroxidase study. *J Comp Neurol* 245:319–341.
- Meek J, Nieuwenhuys R, Elsevier D. 1986b. Afferent and efferent connections of cerebellar lobe C3 of the mormyrid fish *Gnathonemus petersii*: a horseradish peroxidase study. *J Comp Neurol* 245:342–358.
- Metzner W. 1999. Neural circuitry for communication and jamming avoidance in gymnotiform electric fish. *J Exp Biol* 202:1365–1375.
- Murakami T, Ito H, Morita Y. 1986. Telencephalic afferent nuclei in the carp diencephalon, with special reference to fiber connections of the nucleus preglomerulosus pars lateralis. *Brain Res* 382:97–103.
- Prechtl JC, von der Emde G, Wolfart J, Karamursel S, Akoev GN, Andrianov YN, Bullock TH. 1998. Sensory processing in the pallium of a mormyrid fish. *J Neurosci* 18:7381–7393.
- Russell CJ, Bell CC. 1978. Neuronal responses to electrosensory input in the mormyrid valvula cerebelli. *J Neurophysiol* 41:1495–1510.
- Russell CJ, Meyers JP, Bell CC. 1974. The echo response in *Gnathonemus petersii* (Mormyridae). *J Comp Physiol* 92:181–200.
- Striedter GF. 1990. The diencephalon of the channel catfish. II. Retinal, tectal, cerebellar and telencephalic connections. *Brain Behav Evol* 36:355–377.
- Striedter GF. 1991. Auditory, electrosensory and mechanosensory lateral line pathways through the forebrain in channel catfishes. *J Comp Neurol* 312:311–331.
- Szabo T. 1961. Anatomophysiologie de centres nerveux spécifiques de quelques organes électriques. In: Chagas C, del Carvahlo P, editors. *Bioelectrogenesis*. Amsterdam: Elsevier. p 185–201.
- Szabo T. 1983. Cerebellar pathways in the brain of the mormyrid teleost fish. *Acta Morphol Hung* 31:219–234.
- von der Emde G, Prechtl JC. 1999. Anatomical connections of auditory and lateral line areas of the dorsal telencephalon (Dm) in the osteoglossomorph teleost, *Gnathonemus petersii*. *Brain Res* 818:355–367.
- von der Emde G, Gomez Sena L, Niso R, Grant K. 2000. The midbrain precommand nucleus of the mormyrid electromotor network. *J Neurosci* 20:5483–5495.
- Wong CJH. 1997a. Afferent and efferent connections of the diencephalic prepacemaker nucleus in the weakly electric fish, *Eigenmannia virescens*: interactions between the electromotor system and the neuroendocrine axis. *J Comp Neurol* 383:18–41.
- Wong CJH. 1997b. Connections of the basal forebrain of the weakly electric fish, *Eigenmannia virescens*. *J Comp Neurol* 389:49–64.
- Wong CJH. 2000. Electrical stimulation of the preoptic area in *Eigenmannia*: evoked interruptions in the electric organ discharge. *J Comp Physiol A* 186:81–93.
- Wullmann MF, Northcutt RG. 1990. Visual and electrosensory circuits of the diencephalon in mormyrids: an evolutionary perspective. *J Comp Neurol* 297:537–552.