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Central Projections of Octavolateralis Nerves in the Brain of a Soniferous Damselfish (*Abudefduf abdominalis*)

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ABSTRACT

Sounds and hydrodynamic stimuli are important cues detected by the octavolateralis system in fishes. The central organization of auditory, mechanosensory, and vestibular projections is known for only a few phylogenetically diverse fishes, and less is known about projections in derived perciforms that use sounds for acoustic communication. We used neuronal labeling to provide a detailed analysis of octavolateralis endorgan projections in a soniferous perciform that does not have accessory morphological structures to enhance hearing. Octavolateralis nerves terminate ipsilaterally within seven medullary octaval nuclei: caudal (CON) and medial (MON) octavolateralis, anterior (AON), descending (DON), magnocellular (MgON), tangential (TON), and posterior (PON) octaval nuclei, and the eminentia granularis (EG). Anterior and posterior lateral line nerves project to the CON and MON, with dense projections to the EG. Semicircular canal nerves project primarily to ventral regions including the TON, ventral DON, intermediate DON (DONi), and MgON. Otolithic, semicircular canal, and anterior lateral line nerves all project to the MgON, which may serve a sensorimotor integration function. The DONi receives primarily segregated projections from all otolithic and semicircular canal nerves, whereas the ventral DON and TON receive principally utricular and semicircular canal afferents. The AON receives dense lateral and ventral projections from the saccule and utricle, and medial and dorsal projections from the lagena. These projection patterns are similar to those reported for non-sonic perciforms, and indicate the absence of neuroanatomical modifications in first-order octavolateralis nuclei in species that use acoustic communication. Thus patterns of central projections may be conserved among vocal and non-vocal perciforms. J. Comp. Neurol. 512:628–650, 2009.

Indexing terms: auditory; hindbrain; lateral line; medulla; pomacentrid; vestibular

Sound, hydrodynamic, and gravistatic stimuli provide significant cues and signals used by fishes during orientation behaviors, social interactions, and the detection of predators and prey. These important stimuli are detected and encoded by the octavolateralis system, which receives input from the mechanosensory lateral line, vestibular, and auditory endorgans. These receptor systems are all stimulated by excitation of hair cell mechanoreceptors, but with different transduction mechanisms for relevant mechanical stimuli (Coombs and Montgomery, 1999; Popper and Fay, 1999; Braun et al., 2002). The lateral line is found in all fishes and most aquatic amphibians; it consists of superficial neuromasts and subepidermal canals and detects local water movement relative to the skin surface. The three semicircular canals (anterior, posterior, and horizontal) have an orthogonal orientation and detect angular accelerations of the head during movement of the body. The otolithic endorgans consist of three paired maculae (saccule, lagena, utricle) that are mass-loaded with a calcium otolith (or otoconia) and intervening membrane. These endorgans detect particle accelerations via whole-body movements, and in some species indirectly detect sound pressure via excitation of the gas-filled swim bladder and induced local particle motion near the ear. Although many studies have examined the structure and function of the peripheral octavolateralis system in fishes (see Popper and Fay, 1999 for review), relatively few have considered organization of central processing regions (see McCormick, 1992, 1999, for reviews). Comparative organization of central processing regions is particularly important because many close-range behavioral interactions potentially stimulate multiple octavolateralis systems. Thus comparative studies are needed to interpret interactions among these diverse mechanical detection systems, as well as functional adaptations.

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The central organization of primary octavolateralis projections in fish is known for only a small group of phylogenetically diverse species (see McCormick, 1983, 1992, 1999, for reviews). Most studies on octavolateralis organization were performed on less recently derived fishes such as polypteriforms, holosteans, and chondrosteans (McCormick, 1981; 1992; 1999; New and Northcutt, 1984), or those with specialized auditory structures that enhance hearing ability such as otophysans, mormyrids, and clupeids (Bell, 1981; Finger and Tong, 1984; Echteler, 1985; McCormick and Braford, 1994; McCormick, 1997, 2001). Central octavolateralis projections are also known for the less derived batrachoidid vocal midshipman and toadfish (Highstein et al., 1992; Edds-Walton, 1998a; Bass et al., 2000, 2001; Weeg and Bass, 2000; Sisneros et al., 2002). However, complete primary projections of all octavolateralis nerves in more recently derived perciform fishes are limited to cichlids (Meredith and Butler, 1983; McCormick, 1983; O'Marra and McCormick, 1999) and gobies (Northcutt, 1981; Tomchik and Lu, 2005), and none of these previously examined perciform species are yet reported to produce sounds during social interactions for true acoustic communication.

Teleosts are the most diverse fish group, and several species possess anterior projections of the swim bladder that may enhance hearing sensitivity or bandwidth (Coombs and Popper, 1979; Yan et al., 2000; Webb et al., 2006). However, the majority of teleost species are not known to possess any accessory hearing structures. Thus comparative studies on soniferous species that do not possess accessory auditory structures are needed to examine the evolution of octavolateralis processing regions in fishes.

Damselfishes (family Pomacentridae, ~321 species in 28 genera) are one of the best studied groups of soniferous reef fishes and provide an excellent model system to compare central organization of octavolateralis processing regions with non-sonic perciform teleosts. These fish produce context-dependent sounds, show diverse reproductive and territorial behaviors that involve sound and hydrodynamic stimuli, and do not possess special adaptations to enhance detection of the sound pressure component of sound stimuli (Zelick et al., 1999; Bass and McKibben, 2003; Myrberg and Ladich, 2006). Damselfishes are also one of the few teleost groups for which considerable information is available on the agonistic and reproductive function of various sounds, signal content, and significance of different acoustic characteristics (e.g., frequency, number of pulses) in naturally behaving fishes (see reviews by Ladich and Myrberg, 2006; Myrberg and Ladich, 2006). Damselfishes differ from the vocal midshipman and toadfish in that they do not possess intrinsic swim bladder muscles to generate loud, long-duration "hums" and "boat-whistles," are not known to possess complex vocal-acoustic circuitry in the brain that includes a large medullary sonic motor nucleus, and are more recently derived (Nelson, 1994; Bass and McKibben, 2003). The Hawaiian sergeant fish *Abudefduf abdominalis* produces low-frequency, low-intensity sounds associated with reproductive and agonistic behaviors, and has a hearing ability that matches the frequency characteristics of sounds produced by naturally behaving wild fish (Maruska et al., 2007). Thus there is evidence for coevolution of sender and receiver systems to maximize information transfer for acoustic communication that involves octavolateralis processing during social interactions. However, there are

Abbreviations

4v	fourth ventricle
In	cranial nerve I, olfactory nerve
IIn	cranial nerve II, optic nerve
IIIn	cranial nerve III, oculomotor nerve
IVn	cranial nerve IV, trochlear nerve
Vn	cranial nerve V, trigeminal nerve
VIIn	cranial nerve VII, facial nerve
VIIIn	cranial nerve VIII, octavus nerve
IXn	cranial nerve IX, glossopharyngeal nerve
Xn	cranial nerve X, vagus nerve
A	asteriscus otolith
ac	anterior semicircular canal
acn	anterior semicircular canal nerve
ALLd	anterior lateral line nerve, dorsal branch
ALLn	anterior lateral line nerve
ALLv	anterior lateral line nerve, ventral branch
AON	anterior octaval nucleus
CC	cerebellar crest
CE	cerebellum
CON	caudal octavolateralis nucleus
DON	descending octaval nucleus
DONdm	dorsomedial division of the descending octaval nucleus
DONi	intermediate division of the descending octaval nucleus
DONv	ventral division of the descending octaval nucleus
EG	eminentia granularis
FL	facial lobe
hc	horizontal semicircular canal
hcn	horizontal semicircular canal nerve
HYP	hypothalamus
iaf	internal arcuate fibers
IO	infraorbital lateral line canal
L	lapillus otolith
La	lagena
Ln	lagenar nerve
M	medulla
MgON	magnocellular octaval nucleus
MgONd	dorsal division of the magnocellular octaval nucleus
MgONv	ventral division of the magnocellular octaval nucleus
mlf	medial longitudinal fasciculus
mn	macula neglecta
MON	medial octavolateralis nucleus
OB	olfactory bulb
OC	otic lateral line canal
OEN	octavolateralis efferent nucleus
OM	opercular mandibular lateral line canal
pc	posterior semicircular canal
pcn	posterior semicircular canal nerve
PLLn	posterior lateral line nerve
PON	posterior octaval nucleus
RF	reticular formation
S	sagitta otolith
sgt	secondary gustatory tract
Sn	saccular nerve
SN	superficial neuromast
SO	supraorbital lateral line canal
SOd	dorsal secondary octaval nucleus
SOv	ventral secondary octaval nucleus
ST	supratemporal lateral line canal
T	tectum
TEL	telencephalon
TON	tangential octaval nucleus
TrC	trunk lateral line canal
Un	utricular nerve
Ut	utricle
VIIc	central tract of the facial nerve
VIIm	facial motor nucleus
Vde	descending tract of the trigeminal nerve
VL	vagal lobe
Vm	trigeminal motor nucleus
Xm	vagal motor nucleus

no published studies on the central projections of octavolateralis nerves or medullary organization in the brain of this or any other damselfish species.

The purpose of this study was to conduct a detailed analysis of octavolateralis endorgan projections in a soniferous perciform fish that does not possess special adaptations to enhance hearing, which may influence organization of central auditory, vestibular, or mechanosensory pathways. We show that octavolateralis nerves in a soniferous damselfish terminate ipsilaterally within seven first-order medullary octaval nuclei with similar projection patterns to those reported for non-sonic perciform species. These results indicate the absence of a gross neuroanatomical difference in the first-order octavolateralis processing nuclei in a species that uses acoustic communication for intraspecific social interactions, and also that patterns of central octavolateralis projections may be conserved among vocal and non-vocal perciforms. Future studies are needed to test whether there are anatomical specializations at higher levels, or differences in physiological processing mechanisms among soniferous and non-soniferous species.

MATERIALS AND METHODS

Animals, tissue preparation, and general neuroanatomy

Adult Hawaiian sergeant fish *Abudefduf abdominalis* were collected via hook and line from Kaneohe Bay, Oahu, transported to the lab, and either perfused immediately or maintained in flow-through aquaria for 24 hrs prior to use in experiments. A total of 40 fish were used in this study (115-130 mm standard length) for both general neuroanatomy and nerve labeling experiments. Collection, maintenance, and surgical and perfusion procedures for fish used in this study were approved by the University of Hawaii Institutional Animal Care and Use Committee.

Fish were anesthetized with MS-222 (~0.005%) and measured for standard length (SL) and total length (TL) to the nearest 0.5 mm, as well as body weight (BW) to the nearest 0.1 g. Fish used for general neuroanatomical examination were perfused transcardially with 0.9% heparinized saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). Brains were removed, postfixed in 4% paraformaldehyde in 0.1 M PB at 4°C for 12 hours, rinsed in 0.1 M PB, and cryoprotected overnight in 30% sucrose in 0.1 M PB prior to sectioning. Cryoprotected brains were then embedded in Histoprep mounting media (Fisher Scientific, Fair Lawn, NJ) and sectioned in the transverse plane at 24 μm with a cryostat.

Serial sections were collected onto chrom/alum-coated slides, dried flat overnight at room temperature, soaked in distilled water for 10 minutes, stained with 0.5% cresyl violet acetate, dehydrated in an ethanol series (50–100%), cleared in toluene, and coverslipped with Cytoseal 60 mounting media (Richard Allen Scientific, Kalamazoo, MI). Several individuals were also used to examine the peripheral organization of the lateral line system (n = 4) and general structure and organization of the brain, inner ear, and cranial nerves (n = 4). The distribution of lateral line canals and superficial neuromasts was examined by gross dissection and application of a methylene blue solution (~0.5–1.0%) to the skin and scales, and injections into the canals. The structure of the inner ear, brain, and cranial nerves was examined by gross dissection and repeated application of a saturated solution of oil red O stain (Sigma-Aldrich, St. Louis, MO) in 99% isopropyl alcohol followed by rinses with 70% alcohol and then distilled water.

Central projections of octavolateralis nerves

Octavolateralis cranial nerves (anterior and posterior lateral line nerves, saccular, lagenar, utricular, and semicircular canal nerves) were labeled to determine the central projections of lateral line, vestibular, and auditory information in the Hawaiian sergeant fish. Primary projections of the macula neglecta were not examined in this study. In addition, projections of six other cranial nerves (olfactory nerve [In], optic nerve [IIn], trigeminal nerve [Vn], facial nerve [VIIn], glossopharyngeal nerve [IXn], and vagus nerve [Xn]) were labeled to identify their primary central target regions for landmark and labeling purposes. A combination of neurobiotin fills on live animals (anterior lateral line nerve [ALLn]; posterior lateral line nerve [PLLn], IIn, Vn, VIIn) and DiI fills on fixed tissue (ALLn, PLLn, saccular nerve [Sn], lagenar nerve [Ln], utricular nerve [Un], anterior canal nerve [can], posterior canal nerve [pcn], horizontal canal nerve [hcn], In, IXn, Xn) was used as described below.

Neurobiotin fills

Fish used for in vivo surgical experiments were anesthetized with MS-222, lightly clamped to a fish holder positioned in a shallow tank, and ventilated through the mouth with aerated anesthetic seawater. For ALLn (n = 4), Vn (n = 4), and VIIn (n = 4) fills, the left eye was surgically removed by transecting the optic nerve and ocular muscles, the orbit was dried, and a piece of Parafilm was placed beneath a single nerve branch inside the orbit. The exposed nerve was then cut, and several crystals of Neurobiotin tracer (Vector, Burlingame, CA) were applied directly to the surface of the proximal stump and allowed to be absorbed by the axons for ~5 minutes. A second piece of Parafilm was then glued on top to cover the exposed nerve, and the orbit was filled with Gelfoam and sealed with Parafilm and Vetbond (3M, Minneapolis, MN). The PLLn (n = 6) was exposed by removal of the first trunk lateral line scale and the underlying epidermal layer, followed by tracer application and sealing as described above. For all nerve fill surgical procedures, fish were revived in fresh seawater and returned to holding aquaria for recovery (average surgical time = 15 minutes).

Following survival times of 5–7 days, fish were deeply anesthetized with MS-222 and transcardially perfused with 4% paraformaldehyde in 0.1 M PB with 1% glutaraldehyde. Brains were removed, postfixed for 12 hours, rinsed in 0.1 M PB, and cryoprotected in 30% sucrose in 0.1 M PB overnight prior to sectioning. Brains were then sectioned serially in the transverse plane at 40 μm with a cryostat, collected in Coors (Golden, CO) spot plate wells that contained 0.4% Triton-X 100 in 0.05 M phosphate-buffered saline (PBS), and soaked for 0.5–1 hour. Floating sections were then transferred with a paintbrush to wells containing avidin-biotin complex (ABC Vectastain Elite Kit, Vector) for 3 hours at room temperature in a sealed humidified chamber on a shaker table (70–90 rpm), rinsed in 0.1 M PBS, reacted with a diaminobenzidine (DAB) chromogen substrate kit with nickel chloride intensification (Vector) for 6 minutes, transferred to 0.1 M PBS to stop the

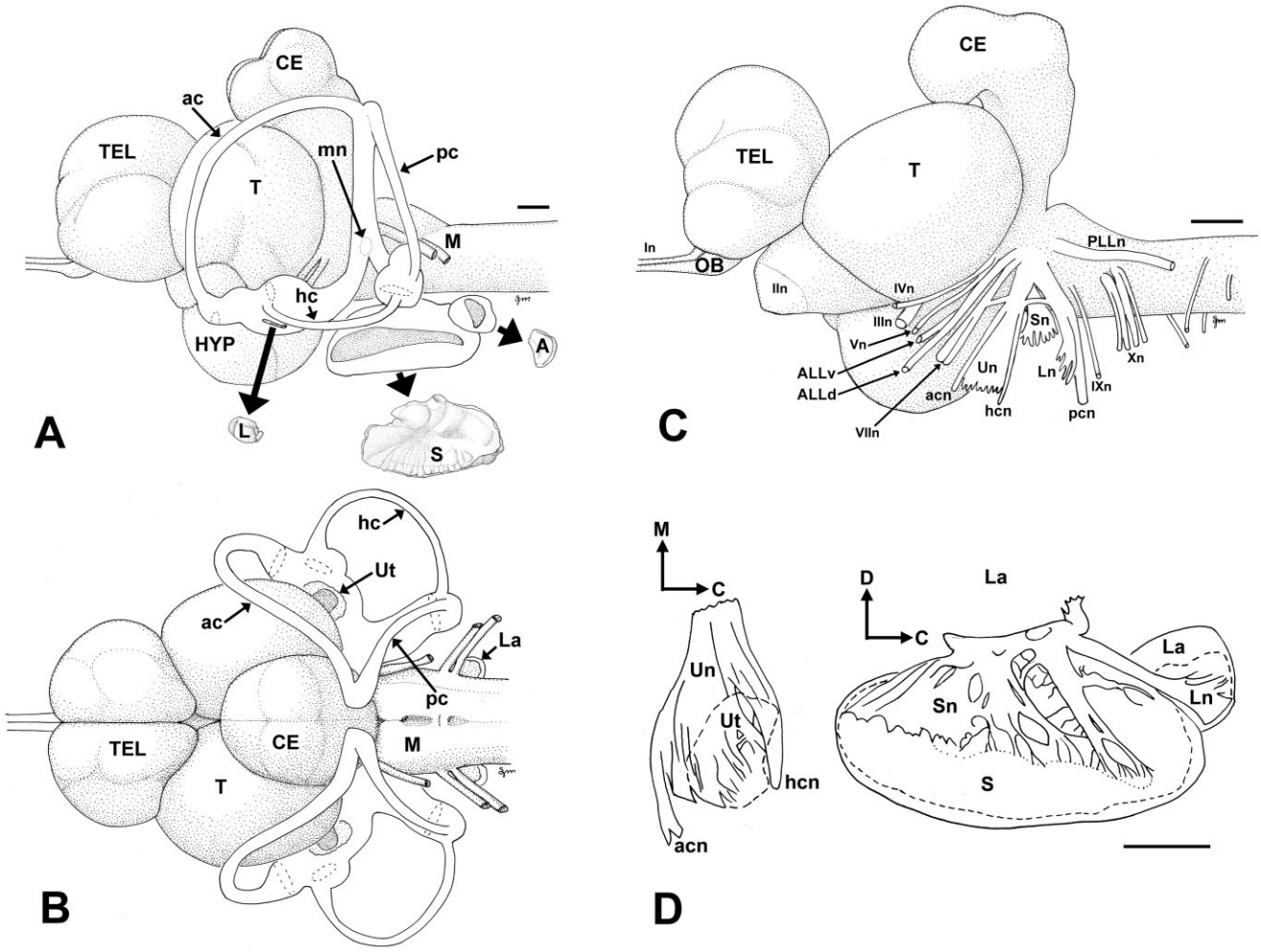

Figure 1.
The brain and inner ear of the Hawaiian sergeant fish *Abudefduf abdominalis*. **A,B:** Left lateral (A) and dorsal (B) views of the brain and inner ear show the location of the semicircular canals and otolithic endorgans. In A, the position of the macula is outlined and shaded within each otolithic endorgan, and the removed otolith is illustrated below (large arrows). Dashed lines represent the location of the crista ampullaris of each semicircular canal, and the dotted line represents the position of the macula neglecta (mn). **C:** Left lateral view of the brain shows the position of the cranial nerves labeled in this study. **D:** Innervation of the three otolithic endorgans. The anterior canal nerve (acn) and horizontal canal nerve (hcn) are closely associated with the large utricular nerve (Un). The saccule (S) is innervated by the broad saccular nerve (Sn) with multiple branches that span the length of the sensory macula (dotted line). The lagena (La) is innervated by the separate lagenar nerve (Ln) that is closely associated with the posterior canal nerve (not shown). Arrows indicate medial (M), caudal (C), and dorsal (D) positions. Dashed lines indicate the approximate locations of each otolith within the membranous otolithic sac. See list for other abbreviations. The illustrations depicted in A and B are from the same brain specimen, whereas C is of a different brain. Scale bar = 1 mm in A (also applies to B), C,D.

reaction, soaked in distilled water for 10 minutes, and then mounted on chrom/alum-coated slides. Slides were then dried overnight at room temperature and counterstained with 0.1% methyl green or 0.5% cresyl violet as above. In some cases, Neurobiotin label was visualized with Texas Red Avidin D (Vector), mounted with Vectashield (Vector), and viewed on a Zeiss Axioskop 2 fluorescent microscope. Line drawings were made with a drawing tube attached to an Olympus BH2 microscope.

Fixed tissue DiI fills

Projections of the auditory and vestibular cranial nerves were labeled in fixed tissue with Neurotrace DiI tissue-labeling paste (Invitrogen, Carlsbad, CA) because auditory endorgans are located deep within the otic capsule beneath the hindbrain and are difficult to access by live surgery methods (Fig. 1). Fish were anesthetized and perfused as described above for general neuroanatomy, and the brain was removed from the cranium with the auditory nerves and endorgans attached. Preliminary experiments showed that the octavolateralis nerves in the Hawaiian sergeant fish show only ipsilateral projections to the hindbrain, so a different nerve was labeled on contralateral sides of each brain. The saccular (n = 4), lagenar (n = 2), utricular (n = 3), ALLn (n = 1), PLLn (n = 1), and semicircular canal nerves (n = 3 each for hcn, acn, and pcn) were each individually labeled. In addition, several combination fills were performed: utricle with semicircular canal

nerves (n = 2), saccular and lagenar nerves together (n = 2), saccular and utricular nerves together (n = 1), all of nerve VIII (n = 3), and ALLn with VIIn and Vn together (n = 2). In addition to octavolateralis nerves, In, Vn, VIIn, IXn, and Xn were also labeled with DiI to identify regions for labeling purposes. The target nerve was cut and DiI tissue-labeling paste applied to the proximal stump. The labeled nerve was sealed with 4% agar to prevent the paste from dislodging and to minimize spread of tracer.

Brains were then submerged in 4% paraformaldehyde in 0.1 M PB and incubated in the dark at room temperature for 12 weeks to allow transport of dye into the brain, rinsed in 0.1 M PB, cryoprotected in 30% sucrose, sectioned at 40 μm in the transverse plane with a cryostat, mounted on chrom/alum-coated slides, and immediately viewed and photographed under a fluorescent microscope (Texas Red filter). Immediate examination and photography was necessary because DiI tracer labels the lipid bilayer of neurons (rather than being taken up by active transport into the axons) and dissolves quickly out of the neurons when sectioned with a cryostat. Following fluorescent photography, slides were dried overnight, counterstained with 0.5% cresyl violet acetate, dehydrated in an ethanol series (50–100%), cleared in toluene, and coverslipped with Cytoseal 60 (Richard Allen Scientific). A second set of photographs was then taken from the Nissl-stained slides and overlaid on the fluorescent image to facilitate identification of the primary projections from each nerve to the octavolateralis and other nuclei. All photomicrographs were adjusted for brightness and contrast, and sharpened with Adobe Photoshop software.

The rostrocaudal extent of each octavolateralis nucleus was determined in seven fish as the product of the number of sections that contained a nucleus and section thickness. Mean somata size was estimated by measurement of the major and minor cell profile axes of six randomly chosen cells per nucleus in seven fish and are reported as $\bar{x}$ major axis μm × $\bar{x}$ minor axis μm. Measurements were taken within the same general region of each nucleus, and fish size differed by ≤2 mm among individual fish. Thus, variation in brain size was assumed to be small, and we did not correct for size allometries in measurements of brain regions. Somata within the PON and CON were not measured because the boundaries of these nuclei are not as distinct as the other octavolateralis nuclei.

RESULTS

Otolithic endorgan, semicircular canal, and peripheral lateral line organization

The Hawaiian sergeant fish has three bilaterally paired otolithic endorgans (saccule, lagena, and utricle) and semicircular canals (anterior, horizontal, and posterior; Fig. 1). The saccule is the largest endorgan, located in the otic capsule ventrolateral to the medulla, and is oriented in the dorsoventral axis. The lagena is smaller, located immediately caudal and slightly dorsal to the saccule, and also oriented along the dorsoventral axis. The utricle is intermediate in size between the lagena and saccule, closely associated with the crista ampullaris of the horizontal and anterior semicircular canals, and oriented in the horizontal plane. The three semicircular canals are oriented orthogonal to each other and have contiguous endolymph. The posterior and anterior canals are vertical and join together at the common crus positioned at the caudal edge of the cerebellum. The macula neglecta organ (a hair-cell-based sensory macula without an overlying cupula or otolith) is located in the common crus that joins the anterior and posterior canals (Fig. 1). The horizontal canal is located in the horizontal plane, and the ampulla is closely associated with the utricle.

The Hawaiian sergeant fish lateral line includes superficial neuromasts located on the skin surface and a system of subepidermal canals on the head and trunk (Fig. 2). Many cephalic lateral line canals and a trunk canal extend from the operculum to the caudal edge of the dorsal fin (Fig. 2A). There is extensive branching of canal tubules to multiple pore openings on each scale (Fig. 2B). Superficial neuromasts are located dorsolaterally along the trunk canal with one to two superficial neuromasts on each lateral line trunk scale intermixed within the canal tubules and pores (Fig. 2A,C–D). There is also a line of superficial neuromasts along the body from the end of the trunk canal to the caudal peduncle, where one to two superficial neuromasts are located on each of about 8–10 scales (Fig. 2A). We were unable to visualize superficial neuromasts on the head with our staining technique due to the natural dark pigmentation and thus cannot comment on their distribution or innervation. The head canals are innervated by the ALLn and the trunk canal and superficial neuromasts on the body by the PLLn.

Octavolateralis cranial nerves project ipsilaterally to seven first-order medullary nuclei and the eminentia granularis (EG) of the cerebellum. The location and organization of each nucleus will be described first, followed by the central projections of each nerve.

Cytoarchitecture of medullary octavolateralis nuclei

The anterior octaval nucleus (AON) is the smallest and most rostral of the octavus column; it is located ventral to the medial octavolateralis nucleus (MON) and dorsomedial to the magnocellular octaval nucleus (MgON) at the entry level of nerve VIII and ALLn (Figs. 3, 5A). The AON is composed primarily of small spherical or fusiform cells (14 × 7 μm; Fig. 4), begins just rostral to the entrance of the ALLn and VIIn, and ends at the rostral border of the MgON. The AON has a mean rostrocaudal extent of 297 ± 39 SD μm in the dorsolateral medulla.

The MgON is located along the dorsal border of the descending tract of the trigeminal nerve at the level of nerve VIII entry to the medulla, and in the transition region between the rostral DON and caudal AON (Figs. 3, 5A, 6C,D). The MgON has a mean rostrocaudal extent of 463 ± 39 SD μm, and the dorsal region (MgONd) is composed of large fusiform or multipolar somata (41 × 16 μm; Figs. 4, 6C,D) that appear interspersed with the incoming nerve VIII primary afferents (Figs. 5A, 6C,D). The MgON also extends ventrolaterally around the descending tract of the trigeminal nerve to an area just dorsal to the tangential octaval nucleus (TON) and anterior to the DONv. This ventral MgON (MgONv) region contains both large somata similar to the MgONd and smaller somata (15 × 8 μm).

The TON is the most lateral of the octavus column, situated in close proximity to the brain edge, and located ventral to the DON, MgONv, and entry level of nerve VIII (Figs. 3, 5A, 6C,E). The TON is an almost spherical nucleus with small to medium-

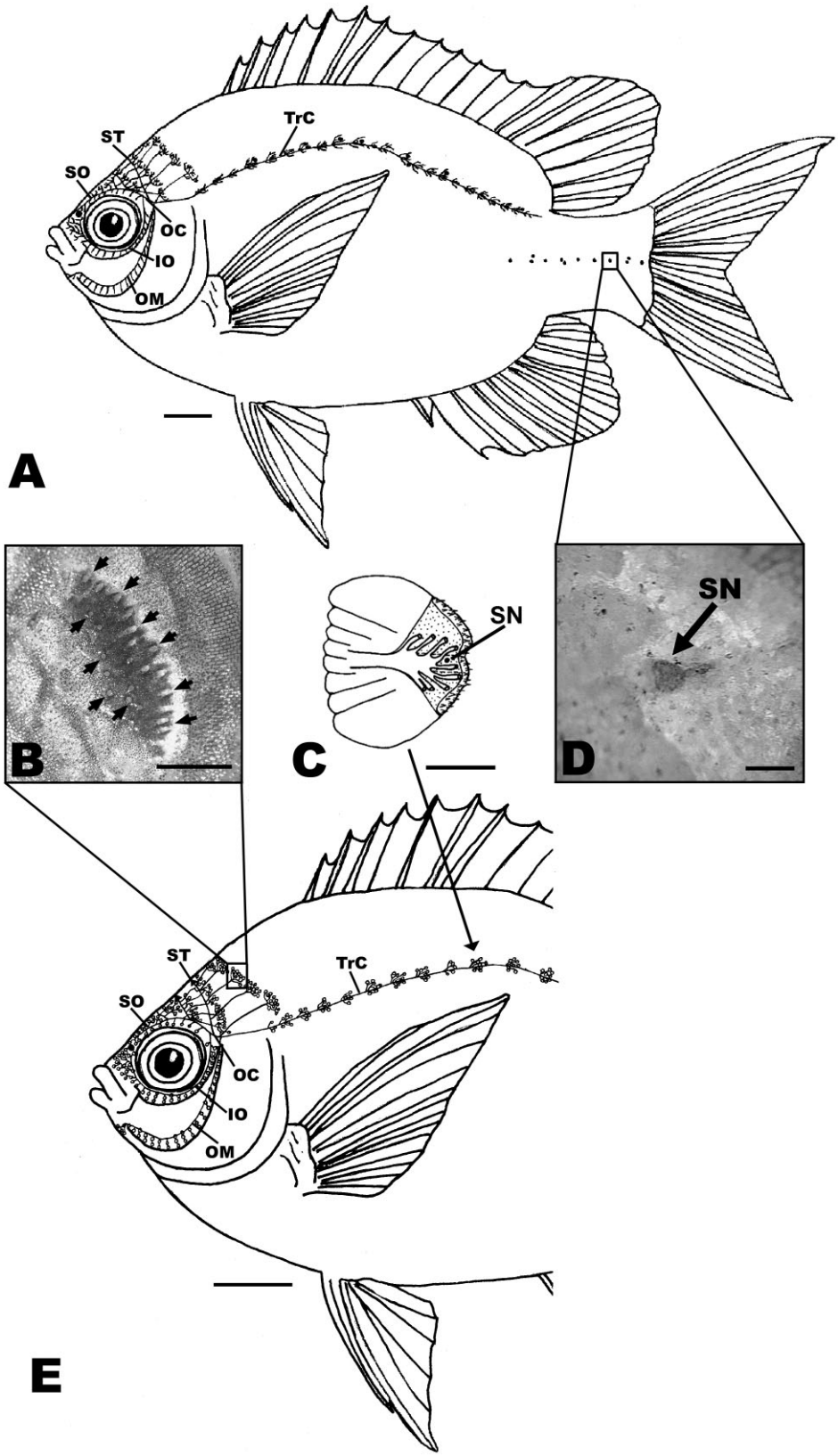

Figure 2.
The mechanosensory lateral line system in the Hawaiian sergeant fish *Abudefduf abdominalis*. **A:** Lateral view shows the distribution of lateral line canals (lines) and superficial neuromasts (dots). Canals on the head are composed of the opercular mandibular (OM), supratemporal (ST), supraorbital (SO), otic (OC), and infraorbital (IO) canals. The trunk canal (TrC) extends from the operculum to the caudal edge of the dorsal fin near the tail. **B:** Photograph of a single cephalic lateral line scale to illustrate the branching of tubules that terminate in multiple pores (arrows) on each scale. **C:** Schematic drawing of a single trunk lateral line canal shows multiple tubules that terminate in pores and a single superficial neuromast (SN) between the pores. **D:** Photograph of a single superficial neuromast from the caudal peduncle. **E:** Lateral view of the head shows the location of lateral line canals (lines) and pores (open circles) on each canal subsection. Scale bar = 1 cm in A,E; 5 mm in B,C; 1 mm in D.

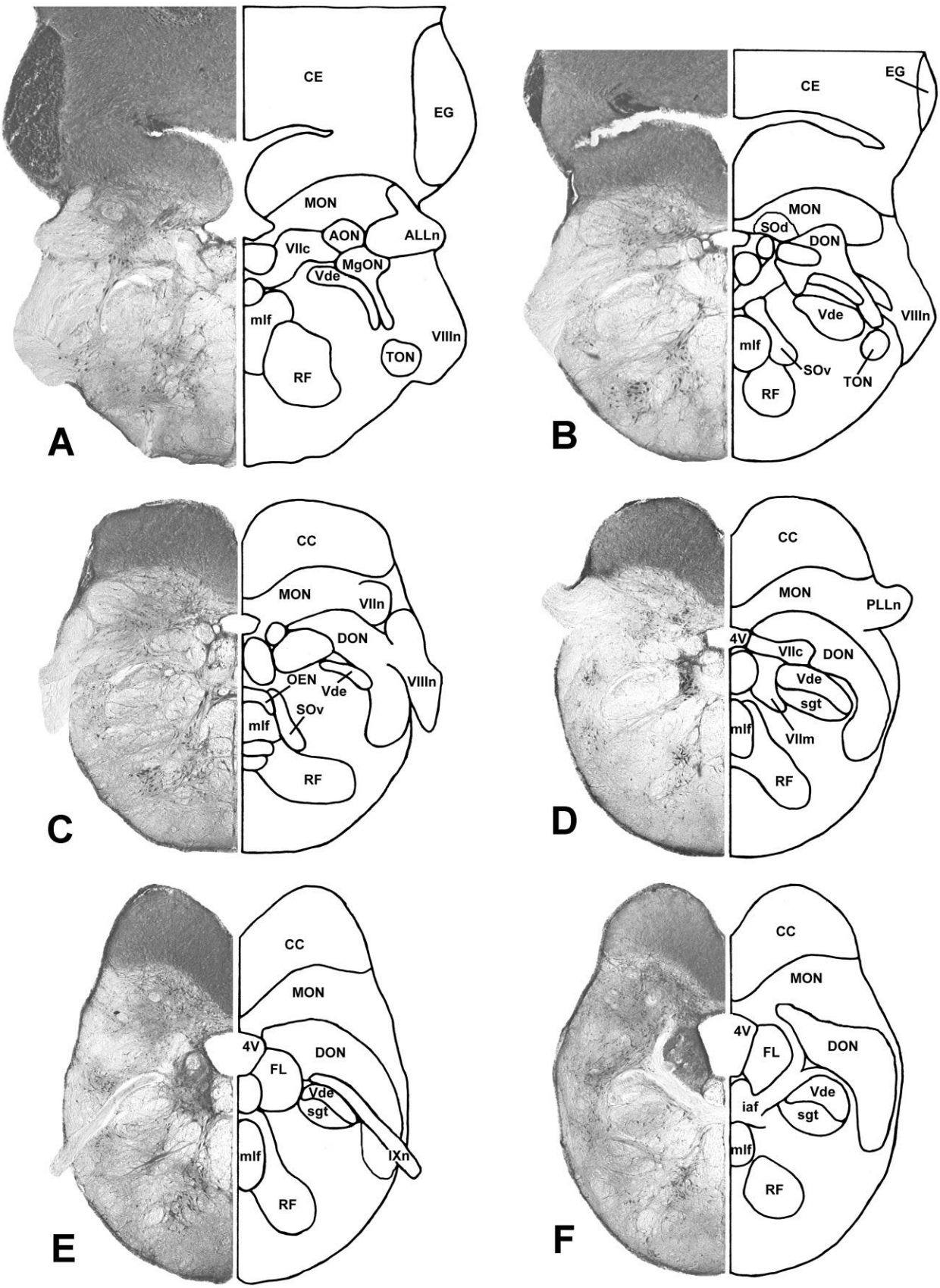

Figure 3 (Continued)

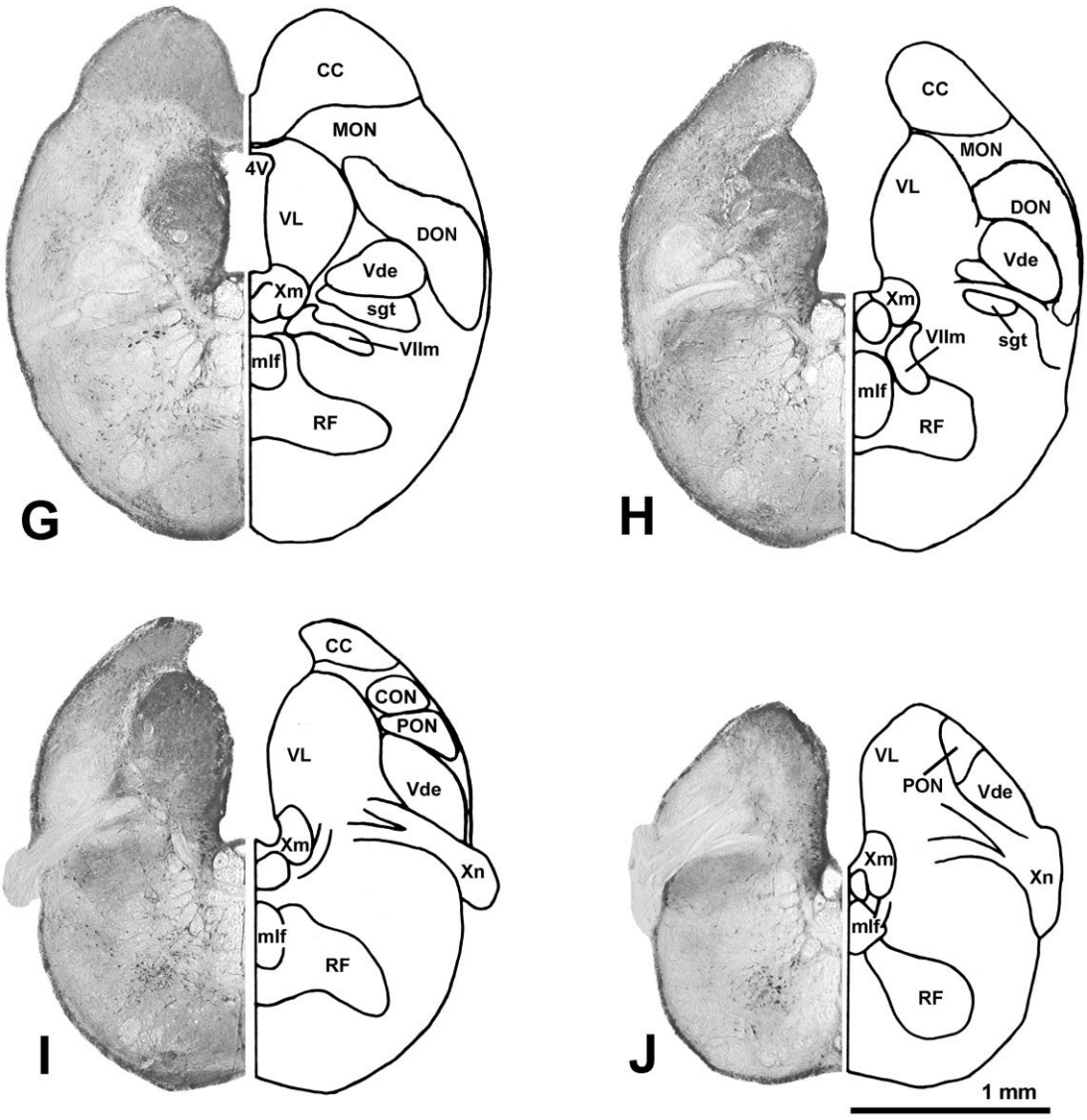

Figure 3.
Photomontages of representative transverse sections from rostral (A) to caudal (J) levels through the hindbrain of the Hawaiian sergeant fish *Abudefduf abdominalis*. Brain outlines and nuclear boundaries were traced from the Nissl-stained left side of the brain and horizontally inverted on the right. Nuclei locations were determined based on a combination of all nerve fills conducted in this study and comparisons with other fish species. See list for abbreviations. Scale bar = 1 mm in J (applies to A–J).

sized round somata (15 × 12 μm) (Figs. 4, 6C,E) and has a mean rostrocaudal extent of 486 ± 49 SD μm.

The DON is the largest nucleus within the octaval column and has a mean rostrocaudal extent of 1,291 ± 60 SD μm (Fig. 3). It is positioned ventral to the MON and spans the region between the MgON and rostral posterior octaval nucleus (PON) along the rostrocaudal axis (Figs. 3, 5B). The DON changes shape in cross section throughout its length and is subdivided into dorsomedial (DONdm), intermediate (DONi), and ventral (DONv) regions similar to that described for the sleeper goby (Tomchik and Lu, 2005) (Fig. 5B). The DONdm is located close to the dorsolateral wall of the fourth ventricle and ventral to the MON, and contains medium to large fusiform and spherical somata (20 × 10 μm). These somata extend dorsal dendrites into the cerebellar crest and ventrolateral dendrites into the DONi (Figs. 5B,D). The DONi is the largest region and located dorsal and lateral to the descending tract of the trigeminal nerve. The DONi contains regions of sparse cells as well as regions of clustered neurons that include primarily small to medium-sized (15 × 9 μm) fusiform somata (Figs. 4, 5B). The DONv is considerably smaller and is located ventrolateral to the DONi, but also has regions of sparse cells (16 × 9 μm) and clustered somata (Fig. 5B,E).

The DONdm is replaced rostrally by the dorsal secondary octaval nucleus (SOd). Neurons in the SOd are fusiform or spindle-shaped with distinct dorsal dendrites that extend into the overlying cerebellar crest (Fig. 5A,C). Ventral dendrites

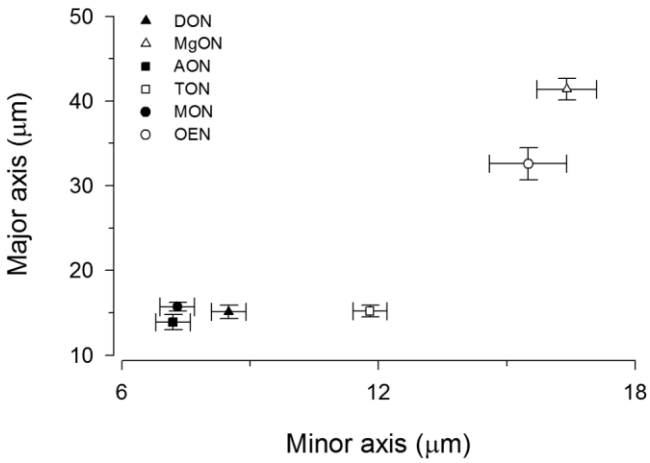

Figure 4.
Somata size within the octavolateralis nuclei of the Hawaiian sergeant fish *Abudefduf abdominalis*. The magnocellular octaval nucleus (MgON) and octavolateralis efferent nucleus (OEN) contain large fusiform somata. The anterior octaval nucleus (AON), medial octavolateralis nucleus (MON), and intermediate descending octaval nucleus (DON) contain smaller fusiform somata, whereas the tangential octaval nucleus (TON) is composed of medium-sized spherical cells. Data are plotted as $\bar{x} \pm$ SE of measurements of the major and minor axes of each cell (n = 6 cells, 7 fish).

extend ventrolaterally into the dorsal region of the DONi (Fig. 5C). In more rostral sections, the SOd is replaced by the MON.

The MON receives the greatest projections from the lateral line nerves, is located ventral to the cerebellar crest along most of its length, and extends approximately 1,823 ± 56 μm from the caudal octavolateralis nucleus (CON) in the caudal hindbrain rostrally to the level of the AON in the anterior hindbrain (Fig. 3). The afferent termination region of the MON is composed primarily of small fusiform cells (16 × 7 μm; Fig. 4) and is bordered dorsally by the principal cell layer that contains larger crest cells with dorsal dendritic extensions into the overlying cerebellar crest.

The octavolateral efferent nucleus (OEN) is located along the midline, dorsal to the medial longitudinal fasciculus and closely associated with the facial motor nucleus, reticular formation, and internal arcuate fibers (Figs. 3, 6A,B). The OEN has large bipolar neurons (33 × 16 μm) with long processes that extend ventrolaterally (Figs. 4, 6B). Because this nucleus is located on the midline, some cell bodies have processes that cross the midline to join the ventrolateral dendritic tract on the contralateral side (Figs. 6A,B). The OEN is the source of efferent input to the octavolateralis endorgans and has a mean rostrocaudal extent of 303 ± 21 SD μm. There are no obvious subdivisions of the OEN (i.e., rostral and caudal divisions).

The PON and CON are the most caudal of the octaval column and have a mean rostrocaudal extent of about 300–500 μm, but are more loosely organized than the other octavolateralis nuclei (Fig. 3). The PON is located ventral to the mechanosensory CON along the dorsolateral edge of the medulla and extends more caudally than the CON. The CON is a small lateral line termination region located caudal to the MON and dorsal to the PON in the region of vagal nerve entry to the hindbrain.

The EG of the cerebellum also receives primary projections from many octavolateralis endorgans. The EG is a region of small, densely packed granule cells at the lateral edge of the caudal cerebellum that expands medially in cross-sectional size as it joins the granule region of the corpus of the cerebellum in more rostral sections (Fig. 3). The granule cell axons from this region form the majority of the cerebellar crest that overlays the MON in the hindbrain.

Central projections of octavolateralis nerves

Lateral line nerves. The ALLn enters the brain as separate dorsal (ALLd) and ventral (ALLv) branches. The ALLn enters the medulla closely associated with the trigeminal and facial nerves in the region between the lateral ventrocaudal tectum and dorsocaudal hypothalamus, and anterior to the PLLn. The PLLn projects to the brain from a caudal direction and enters the medulla at a dorsal position just beneath the cerebellar crest that is caudal to nerve VIII and dorsal to nerve IX. The ALLn and PLLn both terminate exclusively ipsilateral within the CON and MON in the medulla, and in the EG (Table 1; Figs. 7–9). ALLn afferents project primarily ventral and PLLn afferents primarily dorsal within the MON, but it is possible that there are also regions of overlap. Some sparse label from the ALLn (but not PLLn) occurs in the dorsal portion of the MgON (Fig. 8D). ALLn and PLLn projections also appear to overlap within the EG (Figs. 7–9). In all cases in which the ALLn was filled in vivo with Neurobiotin, many granule cells within the lateral EG were also labeled (Fig. 7A,B). These granule cells were not labeled in DiI fills in fixed tissue and thus may represent transneuronal label with the low molecular weight Neurobiotin tracer. Aside from this difference, the termination regions of the ALLn and PLLn were similar among both Neurobiotin- and DiI-labeled tissue.

Saccular nerve. The saccular nerve is relatively short, but is large and flattened to extend across the rostrocaudal length of the macula (Fig. 1D). There is extreme branching of the saccular nerve at the sensory macula, which then converges with other adjacent nerve VIII branches dorsally near the entrance to the medulla. Saccular primary afferents terminate ipsilaterally within the AON, DON, MgON, and PON, as well as the EG (Table 1; Figs. 10, 11). Saccular afferents terminate primarily in the dorsal region of DONi, with heaviest projections to the rostral portion of this nucleus. Labeled saccular afferents come close to, but do not reach the somata within the DONdm near the ventricle, although it is possible that there are synaptic connections with DONdm dendrites (see Discussion). There was also some sparse label within the DONv, but this was not consistent among fish. The lateral and ventral regions of the AON, as well as the dorsal and ventral MgON, also receive dense projections from the saccular nerve (Fig. 11). Saccular afferents were not observed in the TON or in the mechanosensory CON or MON. In several cases, saccular nerve fills also showed projections in the vicinity of the Mauthner neuron.

Lagenar nerve. The lagenar nerve is closely associated with the posterior canal nerve at the periphery, and with the saccular nerve as it enters the medulla. Lagena primary afferents terminate ipsilaterally within the AON, DON, MgON, and PON, as well as the EG (Table 1; Figs. 10, 12). Lagena afferent projections are most prominent in the dorsal portion of the DONi and are most dense in the region caudal to the saccular inputs. Lagenar afferents show sparse projections to the

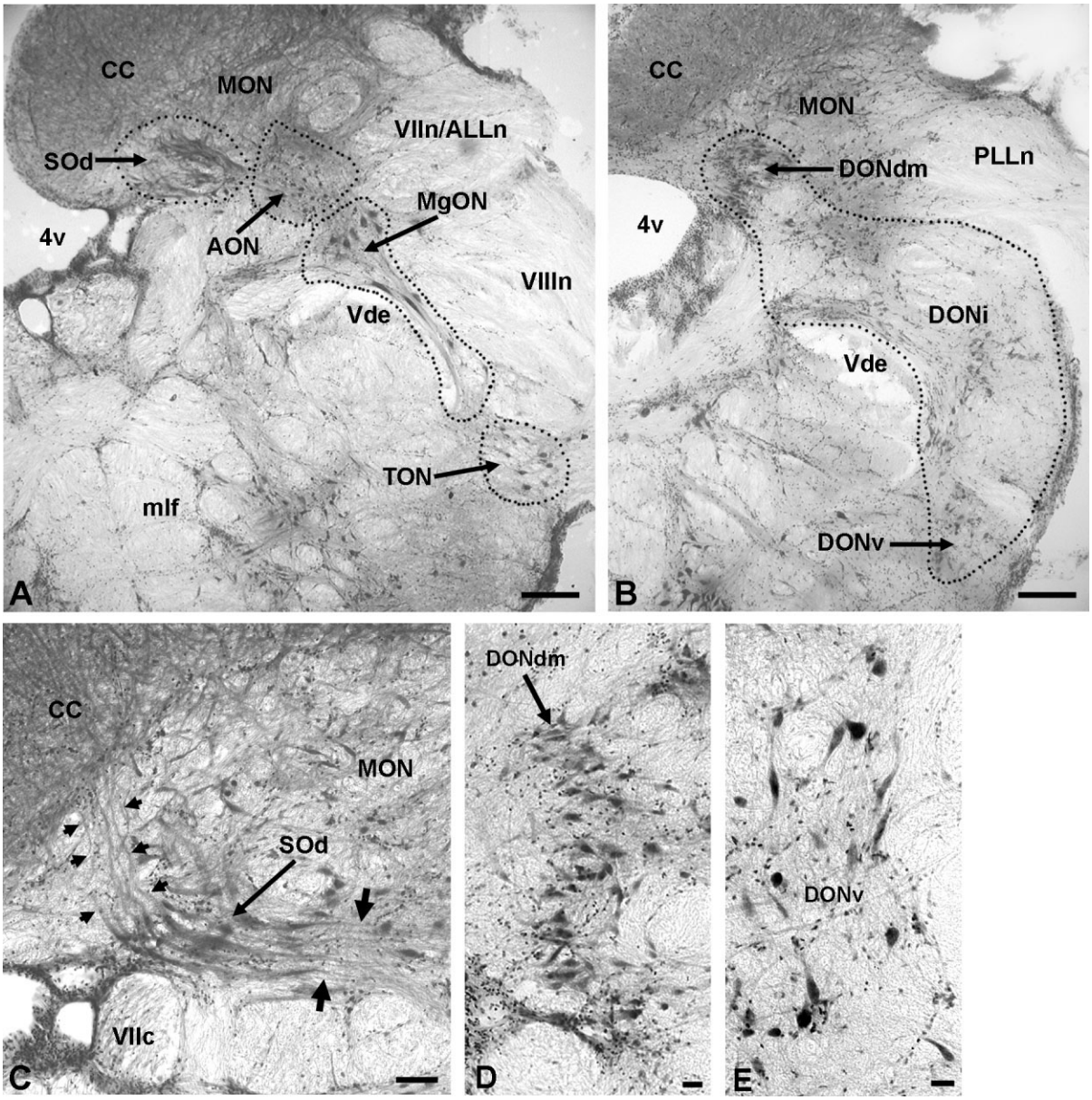

Figure 5.
Organization of octaval nuclei in the hindbrain of the Hawaiian sergeant fish *Abudefduf abdominalis*. **A:** Representative transverse section through the rostral hindbrain shows the locations of the anterior octaval nucleus (AON), magnocellular octaval nucleus (MgON), tangential octaval nucleus (TON), and dorsal secondary octaval nucleus (SOd). **B:** More caudal transverse section shows the dorsomedial (DONdm), intermediate (DONi), and ventral (DONv) divisions of the descending octaval nucleus. **C:** Transverse section through the SOd shows the large fusiform somata with dorsal dendrites (small arrowheads) that extend into the cerebellar crest (CC) and ventrolateral dendrites (large arrowheads) that extend toward the DONi. **D:** Higher magnification of the DONdm shows densely packed fusiform somata. **E:** Higher magnification of the caudal DONv shows a region of diffuse fusiform and spherical somata. Scale bar = 100 μm in A,B; 50 μm in C; 10 μm in D,E.

DONv, moderate to heavy projections to both the dorsal MgON and dorsal and medial AON, and sparse projections to the TON. No projections of the lagena to the CON or MON were observed.

Utricular nerve. The large utricular nerve is closely associated with the smaller anterior and horizontal semicircular canal nerves and enters the medulla in a dorsal position within the nerve VIII complex. Utricular nerve afferents terminate ipsilaterally within the AON, DON, MgON, TON, and PON, as well as the EG (Table 1; Figs. 10, 13). Projections in the DON are concentrated lateral and dorsal in the DONi, and a heavy projection to the DONv is also evident (Figs. 10, 13). Utricular afferents also show dense projections to the lateral and ventral AON, ventral MgON, and TON. Utricular projections to the EG are also denser than those observed from saccular or lagenar fills. In several cases, utricular nerve fills also labeled

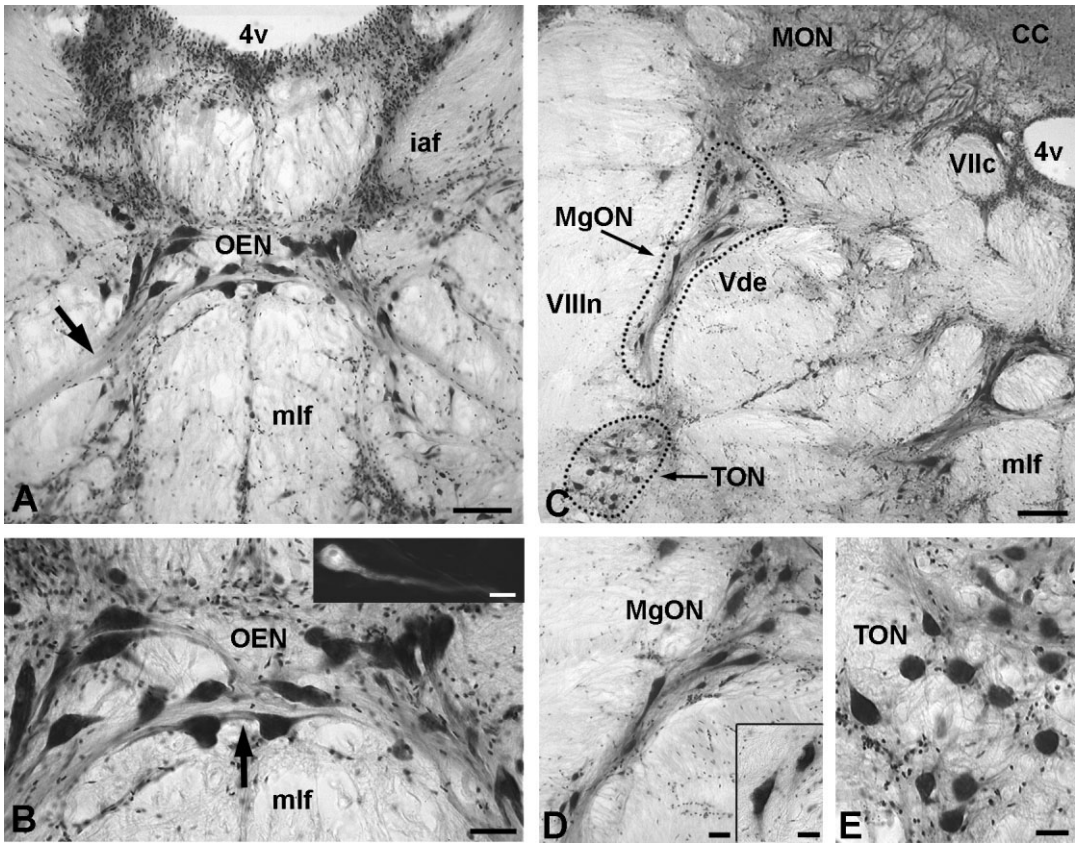

Figure 6.
Organization of primary octavolateralis nuclei in the hindbrain of the Hawaiian sergeant fish *Abudefduf abdominalis*. **A:** Transverse section through the hindbrain at the level of the octavolateralis efferent nucleus (OEN). The OEN is located along the midline and has large bipolar neurons with long processes that extend ventrolaterally (arrow). **B:** Higher magnification of the OEN pictured in A shows some neurons with processes that extend across the midline (arrow). Inset shows a single labeled efferent neuron in the OEN after DiI application to the utricular nerve. **C:** Representative transverse section through the hindbrain shows the location of the magnocellular octaval nucleus (MgON), tangential octaval nucleus (TON), and medial octavolateralis nucleus (MON). Approximate boundaries of each nucleus are outlined. **D:** Higher magnification of the MgON shown in C. Inset shows two large fusiform MgON neurons. **E:** Higher magnification of the TON shown in C illustrates the medium-sized spherical neurons in the TON. Scale bar = 100 μm in A,C; 25 μm in B; 20 μm in B, inset; 25 μm in D; 15 μm in D, inset; 15 μm in E.

TABLE 1. Summary of Auditory, Lateral Line, and Vestibular Nerve Projections to Octavolateralis Nuclei in the Hawaiian Sergeant Fish, *Abudefduf abdominalis*[1]

	ALLn	PLLn	acn	hcn	pcn	Un	Sn	Ln
AON	–	–	?	?	?	++	+++	++
DONdm	–	–	–	–	–	–	–	–
DONi	–	–	+	+	+	+++	+++	+++
DONv	–	–	++	+++	–	+++	+	+
MgON	+	–	++	+	+	++	++	++
TON	–	–	–	+++	–	+++	?	+
MON	+++	+++	–	–	–	–	–	–
CON	+	+	–	–	–	–	–	–
PON	–	–	–	–	–	+	+	+
EG	+++	+++	–	–	–	+++	+	+

[1]+++, dense projections; ++, moderate projections; +, sparse projections; –, no projections; ?, possible projections. For abbreviations, see list.

OEN neurons (Fig. 6B, inset) and had projections in the vicinity of the Mauthner neuron. There were no utricular nerve projections observed to the CON or MON.

Semicircular canal nerves. The crista ampullaris of each semicircular canal is innervated by a separate and distinct small-diameter nerve. The anterior and horizontal canal nerves are found on either side of the larger utricular nerve. The posterior canal nerve is closely associated with the lagenar nerve. All semicircular canal nerves enter the brain with the other nerve VIII components, and the majority of terminations are caudal to this point. The central projection pattern for each semicircular canal nerve is distinct (Fig. 14), with little overlap among them except possibly in the rostral DONi and MgON. Compared with the otolithic endorgan projections to the DONi, semicircular canal afferent projections appear less dense and show greater termination in caudal regions. Projections from all three canal nerves are observed to the DONi, where anterior canal afferents are most numerous and located more dorsally than posterior and horizontal canal afferents (Fig. 14B). In the DONv, horizontal canal afferents terminate in a ventral region that extends beneath the entry of IXn, whereas anterior canal afferents terminate primarily dorsal to IXn (Fig. 14A,B). The horizontal canal nerve shows dense projections to the DONv and TON (Fig. 10), and together with the utricular nerve appears to make up the full volume of the

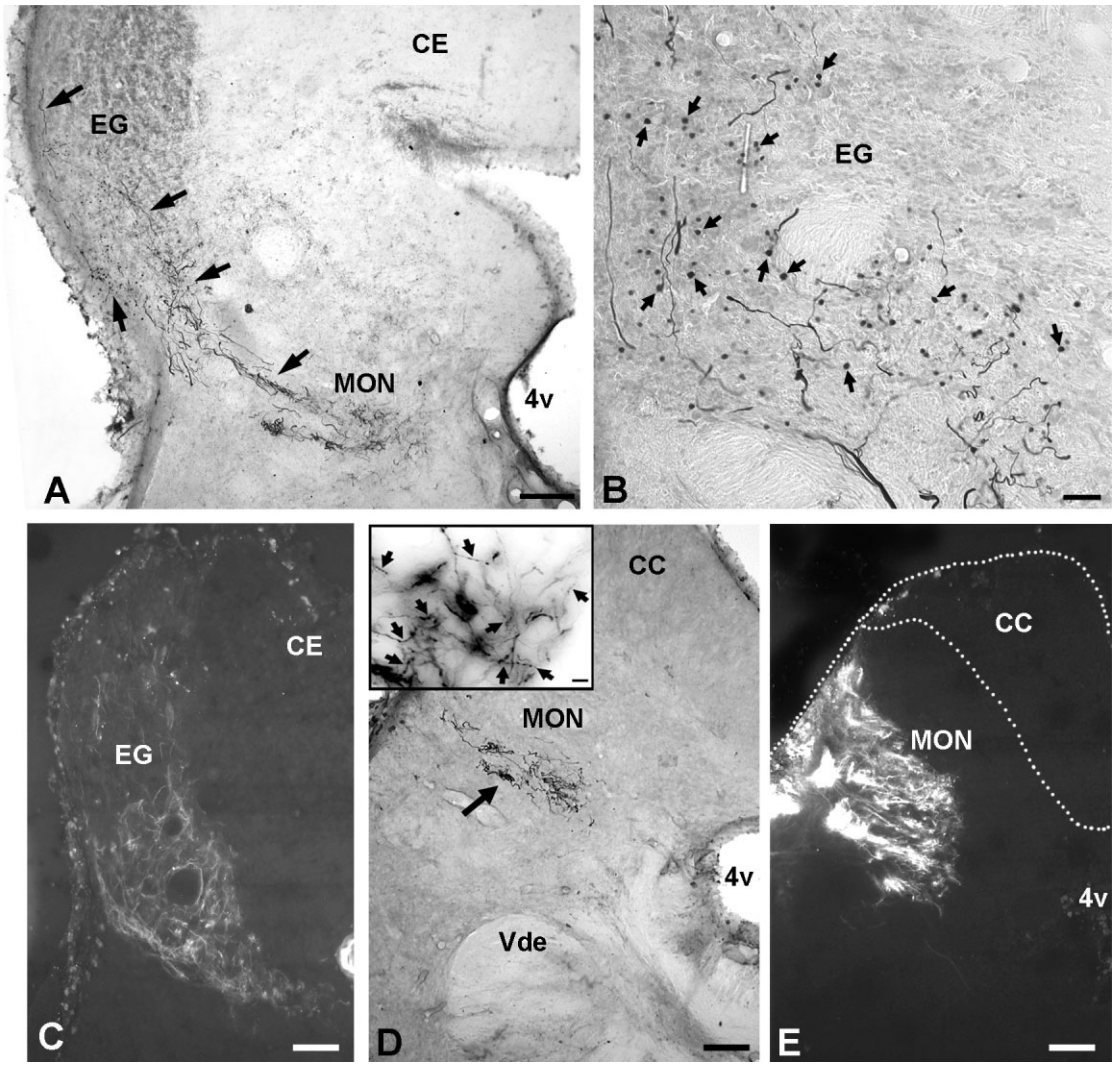

Figure 7.
Projections of lateral line nerves to the medial octavolateralis nucleus and eminentia granularis in the Hawaiian sergeant fish *Abudefduf abdominalis*. **A:** Neurobiotin label (arrows) of the anterior lateral line nerve projection to the eminentia granularis (EG). **B:** Higher magnification of neurobiotin projections in A show labeled granule cells in the EG (arrows). **C:** DiI label of the posterior lateral line projections to the eminentia granularis. **D:** Neurobiotin label (arrow) of the anterior lateral line nerve to the medial octavolateralis nucleus (MON) in the hindbrain. Inset shows a higher magnification of beaded terminals (arrows) from a DiI-labeled ALLn (image was inverted) in a similar region of the MON from a different fish. **E:** DiI label of the posterior lateral line nerve to the MON. Dotted line indicates the brain edge and outline of the cerebellar crest (CC). See list for other abbreviations. Scale bar = 100 μm in A,C–E; 30 μm in B; 10 μm in D, inset.

TON. Projections from acn and pcn were not observed to the TON. The anterior canal nerve has moderate projections to the DONi and to both the dorsal and ventral MgON (Fig. 14B–D). Posterior canal nerve afferents had the weakest of all semicircular canal nerve labels, and only sparse projections to the DONi and MgON were observed. Projections to the PON, CON, MON, and EG were not observed from any semicircular canal nerve (Table 1).

Summary of other octavolateralis projections. Direct projections from octavolateralis nerves to the reticular formation were not observed in the Hawaiian sergeant fish, but utricular fills revealed fibers that extended ventromedially toward the reticular formation from the DONv (Fig. 10B). Strong label of octavolateralis efferent neurons was only evident in a few cases when the utricular nerve was labeled alone or in combination with another octaval nerve (Fig. 6), but weak or incomplete label was observed in several other cases (PLLn, ALLn, Sn, Ln). Afferent projections of the saccule and utricle to the Mauthner cell region were observed only in a few cases. This inconsistent label to the OEN and Mauthner cell is likely due to the labeling techniques that resulted in insufficient transport time, limited dye uptake, or other problems related to application and uptake of the tracer (e.g., contact of nerve stump with vetbond or seawater prior to complete neurobiotin crystal uptake). Projections to the CON and PON were sparse, and the boundaries of these nuclei were less distinct than others. However, projections to the PON were heaviest from utricular fills and to the CON from both ALLn and PLLn fills.

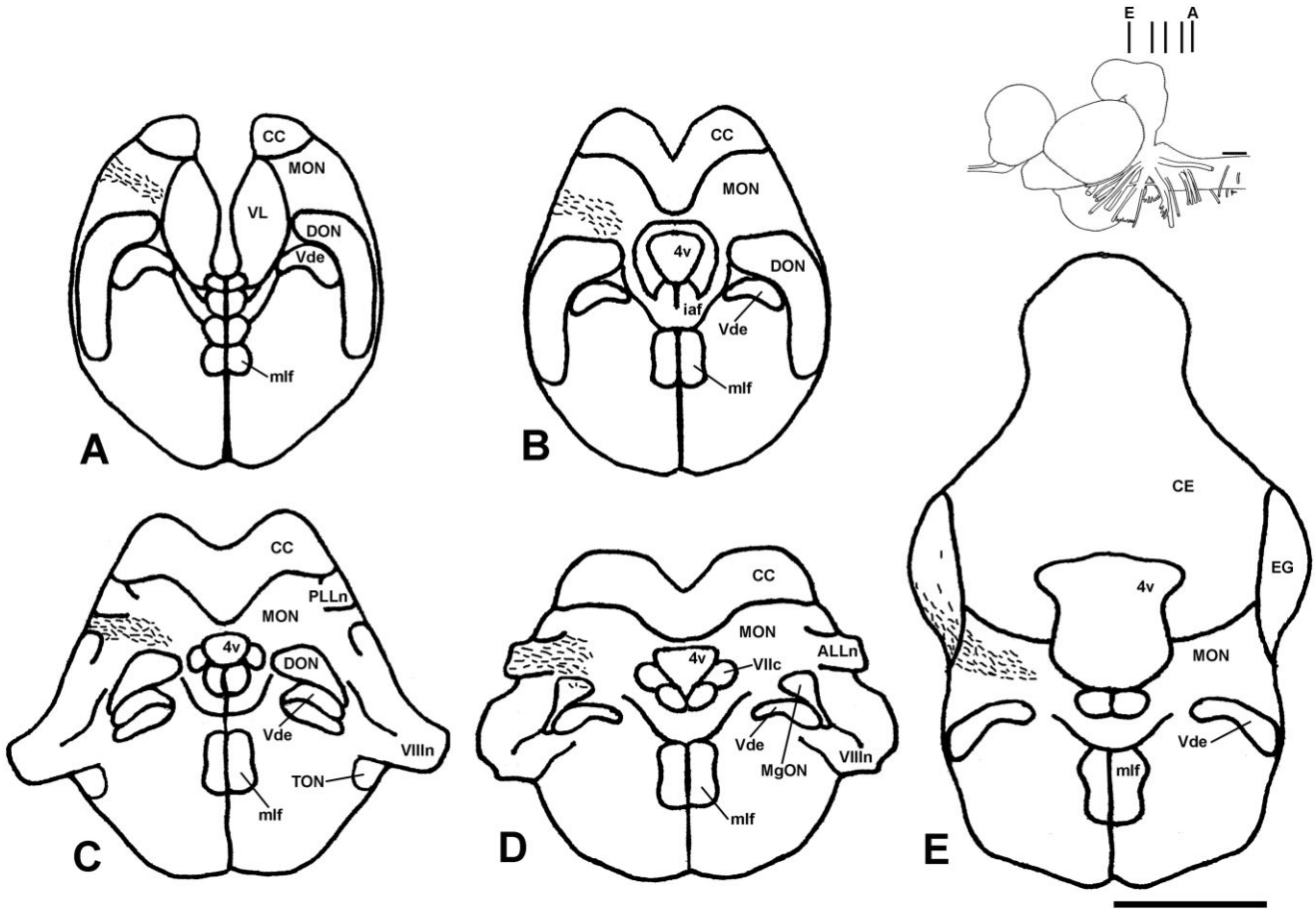

Figure 8.
Line drawings of representative serial transverse sections from caudal (A) to rostral (E) levels show the projections of the anterior lateral line nerve in the Hawaiian sergeant fish *Abudefduf abdominalis*. The anterior lateral line nerve (ALLn) projects primarily to the medial octavolateralis nucleus (MON) and eminentia granularis (EG). Brain inset shows the approximate location of each section. See list for abbreviations. Scale bar = 1 mm in E (applies to A–E).

Direct projections to the EG were also evident from all lateral line and octaval nerves, with the heaviest innervation from the ALLn, PLLn, and utricular nerve. Projections to the EG were generally more caudal and lateral for lateral line nerves, and were rostral and medial for octaval nerves.

DISCUSSION

The central projections of octavolateralis nerves in the soniferous Hawaiian sergeant fish are similar to the few other non-sonic perciforms examined (Northcutt, 1979; Meredith and Butler, 1983; McCormick, 1983; Meredith et al., 1987; Tomchik and Lu, 2005). The similarities include ipsilateral projections of all octavolateralis nerves to the seven identified medullary nuclei, minimal overlap between octaval and lateral line information, and no direct projections to the DONdm somata. The absence of anatomical first-order octavolateralis nuclei specializations among the soniferous damselfish and the few non-soniferous perciforms examined indicates conserved acoustic neural circuits at this gross anatomical level. In contrast, species with specialized auditory structures show some contralateral projections, moderate overlap between octaval and lateral line projections, and direct octaval projections to the DONdm somata (Bell, 1981; McCormick and Braford, 1994; McCormick, 1997, 1999). The biological consequences of these variations among species with different auditory peripheries are unknown, and future comparative studies are needed to test whether they are related to phylogeny or functional adaptations driven by auditory-related selective pressures.

Descending octaval nucleus

The organization of and projections to the DON in the Hawaiian sergeant fish are most similar to those described for the sleeper goby and oscar (Meredith and Butler, 1983; Tomchik and Lu, 2005), both of which are perciform species that do not have specialized structures to detect sound pressure. Afferent projection patterns and relative size of the DONdm differ among fishes with and without accessory hearing structures. The DONdm is large in species that detect sound pressure via accessory hearing structures such as otophysans, mormyrids, and clupeids, and is small in nonteleosts and teleosts that primarily detect the particle motion component

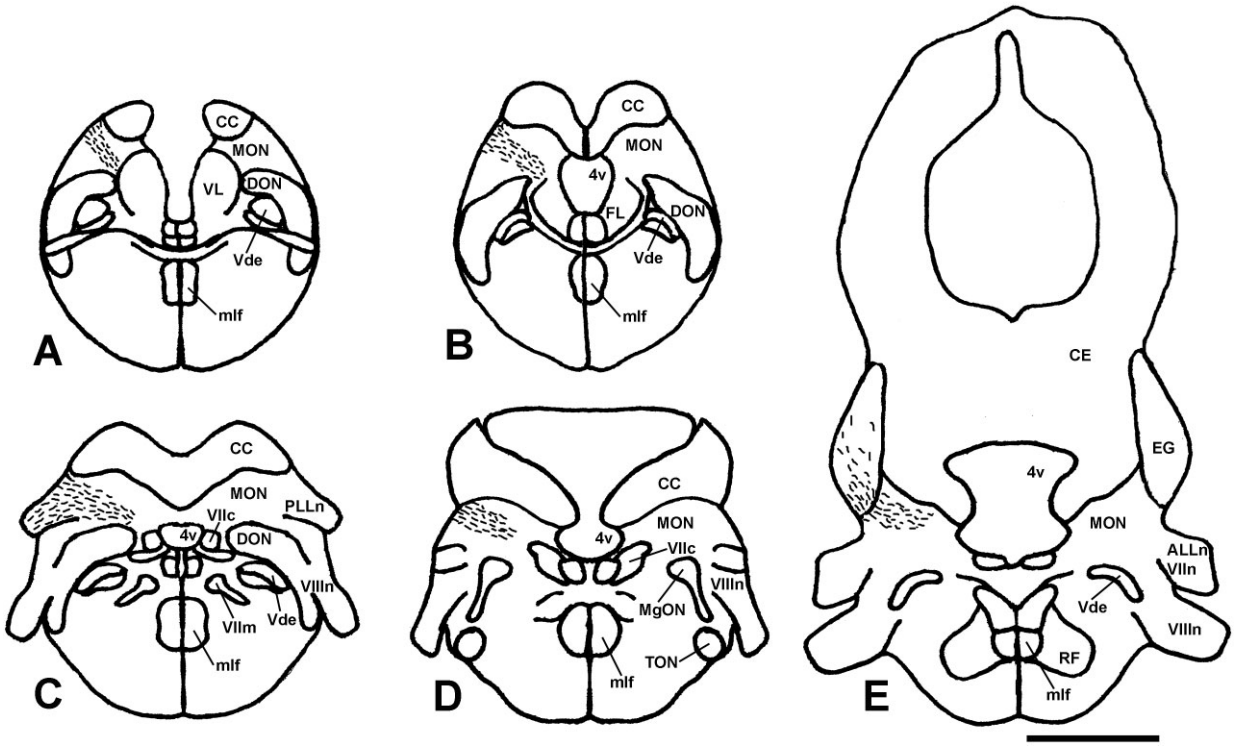

Figure 9.
Line drawings of representative serial transverse sections from caudal (A) to rostral (E) levels show the projections of the posterior lateral line nerve in the Hawaiian sergeant fish *Abudefduf abdominalis*. The posterior lateral line nerve (PLLn) projects primarily to the medial octavolateralis nucleus (MON) and eminentia granularis (EG). Brain inset in Fig. 8 shows the approximate location of each section. See list for abbreviations. Scale bar = 1 mm in E (applies to A–E).

of sound (McCormick, 1999). Thus the DONdm is thought to be a major auditory zone that is particularly distinct in fishes that detect sound pressure via accessory structures, and primary afferent projection patterns seem to correlate with the relative contribution of auditory endorgans to hearing in different species (McCormick, 1992, 1999). For example, in species in which the saccule likely serves a dominant function in hearing, saccular projections are found more dorsomedially in the DON than utricular and lagenar afferents (McCormick, 1983, 1997, 1999; McCormick and Braford, 1993, 1994). In contrast, clupeid fishes that have auditory bullae associated with the utricle (indicative of a primary role in audition) show utricular projections to the medial DONdm, with lagenar and saccular input more lateral (Meredith, 1985; McCormick, 1997). Although the DONdm exists in fishes both with and without accessory hearing structures, the relative size, organization, and projections from different endorgans may reflect functional specializations among species. Tomchik and Lu (2005) proposed that input to the ventrolateral dendrites of the DONdm neurons carries particle motion information (in all fishes) and direct input to the DONdm somata carries sound pressure information (in fishes with specializations). Thus DONdm neurons in species with accessory hearing structures may function in sound localization (i.e., phase model; Rogers et al., 1988) by comparing inputs from direct particle motion (via ventrolateral dendrites) with the indirect particle motion generated by pressure waves acting on the coupled swim bladder (via direct inputs to DONdm somata). Future studies in fishes with different types of accessory hearing structures are needed to test this hypothesis. It should also be noted that a dorsolateral division of the DON (DONdl) was described in batrachoidids and may represent a migrated cell population of the DONdm in which auditory and lateral line information is integrated (Weeg and Bass, 2000; Bass et al., 2000, 2001). Future studies should therefore also carefully examine putative auditory-mechanosensory integration at the level of the DON in fishes.

The DON receives projections from all octaval afferents in the Hawaiian sergeant fish, with the most overlap within the dorsal DONi. Otolithic projections to the DONi appear mostly segregated, with the densest projections dorsal and rostral from the saccule, dorsal and caudal from the lagena, and dorsal and lateral from the utricle. The most overlap was observed in the dorsal DONi throughout its rostrocaudal extent, and it was difficult to discern distinct patterns from each endorgan with our label technique. Previous studies across fish taxa show primarily segregated (with some overlap) otolithic endorgan projections within the DON (Meredith and Butler, 1983; McCormick and Braford, 1994; McCormick, 1999). A complex interdigitating projection pattern from the three otolithic endorgans exists within the DONi of the sleeper goby (Tomchik and Lu, 2005), is not yet described in other fishes, and may represent a topographic map of afferent directional tuning. This complex pattern was observed because of simultaneous labeling of different nerves with multicolor fluorescent dyes in the same individual fish. Our single tracer labels preclude comparisons with a similar complexity in

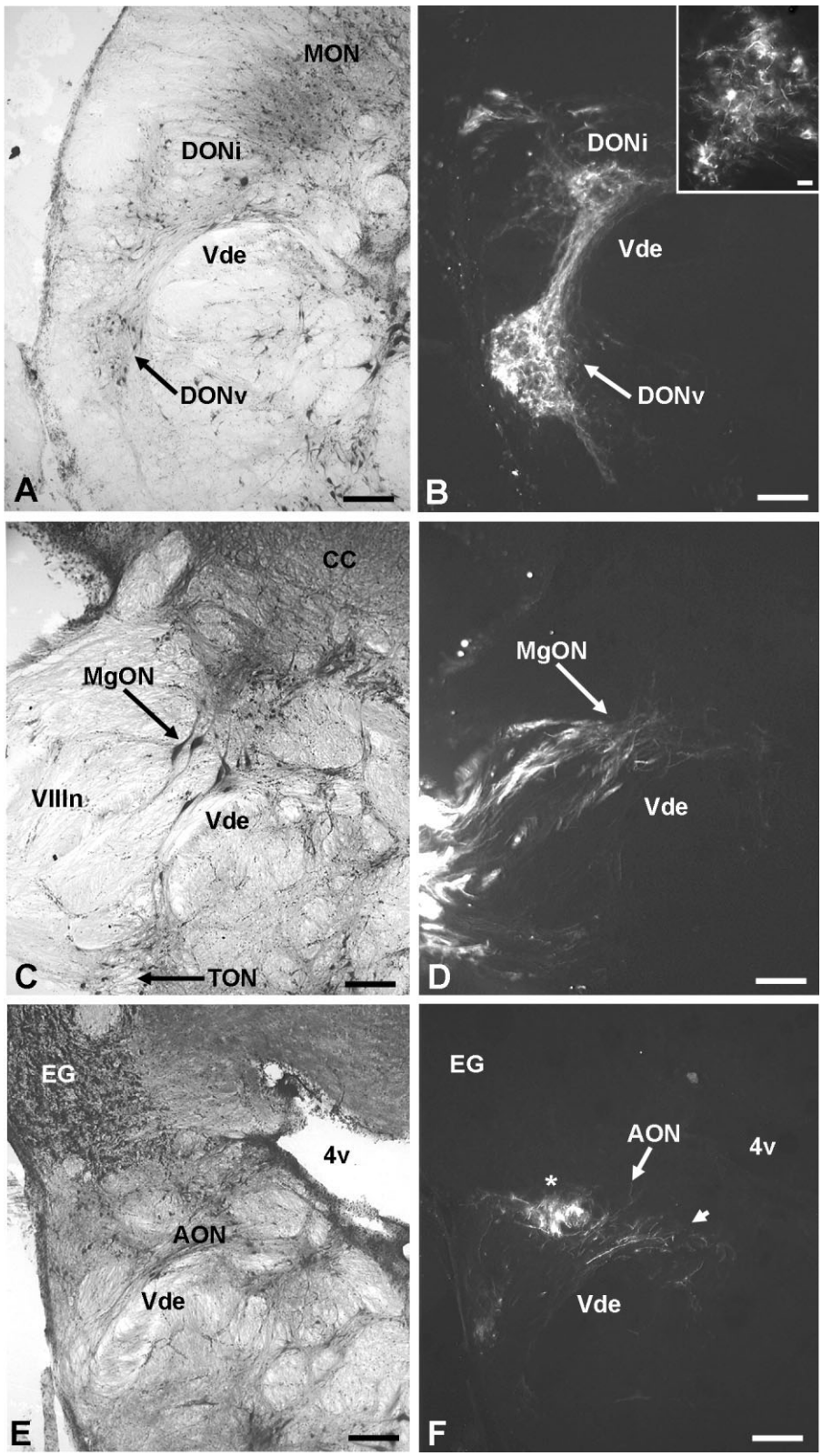

Figure 10.
Representative examples of primary projections from octaval and semicircular canal nerves in the Hawaiian sergeant fish *Abudefduf abdominalis*. Right side shows a representative DiI fill, and left side shows the identical section stained with cresyl violet. **A,B:** Projection of a combined fill of the utricular and horizontal and anterior semicircular canal nerves to the intermediate (DONi) and ventral (DONv) divisions of the descending octaval nucleus. **Inset in B:** Higher magnification of a more caudal utricular termination region in the DONi. **C,D:** Projection of the saccular nerve to the magnocellular octaval nucleus (MgON). **E,F:** Projection of the lagenar nerve to the anterior octaval nucleus (AON). Asterisk and arrowhead on F indicate the nerve tract and termination region, respectively. See list for other abbreviations. Scale bar = 100 μm in A–F; 20 μm in B, inset.

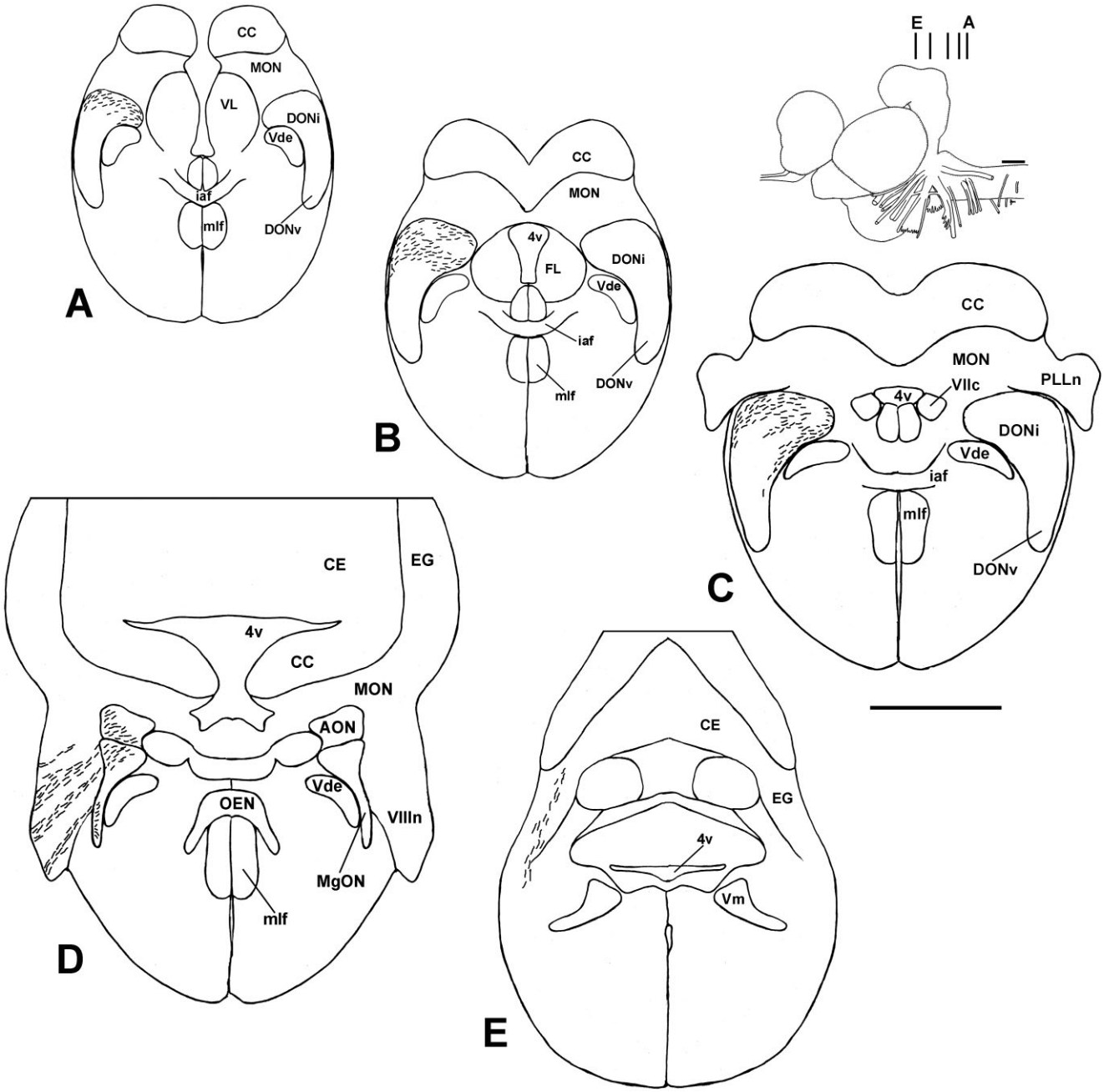

Figure 11.
Line drawings of representative serial transverse sections from caudal (A) to rostral (E) levels show the projections of the saccular nerve in the Hawaiian sergeant fish *Abudefduf abdominalis*. The saccular nerve projects to the posterior, descending (DON), magnocellular (MgON), and anterior (AON) octaval nuclei, and the eminentia granularis (EG). Brain inset shows the approximate location of each section. The top portion of the cerebellum is truncated or not shown in some cross sections. See list for abbreviations. Scale bar = 1 mm in C (applies to A–E).

the Hawaiian sergeant fish, but it is possible that this complex pattern also exists. Comparative studies that use multiple simultaneous labeling, confocal microscopy, and 3D reconstruction are needed to examine the function and evolution of the DON in fishes.

The DON is also the major ascending pathway to the auditory midbrain in many fishes, and labeling studies show ascending projections from the DONi and DONdm to the nucleus centralis of the torus semicircularis (primary midbrain auditory region) (O'Marra and McCormick, 1999; McCormick, 1999; Bass et al., 2000, 2001; Yamamoto and Ito, 2005). The ventral dendrites of the DONdm somata extend into the DONi, likely receive afferent input from all three otolithic endorgans, and may perform complex transformations prior to sending information to the midbrain (McCormick, 1999; Tomchik and Lu, 2005). For example, resting discharge rate, phase-locking

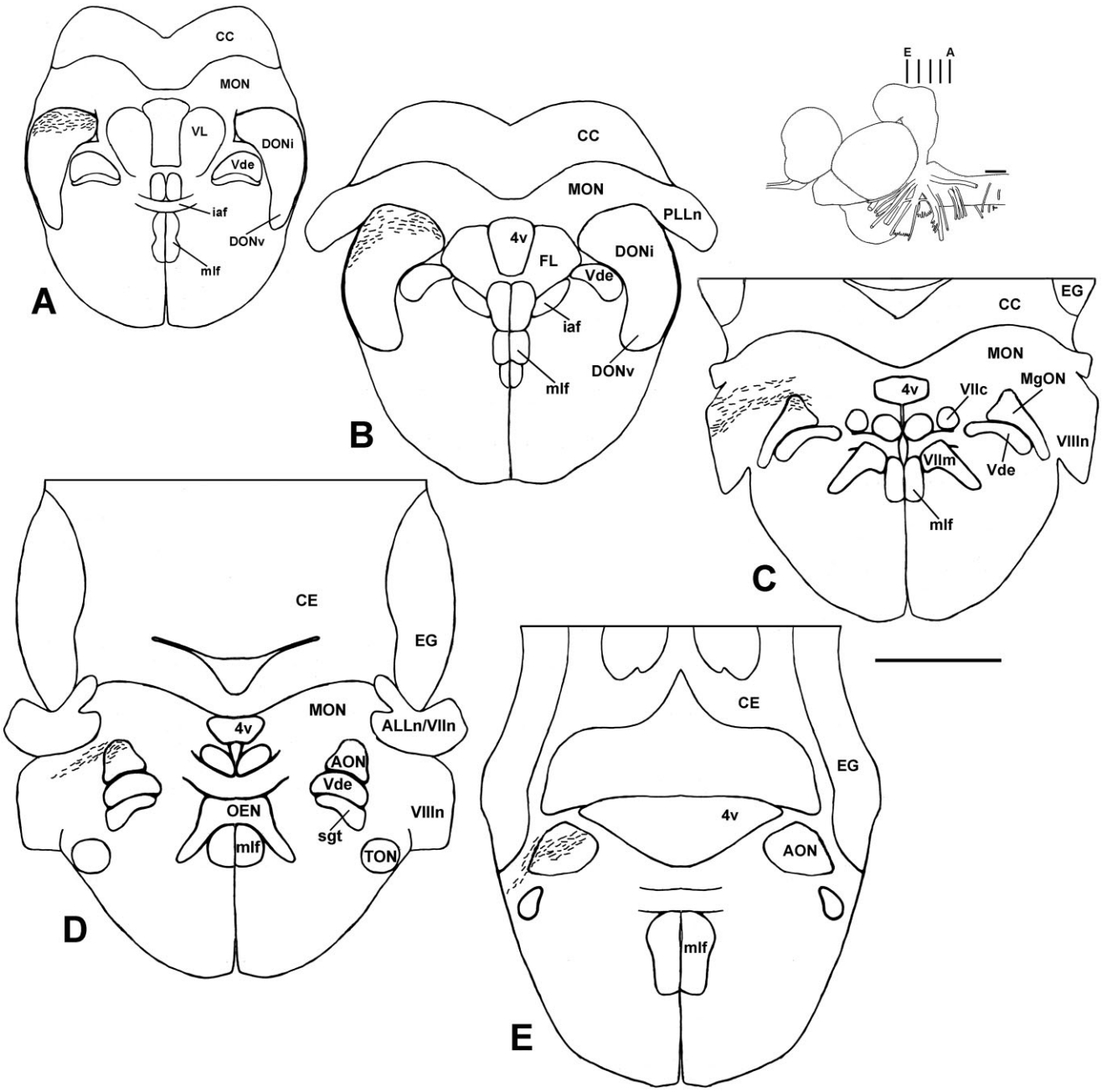

Figure 12.
Line drawings of representative serial transverse sections from caudal (A) to rostral (E) levels show the projections of the lagenar nerve in the Hawaiian sergeant fish *Abudefduf abdominalis*. The lagenar nerve projects to the posterior, descending (DON), magnocellular (MgON), and anterior (AON) octaval nuclei, and the eminentia granularis (EG). Brain inset shows the approximate location of each section. The top portion of the cerebellum is truncated or not shown in some cross sections. See list for abbreviations. Scale bar = 1 mm in C (applies to A–E).

ability, temporal coding, and directional and frequency tuning often differ between the auditory periphery or hindbrain and the midbrain in several fishes (Lu and Fay, 1993; Crawford, 1993; Bodnar and Bass, 1997; Edds-Walton and Fay, 2005; Kozloski and Crawford, 2000; Bass et al., 2001). Although the hindbrain-toral connections are not known in the Hawaiian sergeant fish, single-cell recordings in both the auditory torus semicircularis and DON indicate that similar transformations may occur in this species (Maruska, 2007).

Anterior octaval nucleus

The AON in the Hawaiian sergeant fish receives projections from all octaval nerves. Within the AON, lagenar afferents terminated dorsal and medial, whereas saccular and utricular

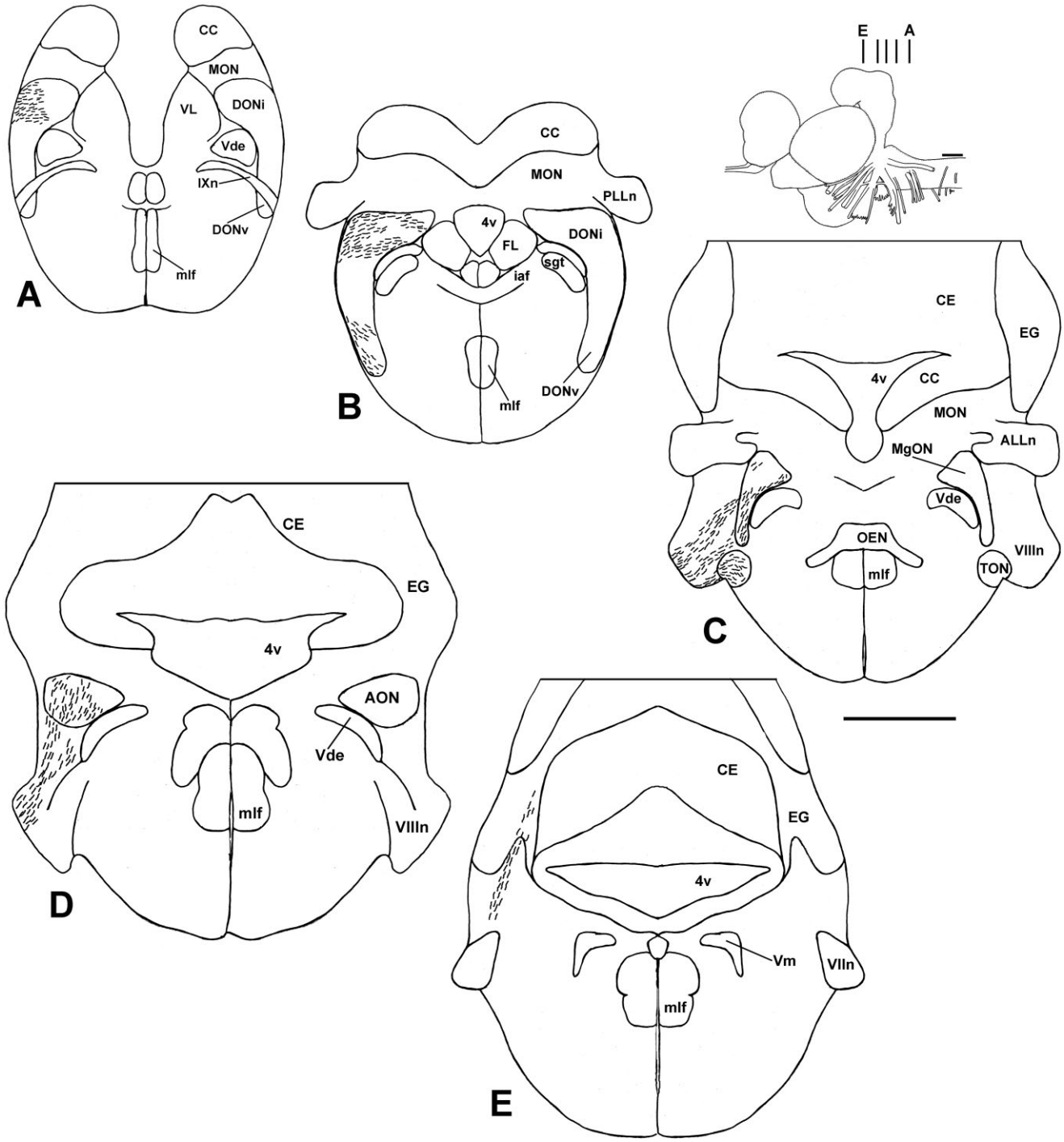

Figure 13.
Line drawings of representative serial transverse sections from caudal (A) to rostral (E) levels show the projections of the utricular nerve in the Hawaiian sergeant fish *Abudefduf abdominalis*. The utricular nerve projects to the posterior, descending (DON), magnocellular (MgON), tangential (TON), and anterior (AON) octaval nuclei, and the eminentia granularis (EG). Brain inset shows the approximate location of each section. The top portion of the cerebellum is truncated or not shown in some cross sections. See list for abbreviations. Scale bar = 1 mm in C (applies to A–E).

afferents terminated more lateral and ventral. The AON receives afferent projections from octaval nerves in other fishes (Meredith and Butler, 1983; Highstein et al., 1992; McCormick and Braford, 1994; McCormick, 1999; Tomchik and Lu, 2005), projects to the nucleus centralis of the torus semicircularis in some species with accessory hearing structures, and thus

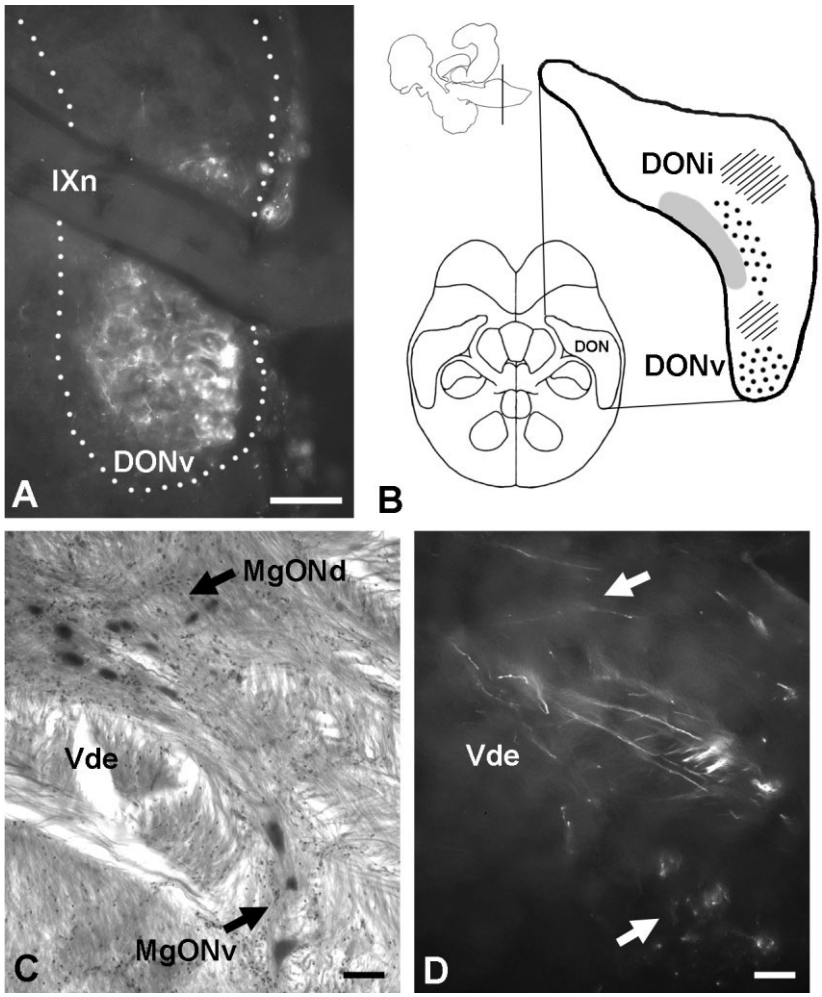

Figure 14.
Primary projections of semicircular canal nerves in the Hawaiian sergeant fish *Abudefduf abdominalis*. **A:** Projection of the horizontal canal nerve to the ventral descending octaval nucleus (DONv) beneath IXn. **B:** Schematic section through the caudal DON shows the distinct primary projection regions of the horizontal (dots), posterior (shaded gray), and anterior (hatched) canal nerves to the intermediate (DONi) and ventral (DONv) descending octaval nuclei. Brain inset at upper left shows the approximate location of the cross section. **C,D:** Projection of the anterior semicircular canal nerve to the dorsal (MgONd) and ventral (MgONv) magnocellular octaval nuclei. D shows the DiI fill and C shows the identical section stained with cresyl violet. Medial is to the left. Arrows in D indicate the position of MgONd and MgONv as in C. Vde, descending tract of the trigeminal nerve. Scale bar = 50 μm in A; 30 μm in C,D.

likely functions in auditory processing (Bell, 1981; Echteler, 1984; Finger and Tong, 1984; Braford et al., 1993; Yamamoto and Ito, 2005). However, in some hearing specialists and non-specialized fishes such as batrachoidids, the AON sends few to no projections to the auditory torus (McCormick and Hernandez, 1996; O'Marra and McCormick, 1999; Edds-Walton, 1998b; Bass et al., 2000, 2001; McCormick, 2001). In the perciform sleeper goby, the lateral AON receives dominant input from the saccule and utricle and may serve an auditory function, whereas the ventral AON is thought to process primarily vestibular information from all three otolithic endorgans (Tomchik and Lu, 2005). However, the absence of projections to the AON from semicircular canal nerves in the present and previous studies (e.g., Tomchik and Lu, 2005) indicate that it may be more involved in auditory processes including sound localization. We were unable to divide the AON in the Hawaiian sergeant fish into clear subdivisions, but we did observe similar lateral AON projections from the saccule and utricle, and medial-ventral projections from all three endorgans. Therefore, different subdivisions of the AON may serve either auditory, vestibular, or gravistatic relays in fishes, and the existence of the AON-toral pathway may vary among species.

Magnocellular octaval nucleus

The MgON contains large somata and receives projections from all octaval and semicircular canal nerves and some sparse innervation from the ALLn in the Hawaiian sergeant fish. The dorsal MgON also receives intermingled projections from all octaval and semicircular canal endorgans in the sleeper goby and toadfish (Highstein et al., 1992; Tomchik and Lu, 2005) and may function as an initial integration and complex processing center. The MgON does not appear to pro-

vide ascending information to the midbrain (Highstein et al., 1992), but it does project to the spinal cord via the vestibulospinal tract in several fishes (Highstein et al., 1992; McCormick, 1999) and may function in sensorimotor integration. The large dorsal MgON somata in the Hawaiian sergeant fish are also known to receive gonadotropin-releasing hormone (GnRH) varicose axons (Maruska, 2007), which may serve to modulate sensory and sensorimotor processing that is important for social behaviors. However, the function and interconnections of the MgON in fishes requires further study.

Tangential octaval nucleus

The TON is found only in teleost fishes and is suggested to serve a vestibular function (McCormick, 1999). The TON in the Hawaiian sergeant fish and most other teleosts receives input primarily from the utricle and semicircular canals (Meredith and Butler, 1983; McCormick, 1999; Tomchik and Lu, 2005). The known outputs from the TON to the spinal cord and oculomotor nucleus in some fishes also indicate a vestibular function (Bell, 1981; Torres et al., 1992). In the sleeper goby, the TON receives dense projections from endorgans that are oriented in the same plane (e.g., utricle and horizontal canal) and may be part of a vestibular processing unit along with the DONv and ventral MgON (Tomchik and Lu, 2005). Our observation in the damselfish of heavy TON input from the utricle and horizontal canal supports this idea, but further physiological and behavioral studies are needed to determine the function of the TON in teleosts.

Medial octavolateralis nucleus

The MON in the Hawaiian sergeant fish receives afferent projections from both the ALLn and PLLn, but lacks innervation from any octaval endorgans. The MON receives varying degrees of octaval input in some fish species (McCormick and Braford, 1993, 1994; McCormick, 1999), but octaval projections are absent in others including the Hawaiian sergeant fish (McCormick, 1999; Highstein et al., 1992; Tomchik and Lu, 2005; this study). Some fishes also show lateral line input to the MgON and DON (McCormick, 1999). The Hawaiian sergeant fish also showed some ALLn projections to the dorsal MgON, but other species such as the sleeper goby (Tomchik and Lu, 2005), rainbow trout (Schellart et al., 1992), and Florida gar (Song and Northcutt, 1991) all lack lateral line input to the MgON. These differences among diverse fish species preclude conclusions from the limited data, and further comparative studies are needed to resolve these innervation differences. In some species, these regions may be involved in central integration of auditory, vestibular, and lateral line information that is required for multisensory guidance of behaviors with strong directional components such as predator-prey interactions and courtship (Braun et al., 2002).

Similar to other species examined (McCormick, 1989; Tomchik and Lu, 2005), PLLn afferents terminate primarily dorsally and ALLn afferents ventrally within the Hawaiian sergeant fish MON, although there may be considerable overlap. The Hawaiian sergeant fish contains both superficial and canal neuromasts, and thus lateral line nerves contain velocity and acceleration information (Kroese and Schellart, 1992). In most fishes, head neuromasts are represented medially or ventromedially and trunk neuromasts laterally or dorsolaterally within the MON (McCormick, 1989). This projection pattern is consistent with the general termination regions for the anterior (head neuromasts) and posterior (trunk neuromasts) lateral line nerves in the Hawaiian sergeant fish, but further studies are needed to refine somatotopic projections in this species.

Octavolateralis efferent nucleus

Octavolateralis efferent neurons are important modulators of peripheral hair cell mechanoreceptor systems (Russell and Roberts, 1972; Flock and Russell, 1976) and are activated during arousal by several systems including input from octavolateralis and vocal pathways (Klinke and Schmidt, 1970; Roberts and Russell, 1972; Highstein and Baker, 1985; Tricas and Highstein, 1991; Weeg et al., 2005). The OEN in the Hawaiian sergeant fish is a single, centrally located nucleus similar to that described in the sleeper goby (Tomchik and Lu, 2005). Like the sleeper goby, the Hawaiian sergeant fish also does not show distinct rostrocaudal divisions of the OEN found in some other fishes (goldfish: Zottoli and van Horne, 1983; toadfish: Highstein and Baker, 1986; Roberts and Meredith, 1989; midshipman: Bass et al., 1994). Although labeled OEN neurons were identified in only a few experiments with the Hawaiian sergeant fish (e.g., utricular fills), studies in other species indicate that the OEN supplies efferent innervation to all lateral line and octaval organs (Roberts and Meredith, 1989; Schellart et al., 1992; Tomchik and Lu, 2005), and possibly some individual OEN neurons that project to multiple endorgans (Tomchik and Lu, 2005). The sparse OEN label in the present study is likely due to the labeling techniques rather than lack of innervation, but further studies that target these neurons in the Hawaiian sergeant fish are needed to determine the relative efferent innervation to different endorgans.

Eminentia granularis

Primary afferent neurons from all octaval and lateral line nerves in the Hawaiian sergeant fish also project to the EG. The EG (a subdivision of the vestibulolateral lobe) is a cerebellar structure that contains small, densely packed granule cells whose axons form the cerebellar crest and are known to influence octavolateralis processing (Montgomery et al., 1995). Acoustic responses were recorded from the EG in the carp (Echteler, 1985), and previous projection studies suggest roles in both vestibular and lateral line processing (Bell, 1981; Meredith and Butler, 1983; McCormick, 1989). One major difference between our damselfish and the sleeper goby is the lack of abundant lateral line projections to the EG in the latter species (Tomchik and Lu, 2005). The mechanosensory lateral line system of the Hawaiian sergeant fish contains both superficial neuromasts and a complex canal system on the head and trunk. In contrast, the sleeper goby has an overall reduced lateral line mechanosensory system including the absence of a trunk canal (Tomchik and Lu, 2005). Thus the differences in mechanosensory projections to the EG may be related to species-specific lateral line organization and function, but this requires further comparative study. Nevertheless, the projections from all octaval and lateral line endorgans in the Hawaiian sergeant fish indicate that the EG may be involved in auditory, lateral line, and vestibular processing, which may involve sensory reafference used in reduction of self-generated noise (Montgomery and Bodznick, 1994).

In addition to direct projections to the EG, neurons within the DONdm have long dorsal dendrites that extend into the cerebellar crest in the Hawaiian sergeant fish, as well as other

fishes (Bell, 1981; Finger and Tong, 1984; McCormick, 1992; McCormick and Braford, 1993, 1994). However, primary octaval projections in the Hawaiian sergeant fish, and other species that do not possess accessory hearing structures such as the sleeper goby and oscar, appear to terminate in the DONi prior to reaching the DONdm (Meredith and Butler, 1983; Tomchik and Lu, 2005; this study). DONdm somata in the sleeper goby and other fishes have ventrolateral dendrites that extend into the DONi and likely synapse with afferents from the otolithic endorgans (Bass et al., 2000, 2001; O'Marra and McCormick, 1999; Tomchik and Lu, 2005). In fact, DONdm neurons were transneuronally labeled following nerve VIII labels in the midshipman (Bass et al., 2000). Thus in species that lack direct primary octaval input to the DONdm somata, information from multiple endorgans can still reach the neurons via the ventral dendrites, whereas the dorsal dendrites receive input from the granule cells in the cerebellar crest. Additional comparative anatomical and physiological studies are needed to determine the role of the EG in acoustic processing.

Functional and evolutionary considerations

The AON, DON, and PON in the Hawaiian sergeant fish receive projections from the three otolithic endorgans; thus it is likely that all are involved in auditory processing, as demonstrated for the sleeper goby (Lu et al., 1998, 2003, 2004; Tomchik and Lu, 2005). The DONi is a large nucleus that appears to contain regions of both segregated *and* overlapping (e.g., dorsal DONi) octaval and semicircular canal inputs. The DONi may provide both auditory and vestibular information to higher processing centers, and future studies are needed to test whether there are functional subregions within this nucleus. In addition, a distinct division of the DON (rostral intermediate division of DON [DOri]) that contains neurons transneuronally labeled by both nerve VIII and occipital nerve roots that innervate sonic swim bladder muscles was identified in batrachoidids, which also suggests there may be subsets of DON neurons that integrate auditory and vocal information in some species (Bass et al., 1994, 2000, 2001). The PON does not appear to project to the spinal cord or the midbrain in fishes, so its function in auditory processing remains unknown (McCormick, 1999). The MgON receives relatively dense projections from most octavolateralis nerves, and MgON somata show close association with GnRH-immunoreactive varicose axons in the Hawaiian sergeant fish (Maruska, 2007). This evidence, together with the known connections between the MgON and the spinal cord in other fishes (Highstein et al., 1992; McCormick, 1999), indicates a role in sensorimotor processing or multimodal integration. Similar to the sleeper goby (Tomchik and Lu, 2005), the ventral MgON, DONv, and TON of the Hawaiian sergeant fish receive prominent projections from the semicircular canals and utricle and thus may serve a strong vestibular or gravistatic function. The CON and MON receive exclusive projections from the ALLn and PLLn and therefore serve a mechanosensory function in the Hawaiian sergeant fish.

The octavolateralis projection patterns in the Hawaiian sergeant fish are similar to that described for the perciform sleeper goby and cichlids (Meredith and Butler, 1983; McCormick, 1983; Tomchik and Lu, 2005), which all differ from those of species with accessory hearing structures such as goldfish and mormyrids (Bell, 1981; McCormick and Braford, 1994; McCormick, 1999). The common themes of octavolateralis projections in the perciform fishes is the ipsilateral input, minimal overlap between octaval and lateral line projections in the hindbrain (similar to non-teleosts), and absence of primary projections to the DONdm somata (Meredith and Butler, 1983; McCormick, 1983; McCormick, 1999; Tomchik and Lu, 2005). However, until more species are examined and the polyphyletic perciform phylogeny is further resolved, it is difficult to conclude whether these characters are related to phylogeny or the structure of the auditory periphery. Additional studies that examine the primary projections of octavolateralis nerves and medullary cytoarchitecture in perciform species that can detect the pressure component of sound stimuli (fishes with swim bladder extensions such as some chaetodontids; Webb et al., 2006) should help to clarify this issue. The similarities in primary projections between the soniferous damselfish and the previously examined non-vocalizing perciform fishes (e.g., sleeper goby; oscar) suggest an absence of anatomical specializations evolved for intraspecific acoustic communication at the first-order processing level in perciform fishes. However, there are some members of cichlid and goby families that do use sounds for acoustic communication (Amorim, 2006). Therefore, differences in central projections may not exist among different species within a single family that contains both soniferous and non-soniferous representatives. In a parallel example, patterns of otolithic endorgan inputs in a soniferous otophysan catfish *Arius felis* are similar to those of other non-soniferous otophysans (McCormick, 2001). However, given that the perciform fishes are not monophyletic (Elmerot et al., 2002), there are large-scale phylogenetic effects that make the causal factors difficult to interpret. It is also possible that there are anatomical specializations at higher levels, or differences in physiological processing mechanisms among soniferous and non-soniferous species. Future studies on additional species with close phylogenetic relationships are needed to test such hypotheses on the selective pressures, constraints, and evolution of central auditory circuitry in perciform fishes.

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