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Innervation of lateral line system in the medaka, *Oryzias latipes*

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Abstract The peripheral nerves which innervate the lateral line organs (neuromasts) were revealed by an immunohistochemical staining method using anti-neurofilament protein (70K+160K+210K) antibodies in the medaka (*Oryzias latipes*). Almost all neuromasts including groove organs in the head region were innervated by the hyomandibular, supraorbital and infraorbital nerve trunks, in which the anterior lateral line nerve (ALLN) was included. A few groove organs received the dorsal and lateral rami of the ALLN. The trunk and tail neuromasts were supplied by the lateral, dorsal and dorsolateral rami of the posterior lateral line nerve (PLLN). The occipital neuromasts were innervated by both the occipital ramus of the PLLN and the dorsal ramus of the ALLN.

Introduction

The lateral line is thought to be a sensory system to detect water displacement (Dijkgraff, 1962) and to detect ion concentrations (Katsuki *et al.*, 1971) in aquatic vertebrates. The system appears to register movement and salt concentration in the water and thus provides valuable sensory information to the swimming animal.

The lateral line system of the medaka (*Oryzias latipes*) has been studied by Yamamoto (1947, 1975), Sato (1955a,b), Iwamatsu *et al.* (1984) and Iwamatsu (1993). All investigators agree that this fish has characteristic large neuromasts (groove organs) in the head and many free or superficial neuromasts (large pit organs of Sato, 1955a; small pit organs of Yamamoto, 1975 and Iwamatsu *et al.*, 1984) over the entire body surface, but no canal organs.

Neuroanatomical knowledge on the lateral line system is essential for future physiological and anatomical studies to understand the behavior of this fish. However, as pointed out by Yamamoto (1975), its innervation has not yet been worked out. It is technically difficult to dissect macroscopically the innervating nerves because of the small size of the medaka.

We have developed a method in which nerve fibers in both peripheral and central nervous systems of the medaka were stained as whole-mount specimens (Ishikawa *et al.*, 1986; Ishikawa, 1992; Ishikawa and Iwamatsu, 1993; Ishikawa and Hyodo-Taguchi, 1994). By using this method, the innervation of the lateral line system is studied in the present paper.

Materials and Methods

Materials

Adult or young-adult fish of d-rR strain of the medaka (*Oryzias latipes*) were used. In order to show the basic pattern of distribution of the neuromasts, newly hatched fry (stage 34/35 of Matui, 1949; or stage 39/40 of Iwamatsu, 1993) of an albino strain, *bi-3R* strain, were used. To show the gross anatomy of the brain, an adult fish of a wild-type strain, HB12A (Hyodo-Taguchi and Sakaizumi, 1993), was used.

Anti-bovine neurofilament protein (70K+160K+210K) monoclonal antibodies (anti-NFP antibodies) from Cosmo Bio. Co., Ltd. (Tokyo), horseradish peroxidase (HRP)-conjugated affinity-purified sheep anti-mouse IgG from Cappel Laboratories, Inc. (Westchester), and saponin from Merck (Darmstadt) were used.

Whole-mount staining of neuromasts

Adult and young-adult fish were fixed in Zamboni's solution (Stefanini *et al.*, 1967) for 1 day at 4°C. They were washed in phosphate-buffered saline (PBS) for 3–10 days at 4°C. In order to permeabilize the samples, they were incubated in freshly prepared 1.5% Triton X-100, 1.5% saponin and 8% sucrose for 1 day at 4°C. After washing in PBS, they were soaked in 1% periodic acid for 0.5–1 hr to block endogenous peroxidase activity. The samples were immunohistochemically stained by the indirect HRP-labeled antibody method using the anti-NFP antibodies (Ishikawa *et al.*, 1986). The neuromasts of the fry were stained according to the previously reported method (Ishikawa and Hyodo-Taguchi, 1994).

Neuromasts in adult fish were also stained by immersing live fish in 0.002% methylene blue for 2 days according to the method of Yamamoto (1975).

Whole-mount nerve staining

The peripheral nerves of young-adult fish were stained twice using anti-NFP antibodies according to the method of Ishikawa *et al.* (1986).

Nomenclature of lateral lines and cranial nerves

I adopted most of Sato's (Sato, 1955a) and Yamamoto's (Yamamoto, 1975) nomenclature for the lateral lines of the medaka, and followed Ariëns Kappers *et al.* (1936), Northcutt and Davis (1983), Puzdrowski (1988), and Ito and Yoshimoto (1991) in the nomenclature of cranial nerves of the teleost fish.

Results

Cranial nerves

Prior to describing the innervation of the lateral line system, I provide a brief description of the cranial nerves in the medaka (Fig. 1). There were 12 pairs of cranial nerves, as in usual teleost fish. The lateralis nerves entered the rhombencephalon ventral to the cerebellar crest, as two bundles, namely, anterior (ALLN) and posterior (PLLN) lateral line nerves. The ALLN was situated close to the facial nerve (VII), while the PLLN was positioned close to the roots of glossopharyngeal (IX) and vagal (X) nerves.

Neuromasts

The neuromasts were stained intensively by the present immunohistochemical staining method using anti-NFP antibodies (Fig. 2). A similar pattern of the distribution of neuromasts was obtained by methylene blue staining, although more neuromast-like structures were stained especially in the belly (data not shown). However, the color of the structures stained by the latter method was so faint that it was often difficult to distinguish neuromasts from other types of cells. Hence, the former method was used for further experiments.

The neuromasts were arranged in definite lines, though their number in each line exhibited considerable variations depending on the size of the medaka, the individual, and even on opposite sides of the same specimen.

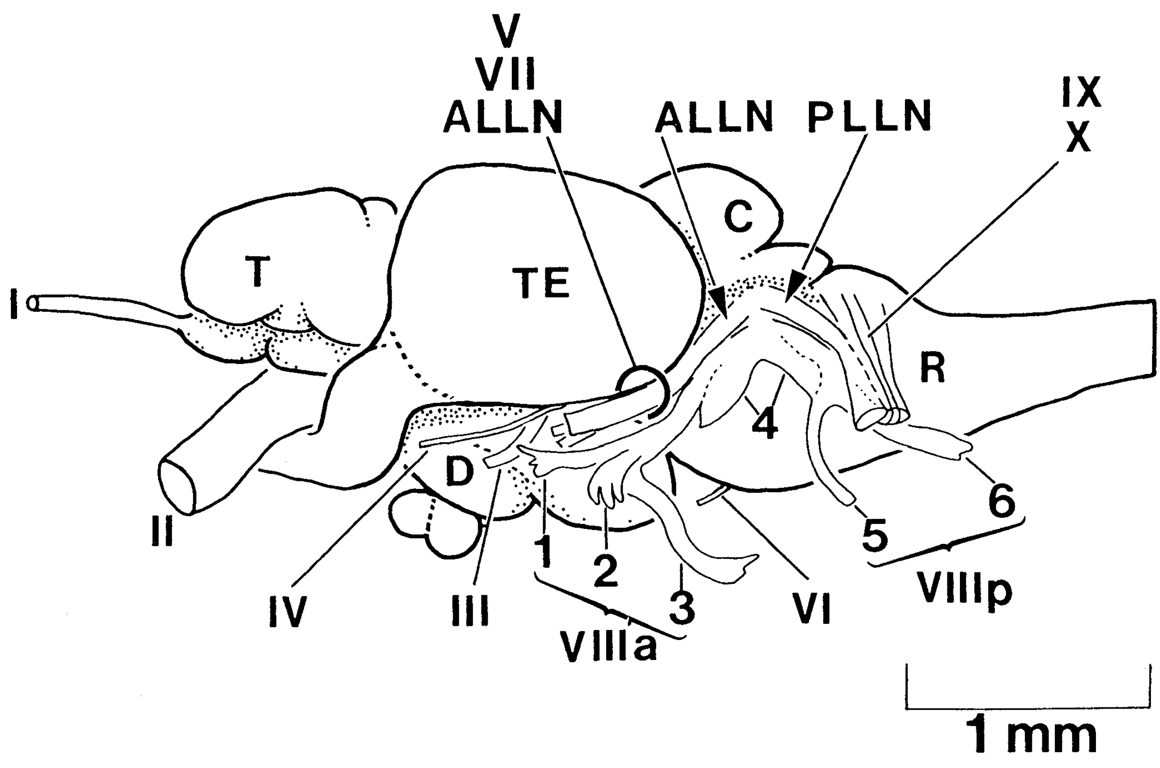

Fig. 1. Left lateral view of the brain of medaka. The lateralis nerves (ALLN and PLLN) and other cranial nerves are shown. In this specimen, the pineal body is lost. I, olfactory nerve; II, optic nerve; III, oculomotor nerve; IV, trochlear nerve; V, trigeminal nerve; VI, abducens nerve; VII, facial nerve; 1–6, octavus nerve (VIIIa, anterior ramus; VIIIp posterior ramus); ALLN, anterior lateral line nerve; PLLN, posterior lateral line nerve; IX, glossopharyngeal nerve; X, vagal nerve, C, cerebellum; D, diencephalon; R, rhombencephalon; T, telencephalon; TE, tectum mesencephali. Scale bar = 1 mm.

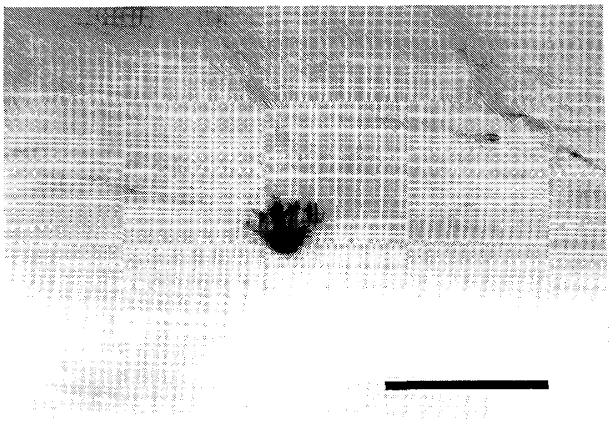

Fig. 2. A neuromast, which is the first one in the ventral-tail line, stained by the present immunohistochemical method using anti-NFP antibodies. The left lateral aspect of a stained fry is shown. Note that the neuromast protrudes from the epidermis and is not sunk in the fry. Scale bar = 0.05 mm.

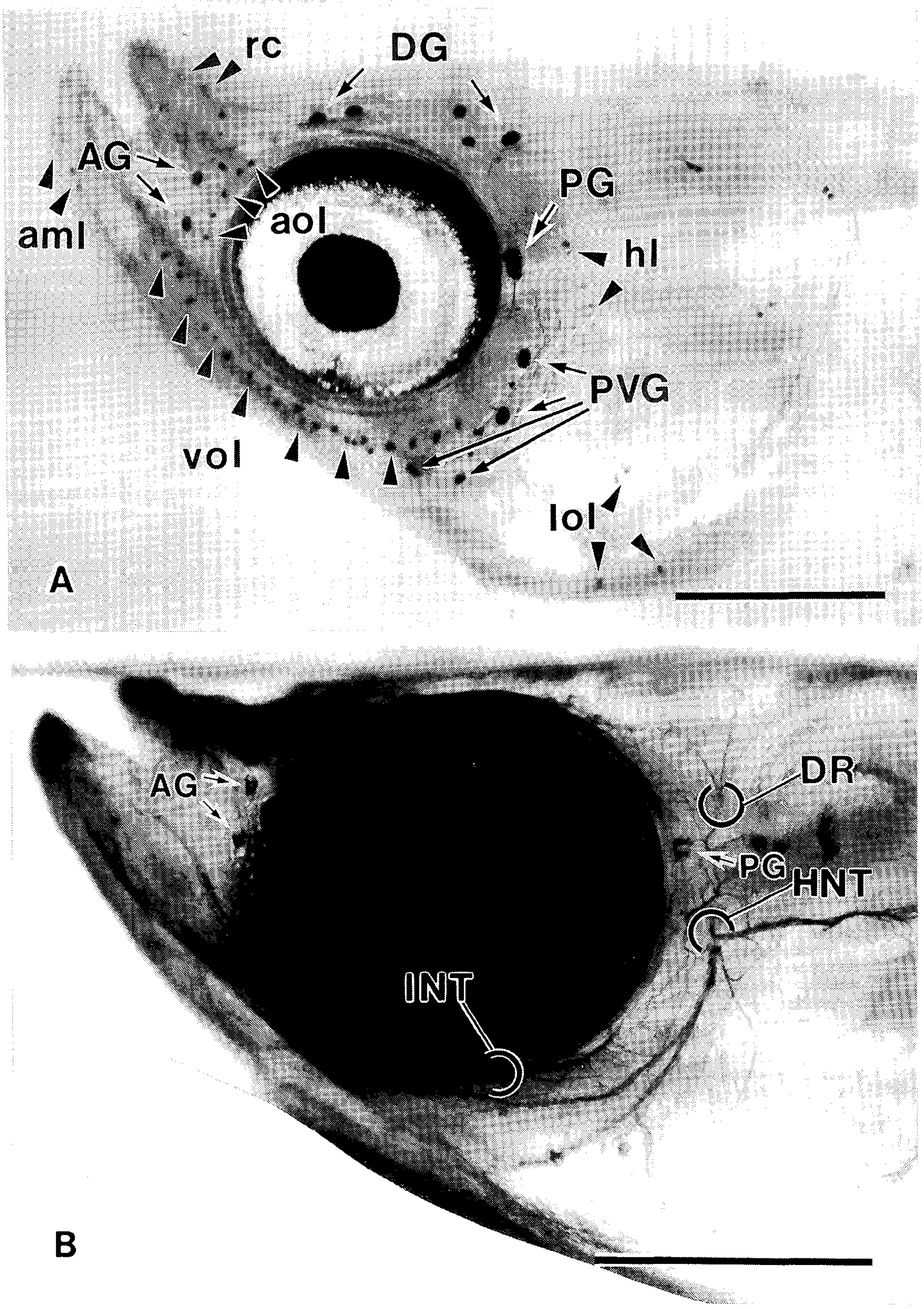

Fig. 3. The distribution of head neuromasts (A) and peripheral nerves (B) stained by the present methods. The left lateral view of the head region of a young-adult fish is shown. AG, anterior group; aml, anterior mandibular line; aol, antorbital line; DG, dorsal group; DR, dorsal ramus of the ALLN, hl, horizontal line; HNT, hyomandibular nerve trunk; INT, infraorbital nerve trunk; lol, lower opercular line; PG, posterior groove organ; PVG, posteroventral group; rc, rostral commissure; vol, ventral orbital line. Scale bars = 1 mm.

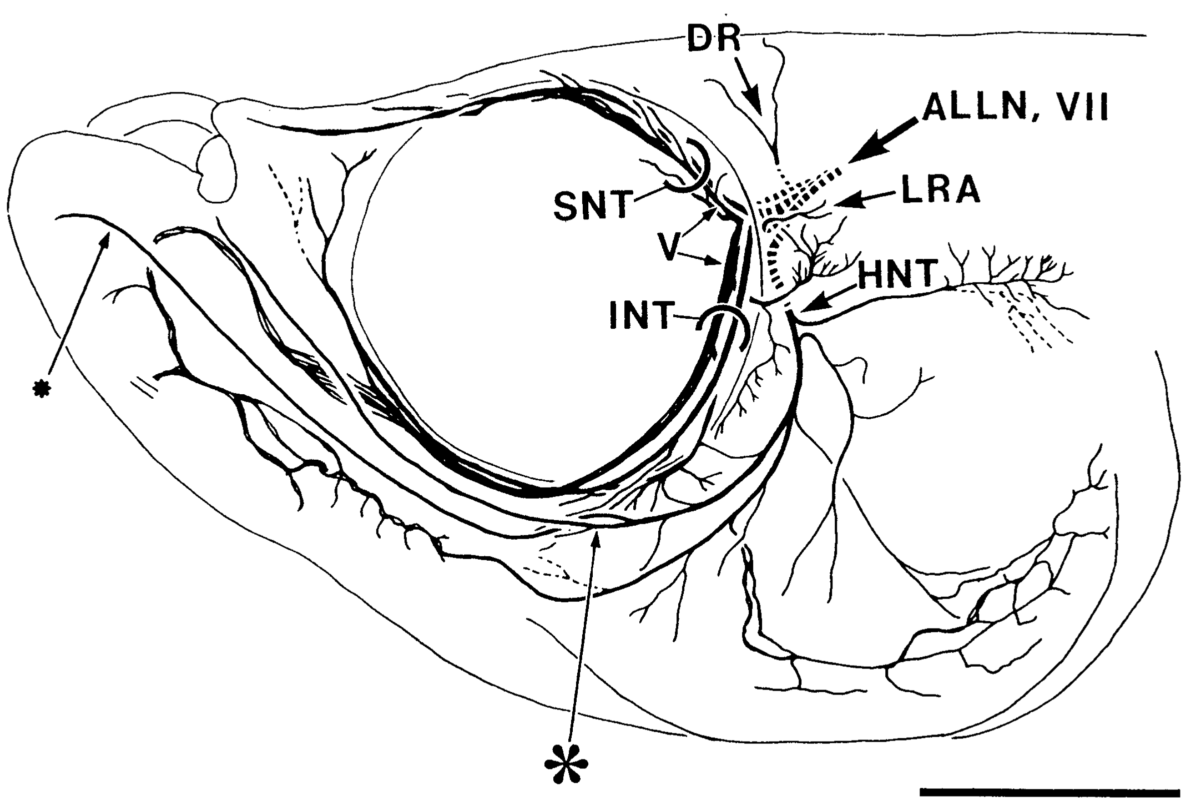

Fig. 4. Peripheral pattern of cranial nerves in the head region. The nerve-stained sample (Fig. 3B) was traced, after removing the eyes. Left ventrolateral aspect is shown. Nerve branches to the ventral orbital line (large asterisk) and to the anterior mandibular line (small asterisk) were indicated by the asterisks. ALLN, anterior lateral line nerve; DR, dorsal ramus of the ALLN; HNT, hyomandibular nerve trunk; INT, infraorbital nerve trunk; LRA, lateral ramus of the ALLN; SNT, supraorbital nerve trunk; V, trigeminal nerve; VII, facial nerve. Scale bar = 1 mm.

Head region

There were 12–13 groove organs on each side of the head of young-adult fish (Fig. 3A): two anterior (AG, anterior group) to, five to six dorsal (DG, dorsal group) to, one posterior (PG, posterior groove organ) to, and four posteroventral (PVG, posteroventral group) to the orbit.

Nerve staining showed that the ALLN, trigeminal nerve (V) and facial nerve (VII) are interconnected in the periphery, and these cranial nerves form three nerve trunks in the periorbital region, namely, the supraorbital, infraorbital and hyomandibular nerve trunks (Figs. 3B and 4). The dorsal, anterior, and posteroventral groups were innervated by the supraorbital, infraorbital, and hyomandibular nerve trunks, respectively. The caudalmost groove organ in the dorsal group was supplied by the dorsal ramus of the ALLN. The posterior groove organ was innervated by the lateral ramus of the ALLN.

About 60 ordinary neuromasts were present on each side of the head of young-adult fish (Fig. 3A). They formed 6 lines. A line (vol, ventral orbital line) ran along the ventral margin of the orbit and was comprised of 20–30 neuromasts. This line may correspond to the anterior part of the lower opercular line and the whole preopercular line of Sato (1955a). Anterior to this line, about 6 neuromasts (aml, anterior mandibular line of Sato, 1955a) were found in the anteroventral surface of the lower jaw. Posterior to the posterior groove organ, about 5 neuromasts (hl, horizontal line of Sato, 1955a) were scattered. About 6 neuromasts (lol, lower opercular line of Sato, 1955a) were arranged on the lower margin of the operculum. Immediately anterior to the orbit, 6 neuromasts (aol, antorbital line of Sato, 1955a) were observed. In the snout, about 5 neuromasts formed a line (rc, rostral commissure of Sato, 1955a).

The anterior mandibular and lower opercular lines were innervated by the hyomandibular nerve trunk (Figs. 3B and 4). The ventral orbital line was innervated by the same nerve trunk, though I could not rule out the possibility that the nerve branches from the infraorbital nerve trunk also contribute to the innervation of this line. The horizontal line received both the hyomandibular nerve trunk and the lateral ramus of the ALLN. The antorbital line was supplied by the infraorbital nerve trunk. The rostral commissure was innervated by the supraorbital nerve trunk.

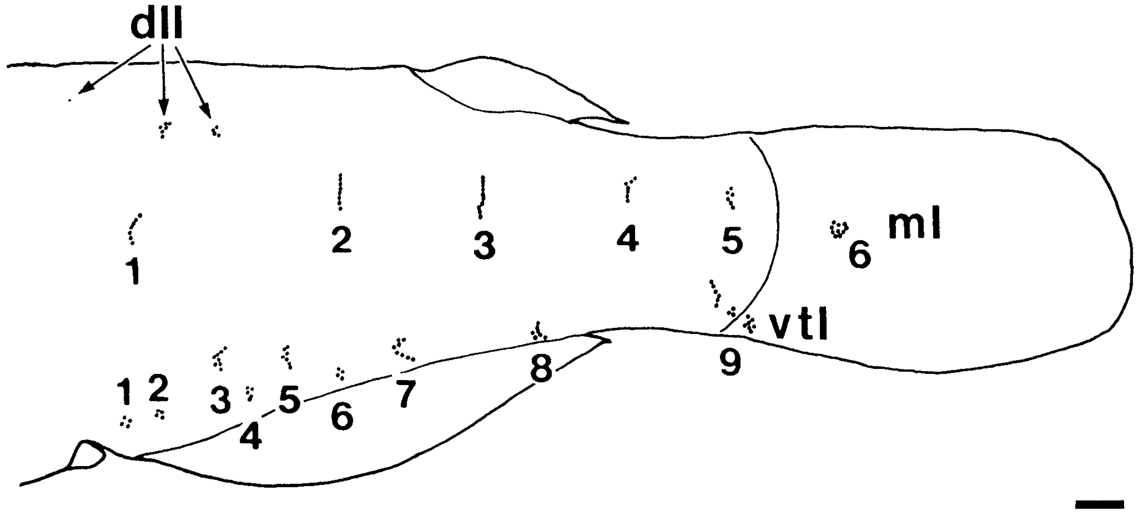

Fig. 5. The distribution of neuromasts in the tail of adult fish which was stained by the present method. A neuromast-stained sample was traced. Left lateral view. Neuromast groups in two lines are numbered. dll, dorsolateral line; ml, midbody line; vtl, ventral-tail line. Scale bar = 1 mm.

Occipital, trunk and tail regions

In the adult fish, neuromast groups, each of which was composed of 2–10 neuromasts, formed each line (Fig. 5, see also Fig. 14 of Ishikawa, 1990). There were 40–50 neuromast groups on each side. In the fry, the neuromasts were decreased in number compared with the adult fish, and single neuromasts were arranged along each line (Fig. 6). However, there was little difference between the fry and adult fish, in terms of the basic pattern of the distribution of the neuromasts. Five lines were found on each side of the trunk and tail: a midbody (ml), a belly (bl), a ventral-tail (vtl), a dorsal (dl) and a dorsolateral (dll) line, each of which ran from anterior to posterior.

Concerning the distribution of neuromasts in the trunk and tail regions, I confirmed the results of Yamamoto (see Fig. 10-2 of Yamamoto, 1975): The midbody line and ventral-tail line correspond to the posterior part of the dorsolateral row and the posterior part of the ventrolateral row of Yamamoto (1975), respectively. The belly line is identical to the ventral row and the anterior part of the ventrolateral row of Yamamoto (1975). The dorsal line and the dorsolateral line correspond to the dorsal row and the anterior part of the dorsolateral row of Yamamoto (1975), respectively.

Nerve stainings of young-adult fish revealed that the midbody and ventral-tail lines were supplied by the lateral ramus (LRP) of the PLLN (Figs. 7 and 8). The dorsal and dorsolateral lines were innervated by the dorsal (DR) and dorsolateral rami (DLR) of the PLLN, respectively. I could not trace the peripheral nerves to the belly line. The neuromasts of this line may be innervated by either ventral ramus (rami) of the PLLN or ventral branch(es) from the lateral ramus of the PLLN.

Anterior to the dorsal line, one line (ol, occipital line) was observed in the occipital region (Figs. 7 and 8). In this line, a few groups of neuromasts were scattered on the dorsal surface of the head and dorsolateral surface of the otic vesicle. This line corresponds to the supratemporal commissure of Sato (1955a). The occipital line was innervated by the occipital ramus (OR) of the PLLN. The anteromost neuromasts in this line also received branches from the dorsal ramus of the ALLN.

Discussion

The present study revealed the innervation of the lateral line system of the medaka and the courses of nerve fibers in the periphery. This study provides a neuroanatomical background for future studies on the lateral line system of this useful laboratory fish.

As in other teleost fish (McCormick, 1983), the lateral line system seems to be innervated by ALLN and PLLN in the medaka: In the head region, the most neuromasts were innervated by the ALLN, among peripheral branches of which the hyomandibular nerve trunk was the most well-developed. In the trunk and tail regions, they were supplied by the rami of the PLLN, among which the lateral ramus of the PLLN was the most prominent innervating nerve.

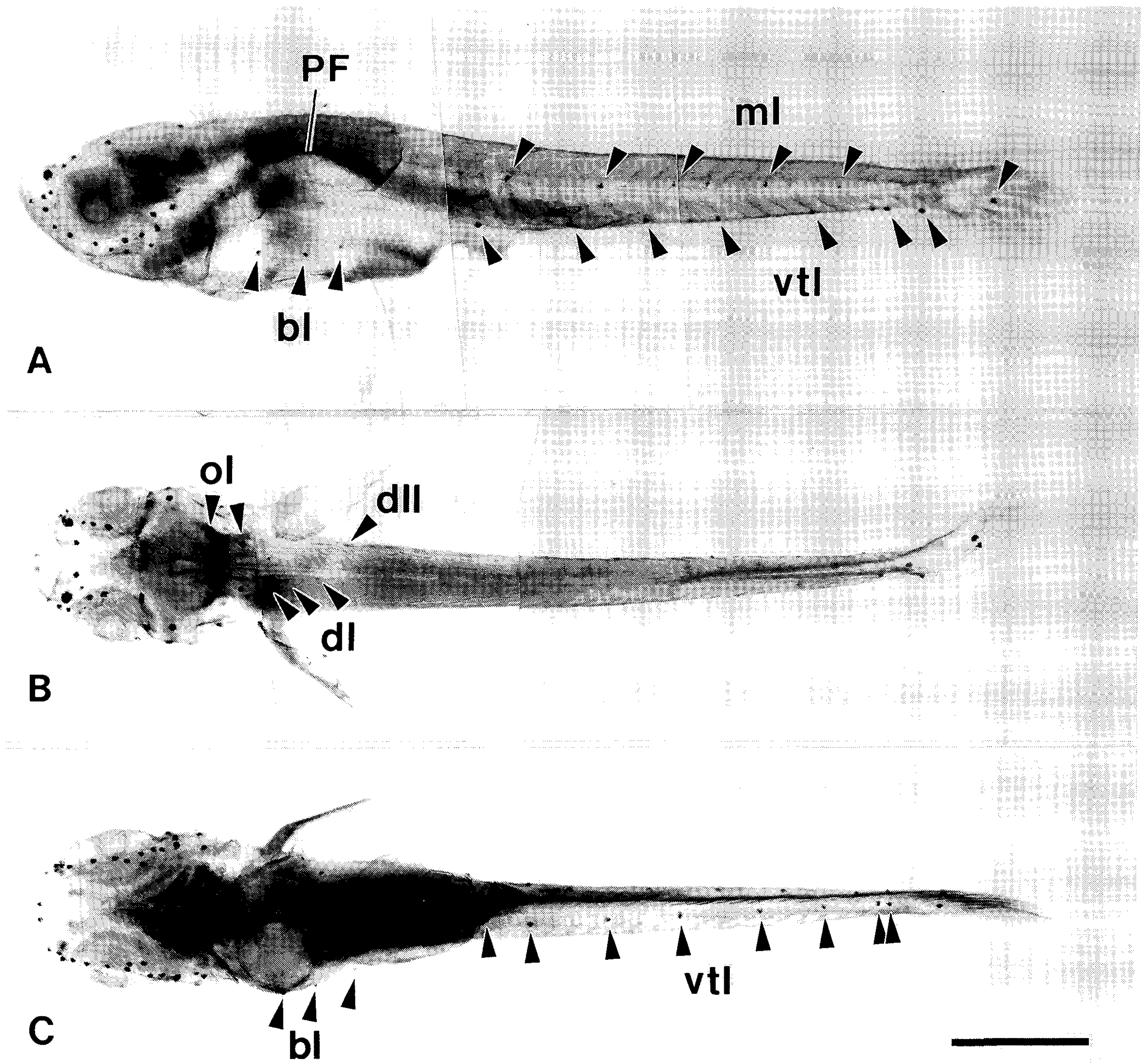

Fig. 6. The distribution of neuromasts in the fry which was stained by the present method. The left lateral (A), dorsal (B), and ventral (C) views. bl, belly line; dl, dorsal line; ol, occipital line; PF, pectoral fin. For other abbreviations, see Fig. 5. Scale bar = 0.5 mm.

Concerning the nomenclature of the lateral lines in the medaka, differences are noted between the present results and those of Sato (1955a). The groove organs in the periorbital region have been thought to be supra- and infraorbital lines (Sato, 1955a). However, the posteroventral group of the groove organs was found to be innervated by the hyomandibular nerve trunk in the present study. Hence, these groove organs do not correspond to the infraorbital line or the supraorbital line, but rather to part of the cheek line (the horizontal line plus vertical line) of other teleost fish.

Although there were no canal organs in the medaka, numerous free (superficial) neuromasts were distributed all over the surface from the head to the tail. Thus, the lateral line system must certainly be of great importance in the life of this fish. This sensory detector may be used not only in swimming but perhaps also in many other types of behavior such as fighting, mating, and schooling.

In this connection, it is of interest to know the central processing of lateralis inputs in the brain. Experimental studies should be performed to determine whether the projection of the lateralis impulses to the central nervous system is somatotopically organized. Experimental hodological studies in the brain should be also performed in the future.

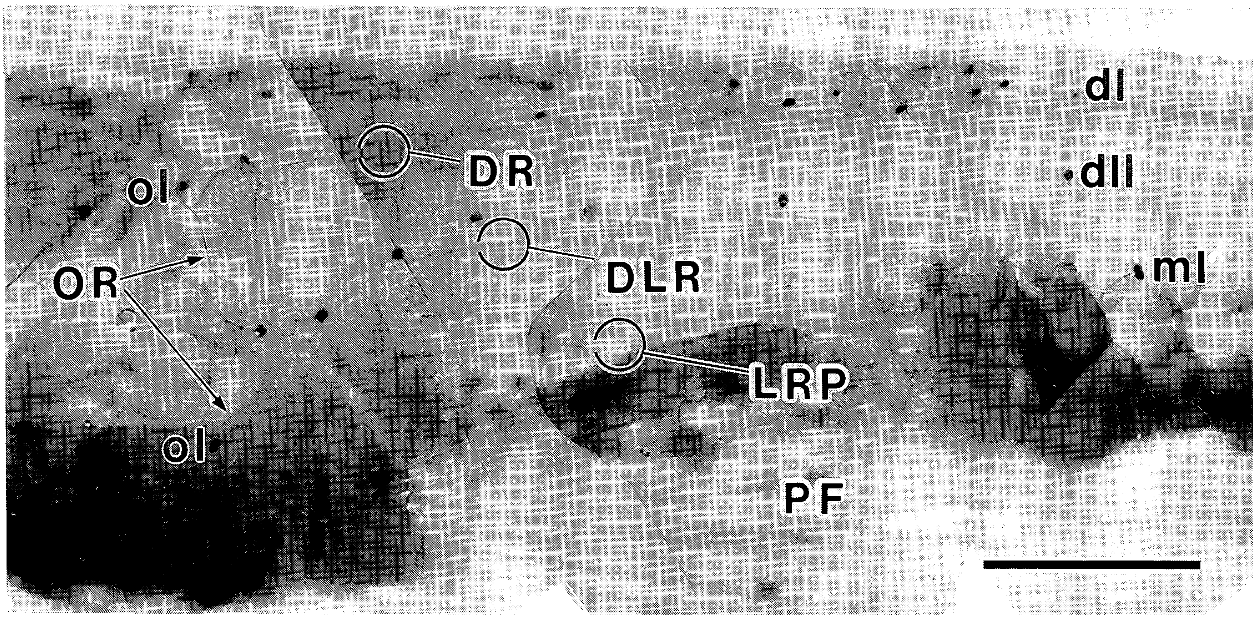

Fig. 7. Left dorsolateral aspect of the occipital and trunk regions of the young-adult fish which was stained by the present method. Note that the neuromasts in each line were innervated by small collateral nerves from each ramus. dl, dorsal line; dll, dorsolateral line; DLR, dorsolateral ramus of the PLLN; DR, dorsal ramus of the PLLN; LRP, lateral ramus of the PLLN; ml, midbody line; ol, occipital line; OR, occipital ramus of the PLLN; PF, pectoral fin. Scale bar = 1 mm.

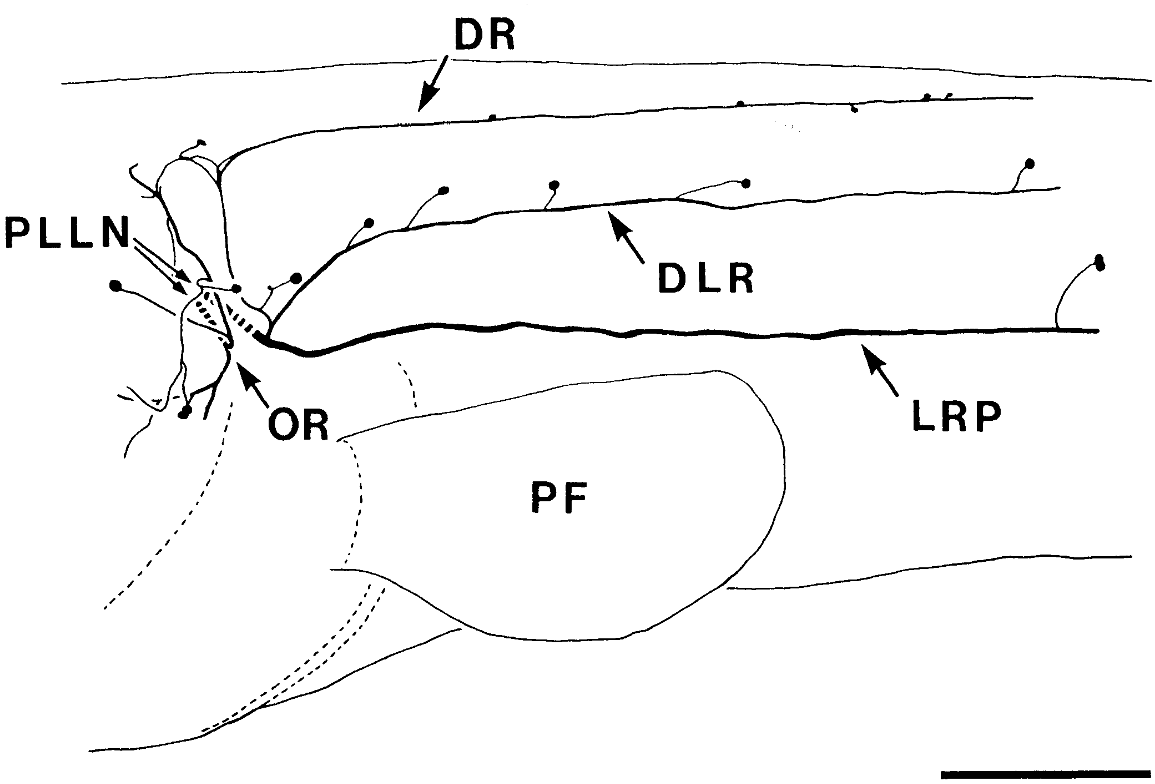

Fig. 8. Peripheral pattern of the PLLN and distribution of neuromasts in the occipital and trunk regions. The stained sample (Fig. 7) was traced. PLLN, posterior lateral line nerve. For other abbreviations, see Fig. 7. Scale bar = 1 mm.

Acknowledgments

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