Original Paper

Brain, Behavior and Evolution

Brain Behav Evol 2000;56:14–28

Sonic/Vocal Motor Pathways in Squirrelfish (Teleostei, Holocentridae)

Bruce A. Carlson Andrew H. Bass

Department of Neurobiology and Behavior, Cornell University, Ithaca, N.Y., USA

Key Words

Teleost · Squirrelfish · Sonic motor nucleus · Vocalization · Brainstem

Abstract

Similar to many teleost fish, squirrelfish (family Holocentridae) produce vocalizations by the contraction of muscles that lead to vibration of the swimbladder. We used biotinylated compounds to identify the position and extent of vocal motor neurons in comparison to additional motor neuron groups, namely those of red and white dorsal epaxial muscle and opercular muscle that are located adjacent to or near the sonic muscle. The sonic motor nucleus (SMN) was located in the caudal medulla and rostral spinal cord in a ventrolateral position with dendrites extending dorsally in a dense bundle along the lateral edge of the medulla and axons exiting via ventral occipital nerve roots. Transneuronal transport of biocytin identified premotor neurons within the SMN and in the medially adjacent reticular formation that projected to the contralateral SMN and more rostrally to the octavolateralis efferent nucleus and nucleus praeeminentialis, suggesting interactions between vocal and octavolateralis systems as seen in other teleosts. Motor neurons innervating the red and white dorsal muscle formed a loose aggregate in the dorsal motor column, adjacent to the medial longitudinal fasciculus, sending fibers bilaterally throughout the spinal cord with axons exiting via ventral spinal nerve roots. Opercular motor neurons were located within the facial motor nucleus. The anatomical characteristics of the SMN of squirrelfish, a representative member of the order Beryciformes, are similar to those of representative members of the closely related order Scorpaeniformes, but diverge from the SMN of more distantly related orders of paracanthopterygiian and ostariophysan teleosts. These results therefore suggest a possible homology among the SMNs of acanthopterygiian fishes.

Introduction

Numerous groups of actinopterygiian fish produce vocalizations that are important for social interactions both within and between species, including osteoglossomorphs, ostariophysids, paracanthopterygiians, and acanthopterygiians [Myrberg, 1981; Ladich, 1997]. In many cases, sound production is generated by the rhythmic contraction and expansion of the swimbladder [Tower, 1908; Winn and Marshall, 1963]. Other mechanisms also exist, including membrane 'drumming' [Salmon et al., 1968], rubbing of pectoral spines [Schachner and Schaller, 1981], snapping of pectoral tendons [Kratochvil, 1978], and grinding of pharyngeal teeth [Burkenroad, 1930; Lanzing, 1974] or vertebrae [Fish, 1953; Tavolga, 1971]. The diversity of mechanisms and the apparent lack of phylogenetic patterns suggest that sound-production has evolved independently in several groups, though it is likely that many of the sound producing structures are homologous in origin [Bass, 1989; Bass and Baker, 1991, 1997; Ladich and Bass, 1998].

The central neuroanatomy of swimbladder-based sonic motor systems have been studied in many groups of teleosts,

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/journals/bbe

Andrew H. Bass
Department of Neurobiology and Behavior, Cornell University
Ithaca, NY 14853 (USA)
Tel. +1 (607) 254-4372, Fax +1 (607) 254-4308
E-Mail ahb3@cornell.edu

including mormyrid electric fish [Bass, 1985; Bass et al., 1986], Batrachoidiformes [Demski et al., 1973; Fine et al., 1984; Bass, 1985; Bass et al., 1994], Scorpaeniformes [Bass, 1985; Finger and Kalil, 1985; Bass and Baker, 1991; Ladich and Bass, 1998; Yoshimoto et al., 1999], and Siluriformes [Ladich and Fine, 1994; Ladich and Bass, 1996; 1998]. The sonic motor nucleus extends from the caudal medulla into the rostral spinal cord as a midline structure in batrachoidids and mochokid and doradid catfishes, a lateral, paired structure in ariid catfishes (both midline and lateral structures are found in pimelodid catfishes), and a paired, ventrolateral structure in triglids, cottids, and scorpaenids [Ladich and Bass, 1998; Yoshimoto et al., 1999]. In batrachoidids, scorpaenids, and mochokid catfish, premotoneurons and connections with the auditory/lateral line circuitry have also been identified [Bass and Baker, 1990; Bass et al., 1994, 2000; Ladich and Bass, 1996; Yoshimoto et al., l999]. Axons of sonic motoneurons exit via occipital nerve roots (homologue of the tetrapod hypoglossal nerve) to innervate sonic muscle, which is derived from occipital somites [Bass and Baker, 1997].

Although swimbladder-based sonic motor systems are found in numerous acanthopterygiian fish [Fish and Mowbray, 1970], studies of the central neuroanatomy of vocal pathways have been limited to scorpaeniforms [Bass and Baker, 1991; Ladich and Bass, 1998; Yoshimoto et al., 1999]. From a comparative viewpoint, studies of additional orders of acanthopterygiians are essential to further elucidate the evolutionary origins of teleost sonic mechanisms and speculate on the importance of phylogeny and adaptation as related to functional differences among species [Bass, 1989; Bass and Baker, 1991; Ladich and Bass, 1998]. The goal of the present study was to analyze the sonic motor system in squirrelfish (Beryciformes, Holocentridae).

Holocentrids constitute a highly successful teleost lineage, being found circumtropically and comprising at least 70 species [Shimizu and Yamakawa, 1979; Myers, 1989; Lieske and Myers, 1996]. Of the few holocentrid species examined, all produce sounds consisting of 'grunts' produced either singly or in bursts, each grunt being made up of between 2–8 individual pulses with spectral components between about 75–600 Hz [Moulton, 1958; Winn and Marshall, 1963; Winn et al., 1964; Fish and Mowbray, 1970; Bright and Sartori, 1971]. Although the exact function of sound production in squirrelfish remains unclear, evidence suggests that it may be involved in courtship, aggression, and predator deterrence [Herald and Dempster, 1957; Moulton, 1958; Winn et al., 1964; Bright and Sartori, 1971].

The sonic muscles originate bilaterally from the skull, just rostral and dorsal to the auditory bulla and attach to the first 2–3 ventral ribs, which are flattened and connected to the swimbladder (fig. 1A) [Nelson, 1955; Winn and Marshall, 1963]. The caudal end of the sonic muscle extends past the second rib to terminate directly on the swimbladder (fig. lA). Contraction of the muscles results in swimbladder vibration and the fundamental frequency of the sound (ca. 75–85 Hz) is determined in a one-to-one manner by the firing rate of the motor axons innervating the sonic musculature [Gainer et al., 1965].

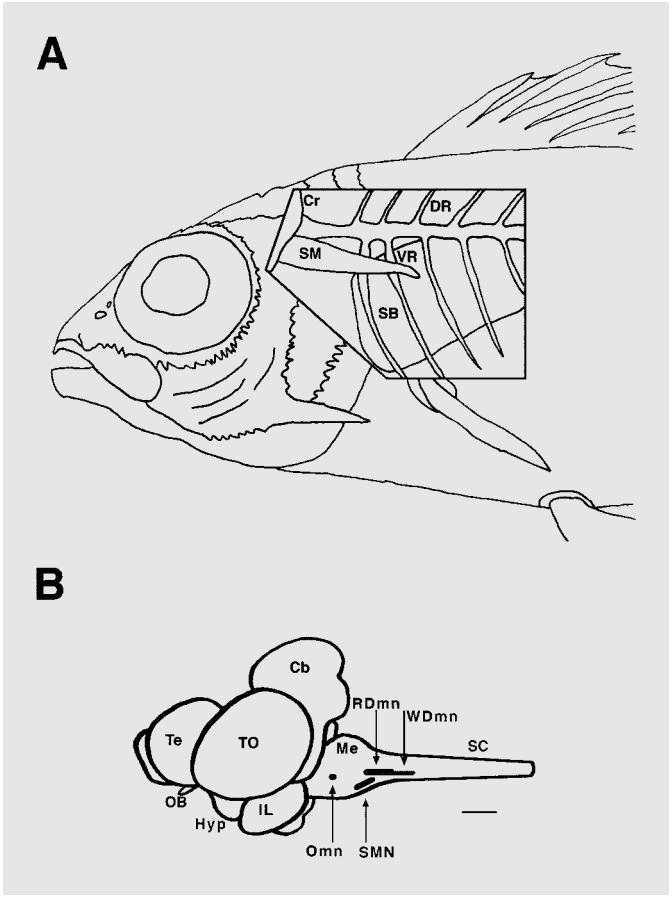


Fig. 1. A Line drawing showing the location of the structures involved in sound production in holocentrids (shown here is *Sargocentron cornutum*). The sonic muscle (SM) originates at the cranium (Cr) and inserts on the first 2–3 ventral ribs (VR), which attach to the swimbladder (SB). Contraction of the sonic muscle leads to vibration of the swimbladder. Other abbreviations: DR = Dorsal ribs. **B** Line drawing of a lateral view of the brain of *S. cornutum*, showing the approximate rostral-caudal extent of opercular motor neurons (Omn); sonic motor nucleus (SMN); red dorsal motor neurons (RDmn); white dorsal motor neurons (WDmn). Other abbreviations: Cb = Cerebellum; Hyp = hypothalamus; IL = inferior lobe of the hypothalamus; Me = medulla; OB = olfactory bulb; SC = spinal cord; Te = telencephalon; TO = optic tectum. Bar scale = 100 µm.

The swimbladder also appears to be involved in hearing. Within the subfamily Myripristinae, there is a tight coupling between the rostral end of the swimbladder and the auditory bulla, whereas within the subfamily Holocentrinae, such a connection is lacking, although a secondary, less intimate association is found in two species of *Holocentrus* [Nelson, 1955]. This association permits the swimbladder to function as a pressure transducer to enhance hearing sensitivity. In fact, these three different morphologies correlate with hearing sensitivity, both in terms of threshold and bandwidth; i.e. sensitivity is directly related to the degree of coupling [Coombs and Popper, 1979]. In the present study, we injected two types of biotinylated tracers into the sonic muscles and adjacent muscles to determine the location and morphology of the sonic motor nucleus in Holocentrids. We interpret our findings in the broader context of sound production and sonic/vocal motor pathways in teleost fish.

Materials and Methods

A total of twenty-two fish were used in this study: twelve *Sargocentron xantherythrum* (2.41–3.94 g), four *S. seychellense* (1.92–14.51 g), one *S. cornutum* (4.28 g), one *Neoniphon sammara* (4.03 g), and four *Holocentrus rufus* (151.3–172.0 g). All fish were obtained commercially (*Sargocentron spp.* and *Neoniphon sammara* from Quality Marine, Los Angeles, Calif., and *Holocentrus rufus* from Reef Displays of Florida, Marathon, Fla., USA) and maintained in groups of 2–8 individuals at 26 °C in aerated and filtered marine aquaria. Most individuals were immature, though a few of the larger ones appeared to be just reaching maturity based on gross examination of the gonads. This research was performed under the guidelines established by the National Institute of Health and the Cornell University Institutional Animal Care and Use Committee.

Four groups of muscles were studied, including sonic muscle, red and white dorsal (epaxial) muscle, and opercular muscle. The latter three were included as controls for the possible spread of biotin following labeling of the sonic muscle and for comparative purposes aimed to distinguish other motor neuron populations in the medulla and spinal cord. Surgical and immunohistochemical procedures were similar to those of Bass et al. [1994] and Ladich and Bass [1996, 1998]. Fish were anesthetized with tricaine methanesulfonate (MS 222; 50–150 mg/l). For injections into the sonic and opercular muscles, no incision was necessary as the muscles were easily accessible through the operculum. We did, however, clip the terminal edge of the operculum in order to increase accessibility. For injections into the red and white dorsal muscles, a 2–3 cm incision was made dorsolaterally, about one cm off of the midline. The white muscle was immediately accessible through the incision, whereas the red muscle, which is just ventral, was made accessible by displacing the white muscle.

Small incisions were made into the muscle of interest. Then, using a '00' minutien insect pin, we directly placed crystals of biocytin (Sigma, St. Louis, Mo., USA) or biotin dextran-amine (BDA, 10 kD; Molecular Probes, Eugene, Oreg., USA) into the incision. Previous studies have demonstrated that biocytin, but not BDA, may be transported transynaptically following labeling of peripheral sensory and motor nerves [see Bass et al., 1994]. Incisions were sutured shut and sealed using parafilm and Vetbond (3M, St. Paul, Minn., USA). Animals were then returned to their home aquarium for recovery.

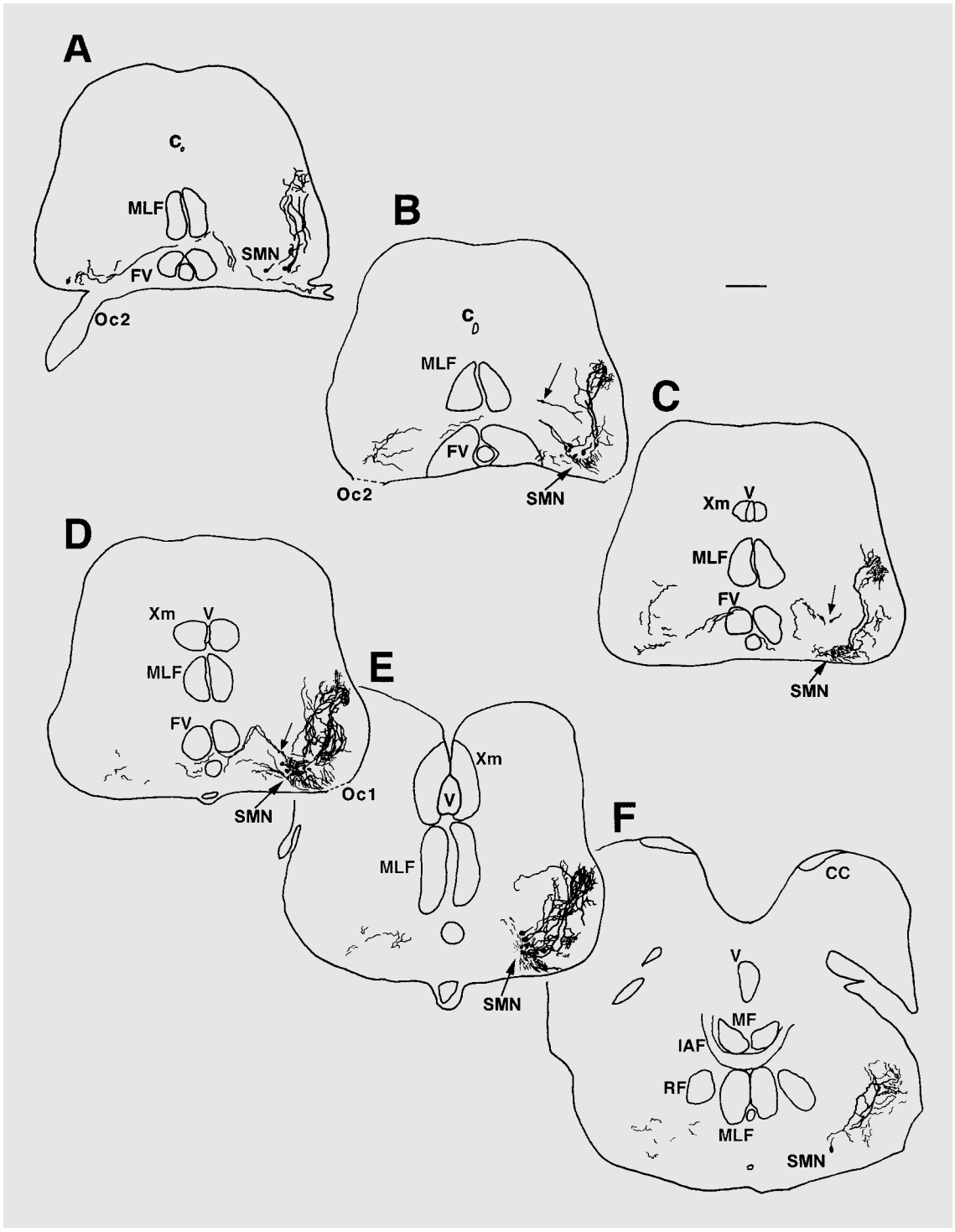
Of the twelve *S. xantherythrum*, we injected four in the sonic muscle, two with biocytin and two with BDA. Four were injected in the red dorsal muscle, three with biocytin and one with BDA. Two were injected in the white dorsal muscle, both with biocytin. One individual received a biocytin application to the opercular muscle and one received a biocytin application to the opercular muscle and contralateral sonic muscle. Of the four *S. seychellense*, three received injections into the sonic muscle, two with biocytin and one with BDA. One received an injection of biocytin into the opercular muscle. The single individuals of *S. cornutum* and *N. sammara* both received an injection of biocytin into the red dorsal muscle. All four *H. rufus* individuals received injections of biocytin into the sonic muscle.

After survival times of 2–4 days, animals were deeply anesthetized in MS 222 (200–300 mg/l) and perfused transcardially with cold marine teleost Ringer's followed by 4% paraformaldehyde/1% glutaraldehyde dissolved in 0.1 *M* phosphate buffer (PB). Brains were removed and postfixed for about 1 h, then stored in 0.1 *M* PB, and finally transferred to 30% sucrose-PB on the night prior to sectioning.

Transverse sections were cut frozen at 50 µm and processed as follows: (1) 30 min incubation in 0.4% Triton-X in phosphate-buffered saline (PBS); (2) 3 h incubation in an avidin-biotinylated horseradish peroxidase (HRP) complex (Elite Kit, Vector Laboratories, Burlingame, Calif., USA); (3) Two 10-min rinses in 0.1 *M* PB; (4) 1–2 min incubation in 0.05% diaminobenzidine (DAB), 0.01% hydrogen peroxide dissolved in 0.2 *M* PB (visualized for reaction product); (5) Two rinses in PB. Sections were then stored in PB until mounted on chrom-alum-subbed slides. Slides were counterstained with cresyl violet, dehydrated in a graded alcohol series, and coverslipped.

In two cases each of biocytin and BDA labeling in *S. xantherythrum* that were similar in body size (2.41–2.83 g), the cross sectional area of neurons was estimated using a micrometer. As the neurons measured were all approximately ellipsoid in shape, we measured the length of the long (L) and short (S) axes of each neuron, and then determined the area using the standard calculation for ellipsoid area $[(0.5L)(0.5S)\pi]$. Only those neurons in every section with a clearly discernible perimeter and at least one neurite were used. Given the small sample size, no statistical analysis was performed.

Fig. 2. Line drawings of transverse sections through the caudal medulla and rostral spinal cord of *S. xantherythrum* following biocytin application to the sonic muscle. Large dots represent the locations of labeled somata; black lines represent the locations of fibers. Dashed lines indicate the position of occipital nerve roots cut during removal of the brain. Small arrows in B–D point to the location of putative premotor neurons located at the dorsomedial edge of the sonic motor nucleus and within the reticular formation. **A**–**B** Caudal extent of sonic motor nucleus, where primary efferents enter the second occipital nerve; note projections to contralateral sonic motor nucleus (SMN). **C**–**E** Center of SMN, at the level of the vagal motor nucleus; note primary efferents entering the first occipital nerve in **D**. **F** Rostral extent of SMN, at the caudal end of the cerebellar crest and internal arcuate fibers. Abbreviations: C = Central canal; CC = cerebellar crest; FV = ventral fasciculus; IAF = internal arcuate fibers; MF = Mauthner fibers; MLF = medial longitudinal fasciculus; Ocl/ Oc2 = occipital nerve roots one and two; RF = reticular formation; V = fourth ventricle; Xm = vagal motor nucleus. Distance between sections = 150 µm. Bar scale = 200 µm.


A
C
MLF
SMN
FV
Oc2
B
C
MLF
FV
Oc2
SMN
C
Xm
V
MLF
FV
SMN
D
Xm
V
MLF
FV
Oc1
SMN
E
Xm
V
MLF
SMN
F
CC
V
MF
IAF
RF
MLF
SMN

2

Results

Application of tracer to the various muscles led to labeling in distinct areas of the medulla and spinal cord for each muscle group (fig. 1B). The sonic motor nucleus (SMN) was located ventrolaterally, approximately at the junction between the rostral spinal cord and caudal medulla. Generally speaking, the motor neurons innervating the red (RDmn) and white (WDmn) dorsal muscles were localized at the rostral end of the spinal cord within the dorsal motor column and partially overlapping, the former extending anterior to the latter. Opercular motor neurons (Omn) were located in the facial motor nucleus (nVIIm) of the medulla, arising just rostral to the caudal end of the cerebellum. Tracer application to a given muscle did not result in marked staining of any other motor neuron groups, indicating that label did not spread between adjacent muscles. We did not observe any obvious differences between species in the patterns of labeling for any of the motor neuron groups studied.

Sonic Motor Nucleus

Application of either biocytin or biotin-dextran amine (BDA) to the sonic muscle resulted in ipsilateral labeling of a paired, ventrolateral sonic motor nucleus (SMN) extending from the rostral spinal cord into the caudal medulla (fig. 2), beginning just anterior to the vagal motor nucleus (Xm, fig. 2E). Labeled axons exited through the paired, ventral occipital nerve roots (Ocl, Oc2; fig. 2A, B, D). Large dendrites from the SMN neurons extended dorsally along the lateral edge of the medulla and rostral spinal cord where they ended in a dense plexus of varicosities (fig. 2, 3A, C). Smaller caliber dendrites also formed varicosities within the SMN and in a region slightly medial of the SMN (fig. 3B). In three cases with biocytin and one with BDA, a small number of cells were labeled within the dorsal motor column, just lateral to the medial longitudinal fasciculus (MLF), throughout the rostral-caudal extent of the SMN (not shown). This labeling was likely due to tracer spread to adjacent red and white dorsal muscles (see below).

Biocytin application resulted in labeled fibers extending across the midline and appearing in the contralateral SMN (fig. 2, 3C). In a few cases, fibers extended rostrally from the SMN along the lateral edge of the ipsilateral medulla to enter the rostral medulla and midbrain (fig. 4). In two cases, labeled fibers and terminals were found in the octavolateralis efferent nucleus (OEN; fig. 4B), located medially between the MLF and fourth ventricle at the level of nucleus medialis (M), and in nucleus praeeminentialis (PE; fig. 3D, 4D), a dorsolateral nucleus located just ventral to the eminentia granularis (EG) of the cerebellum. These rostral and contralateral projections were never observed following labeling with BDA, suggesting that they did not originate from the primary sonic motor neurons, but from premotor neurons. Small, fusiform-shaped cells were found in the medial portion of the SMN and dorsomedially within the medial reticular formation that projected ventrolaterally to the ipsilateral SMN and to the contralateral SMN via small fiber bundles located either just ventral or just dorsal to the ventral fasciculi (FV; fig. 2A–D, 3C). Cells with these characteristics were not observed after labeling with BDA, making them likely candidates for premotor neurons.

In *Sargocentron xantherythrum*, neurons labeled with biocytin ranged in size from 18.85 μm^2 to 730.98 μm^2 (n = 2 animals; fig. 5A, B), whereas those labeled with BDA ranged in size from 47.12 μm^2 to 703.72 μm^2 (n = 2 animals; fig. 5C, D). Thus, the range of sizes extended into smaller neurons after labeling with biocytin as compared to BDA, supporting the notion that smaller premotor neurons were labeled transynaptically with biocytin.

Non-Sonic Motor Neuron Groups

Application of biocytin and BDA to both the red and white dorsal (epaxial) muscle resulted in dense labeling of motor neurons within the dorsal motor column in the rostral spinal cord. There was extensive overlap in the locations of red (RDmn) and white (WDmn) dorsal motor neurons, the former extending anterior to the latter. Figure 6A–D shows the labeling pattern following biocytin injection into the red dorsal muscle. The strongest labeling occurred in a group of variable-sized motor neurons located immediately lateral to the MLF (fig. 3E, 6B–D). The dendrites of these neurons projected to the lateral edge of the spinal cord and medulla to form a dense network of fibers in the lateral funiculus. The axons usually coursed medially and then headed ventral, either going between the two MLFs or through the ipsilateral MLF, to exit via ventral spinal motor nerves (SpN; fig. 3E, 6C). A smaller number of axons headed directly ventrolateral to the ventral nerve roots.

After injections into the red dorsal muscle, a second group of smaller labeled neurons were located in a slightly more ventrolateral position, caudal to the larger motor neurons in general, but showing extensive overlap (fig. 3E, 6A–C). Fibers from these cells projected bilaterally to the same region as the dendrites from the larger neurons. In a few cases, these cells were found on the contralateral side as well (fig. 3E). In all cases, numerous fibers were seen on the contralateral side (fig. 3E, 6A–D), with some exiting the ventral nerve roots (not shown). There were no differences in the pattern of labeling resulting from application of either

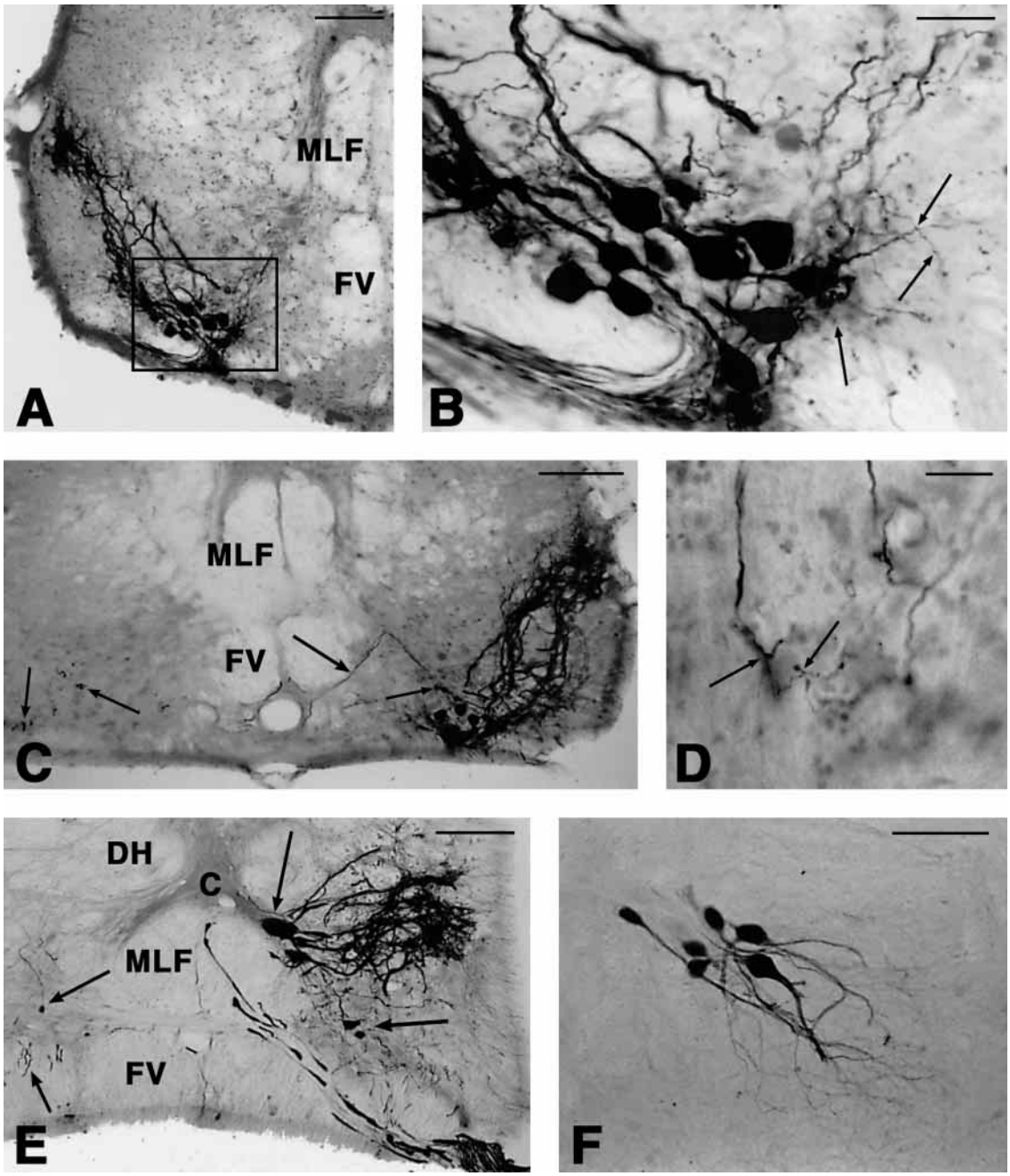


Fig. 3. Photomicrographs of transverse sections in the rostral spinal cord and hindbrain in *Sargocentron xantherythrum* (**A**–**D**) and *S. cornutum* (**E**, **F**). **A** Sonic motor nucleus labeled with BDA. **B** Magnified view of region in (**A**) enclosed by box; arrows note the location of dendritic varicosities located medial and within the nucleus. **C** Sonic motor nucleus labeled with biocytin; small arrow denotes the location of putative premotor neurons, which give rise to medially-directed fibers (large arrow) that result in labeling of fibers in the contralateral sonic motor nucleus (medium arrows). **D** Labeled fibers and terminals in nucleus praeeminentialis, following biocytin labeling of the sonic motor nucleus. Arrows denote axon branches leading to terminals overlying somata of counterstained cells. **E** Motor neurons in the dorsal motor column of the spinal cord labeled following injection of biocytin into the red dorsal muscle. Large arrow denotes location of large cells just lateral to the MLF. Medium arrows indicate bilateral labeling of small, ventrolateral cells, and small arrow indicates the position of contralateral labeled fibers. **F** Opercular motor neurons and dendrites labeled with biocytin, located within the facial motor nucleus. Abbreviations as in figures 2 and 6. Bar scale = 100 μm (**A**, **C**, **E**, **F**); 50 μm (**B**); 20 μm (**D**).

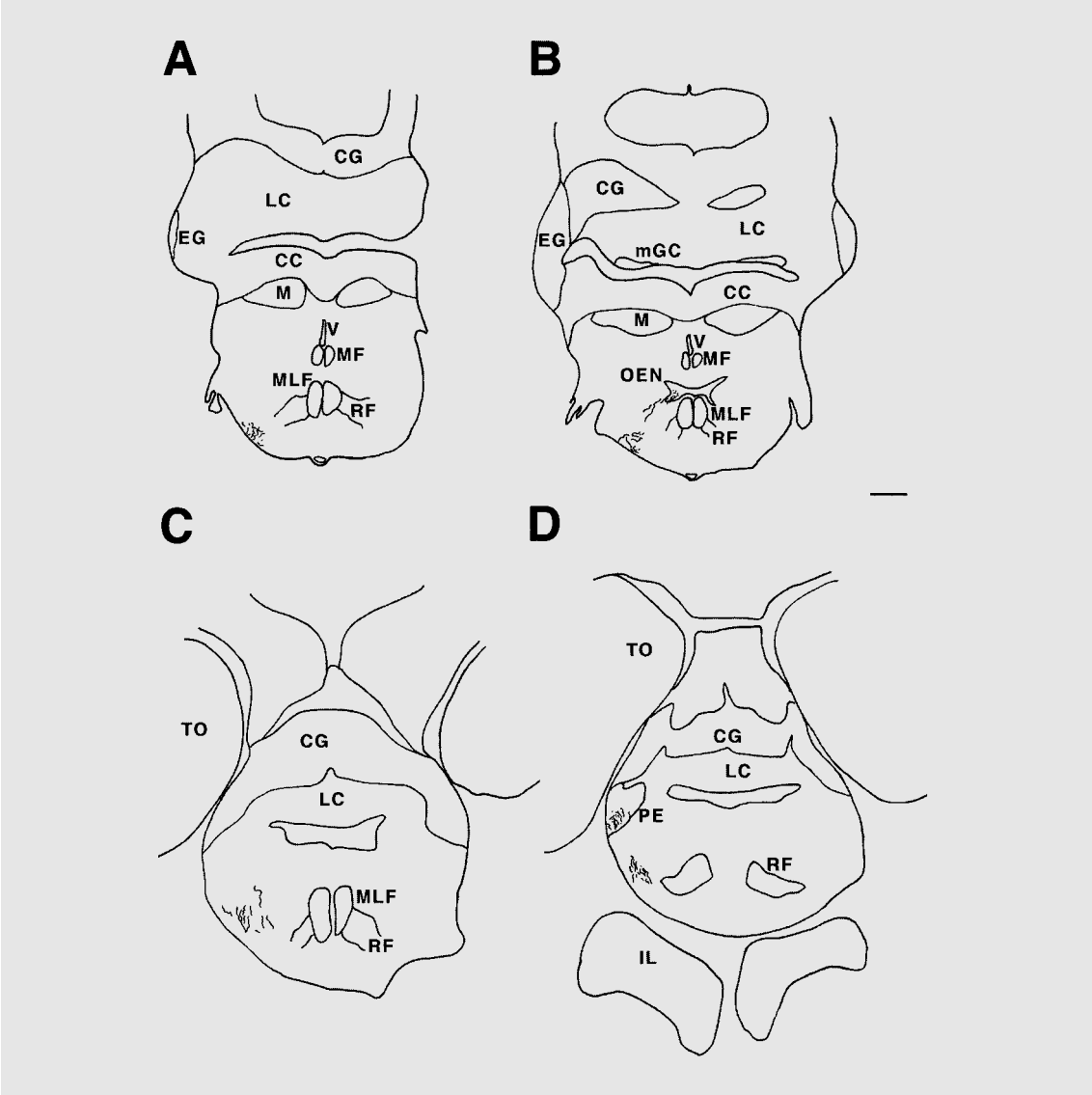


Fig. 4. Line drawings of transverse sections through the rostral medulla of *S. xantherythrum* following biocytin application to the sonic muscle. Black lines and dots represent the location of fibers and terminals, respectively. **A** Ascending fibers from the sonic motor nucleus at the ventrolateral edge of the brainstem, at the level of nucleus medialis and the caudal edge of the eminentia granularis. **B** Projections to the octavolateralis efferent nucleus at the level of nucleus medialis. **C** Ascending fibers at the lateral edge of the brainstem, at the level of the granule cell layer of the corpus of the cerebellum. **D** Ascending fibers and terminals in nucleus praeeminentialis, at the level of the caudal extent of the optic tectum and inferior lobes. Abbreviations: CC = Cerebellar crest; CG = granule cell layer of corpus of the cerebellum; EG = eminentia granularis; IL = inferior lobe of the hypothalamus; LC = caudal lobe of the cerebellum; mGC = medial granule cells; M = nucleus medialis; MF = Mauthner fibers; MLF = medial longitudinal fasciculus; OEN = octavolateralis efferent nucleus; PE = nucleus praeeminentialis; RF = reticular formation; TO = optic tectum; V = fourth ventricle. Distance between sections = 250 μm. Bar scale = 300 μm.

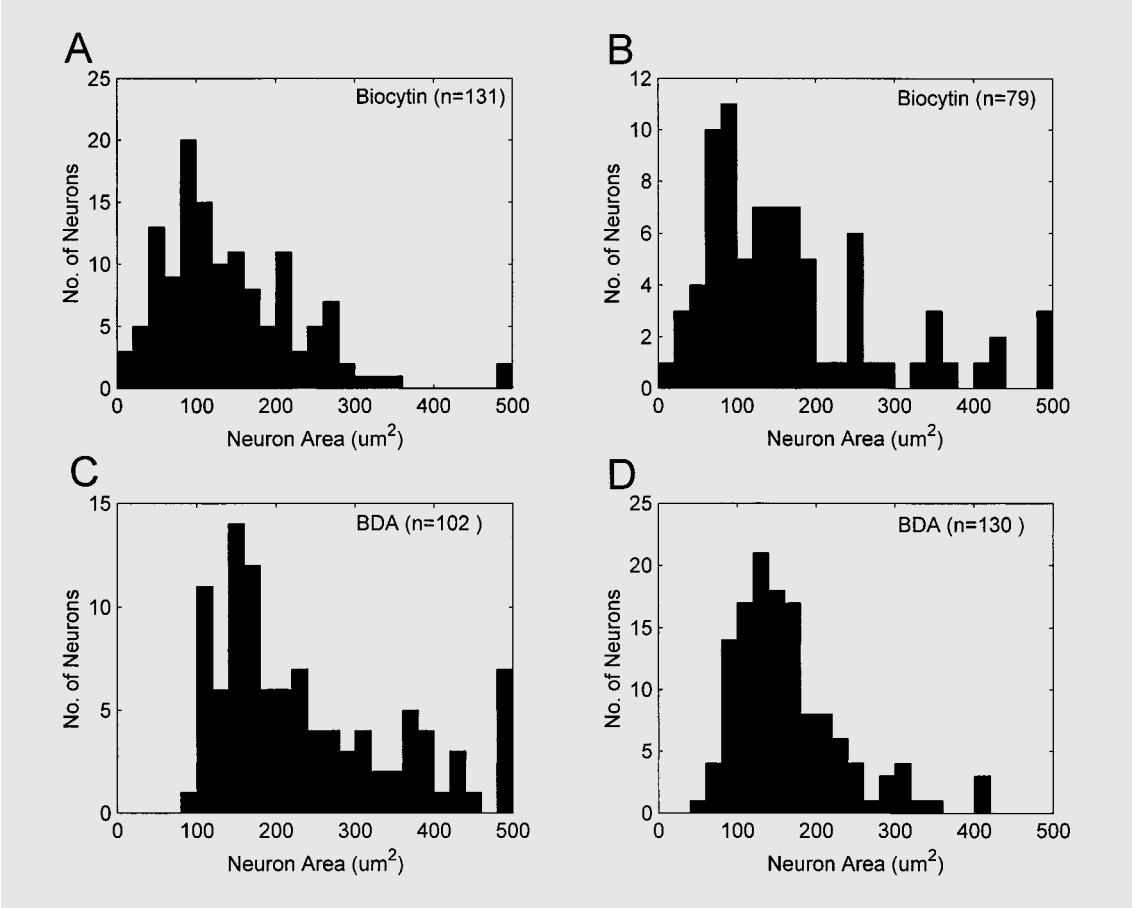


Fig. 5. Histograms of cross-sectional areas of labeled neurons in four individuals of *Sargocentron xantherythrum*, two (**A**, **B**) following biocytin labeling, and two (**C**, **D**) following BDA labeling. Note smaller neurons labeled with biocytin, but not BDA.

BDA or biocytin, indicating that these cells are all primary motor neurons.

Opercular motor neurons (Omn) were localized in the rostral medulla at the level of nucleus medialis (M) within the ipsilateral facial motor nucleus (nVIIm) following biocytin application (fig. 6E). Only a small number of spherical-shaped cells were labeled with dendrites extending ventrolaterally within the nucleus (fig. 3F, 6E). The axons of these motor neurons projected rostrally, first in a medial direction to pass through the Mauthner fibers (MF; fig. 6F), and then laterally (fig. 6G) to eventually exit through the ventral facial nerve (not shown).

Discussion

Comparison with Other Teleosts

The sonic motor nucleus (SMN) of holocentrids bears a remarkable similarity to that of the scorpaeniform fishes that have been studied (fig. 7). In holocentrids, cottids, triglids, and scorpaenids, the SMN is a paired, ventrolateral structure (SMNv) that sends dendrites dorsally along the lateral margin of the medulla to end in a dense tuft of fibers, whereas axons exit via ventral occipital nerve roots [Bass, 1985; Finger and Kalil, 1985; Bass and Baker, 1991; Ladich and Bass, 1998; Yoshimoto et al., 1999]. Thus, among the acanthopterygiian fishes that have been examined, the cytoarchitecture of the SMN follows a common pattern, suggesting conservation in its structure. This pattern differs, however, from that seen in the more distantly related Batrachoidi-

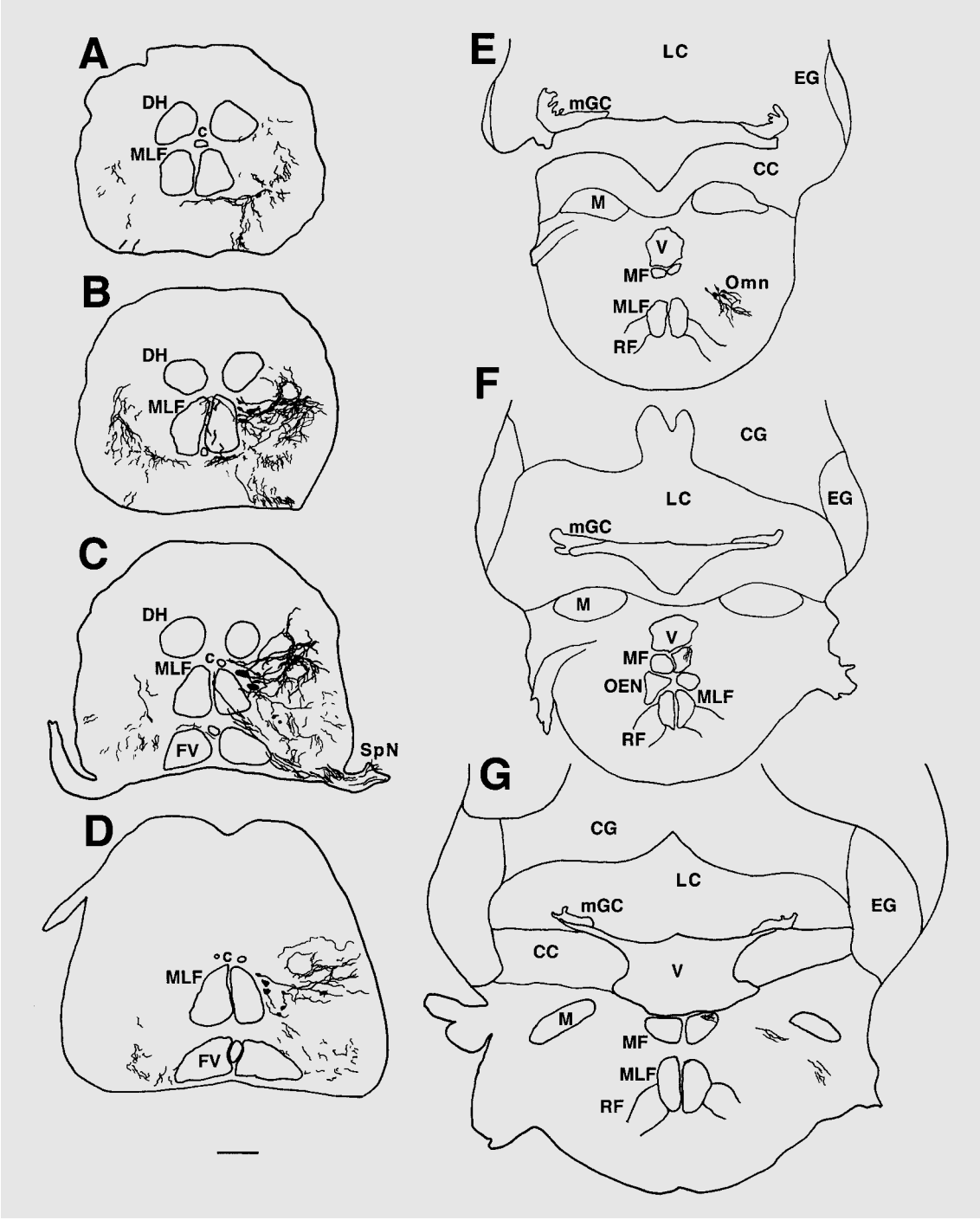


Fig. 6. Line drawings of transverse sections through the rostral spinal cord (**A**–**D**) and rostral medulla (**E**–**G**) of *S. cornutum* following biocytin application to the red dorsal muscle (**A**–**D**) and the opercular muscle (**E**–**G**). Large black dots and black lines represent the location of cells and fibers, respectively. **A** Caudal extent of labeling with bilateral fibers and small cells located ventrolateral to the medial longitudinal fasciculus. **B**–**D** Large cells appear further rostral, immediately lateral to the medial longitudinal fasciculus; note primary efferents entering through ventral spinal root and bilateral labeling of small ventrolateral cells in (**C**). **E** Opercular motor neurons located within the facial motor nucleus, at the level of nucleus medialis and the caudal extent of the eminentia granularis. Further rostral, labeled axons course medially through the Mauthner fibers (**F**), then head ventrolateral (**G**), before exiting through the facial motor nerve (not shown). See legend for figures 2 and 4 for most abbreviations; others: DH = dorsal horn; Omn = opercular motor neurons; SpN = spinal motor nerve. Distance between sections = 1200 µm (**A**–**D**); 250 µm (**E**–**G**). Bar scale = 200 µm.

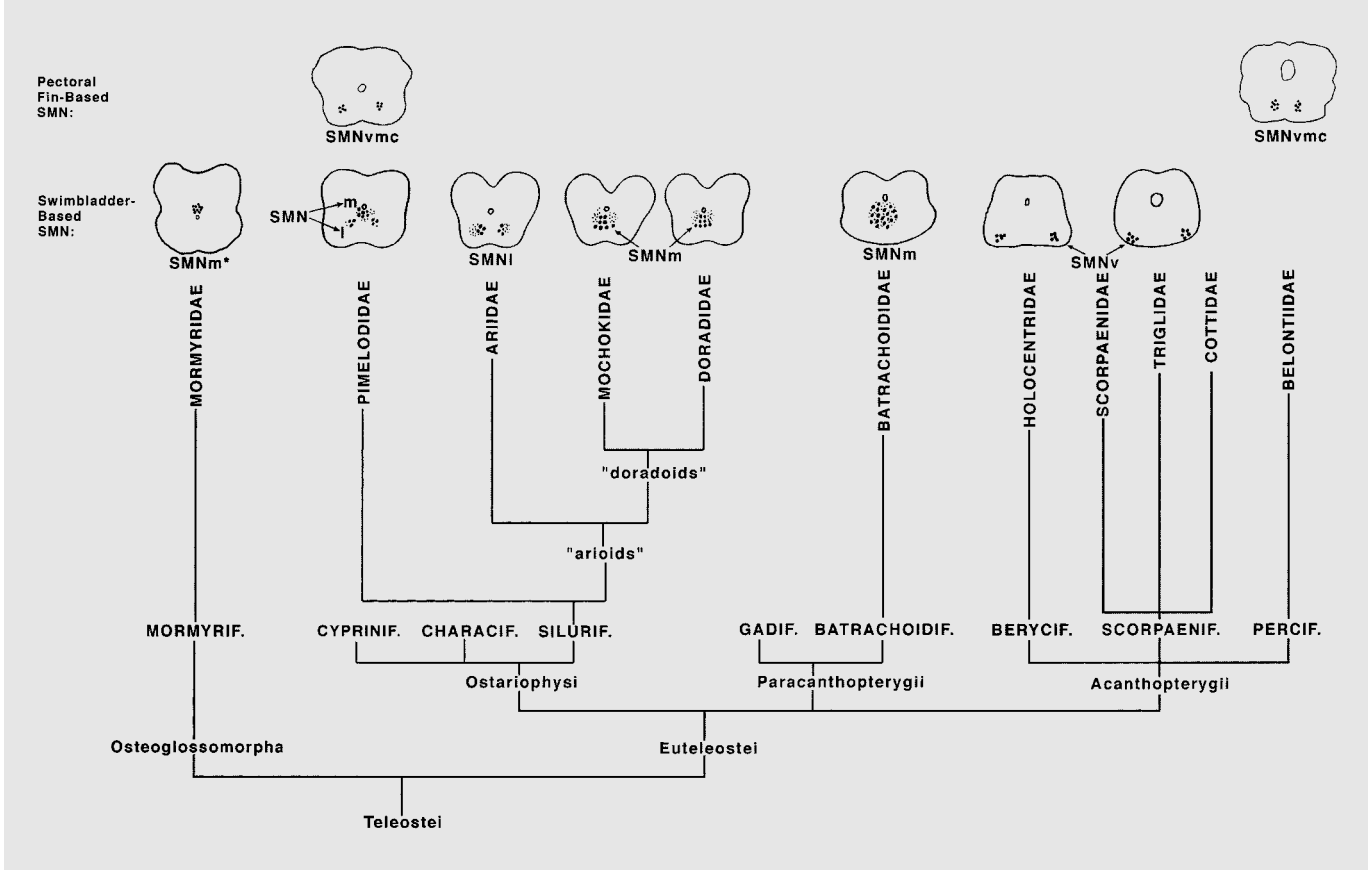


Fig. 7. Cladistic relationships among teleostean families with swimbladder-based and pectoral-fin based vocalization and identified sonic motor neurons. There are four patterns of sonic motor nuclear (SMN) organization: medial SMN (SMNm), lateral SMN (SMNl), ventrolateral SMN (SMNv), and ventral motor column SMN (SMNvmc). SMNm occurs in Siluriformes and Batrachoidiformes. SMNl occurs only in Siluriformes. SMNv is found exclusively in acanthopterygiians. SMNvmc is found in all pectoral fin-based sonic motor systems studied, including belontiids and pimelodids. *The only mormyriform studied is not known to be sonic, and the SMNm is therefore putative. Abbreviations: BATRACHOIDIF. = Batrachoidiformes; BERYCIF. = Beryciformes; CHARACIF. = Characiformes; CYPRINIF. = Cypriniformes; GADIF. = Gadiformes; MORMYRIF. = Mormyriformes; PERCIF. = Perciformes; SCORPAENIF. = Scorpaeniformes; SILURIF. = Siluriformes. Modified after Ladich and Bass [1998].

formes and Siluriformes (fig. 7), in which the SMN occupies a more medial position, being located either on the midline (SMNm; Batrachoidiformes, Mochokidae, and Doradidae), slightly lateral of the midline (SMNl; Ariidae), or in both positions (Pimelodidae). Ladich and Bass [1998] propose that the SMNl pattern is derived from the SMNm pattern. In these cases, dendrites typically branch extensively either within (SMNm) and/or lateral (SMNm and SMNl) to sonic motor neurons, and their axons exit via ventral occipital and spinal nerve roots. Differences in central anatomy between acanthopterygiians (SMNv) and other teleosts (SMNm and SMNl) may result from either the independent evolution of sonic/vocal circuitry and/or the modification of conserved sonic/vocal motor networks in one or more lineages [see discussions in Bass and Baker, 1991, 1997; Ladich and Bass, 1998].

Within osteoglossomorphs, motor neurons innervating swimbladder muscles in a species of mormyrid *(Brienomyrus brachyistius)* are located on the midline in the caudal medulla and rostral spinal cord, similar to the SMNm, but are dorsal, rather than ventral, to the central canal (fig. 7); [Bass, 1985]. Although this species is not known to produce sound, other species of mormyrids have a rich repertoire of vocal signals and also employ a sonic swimbladder mechanism [Crawford et al., 1986, Crawford and Huang, 1999]. Hence, it remains important to identify the position

and extent of a sonic motor nucleus in a known sonic species of mormyrid. For comparative purposes, we have included a schematic of the position of the swimbladder motoneurons so far identified for a mormyrid (fig. 7).

Convergence in the position and extent of the SMN among acanthopterygiians parallels a similarity in the association of the peripheral sound generating apparatus with skeletal elements. In holocentrids, the sonic muscles insert on the first 2–3 ventral ribs which, in turn, are attached to the swimbladder [Nelson, 1955; Winn and Marshall, 1963]. A similar situation occurs in a scorpaenid, *Sebasticus marmoratus* [Dotu, 1951; Yoshimoto et al., 1999]. In cottids, a swimbladder is lacking, but sound is produced by the contraction of muscles that vibrate the pectoral girdle [Barber and Mowbray, 1956; Bass and Baker, 1991]. In triglids, there is greater intrafamilial variation. Some species have extrinsic muscles that associate with the pectoral girdle, supracleithrum and several vertebrae, whereas others have completely intrinsic muscles that are devoid of any skeletal interactions [Evans, 1973]. However, ontogenetic studies have shown that the intrinsic pattern is derived from the extrinsic pattern; i.e. there are skeletal interactions early during development [Rauther, 1945]. The common features of the central sonic/vocal anatomy in acanthopterygiians may therefore relate, in part, to the involvement of skeletal structures in sound production.

This hypothesis is supported by data from other teleosts. In addition to swimbladder-based mechanisms, teleost sound production may be achieved by rubbing pectoral spines, snapping pectoral tendons, membrane 'drumming', or grinding pharyngeal teeth [Tower, 1908; Fish, 1953; Winn and Marshall, 1963; Salmon et al., 1968; Tavolga, 1971; Lanzing, 1974; Kratochvil, 1978; Schachner and Schaller, 1981]. In addition to swimbladder-based sound production, pimelodid catfish produce sounds by pectoral fin stridulation, and in gouramis (Anabantoidei), sound generation is achieved by the snapping of pectoral tendons. Sound production therefore involves skeletal interactions in these groups, and in each case, the sonic motor neurons are located within a ventrolateral motor column, in this case within the spinal cord proper (fig. 7) [Ladich and Fine, 1992, 1994; Ladich and Bass, 1996].

In Batrachoidiformes, sound production does not involve any skeletal elements, and the SMN follows the midline pattern [Demski et al., 1973; Fine et al., 1984; Bass, 1985; Bass et al., 1994]. Among Siluriformes, both midline and lateral patterns are seen [Ladich and Bass, 1998]. In pimelodids, there are no skeletal associations with the sonic muscles, whereas arioids (ariids, mochokids, and doradids) all have a bony plate, the ramus Mülleri, that couples the sonic musculature to the swimbladder [Ladich and Bass, 1998]. In ariids, the ramus Mülleri is a pointed elastic spring, but in doradoids (mochokids and doradids), it is a disc-shaped structure. These groups have skeletal elements involved in sound production, but they do not possess a ventrolateral SMN. However, the nature of the skeletal interaction differs, in that the ramus Mülleri is a dedicated sound-producing element that appears to have evolved to serve this function much like the swim bladder drumming muscles. As described by Ladich and Bass [1998], the ramus Mülleri appears to be a derived feature that appeared after the evolution of sound production in Siluriformes. By contrast, the skeletal elements seen in acanthopterygiians and pectoral spine-based mechanisms are derivations of skeletal structures that are also important for other functions (the ribs, pectoral girdle, and pectoral spines). Thus, the available evidence suggests that these skeletal elements did not evolve for the purpose of sound production, but were secondarily adapted to serve this function. The difference in peripheral mechanisms may therefore be coupled to different functional requirements and developmental patterns that result in a divergence in central anatomy, i.e. ventrolateral versus midline/lateral positions of the SMN.

As hypothesized by Bass and Baker [1991], the separation of paired midline nuclei may have been coupled with innovation in the design of sonic mechanisms and divergence from an ancestral pattern of direct swimbladder vibration. This may explain the diversity of the SMN position seen in Siluriformes. In addition, it provides a basis for the diversity of peripheral sound producing mechanisms seen in acanthopterygiians, despite the remarkable similarities in the position of the SMN. Clearly, this hypothesis requires further study in both non-acanthopterygiians and acanthopterygiians. Of particular interest are acanthopterygiians that use other skeletal-based sound production mechanisms, such as pharyngeal tooth grinding in cichlids (Cichlidae) and grunts (Haemulidae) [Burkenroad, 1930; Lanzing, 1974], vertebral grinding in seahorses (Syngnathidae) [Fish, 1953], and membrane drumming in triggerfish (Balistidae) [Salmon et al., 1968]. Also of interest are drums (Sciaenidae), that produce sounds with muscles that are not attached to the swimbladder, but are located within the body wall adjacent to the swimbladder [Schneider and Hasler, 1960; Hill et al., 1987].

Comparisons between Motor Neuron Groups

In comparison to the other motor neuron groups examined in this study and others, the SMN is a distinct structure. For example, the motor neurons innervating epaxial muscle (red and white dorsal muscles) are located entirely within

the dorsal motor column (DMC), either directly adjacent to the medial longitudinal fasciculus (MLF) or slightly ventrolateral of this position (fig. 6A–D). In contrast, the SMN is located further rostral, at the ventrolateral edge of the caudal medulla in a column distinct from the DMC (fig. 2). This same pattern is found in the triglid *Prinonotus carolinus* [Bass, 1985; Finger and Kalil, 1985], where motor neurons innervating epaxial musculature are located within the DMC, and those innervating pectoral musculature are located within the ventral motor column (VMC), whereas the sonic motor neurons are located in a distinct, ventrolateral column. In addition, the somata of SMN neurons were a homogeneous group of relatively large, ovoid-shaped neurons organized in a compact, discrete nucleus (fig. 2, 3A–C). Although many of the motor neurons innervating epaxial muscle were also quite large, their somata were multipolar, forming a diffuse cluster of neurons rather than a distinct nucleus (fig. 3E, 6A–D). The SMN gave rise to a distinct, but large dendritic field, composed of numerous varicosities (fig. 2, 3A, C). The epaxial motor neurons, however, gave rise to a large, diffuse dendritic field with no visible varicosities (fig. 3E). In the present study, we also examined the extent of motor neurons innervating the opercular muscles (Omn). These neurons were located far rostral relative to the other motor neuron groups, within the facial motor nucleus (nVIIm). These neurons also differed from the SMN neurons in that they were more elongate and had much less elaborate dendritic arbors with no obvious varicosities (fig. 3F, 6E).

These differences likely relate to the specialized nature of sonic motor neurons. Very high rates of synchronous activation are required for vocalization in holocentrids and batrachoidids [Gainer et al., 1965; Bass and Baker, 1990], and the morphological traits of sonic motor neurons may relate to these functional requirements. Indeed, in all vocal teleosts that have been studied, the SMN is a very distinctive structure, with relatively large motor neurons and extensive anatomical coupling [Bass and Baker, 1990, 1991; Bass et al., 1994; Ladich and Bass, 1998]. Although the physiology of the SMN has not been well studied across different teleost groups, data on Batrachoidiformes indicate that these morphological characteristics do provide for strong coupling, thus enhancing the degree of synchrony across motor neurons [Bass and Baker, 1990]. A similar morphology is found in the command and relay nuclei of electrogenic teleosts, where the same requirements for fast, synchronous activation are found [Bell et al., 1983; Grant et al., 1986; Metzner, 1993]. Bass and Baker [1997] suggest that rhythmic circuits in the hindbrain of sonic/vocal and electrogenic vertebrates are derived from ancestral circuits involved in other rhythmic activities such as respiration and cardiac control. The unique features of the SMN, then, may be paralleled by different evolutionary and embryological origins relative to other motor neuron groups.

Despite functional similarities across groups, namely the contraction of sonic muscles at very high rates, the midline position of the SMN in Batrachoidiformes is linked to relatively massive transneuronal transport of biocytin throughout the hindbrain vocal circuitry when compared to other groups, including holocentrids [Bass et al., 1994]. This indicates a greater degree of anatomical coupling in batrachoidids, which likely depends, in part, on electrical coupling via gap junctions [Bass et al., 1994; also see figure 1 in Bass and Baker, 1997]. Among batrachoidids, sonic motor neurons are physiologically coupled via presynaptic afferents from nearby pacemaker neurons, whose axons ramify extensively throughout both sonic motor nuclei, providing for their synchronous activation which then leads to the simultaneous contraction of both sonic muscles at high rates [Pappas and Bennett, 1966; Bass and Baker, 1990, 1991; Bass et al., 1993, 1994]. In addition, the dendrites of single motor neurons ramify throughout the ipsilateral and contralateral SMN [Bass and Baker, 1990, 1991; Bass et al., 1993; 1994; Fine and Mosca, 1995]. This organizational pattern is linked to a diverse vocal repertoire that includes brief duration grunts and growls as well as long, continuous vocalizations of near constant waveform termed hums or boatwhistles [Gray and Winn, 1961; Brantley and Bass, 1994; Bass et al., 1999]. By contrast, holocentrids only produce grunts [Moulton, 1958; Winn and Marshall, 1963; Winn et al., 1964; Fish and Mowbray, 1970; Bright and Sartori, 1971]. Although these grunts do result from synchronous activation of sonic motor neurons [Gainer et al., 1965], the relatively greater complexity of the batrachoidid vocal repertoire suggests a dependence on their extensive midline-positioned pacemaker-motor neuron circuitry.

Circuitry of the SMN

The results of our study suggest the existence of premotor neurons within or immediately adjacent to the SMN: contralateral and rostral projections were only observed after injection of biocytin, suggesting the involvement of transneuronal labeling although no additional stained nuclei were actually seen. Qualitative observation suggests that small, elongate, medially-situated neurons within the SMN as well as neurons located dorsomedially within the reticular formation are likely candidates as they were only observed following biocytin labeling (fig. 2B–D, 3C). This is supported by the fact that the distributions of neuronal cross-sectional area extended into smaller sizes following

biocytin labeling as compared to BDA labeling (fig. 5). Similarly, in mochokid catfish, one group of premotor neurons are found within the SMN that project bilaterally [Ladich and Bass, 1996]. Recently, Yoshimoto et al. [1999] found bilaterally projecting premotor neurons in a scorpaenid that were located dorsomedial to the SMN, within the reticular formation. In general, premotor neurons may be important in maintaining synchrony between the two nuclei and therefore between the sonic muscles on opposite sides of the body; neurophysiological evidence from batrachoidids supports this role for premotor neurons in the sonic motor system [Pappas and Bennett, 1966; Bass and Baker, 1990]. Vocalization in holocentrids [Winn and Marshall, 1963], like batrachoidids [Skoglund, 1961; Cohen and Winn, 1967], does appear to depend upon the simultaneous activity of both sonic muscles.

In those cases with labeling of vocal motor neurons and putative premotor neurons, terminal labeling was observed in the octavolateralis efferent nucleus (OEN) and nucleus praeeminentialis (PE; fig. 3D, 4B, D), suggesting either direct or indirect coupling of the vocal premotor neurons to these nuclei. Based on our results, it is unclear whether individual premotor neurons project to one or more of these targets (ipsilateral and contralateral SMN, OEN, and PE) via axon collaterals, or whether single premotor neurons are specialized for specific targets. However, some premotor neurons must project to at least the ipsilateral SMN, because labeling of afferents to these targets required transneuronal labeling through the SMN. Intracellular recording and labeling of premotor neurons is necessary to determine precisely the projection patterns of individual cells.

A similar pattern of labeling was observed in mochokid catfish [Ladich and Bass, 1996], and studies in a batrachoidid, the plainfin midshipman, showed a coupling of the hindbrain vocal pacemaker circuitry to the OEN [Bass et al., 1994]. Studies in other teleosts show that the OEN projects to inner ear hair cells and may thereby provide inhibition of auditory responses [Russell, 1971; Furukawa, 1981; Lin and Faber, 1988]. Thus, vocalization may be coupled with inhibition of auditory input such that reafference of an extremely loud signal is avoided [see discussion in Bass et al., 1994].

Nucleus praeeminentialis (PE) is a second-order lateral line nucleus located dorsolaterally in the rostral medulla [see Finger and Tong, 1984; New and He, 1998]. Its function is not quite clear. In electroreceptive teleosts, PE is involved in descending gain control of the electrosensory pathway, allowing the system to operate over a wide range of amplitudes [Bastian, 1986; Bastian and Bratton, 1990; Berman and Maler, 1999]. It seems likely that a similar function may be served for the lateral line system in other teleosts. This is supported by the results of recent studies demonstrating reciprocal connections between PE and lateral line nuclei [McCormick and Hernandez, 1996; Weeg and Bass, 2000]. The projection from the SMN circuitry to PE may play a similar role to that of the SMN-OEN projection in that suppression of the lateral line system may be induced during vocalization.

Sound production is common to many groups of teleost fish. The sounds produced are relatively stereotyped and readily quantified, and the neural structures serving vocalization lend themselves to anatomical and physiological analysis. These features make vocal teleosts excellent model systems for studying neural and behavioral evolution. Clearly, the similarities among sonic and electric motor and sensory systems are indicative of structural requirements for effective communication and sensory processing. However, there are also numerous differences among different groups in the neural substrates for vocalization. These differences are likely related to observable, functional divergence in behavior. Thus, teleostean vocal systems may prove ideal for studying structure-function linkages and evolutionary pressures on communication behavior.

Acknowledgements

The authors would like to thank the members of the Bass lab at Cornell for technical support, especially James Goodson, Margaret Marchaterre, and Matthew Weeg. Walter Wilczynski and two anonymous reviewers provided helpful comments on the manuscript. Research support was provided by the National Institutes of Health (DC00092 to AHB). BAC was supported by a National Science Foundation Predoctoral Fellowship.

References

Barber, S.B., and W.H. Mowbray (1956) Mechanism of sound production in the sculpin. Science, *124:* 219–220.

Bass, A.H. (1985) Sonic motor pathways in teleost fishes: a comparative HRP study. Brain Behav. Evol., *27:* 115–131.

Bass, A.H. (1989) Evolution of vertebrate motor systems for acoustic and electric communication: peripheral and central elements. Brain Behav. Evol., *33:* 237–247.

Bass, A.H., and R. Baker (1990) Sexual dimorphisms in a vocal control system of a teleost fish: morphology of physiologically identified neurons. J. Neurobiol., *21:* 1155–1168.

Bass, A.H., and R. Baker (1991) Evolution of homologous vocal control traits. Brain Behav. Evol., *38:* 240–254.

Bass, A.H., and R. Baker (1997) Phenotypic specification of hindbrain rhombomeres and the origins of rhythmic circuits in vertebrates. Brain Behav. Evol., *50(suppl 1):* 3–16.

Bass, A.H., D.A. Bodnar, M.A. Marcheterre (2000) Midbrain acoustic circuitry in a vocalizing fish. J. Comp. Neurol., *419:* 505–531.

Bass, A.H., M.A. Marchaterre, and R. Baker (1993) Transneuronal biocytin delineates species differences in a brainstem vocal-acoustic circuit in sound producing fish. Soc. Neurosci. Abstr., *19:* 1202.

Bass, A.H., M.A. Marchaterre, and R. Baker (1994) Vocal-acoustic pathways in a teleost fish. J. Neurosci., *14(7):* 4025–4039.

Bass, A.H., N. Segil, and D.B. Kelley (1986) Androgen binding in the brain and electric organ of a mormyrid fish. J. Comp. Physiol. A, *159:* 535–544.

Bastian, J. (1986) Gain control in the electrosensory system mediated by descending inputs to the electrosensory lateral line lobe. J. Neurosci., *6:* 553–562.

Bastian, J., and B. Bratton (l990) Descending control of electroreception. I. Properties of nucleus praeeminentialis neurons projecting indirectly to the electrosensory lateral line lobe. J. Neurosci., *10:* 1226–1240.

Bell, C.C., S. Libouban, and T. Szabo (1983) Pathways of the electric organ discharge command and its corollary discharges in mormyrid fish. J. Comp. Neurol., *216:* 327–338.

Berman, N.J., and L. Maler (1999) Neural architecture of the electrosensory lateral line lobe: adaptations for coincidence detection, a sensory searchlight and frequency-dependent adaptive filtering. J. Exp. Biol., *202:* 1243–1253.

Brantley, R.K., and A.H. Bass (1994) Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish *Porichthys notatus* Girard (Teleostei, Batrachoididae). Ethology, *96:* 213–232.

Bright, T.J., and J.D. Sartori (1971) Sound production by the reef fishes *Holocentrus coruscus, Holocentrus rufus,* and *Myripristis jacobus* (family Holocentridae). Hydrolab. J., *1:* 21–29.

Burkenroad, M.D. (1930) Sound production in the Haemulidae. Copeia, *1:* 17–18.

Cohen, M., and H. Winn (1967) Electrophysiological observations on hearing and sound production in the fish, *Porichthys notatus.* J. Exp. Zool., *165:* 355–369.

Coombs, S., and A.N. Popper (1979) Hearing differences among Hawaiian squirrelfish (family Holocentridae) related to differences in the peripheral auditory system. J. Comp. Physiol. A., *132:* 203–208.

Crawford, J.D., and X. Huang (l999) Communication signals and sound production mechanisms of mormyrid electric fish. J. Exp. Biol., *202:* 1417–1426.

Crawford, J.D., M. Hagedorn, and C.D. Hopkins (1986) Acoustic communication in an electric fish *Pollimyrus isidori* Mormyridae. J. Comp. Physiol. A, *159:* 297–310.

Demski, L., J. Gerald, and A. Popper (1973) Central and peripheral mechanisms of teleost sound production. Amer. Zool., *13:* 1141–1167.

Dotu, Y. (1951) On the sound producing mechanisms of a scorpaenoid fish. Kyushu Imp. Univ. Dept. Agr. Bull. Sci., *13:* 286–288.

Evans, R.R. (1973) The swimbladder and associated structures in western Atlantic sea robins (Triglidae). Copeia, *1973:* 315–321.

Fine, M.L., and P.J. Mosca (1995) A golgi and horseradish peroxidase study of the sonic motor nucleus of the oyster toadfish. Brain Behav. Evol., *45:* 123–137.

Fine, M., D. Economos, R. Radtke, and J. McClung (1984) Ontogeny and sexual dimorphism of the sonic motor nucleus in the oyster toadfish. J. Comp. Neurol., *225:* 105–110.

Finger, T.E., and K. Kalil (1985) Organization of motoneuronal pools in the rostral spinal cord of the sea robin, *Prionotus carolinus.* J. Comp. Neurol., *239:* 384–390.

Finger, T.E., and S.L. Tong (1984) Central organization of eighth nerve and mechanosensory lateral line systems in the brainstem of Ictalurid catfish. J. Comp. Neurol., *229:* 129–151.

Fish, M.P. (1953) The production of underwater sounds by the northern seahorse *Hippocampus hudsonius.* Copeia, *1953:* 98–99.

Fish, M.P., and W.H. Mowbray (1970) Sounds of Western North Atlantic Fishes. Johns Hopkins Press, Baltimore.

Furukawa, T. (1981) Effects of efferent stimulation on the saccule of goldfish. J. Physiol., *315:* 203–215.

Gainer, H., K. Kusano, and R.F. Mathewson (1965) Electrophysiological and mechanical properties of squirrelfish sound-producing muscle. Comp. Biochem. Physiol., *14:* 661–671.

Grant, K., C.C. Bell, S. Clausse, and M. Ravaille (1986) Morphology and physiology of the brainstem nuclei controlling the electric organ discharge in mormyrid fish. J. Comp. Neurol., *245:* 514–530.

Gray, G.A., and H.E. Winn (1961) Reproductive ecology and sound production of the toadfish *Opsanus tau.* Ecology, *42:* 274–282.

Herald, E.S., and R.P. Dempster (1957) Courting activity in the white-lined squirrelfish. Aquar. J., *28:* 43–44.

Hill, G.L., M.L. Fine, and J.A. Musick (1987) Ontogeny of the sexually dimorphic sonic muscle in three sciaenid species. Copeia, *1987:* 708–713.

Kratochvil, H. (1978) Der Bau des Lautapparates vom knurrenden Gurami (*Trichopsis vittatus* Cuvier & Valenciennes) (Anabantidae, Belontiidae). Zoomorphologie, *91:* 91–99.

Ladich, F. (1997) Agonistic behavior and significance of sounds in vocalizing fish. Mar. Fresh. Behav. Physiol., *29:* 87–108.

Ladich, F., and A.H. Bass (1996) Sonic/vocal acousticolateralis pathways in teleost fishes: a transneuronal biocytin study in mochokid catfish. J. Comp. Neurol., *374:* 493–505.

Ladich, F., and A.H. Bass (1998) Sonic/vocal motor pathways in catfishes: comparisons with other teleosts. Brain Behav. Evol., *51:* 315–330.

Ladich, F., and M.L. Fine (1992) Localization of pectoral fin motoneurons (sonic and hovering) in the croaking gourami *Trichopsis vittatus.* Brain Behav. Evol., *39:* 1–7.

Ladich, F., and M.L. Fine (1994) Localization of swimbladder and pectoral motoneurons involved in sound production in pimelodid catfish. Brain Behav. Evol., *44:* 86–100.

Lanzing, W.J.R. (1974) Sound production in the cichlid *Tilapia mozambica.* Peters J. Fish Biol., *6:* 341–347.

Lieske, E., and R. Myers (1996) Coral Reef Fishes. Princeton Univ. Press, Princeton, NJ.

Lin, J.W., and D.S. Faber (1988) An efferent inhibition of auditory afferents mediated by the goldfish Mauthner cell. J. Neurosci., *24:* 829–836.

McCormick, C.A., and D.V. Hernandez (1996) Connections of octaval and lateral line nuclei of the medulla in the goldfish, including the cytoarchitecture of the secondary octaval population in goldfish and catfish. Brain Behav. Evol., *47:* 113–137.

Metzner, W. (1993) The jamming avoidance response in *Eigenmannia* is controlled by two separate motor pathways. J. Neurosci., *13:* 1862–1878.

Moulton, J.M. (1958) The acoustical behavior of some fishes in the Bimini area. Biol. Bull., *114:* 357–374.

Myers, R.F. (1989) Micronesian Reef Fishes. Coral Graphics, Guam.

Myrberg, A.A. (1981) Sound communication and perception in fishes. *In* Hearing and Sound Communication in Fishes (ed. by W.N. Tavolga, A.N. Popper, and R.R. Fay), Springer-Verlag, New York, pp. 395–426.

Nelson, E.M. (1955) The morphology of the swimbladder and auditory bulla in the Holocentridae. Fieldiana Zool., *37:* 121–130.

New, J.G., and J. He (1998) Afferent and efferent connections of nucleus praeeminentialis in the channel catfish: a reevaluation. Brain Behav. Evol., *51:* 202–214.

Pappas, G. and M.V.L. Bennett (1966) Specialized junctions involved in electrical transmission between neurons. Ann. N.Y. Acad. Sci., *137:* 495–508.

Rauther, M. (1945) Über die Schwimmblase und die zu ihr in Beziehung tretenden somatischen Muskeln bei den Trigliden und anderen Scleroparei. Zool. Jb. Anat., *69:* 159–250.
Russell, I.J. (1971) The role of the lateral-line efferent system in *Xenopis laevis*. J. Exp. Biol., *54:* 621–641.
Salmon, M., H.E. Winn, and N. Sorgente (1968) Sound production and acoustical behavior in triggerfish. Pac. Sci., *12:* 11–20.
Schachner, G., and F. Schaller (1981) Schallerzeugung und Schallreaktionen beim Antennenwels (Mandim) *Rhamdia sebae*. Val. Zool. Beitr., *27:* 375–392.
Schneider, H., and A.D. Hasler (1960) Laute und Lauterzeugung beim Süsswassertrommler *Aplodinotus grunniens* Rafinesque (Sciaenidae: Pisces). Z. Vergl. Physiol., *43:* 499–517.
Shimizu, T., and T. Yamakawa (1979) Review of the squirrelfishes (subfamily Holocentrinae order Beryciformes) of Japan with description of *Adioryx dorsomaculatus*. Jpn. J. Ichthyol., *26:* 109–147.
Skoglund, C. (1961) Functional analysis of swimbladder muscles engaged in sound production of the toadfish. J. Biophys. Biochem. Cytol., *10:* 187–200.
Tavolga, W.N. (1971) Sound production and detection. *In* Fish Physiology, Vol. 5 (ed. by W.S. Hoar and D.J. Randall), Academic Press, New York, pp. 135–205.
Tower, R. (1908) The production of sound in the drumfishes, the sea-robin and the toadfish. Ann. N.Y. Acad. Sci., *18:* 149–180.
Weeg, M.S., and A.H. Bass (2000) Central lateral line pathways in a vocalizing fish. J. Comp. Neurol., *418:* 41–64.
Winn, H.E., and J.A. Marshall (1963) Sound-producing organ of the squirrelfish *Holocentrus rufus*. Physiol. Zool., *36:* 34–44.
Winn, H.E., J.A. Marshall, and B. Hazlett (1964) Behavior, diel activities, and stimuli that elicit sound production, and reaction to sounds in the longspine squirrelfish. Copeia, *1964:* 413– 425.
Yoshimoto, M., K. Kikuchi, N. Yamamoto, H. Somiya, and H. Ito (1999) Sonic motor nucleus and its connections with octaval and lateral line nuclei of the medulla in a Rockfish, *Sebastiscus marmoratus*. Brain Behav. Evol., *54:* 183– 204.