Spinal nerve innervation to the sonic muscle and sonic motor nucleus in red piranha, *Pygocentrus nattereri* (Characiformes, Ostariophysi).

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ABSTRACT

The red piranha, *Pygocentrus nattereri*, produces sounds by rapid contractions of a pair of extrinsic sonic muscles. The detailed innervation pattern of the sonic muscle of the red piranha was investigated. The sonic muscle is innervated by branches (sonic branches) of the third (S3so), fourth (S4so), and fifth (S5so) spinal nerves. The average total number of nerve fibers contained in the right sonic branches (n = 5; standard length, SL, 71–85 mm) was 151.8 (standard deviation, SD, 28.3). The occipital nerve did not innervate the sonic muscle. The sonic motor nucleus (SMN) in the piranha was identified by tracer methods using wheat germ agglutinin-conjugated horseradish peroxidase; labeled sonic motor neurons were only observed on the side ipsilateral to the sonic muscle injected with the tracer. In the transverse sections, the labeled sonic motor neurons were located in the dorsal zone (mainly large and medium neurons) and in the ventral zone (mainly small neurons) of the ventral horn. In the horizontal sections, the labeled neurons formed a rostrocaudally elongated SMN from the level of the caudal part of the second spinal nerve root to the intermediate region between the fifth and sixth spinal nerve roots. The average number of the labeled neurons (n = 5; SL, 64–87 mm) was 152.6 (SD, 7.3). We conclude that the sonic muscles of the piranha are innervated by approximately 300 sonic motor neurons located only in the spinal cord.

INTRODUCTION

The red piranha, *Pygocentrus nattereri* (Serrasalminae, Characidae, Characiformes, Ostariophysi), is considered a vocalizing fish [Meschkat, 1957]. The vocal sounds are generated by rapid contractions of a pair of extrinsic drumming (sonic) muscles, which are located dorsolateral to the anterior part of the swimbladder [Markl, 1971]. Concerning the innervation of the muscle, Markl [1971] described that the two anterior spinal nerves send fibers to the swimbladder-associated sonic muscles. However, the swimbladder-associated sonic muscles in most of the diverse sonic fishes (e.g., batracoidids, triglids, and even ostariophysian catfish) are innervated by one or two pairs of the occipital nerves [Ladich and Bass, 1998]. The reports available in the literature [Tavolga, 1971; Fine et al., 1977; Ladich, 1991; Ladich and Bass, 1998; Yoshimoto et al., 1999; Carlson and Bass, 2000; Bass and McKibben, 2003; Takayama et al., 2003] indicate that no investigation of the detailed innervation pattern of the sonic muscles of the piranha has been conducted. Also, studies on the distribution of the sonic motor neurons that innervate the sonic muscles via the spinal nerves are lacking. Therefore, the aims of this study were to elucidate the detailed pattern of innervation to the sonic muscle and to determine the distribution of sonic motor neurons that constitute the sonic motor nucleus (SMN) in the red piranha, in order to provide insight into the significance of occipital nerve innervation in other vocalizing fishes.

MATERIALS AND METHODS

Live red piranha (*Pygocentrus nattereri*) were obtained from a local trader (Nagoya, Japan) and kept in the laboratory. A total of 32 fish (standard length, SL, 64–133 mm) were used in this study. The original research reported herein was performed under the official Japanese regulations for research on animals.

A total of 20 individuals were used in studying the innervation pattern of the sonic muscle. Fish were euthanized with an overdose of tricaine methanesulfonate (MS222) and were then fixed in 10% formalin or 4% paraformaldehyde in 0.1 *M* phosphate buffer (PB, pH 7.4) for anatomical and histological observations. After the location and attachment sites of the sonic muscles were confirmed, the innervation pattern of the sonic muscles was examined under a dissection microscope. To trace the nerves, 1% osmic acid was used as a stain. The observations of innervation pattern of the sonic muscles were conducted from the lateral and medial sides of the sonic muscle. We followed the nomenclature for the nerves as described by Parenti and Song [1996], although we used the term "occipital nerve", rather than "spino-occipital nerve," to avoid confusion between occipital nerve elements and spinal nerve elements. The occipital nerve was defined as the nerve emerging from the cranial cavity through a pair of foramina on the occipital cranium. The first spinal nerve was defined as emerging from the first free vertebra, the second spinal nerve emerged from the second free vertebra, and so on. In the piranha, a nerve emerging between the occipital ring and the first free vertebra was observed. This nerve is characteristic of the Ostariophysi and was termed the “occipito-spinal nerve” by Fürbriger [1897]. However, this nerve was considered to be a spinal nerve element because its root was near the root of the first spinal nerve rather than near that of the occipital nerve. Consequently, it was termed “spinal nerve zero” in the present study, to avoid confusing it with occipital nerve elements. Moreover, the term “sonic branch” was used to designate the nerve branches innervating the sonic muscle. To precisely confirm the foramina through which these nerves pass, cranial and vertebral structures were carefully observed in cleared preparations stained with alizarin red S according to the methods of Taylor [1967].

From the fish used in studying the innervation pattern of the sonic muscle, five specimens were also used in analysis of fibers in the sonic branches. The nerves involving sonic branches were removed from one specimen (SL, 85 mm; female) fixed in 4% paraformaldehyde in 0.1 *M* PB (pH 7.4), post-fixed in 1% osmic acid, and embedded in Epon. Transverse sections (1 μm) were cut and stained with toluidine blue for light microscopy. Considering the deformation that may arise as a result of tissue shrinkage, the fiber diameters were calculated from the circumference divided by π. The circumferences of the nerve fibers were traced on tracing paper, digitized using an image scanner, and measured with NIHImage (Scion Co.). The computed values were sorted into a frequency distribution. Moreover, the nerves involving sonic branches were removed from four specimens (SL, 71–81 mm) fixed in 10% formalin, and transverse sections (1 μm) were made with the same procedure. From these sections, the numbers of fibers of the sonic branches were counted.

The remaining 12 piranha specimens were used to investigate the distribution of the motor neurons innervating the sonic muscle (sonic motor neurons). The fish were anesthetized by immersing them in a solution of 100 mg/l MS222. They were positioned in a device for physical restraint and hydraulic ventilation of fishes under surgery, in which the gills were artificially aerated by circulating water containing 50 mg/l of MS222. To expose the sonic muscle, a wedge-shaped incision was made lateral to the sonic muscle, and the skin was elevated and retracted to the cleithrum. After removing the adipose tissue, 0.5–1 μl of 5% wheat germ agglutinin-conjugated horseradish peroxidase (Toyobo, WGA-HRP) in saline was microinjected into the anterior and posterior parts of the left sonic muscle with a microliter syringe (Hamilton, 7001KH). After the injection, a small volume of Chloromycetin antibiotic ointment (Sankyo Co.) was spread on the sonic muscle, and the skin was replaced and coated with an acrylic adhesive. Then, the fish were revived by flowing water of the fish tank into their gill cavity, and were returned the tank.

After 3 to 4 days, the fish were anesthetized again with MS222 (300 mg/l) and perfused transcardially with saline, followed by a solution of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 *M* PB (pH 7.4). Finally, the fish were perfused with 10% sucrose in 0.1 *M* PB (pH 7.4) at 4°C. The brains and spinal cords were then dissected and immersed in 30% sucrose in 0.1 *M* PB (pH 7.4) and kept at 4°C overnight. The tissues were embedded in Tissue-Tek II O.C.T. compound (Sakura Finetechnical Co.), and 50-μm transverse or horizontal sections were cut on a cryostat. The sections were serially mounted on slides coated with chromium-gelatin solution and dried for 30 min with a fan at room temperature. After immersion in 0.1 *M* PB (pH 7.4) at 4°C overnight, the sections were incubated with tetramethylbenzidine (TMB) medium as described by Mesulam [1982]. The sections were then counterstained with neutral red.

The WGA-HRP-labeled neurons were counted from photomicrographs of the serially sections using NIHImage (Scion corp.). In the counting, two labeled neurons that located the same positions in adjacent sections were counted as one neuron. The soma areas of the labeled cells were measured with the same procedure used for the measurements of the nerve fiber diameters, using NIHImage (Scion corp.). In the two labeled neurons of adjacent sections considered as one neuron in the counting, a labeled cell that had lager soma area was only measured. The measured soma areas were sorted into a frequency distribution.

RESULTS

Sonic Muscle and Bony Structures

The red piranha had a pair of sonic muscles that were located dorsolaterally adjacent to the anterior part of the swimbladder (Fig. 1A). The muscles were extrinsic sonic muscles that originated from the vertebrae and inserted on the ventrolateral region of the anterior swimbladder. Lateral trunk muscles were found to be absent lateral to the anterolateral parts of the sonic muscles (Fig. 1A, B). In this region, the sonic muscle was easily exposed by removing the overlaying skin and adipose tissue. The bony structures of the cranium and anterior vertebrae in the red piranha are shown in Fig. 1C. The occipital nerve and the spinal nerves were identified based on the sites where these nerves emerged from the bony structures (Fig. 1C).

Innervation Pattern of the Sonic Muscle

The sonic muscles of the piranha were found to be innervated by the third, fourth, and fifth spinal nerves (Fig. 2, 3, 4). Very few nerves entered the sonic muscle on its lateral (or outer) surface (Fig. 2). The sonic branches of the third and fourth spinal nerves entered between the sonic muscle and the swimbladder. The sixth spinal nerve lay on the lateral surface of the sonic muscle without providing innervation to the muscle (Fig. 2); thus, it did not appear to contribute to the innervation of the sonic muscle.

The sonic branches mainly entered the sonic muscle from the medial (or inner) side where the muscle is in contact with the swimbladder wall (Fig. 3). The sonic branches of the third and fourth spinal nerves (S3so and S4so) were found to be distributed in the anterior and middle portions of the sonic muscle (Fig. 3). The fifth spinal nerve coursed along the medial (or inner) surface of the sonic muscle, and branches providing innervation to the sonic muscles emerged along its course. The sonic branches of the fifth spinal nerve (S5so) were distributed in the middle and posterior portions of the sonic muscle (Fig. 3). The innervation pattern to the sonic muscle was found to be the same in all specimens used in this study.

The occipital nerve did not innervate the sonic muscle in our red piranha specimens (Fig. 4). The occipital nerve coursed ventrally and merged with the spinal nerve zero and the first spinal nerve (Fig. 4B), and this nerve complex was found to provide innervation primarily to the muscles of the pectoral fin and the hypobranchial region (Fig. 4A).

Analysis of Fibers in the Sonic Branches

Five specimens (SL, 71–85 mm) were used for counting the fibers in the sonic branches. Numbers of the nerve fibers in the sonic branches of the right sonic muscle are shown in Table 1. Representative transverse semi-thin sections (1 μm) of the isolated sonic branches of the third and fourth spinal nerves are shown in Fig. 5A–D. The fibers innervating the sonic muscles are myelinated [Fine and Mosca, 1989; Bass and Baker, 1990]. The myelinated axons were counted and measured as the nerve fibers of the sonic motor neurons. Representative transverse semi-thin sections of the fifth spinal nerve taken from sites before the nerve branches to the sonic muscle and after it traverses the sonic muscle are shown in Fig. 5E and F, respectively. It was difficult to make sections of each sonic branch of the fifth spinal nerve because the branches were too fine and fragile (Fig. 3); thus, the number of fibers in the sonic branches of the fifth spinal nerve were estimated from the difference in the number of fibers between the two nerve sections (Fig. 5E, F). Average total number of fibers in the right sonic branches was 151.8 (standard deviation, SD, 28.3; Table 1). Average fiber numbers of the S3so, S4so, and S5so branches were 36.2 ±3.7, 61.4 ±13.9, and 54.2 ±20.5 (mean ± SD), respectively (Table 1). Therefore, we estimate that roughly 300 nerve axons innervate the paired sonic muscles of the red piranha.

One specimen (SL, 85 mm; female) was also used for measurement of the fiber diameter in the sonic branches. Histograms of the fiber diameters show roughly bimodal distributions in each group of sonic branches (Fig. 6A–C). The diameters of the fibers ranged from 2 to 24 μm when all data were combined (Fig. 6D). Most of the large-diameter fibers were in the sonic branches of the fourth spinal nerve, and most of the small-diameter fibers were in the sonic branches of the fifth spinal nerve.

Distribution of the Sonic Motor Neurons

Application of WGA-HRP to the sonic muscle of the red piranha resulted in ipsilateral labeling of the sonic motor neurons, which were located in the ventral horn of the anterior spinal cord (Figs. 7 and 8). Data of successful cases were obtained from five specimens (SL, 64–87 mm) of 12 fish used for investigating the distribution of the sonic motor neurons. Numbers of the labeled sonic motor neurons by the WGA-HRP injection into the left sonic muscle are shown in Table 2. The average number of the labeled neurons (SL, 64–87 mm) was 152.6 ±7.3 (mean ± SD).

Examples of transverse sections of the spinal cord are shown in Fig. 7. In one piranha specimen (SL, 87 mm; immature), 145 sonic motor neurons were ipsilaterally labeled by the application of WGA-HRP. In transverse sections, the cell bodies of these neurons were ovoid and could be classified into three types based on the size of the soma (Fig. 9). Large (peak, 640 μm^2) and medium (peak, 440 μm^2) type neuronal somata were mainly seen in the dorsal zone of the ventral horn (Fig. 7D–F, closed arrows), while small soma types (peak, 120–160 μm^2) were located in the more ventral area of the ventral horn (Fig. 7D, F, open arrows). No sonic motor neurons were observed in the most medial and ventral area of the ventral horn. In some sections, labeled dendrites arose from the lateral side of the soma and extended laterally (Fig. 7G, H, closed arrowheads). An axon arising from the ventral side of the soma extended ventrally (Fig. 7G, open arrowhead) and entered the ventral root of the spinal nerve through the dorsolateral side of the ventral fasciculus.

An example of a horizontal section of the spinal cord is shown in Fig. 8A and B. In one piranha specimen (SL, 75 mm; female), 142 sonic motor neurons were ipsilaterally labeled by the application of WGA-HRP. In horizontal sections, the somata of these neurons were ovoid, and some had their major axis oriented rostrocaudally. In the section shown in Fig. 8A, axons entering the ventral root of the fifth spinal nerve were observed (arrow). These axons extended from the anterior region to the level of the fifth spinal nerve. In some sections, labeled dendrites arose from the rostral and caudal sides of the soma and extended rostrocaudally or laterally (Fig. 8B).

The distribution of the cell bodies of the sonic motor neurons as seen in a dorsal view is shown in Fig. 8C. The neurons formed a rostrocaudally elongated SMN with a rostrocaudal length of approximately 5000 μm that extended from the caudal level of the second spinal nerve root (about 500 μm anterior to the third spinal nerve root) to the intermediate region between the fifth and sixth spinal nerve roots (about 500 μm posterior to the fifth spinal nerve root).

DISCUSSION

Markl [1971] observed that two anterior spinal nerves send their fibers to the sonic muscles of the red piranha. Our study indicates that the sonic muscles of the red piranha are innervated by the third, fourth, and fifth spinal nerves. The anterior two spinal nerves referred to by Markl [1971] probably correspond to the third and fourth spinal nerves described in our study. The red piranha belongs to the Ostariophysi and possesses a Weberian apparatus that consists of specialized processes of the first three vertebrae [Fink and Fink, 1996]. The differences between Markl's descriptions and those of the present study are presumably attributable to the difficulty in observing and defining the anterior spinal nerves, which are partially obscured by the presence of the Weberian apparatus. Thus, the present study revealed for the first time that the fifth spinal nerve contributes to the innervation of the sonic muscles in the red piranha. The fifth spinal nerve lies on the medial (or inner) surface of the sonic muscle and distributes branches along its course to innervate the sonic muscle. Markl [1971] may have missed the innervation by the fifth spinal nerve simply because the sonic nerve branches are on the medial (or inner) side of the sonic muscle.

The John Dory (*Zeus faber*), one of the few species of sonic fishes whose sonic muscles are known to be innervated solely by spinal nerves, has a membranous window on the body surface at the centro-lateral part of the sonic muscle, which may enhance the transmission of sounds generated by the sonic muscle to the external environment [Onuki and Somiya, 2004]. In the red piranha, window-like areas (a portion lacking the lateral trunk muscle) were also observed at the antero-lateral region of the sonic muscles. As in the case of the dory, the window-like areas of the red piranha possibly facilitate the transmission of the vibrations produced by the sonic muscles to the surrounding water, serving as an acoustic window. Moreover, the similar features of the sonic muscles of the dory and the red piranha, i.e., the positions (bilateral, anterior to the swimbladder), innervation patterns (spinal nerves exclusively), and the presence of the window-like structure, suggest that the sonic muscles of these two species developed through similar processes, although they are not closely related species (Characiformes and Zeiformes). The sonic muscles of these fishes are probably derived from the *obliquus* *superioris* (surface layer of *hypaxialis*) and *obliquus inferioris* (deep layer of *hypaxialis*), as described in the terminology of muscles by Winterbottom [1974].

This is the first report describing the sonic motor nucleus (SMN) in a fish in which the sonic muscles are innervated solely by spinal nerves. The SMN of the red piranha is located at the dorsal and more ventral zones in the ventral horn of the spinal cord. No sonic motor neurons were observed in the most medial and ventral areas of the ventral horn. The SMN also extends from the caudal level of the second spinal nerve root to the intermediate region between the fifth and sixth spinal nerve roots. Based on these results, we propose two hypotheses concerning the characteristics of the SMN in fishes whose sonic muscles are innervated by spinal nerves only: 1) the SMN does not occupy the most medial and ventral zones of the ventral horn because these zones are occupied by the motor neurons for swimming and fin movement, and 2) the SMN is elongated rostrocaudally because multiple spinal nerves are involved in controlling the sonic muscle. To verify these hypotheses, it will be necessary to identify and describe the SMN of other sonic fishes whose sonic muscles are innervated solely by the spinal nerves, such as John Dory [Dufossé, 1874; Onuki and Somiya, 2004] and sciaenid fishes [Ono and Poss, 1982; Vance et al., 2002].

From average of nerve fiber numbers of sonic branches in five piranha specimens (Table 1), the right sonic muscle was innervated by approximately 150 myelinated fibers. Additionally, approximately 150 sonic motor neurons were labeled with WGA-HRP injected unilaterally (Table 2). This implies that the bilateral sonic muscles of the piranha are controlled by about 300 sonic motor neurons. Thus, the number of myelinated fibers is almost equal to the number of labeled sonic motor neurons. These results support the efficacy of estimating the number of sonic motor neurons by simply counting the number of myelinated fibers entering the sonic muscles.

In the sonic fishes whose sonic muscles are innervated by the occipital nerves, the position of the SMN is associated with the synchronous or asynchronous contraction patterns of the bilateral sonic muscles [Bass and Baker, 1991; Ladich and Bass, 1998]. The plainfin midshipman (*Porichthys notatus*) has a midline sonic motor nucleus (SMNm), and its bilateral sonic muscles synchronously contract by electrical coupling of the motor neurons that form an unpaired SMNm [Bass and Baker, 1991]. Kastberger [1981] reported that synchronous contractions of the bilateral sonic muscles in the piranha were evoked by electrical stimulation of the medulla and inferred that the synchronous contraction was caused by electrical coupling of the motor neurons. However, the red piranha possesses a pair of separated, or bilateral, SMN, and no labeled sonic motor neurons extended their dendrites and/or axons to the contralateral side. Therefore, premotor interneurons in the spinal cord may play an important role in the synchronous contraction of the sonic muscles of the piranha. In the rockfish (*Sebastiscus marmoratus*), two populations of premotor neurons (reticular formation and descending octaval nucleus) have been reported [Yoshimoto et al., 1999]. In future studies, it will be necessary to investigate the existence of such neuronal elements in the central vocal pathway of the red piranha.

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Table 1. Numbers of the nerve fibers in the sonic branches of the right sonic muscle.

Specimen	1	2	3	4	5	Average	SD
SL (mm)	71	74	75	81	85	77.5	5.5
Sex	Immature	Immature	Female	Male	Female	—	—
S3so	41	38	36	35	31	36.2	3.7
S4so	73	49	79	57	49	61.4	13.9
S5so	59	32	68	34	78	54.2	20.5
Total	173	119	183	126	158	151.8	28.3

SD, standard deviation; SL, standard length; S3so–S5so, sonic branches in the third, fourth, and fifth spinal nerves.

Table 2. Numbers of the labeled sonic motor neurons (WGA-HRP injection into the left sonic muscle).

Specimen	1	2	3	4	5	Average	SD
SL (mm)	64	75	82	83	87	78.2	7.0
Sex	Immature	Female	Female	Female	Immature	—	—
Sections	Transverse	Horizontal	Horizontal	Horizontal	Transverse	—	—
Labeled neurons	159	142	156	161	145	152.6	7.3

SD, standard deviation; SL, Standard length.

FIGURE LEGENDS

Fig. 1. Diagrammatic illustrations of the sound-producing system (sonic muscle and swimbladder) and related bony structures in the piranha. **A.** Drawing showing the position of the sonic muscle (lateral cutaway view). The gray area circled by the dashed line indicates the portion of the body that lacks lateral trunk muscles (window-like area). **B.** Transverse sectional view on the plane B indicated in A. Arrowheads indicate the positions of the window-like area. **C.** Lateral view of the neurocranium and vertebrae. Some parts of the Weberian apparatus were removed in the specimen for this illustration. Black dots indicate the positions where each nerve emerges from the bony structures. SM, sonic muscle; AS, anterior chamber of the swimbladder; PS, posterior chamber of the swimbladder; OC, occipital nerve; S0, spinal nerve zero; S1–S6, first to sixth spinal nerves; IV–VI, fourth to sixth vertebrae.

Fig. 2. Lateral view of the sonic muscle and the associated nerves (SL, 93 mm; right sonic muscle). Photograph (**A**) and diagrammatic drawing (B) showing the sonic branches of the spinal nerves (S3 and S4, arrowheads) entering between the sonic muscle and the swimbladder. Scale bar, 1 mm. SM, sonic muscle; OC, occipital nerve; S0, spinal nerve zero; S1–S6, first to sixth spinal nerves; R6, rib of sixth vertebra; R, rostral; C, caudal.

Fig. 3. Medial view of the sonic muscle and the associated nerves (SL, 101 mm; right sonic muscle). Photograph (**A**) and diagrammatic drawing (**B**) showing the innervation pattern of the spinal nerves to the sonic muscle. The dark gray nerves indicate distributions of the third and fourth spinal nerves. The light gray nerve indicates the distribution of the fifth spinal nerve. Scale bar, 1 mm. SM, sonic muscle; S2–S7, second to seventh spinal nerves; R5–R8, ribs of the fifth to eighth vertebrae; R, rostral; C, caudal.

Fig. 4. Schematic drawing of the distributions of occipital and spinal nerves. **A.** Lateral cutaway view showing distributions of the occipital nerve and the third, fourth, and fifth spinal nerves. The black nerve indicates the occipital nerve. The dark gray nerves indicate distributions of third, fourth, and fifth spinal nerves. The light gray nerves indicate sonic branches on the medial side of the sonic muscle. **B.** Lateral cutaway view of the spinal cord, spinal nerves, and sonic muscle. The light gray indicates sonic branches innervating the sonic muscle medially. SC, spinal cord; SM, sonic muscle; OC, occipital nerve; S0, spinal nerve zero; S1–S6, first to sixth spinal nerves.

Fig. 5. Transverse semi-thin sections (1 μm) of the spinal nerves having sonic branches (SL, 85 mm; female; right sonic muscle). **A, B.** The sonic branches of the third spinal nerve. **C, D.** The sonic branches of the fourth spinal nerve. **E, F.** Sections of the fifth spinal from before (E) and after (F) the emergence of the branches to the sonic

muscle. **G.** Schematic drawing of the medial view of the right sonic muscle and spinal nerves. Arrowheads with letters indicate the approximate positions of the transverse sections shown in panels A–F. Scale bar, 50 μm. SM, sonic muscle; S3–S5, third to fifth spinal nerves; R, rostral side.

Fig. 6. Frequency distributions of the fiber diameters in the sonic branches (SL, 85 mm; female; right sonic muscle). **A–C.** Histograms of the sonic branches in the third (A), fourth (B), and fifth (C) spinal nerves (S3so–S5so). **D.** Histogram of the three nerves combined.

Fig. 7. Photomicrographs of the labeled sonic motor neurons after WGA-HRP injection into the left sonic muscle (SL, 87 mm; immature). **A–C.** Representative transverse sections of the spinal cord between the third and fourth spinal nerves. The distance between the nerves was 1550 μm. The distances from the third spinal nerve exit to each section (in A–C) were 600, 850, and 1100 μm, respectively. **D–F.** Higher magnifications of sections A–C, respectively. Closed Arrows indicate example of the large and medium sized labeled sonic motor neurons. Open Arrows indicate example of the small sized labeled sonic motor neurons. **G, H.** Higher magnifications of D and F, respectively. Closed arrowheads indicate dendrites of sonic motor neurons. Open arrowhead indicates axon of

sonic motor neuron. Scale bar = 100 µm (A–F); 50 µm (G, H). CC, central canal; DH, dorsal horn; FD, dorsal fasciculus; FL, lateral fasciculus; FV, ventral fasciculus; VH, ventral horn.

Fig. 8. Photomicrographs of labeled sonic motor neurons and the distribution of sonic motor neurons in the horizontal plane (SL, 75 mm; female; WGA-HRP injection into the left sonic muscle). **A.** Representative horizontal section of the spinal cord anterior to the fifth spinal nerve. Arrow indicates labeled axons of sonic motor neurons. These axons entered the ventral root of the fifth spinal nerve. **B.** Higher magnification of A. Arrows indicate labeled sonic motor neurons. **C.** Charting of the cell bodies of the sonic motor neurons projected onto a dorsal view of the caudal brainstem and spinal cord. Generally, each dot indicates a cell body of a sonic motor neuron. Some dots indicate multiple cell bodies overlapping in the dorsal view. Gray area on the spinal cord indicates the sonic motor nucleus (SMN). Gray nerves indicate spinal nerves that contain axons of sonic motor neurons. Scale bar = 100 µm (**A**, **B**); 500 µm (**C**). OB, obex; OC, occipital nerve; S0–S6, zero to sixth spinal nerves.

Fig. 9. Frequency distribution of the soma areas of the labeled sonic motor neurons (SL, 87 mm; immature; injection into the left sonic muscle).

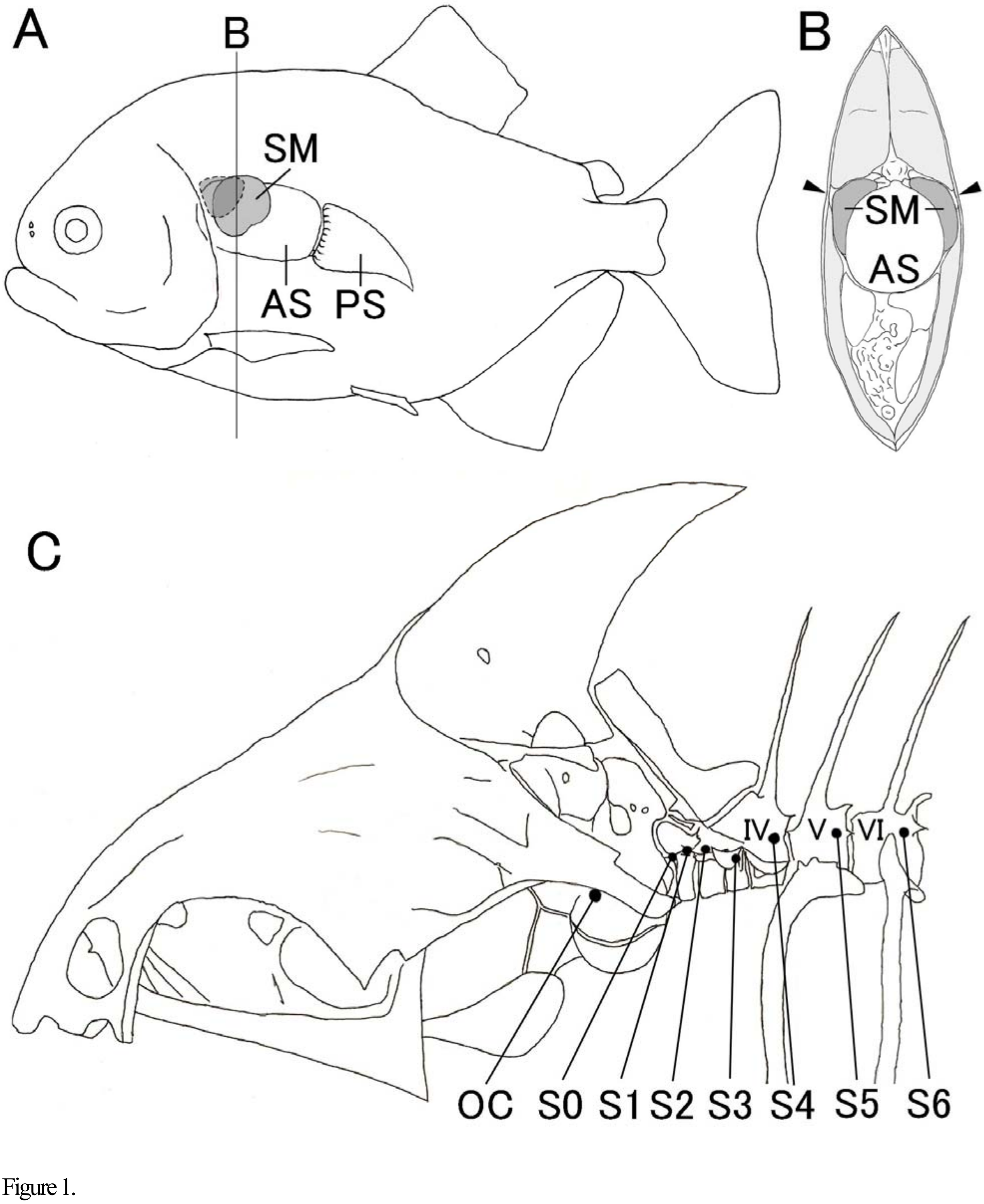


Figure 1.

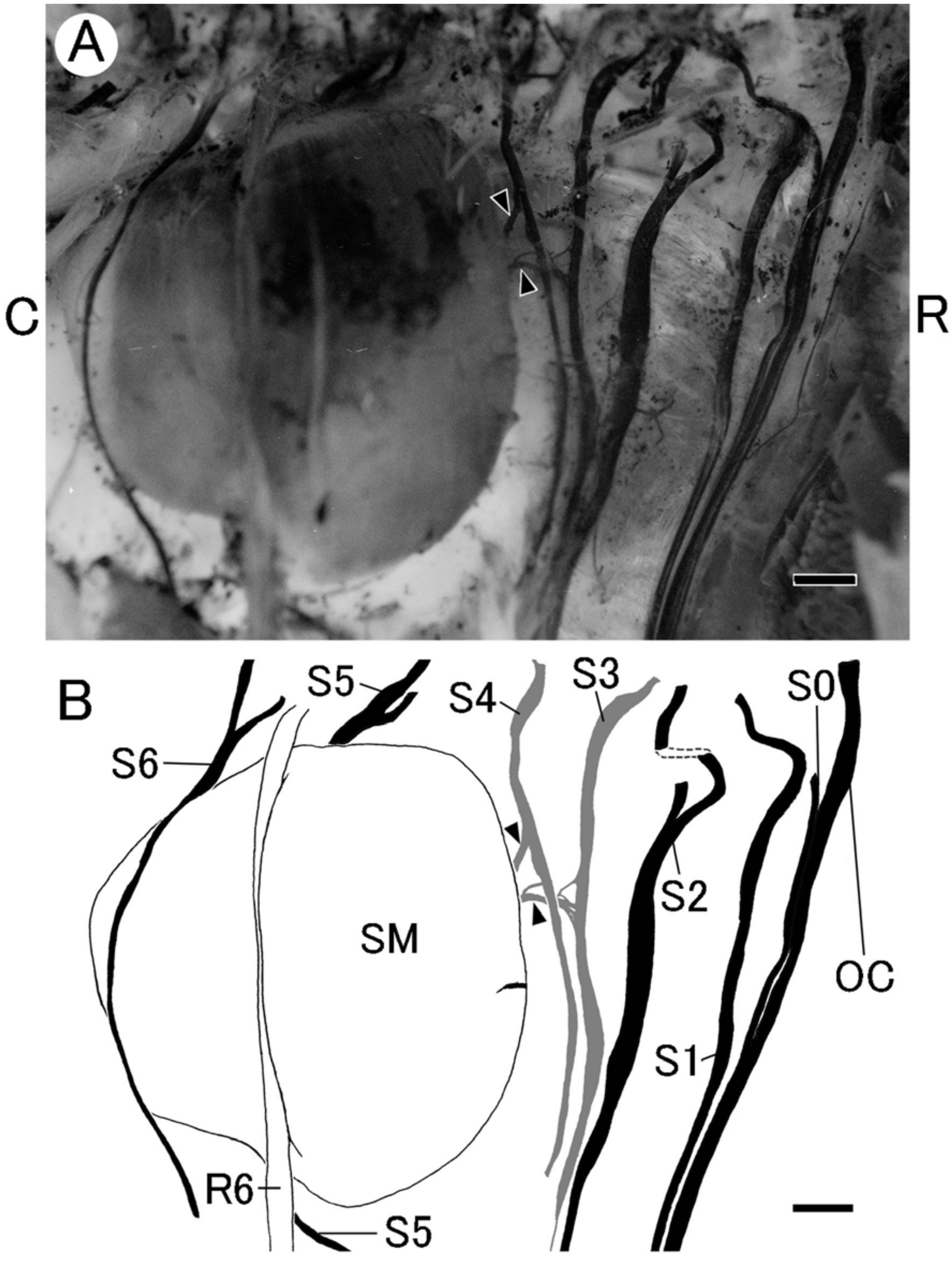


Figure 2.

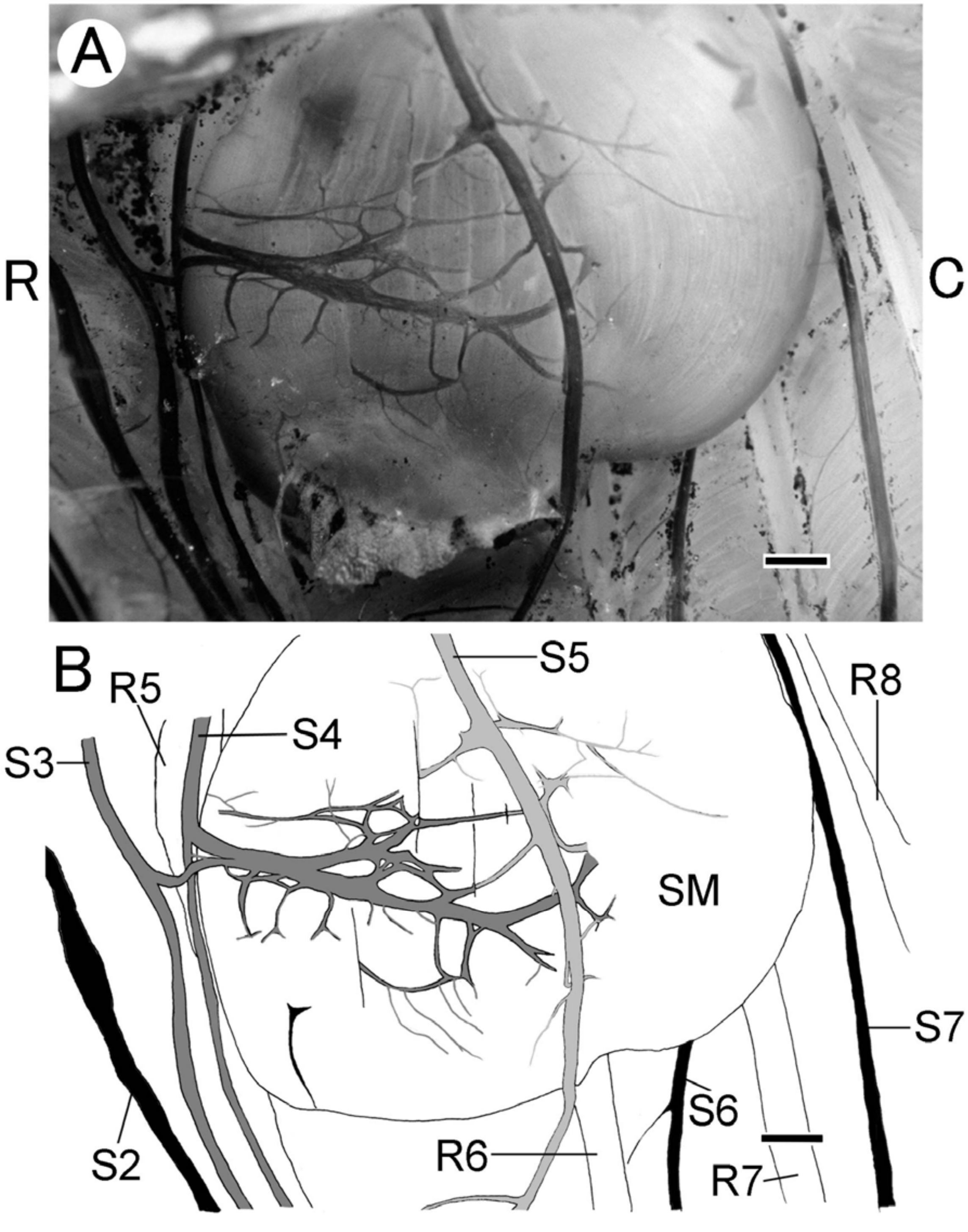
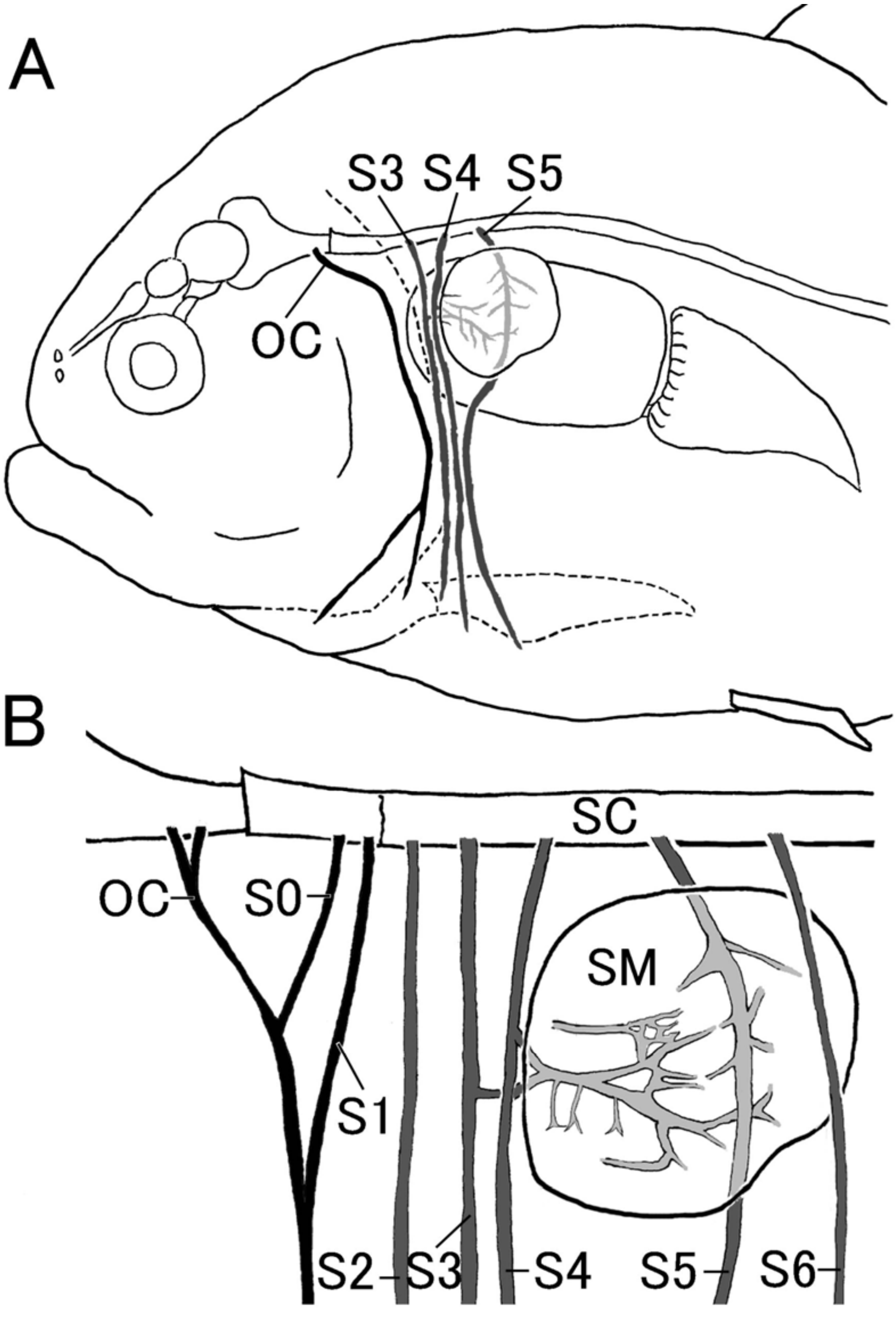


Figure 3.



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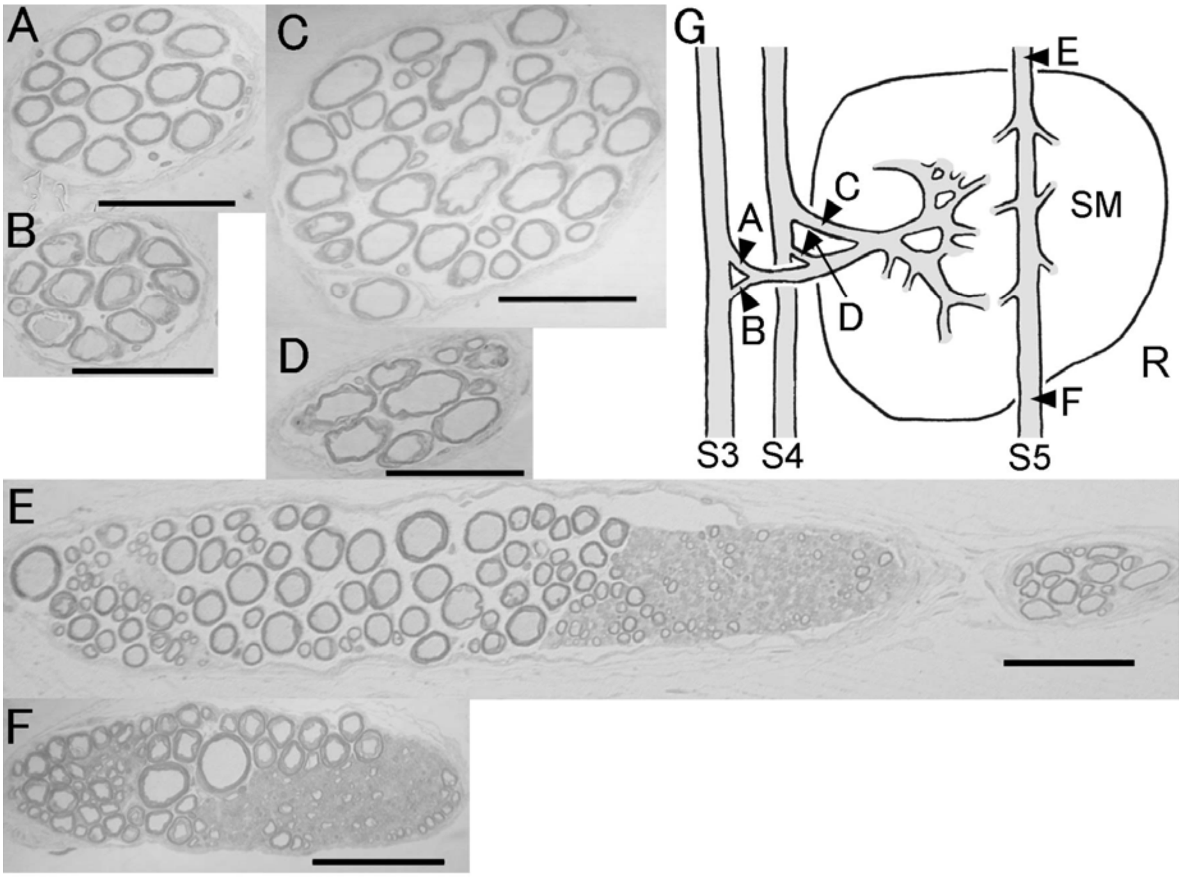

Figure 5.

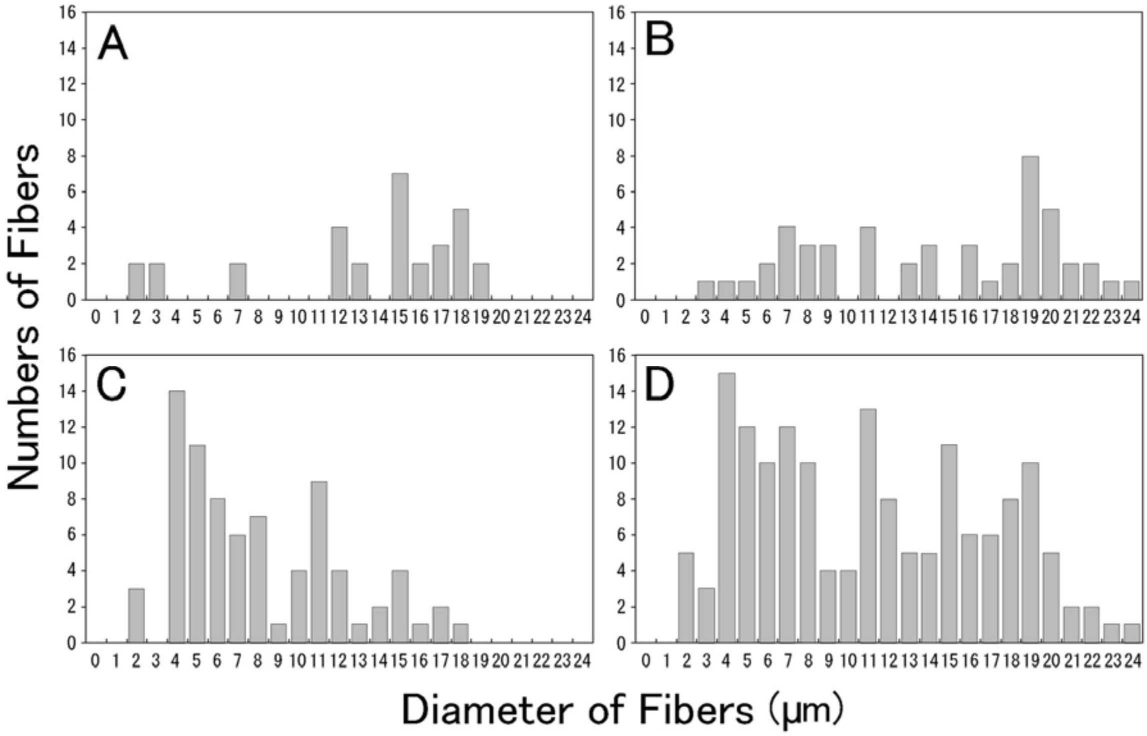

Figure 6.

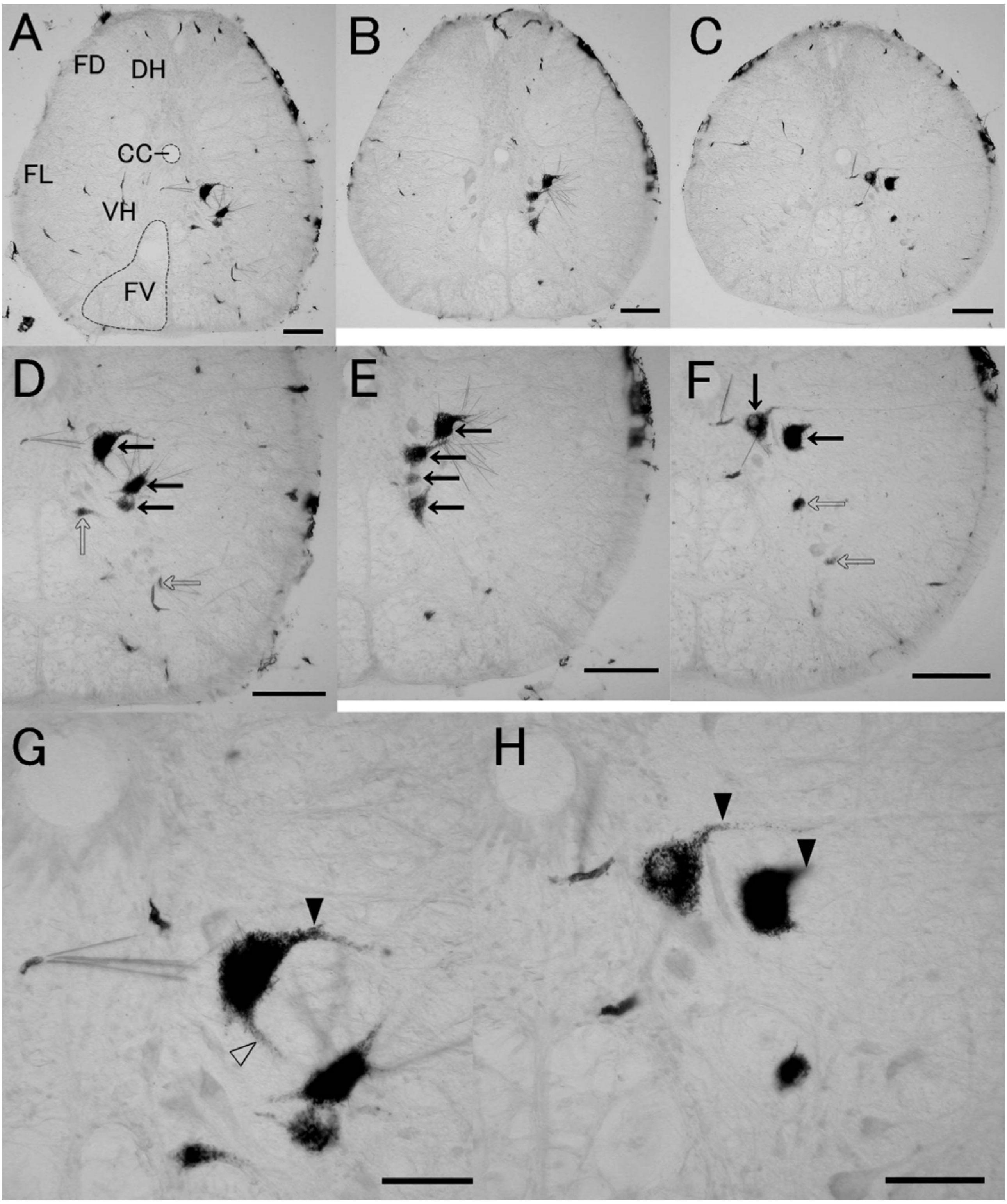

Figure 7.

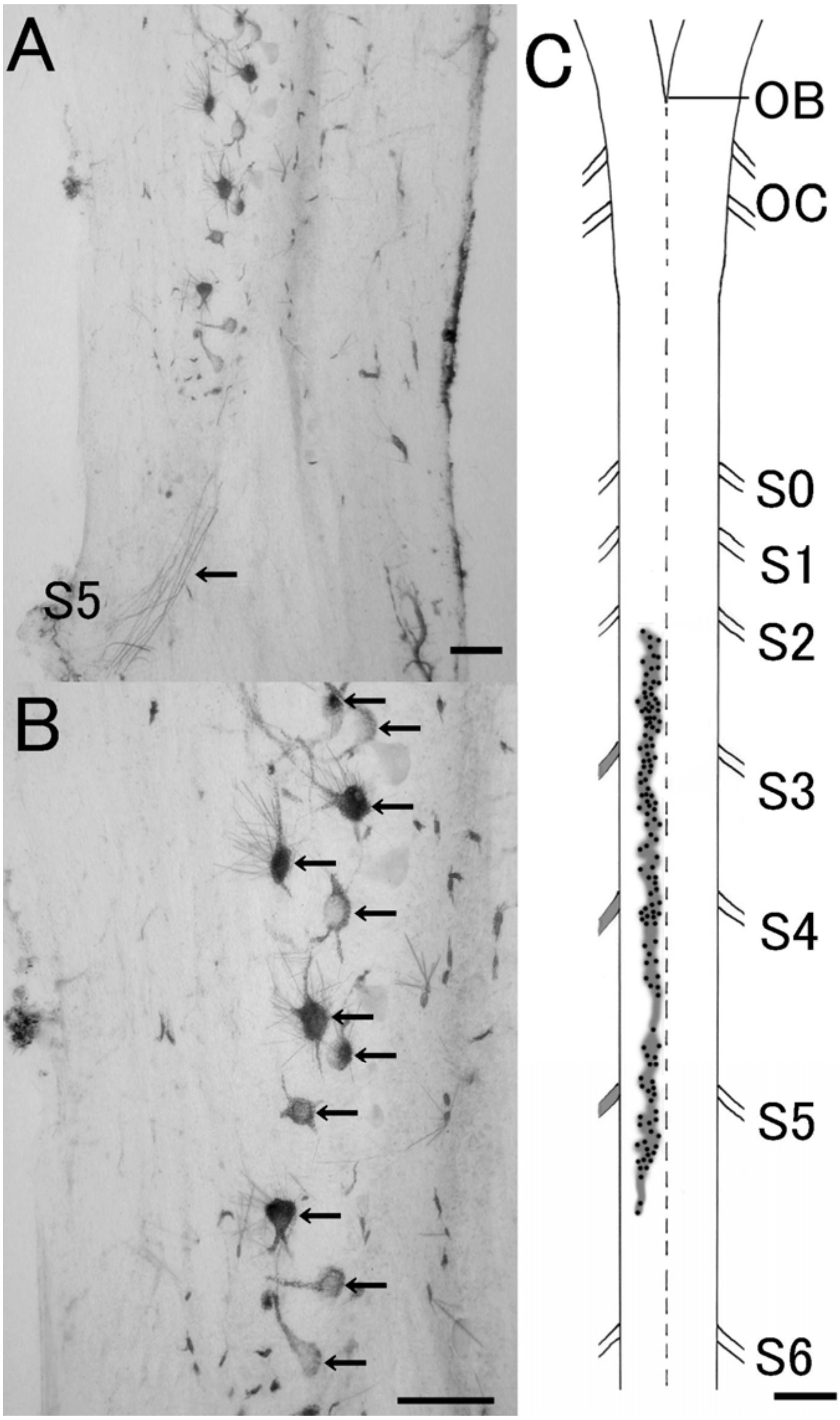

Figure 8.

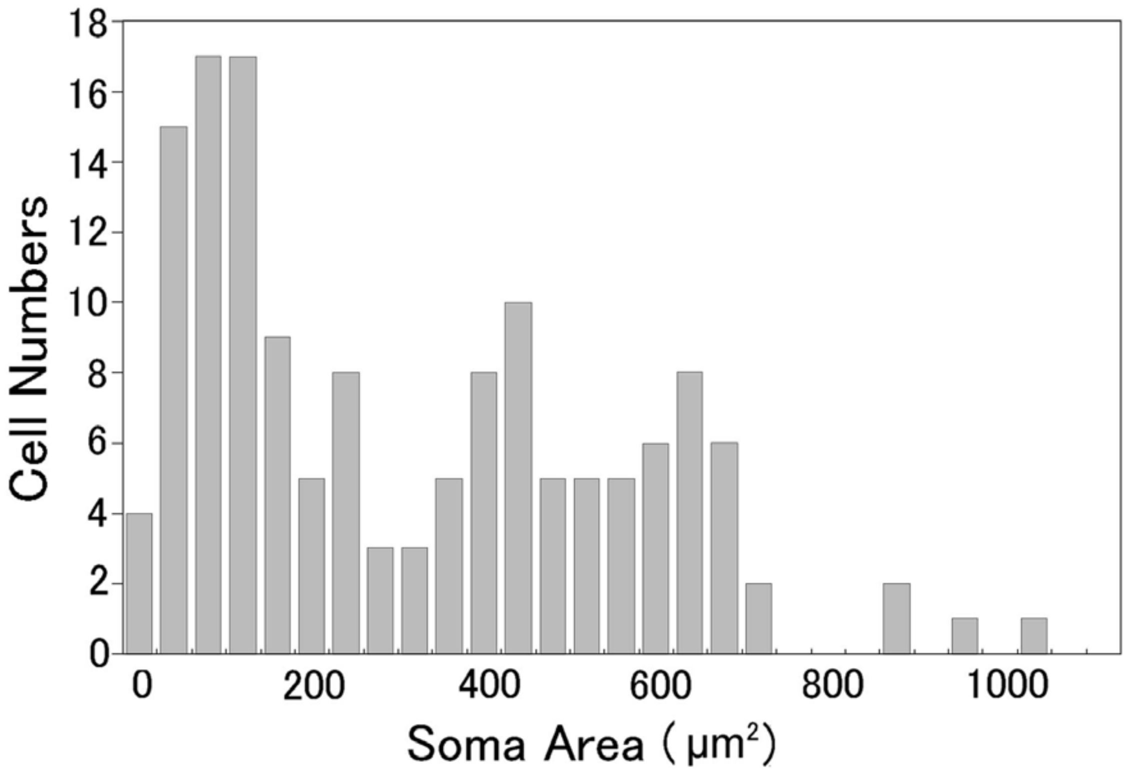
Cell Numbers
0
2
4
6
8
10
12
14
16
18
0
200
400
600
800
1000
Soma Area (μm²)

Figure 9.