Morphological Characteristics of the Lateral Line Receptors of *Hynobius leechii* (Urodela: Hynobiidae)

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Morphological characteristics of the lateral line receptors, specifically the superficial neuromast, the pit organ, and the ampullary organ, are described and compared among larval, metamorphosed, and both female and male adult *Hynobius leechii*. The distribution and morphologies of the lateral line receptors are similar to those of other urodèles. During the transition from a larval to an adult salamander, the number of receptors is generally reduced, but their sizes increase. Other morphological changes also occur, such as the development of a more distinct mantle cell boundary at the epidermal surface of the adult ampullary and pit organs. In particular, we found kinocilia on the sensory hair cells of the ampullary organs of both larval and adult salamanders, and, for the first time, we describe two different morphologies of the pit organ in urodèles. The detailed description of the pit organ represents only the second study of its kind in urodèles. However, our comparison of lateral line receptors between larval and adult salamanders is the first of its kind for salamanders that utilize external fertilization.

Key words: mechanosensory, lateral line, *Hynobius leechii*, neuromast, pit organ, ampullary organ, external fertilization

INTRODUCTION

The mechanosensory system is one of the most primitive sensory systems. A variety of animal taxa have well-developed mechanosensory systems, including invertebrates (Maklakov et al., 2003; Elias et al., 2006; Sane et al., 2007), fish (Montgomery et al., 1997; Maruska and Tricas, 2004; Coombs and Grossman, 2006), amphibians (Lannoo, 1987; Northcutt, 1992; Claas and Dean, 2006; Fritzsch, 2006), and reptiles (Soares, 2002; Westhoff et al., 2005). Previous studies have demonstrated that these systems play a key role in foraging (Lang, 1980; Claas and Dean, 2006), social interactions (Coki et al., 1999; Warkentin, 2005), and mating (Satou et al., 1994; Usuda, 1995; Maklakov et al., 2003). Lateral line receptors, such as the superficial neuromast, the pit organ, and the ampullary organ, have been identified in several urodèle species (Fritzsch, 1988; Himstedt and Fritzsch, 1990; Northcutt, 1992; Northcutt et al., 1995; Hong et al., 1995, 2000). The ampullary and pit organs have relatively recently been discovered in amphibians (Hetherington and Wake 1979; Fritzsch and Münz, 1986; Northcutt and Bleckmann, 1993). The morphology, development, and central projection of the lateral line receptor have been studied in several urodèles (Fritzsch, 1981; Fritzsch and Bolz, 1986; Fritzsch, 1990; Northcutt et al., 1995; Fritzsch et al., 2005), whereas comparative studies of these receptors in larval and adult salamanders are few in number. In particular, pit organ morphology has been studied in only two urodèle species, and the results were inconsistent between the two studies (Northcutt and Bleckmann, 1993; Hong et al., 1995). Furthermore, relatively few morphological studies have been performed on salamanders that externally fertilize their eggs (Lannoo, 1987; Hong et al., 1995, 2000).

*Hynobius* species utilize external fertilization. During courtship displays, male salamanders frequently conduct body undulations that cause water vibrations (Park and Park, 2000). Both male and female *Hynobius* salamanders respond to water vibrations by approaching the site where the undulating male is located (Tanaka, 1987; Usuda, 1995; Jeon et al., 2005). This suggests that the mechanosensory lateral line system may play a role in the perception of a courtship signal during mating. In *Hynobius*, only a single morphological study (Lannoo, 1987) of the neuromast of larval *H. nebulosus* has been conducted, but it only used three individuals. The mechanosensory lateral line system of adult *Hynobius* has never been studied morphologically.

In the present study, we describe the distribution and morphologies of three lateral line receptors of *H. leechii* and compare these among larval, metamorphosed, and both male and female adult salamanders by microscopic observations and histological techniques. As a result, we have discovered kinocilia on the sensory hair cells of the ampullary organ and, for the first time, describe two different morphologies of the pit organ of adult salamanders.

MATERIALS AND METHODS

The salamanders used in this study, including five larval, four adult female, and four adult male *H. leechii*, were collected by using a hand net in a small mountain pond in the research forest of the Kangwon National University located at Dongsan-myeon, Kangwon-
do, South Korea in 2006; specimens were then transported to the laboratory in a refrigerated box (5–10°C; 550×350×350 mm) and immediately used in experiments. Both females and males were in full breeding condition, based on the presence of swollen cloaces in males and eggs in females. From the same pond, we additionally collected 10 larval salamanders to serve as a sample of metamorphosed individuals and housed them in a laboratory aquarium (330×200×250 mm) containing 20 L of aged tap water. We placed several rocks on the bottom of the aquarium to provide hiding places and changed half of the water once per week. The photoperiod followed the local light regime, which was approximately 12D:12L. We fed salamanders chopped earthworms and freshwater Grammas amphipods ad libitum. We collected two metamorphosed individuals at one week and another two one month after metamorphosis. At the end of experiments, we released six metamorphosed individuals that were not used in the study at the same site where we caught them.

For light microscopic observations, we prepared our samples following the Northcutt (1992) method. Briefly, we sacrificed the salamanders by using an overdose of 0.1% tricaine methanesulfonate (MS222, pH 7.6, Sigma) and then fixed them for more than 24 hr in 4% glutaraldehyde (Sigma) in PBS buffer (pH 7.2). We measured the body weight of all individuals to the nearest 0.01 g with an electronic balance (Ohaus) and the SVL (snout-vent length) to the nearest 0.01 cm using an electronic Vernier caliper. We then peeled off the head and body skin under a dissecting microscope. After removing excess melanin on the skin by submersing it in 35% H2O2 for 24–48 hr, we stained the skin by using 0.5% methylenegreen for 30–45 sec and observed the samples under a light microscope.

During the observations, we identified different lateral line receptor types based on the morphological characteristics described in previous studies (Northcutt, 1992; Hong et al., 1995) and counted the number of receptors and measured the size of each different type of receptor. In addition, we determined and recorded the distribution of different lateral line receptors on the surfaces of the heads and bodies of larval and adult salamanders. To measure the size of the receptors, we chose 6–17 receptors of each type for each individual and measured the longest and shortest diameter of each receptor to the nearest 0.1 μm at 400X magnification, by using ImageInside software (v. 2.32). For the size of a receptor, we used the average values of the longest and shortest diameter of the receptor. We arbitrarily selected for measurement receptors in the receptor lines of the neuromast, pit organ, and ampullary organ.

To investigate in detail the morphological characteristics of each type of lateral line receptor, we used a scanning electron microscope (SEM). Skin tissue was washed twice in 0.1 M cacodylate buffer (pH 7.4) and was then serially dehydrated in 50, 70, 90, 100, and 100% ethanol for 30 min at each concentration. The medium was then exchanged with isomyl acetate, twice for 30 min each. The tissues were dried by critical-point drying, mounted on a specimen stub, coated with Au-Pd, and observed under a S-3500N medium was then exchanged with isoamyl acetate, twice for 30 min

Three different lateral line receptors (i.e., the superficial neuromast, the pit organ, and the ampullary organ) were found on the surface of larval, metamorphosed, and both female and male adult salamander heads. We also confirmed the presence of superficial neuromasts on the bodies of larval and adult salamanders. Relationships between the SVL and body weight of salamanders and the number and size of the lateral line receptors were determined using the Spearman correlation test. All statistical analyses were two-tailed and performed using SPSS v. 11.0 (SPSS Inc., Chicago Illinois, USA). Data are presented as mean±SE.

RESULTS

Three different lateral line receptors (i.e., the superficial neuromast, the pit organ, and the ampullary organ) were found on the surface of larval, metamorphosed, and both female and male adult salamander heads. We also confirmed the presence of superficial neuromasts on the bodies of larval and adult salamanders.

The sizes of the neuromast and ampullary organs on the surface of the head were positively correlated with SVL (r=0.621, n=13, P=0.024) and body weight (r=0.588, n=13, P=0.035), whereas the number of pit organs on the surface of the head was negatively correlated with both SVL (r=0.689, n=13, P=0.009) and body weight (r=0.797, n=13, P=0.001). Other comparisons were not significant (P>0.05). Several morphological differences were found between larval and adult salamander receptors. A detailed description of the morphology and distribution of each receptor is given in following sections.

Neuromast Morphology

The elliptical-shaped superficial neuromast was the largest lateral line receptor and consisted of hair cells, supporting cells, and mantle cells (Fig. 1A, B). The hair cells were round. Columnar supporting cells were found under the hair cells, and elongated mantle cells encircled both the supporting and hair cells (Fig. 1A, B). The degree of roundness of neuromasts varied with neuromast location. Highly elliptical neuromasts were found in the neuromast lines that contain only neuromasts, and head skin that contained only neuromasts and ampullary organs by using a dissecting microscope (40X magnification). We could easily discriminate between the neuromast and ampullary organs due to their different sizes in our histological specimens. The prepared skins were paraffin-embedded, sectioned at a 10-μm thickness with a microtome, stained following the Harris hematoxylin-eosin method, and observed under a microscope. We took representative micrographs of each receptor in the neuromast, pit organ, and ampullary organ (400X magnification).

This study was approved by the Korean government. All other experimental procedures followed the guidelines for the use of live amphibians and reptiles in field and laboratory research (ASIH, 2004). During the study, we detected no receptors on the skin surface of the two metamorphosed salamanders examined one month after metamorphosis and excluded from the analyses. For the two metamorphosed individuals examined one week after metamorphosis, we found and described the characteristics of each type of lateral line receptor, but did not apply any statistical analyses due to the small sample sizes (1 or 2).

For statistical analyses, we applied non-parametric analysis due to the small sample size. To compare the number and size of the lateral line receptors among larvae and female and male adults, we used the Kruskal-Wallis test followed by a post-hoc test to detect significant differences (Siegel and Castellan, 1988). We applied the Mann-Whitney U test to compare independent group data sets, such as the number of kinocilia on the sensory hair cells of the pit organ and the ampullary organ and the length of sensory cupulae in the superficial neuromast and pit organ in larval and adult salamanders. Relationships between the SVL and body weight of salamanders and the number and size of the lateral line receptors were determined using the Spearman correlation test. All statistical analyses were two-tailed and performed using SPSS v. 11.0 (SPSS Inc., Chicago Illinois, USA). Data are presented as mean±SE.
were parallel to the rostrocaudal body axis, such as in the middle part of the supraorbital line (Fig. 2). The superficial neuromasts of larval salamanders were slightly more elliptical than those of adult salamanders, but the difference was not statistically significant (P=0.064). The ratio of the longest and shortest diameter of the neuromasts was 1.5±0.07 for larvae, 1.33±0.02 for adult females, and 1.35±0.08 for adult males. The boundary between mantle cells and epidermal cells was more distinct in adult salamanders than in larval salamanders, based on the sunken boundary of the mantle cells below the epidermal surface (Fig. 4A, B). In one female salamander, the superficial neuromasts were recessed into the epidermis, so only the kinocilia and several microvilli protruded onto the surface (Fig. 4F). In that salamander, most of the other lateral line receptors were also found under the epidermis. The size of the superficial neuromasts was significantly different among larval (20.33±5.13 μm, n=4 cells from five individuals) and adult males (9.52±5.13 μm, n=4 cells from two males, Mann-Whitney U test, P=0.008).

**Distribution**

Larval salamanders had an average of 144.40 (n=5, range=101–182) neuromasts (Table 1) on each side of the head. Although the number of neuromasts tended to decrease in adult salamanders, the difference was not significant (P=0.064, Table 1). Metamorphosed salamanders had on average 103.50 (n=2, range=86–121) neuromasts; adult females had 89.5 (n=4, range=62–109), and adult males had 105.5 (n=4, range=99–118) (Table 1).

We confirmed the presence of superficial neuromasts on both the head and the body of larval and adult salamanders. Eight superficial neuromast lines were identified on the surface of the head (Fig. 2B), based on the Northcutt (1992) study: the supraorbital neuromast line (SO), infraorbital line (IO), postotic line (PO), preopercular line (POP), oral line (OR), jugal line (JU), mandibular line (MA), and angular line (A).

The SO extended as a single row from the rostral tip to the orbital orbit, then extended approximately to the middle of the head, parallel to the rostrocaudal body axis. At the rostral tip, the SO branched, perpendicular to the rostrocaudal body axis (Fig. 2A, B). The IO was positioned under the chin, the MA was located parallel to the rostrocaudal body axis. The PO began a short distance from the caudal end of the IO and extended to the rostral end of the main trunk neuromast line at the upper base of the external gill (Figs. 2B, 3). This main PO lay at an approximately 40° angle to the rostrocaudal body axis. In the middle of this line, a line branched and extended dorsally, perpendicular to the main PO. A semicircular POP formed just below the caudal end of the PO and extended ventrally to meet the JU, MA, and gular pit line. The POP was roughly perpendicular to the rostrocaudal body axis. The OR formed along the tip of the lower jaw. At the edge of the mouth, where the OR ended, the JU started and extended as a single row to the POP. In the middle of the epidermis under the chin, the MA was positioned parallel to the rostrocaudal body axis. Rostrally, the MA split into two rows, but caudally, it congregated into a single row. The MA

### Table 1.

<table>
<thead>
<tr>
<th>Individuals used</th>
<th>Metamorphosed (n=1 or 2)</th>
<th>Adult female (n=4)</th>
<th>Adult male (n=4 or 3)</th>
<th>Kruskal-Wallis test among larva and adult female and male, H, P</th>
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<tbody>
<tr>
<td>No. of neuromasts (range)</td>
<td>90.7±3.02 (range=86–121)</td>
<td>76.84 (range=62–109)</td>
<td>61.15 (range=50–90)</td>
<td>0.022</td>
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<tr>
<td>No. of ampullary organs</td>
<td>7.20±0.10 (range=5–7)</td>
<td>6.00±0.10 (range=4–8)</td>
<td>4.00±0.10 (range=2–6)</td>
<td>0.008</td>
</tr>
<tr>
<td>No. of pit organs</td>
<td>14.00±1.00 (range=10–12)</td>
<td>12.00±1.00 (range=8–10)</td>
<td>9.00±1.00 (range=6–8)</td>
<td>0.093</td>
</tr>
<tr>
<td>No. of neuromasts except tail parts</td>
<td>49.2±4.00 (range=42–50)</td>
<td>36.0±4.00 (range=29–47)</td>
<td>24.0±4.00 (range=17–31)</td>
<td>0.008</td>
</tr>
<tr>
<td>No. of neuromasts in the main trunk line except tail parts</td>
<td>10.0±1.00 (range=6–12)</td>
<td>8.0±1.00 (range=4–9)</td>
<td>5.0±1.00 (range=3–7)</td>
<td>0.008</td>
</tr>
<tr>
<td>No. of neuromasts in the ventral line</td>
<td>23.0±2.00 (range=19–27)</td>
<td>20.0±2.00 (range=16–24)</td>
<td>17.0±2.00 (range=14–20)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

**Physical parameters of individuals**

<table>
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<tr>
<th>SVL (cm)</th>
<th>Body weight (g)</th>
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<td>2.59 (2.25–3.00)</td>
<td>0.41 (0.30–0.50)</td>
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<tr>
<td>2.35 (2.7.2–2.8)</td>
<td>0.55 (0.8–0.8)</td>
</tr>
<tr>
<td>6.79 (6.50–7.40)</td>
<td>7.23 (5.72–6.60)</td>
</tr>
<tr>
<td>6.29 (5.72–7.50)</td>
<td>5.75 (3.80–10.90)</td>
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</tbody>
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Fig. 1. Photomicrographs of flat-mounted (A, C, E) and sectioned (B, D, F) skin showing (A, B) a neuromast, (C, D) a pit organ and (E, F) an ampullary organ. Abbreviations: h, hair cell; su, supporting cell; mc, mantle cell; b, basal cell. Bars=30 μm.
finally met the POP caudally. The A was arranged between the IO and OR, perpendicular to the rostrocaudal body axis. The eight lines in the larval salamanders were confirmed at least once in the adult salamanders, although the number of neuromasts in each line was reduced and the lines were not very distinct.

**Superficial neuromasts on the body skin**

The superficial neuromasts on the skin of the body were arranged in three different lines (Fig. 3): the dorsal (DL), main trunk (ML), and ventral (VL) lines. Other types of lateral line receptors were not found on the body surface. The degree of roundness of the superficial neuromasts was similar to that of the superficial neuromasts found on the surface of the head and was not different between larval and adult salamanders: 1.49±0.08 for larvae, 1.41±0.04 for adult females, and 1.47±0.11 for adult males (P=0.625). The size of the superficial neuromasts found on the head and body was similar among larval and adult female and male salamanders: 90.87±6.52 μm for larvae, 96.95±5.95 μm for adult females, and 116.44±7.14 μm for adult males (H₂=5.76, P=0.056, Table 1). The size of the superficial neuromasts of metamorphosed salamanders was similar to that in larval salamanders: 85.25±3.73 μm.

We counted all neuromasts on one side of the body of one larval salamander and found 83 superficial neuromasts. In most samples, counting the number of all neuromasts of an individual was not possible because the tail contained numerous lipid droplets and was very fragile; furthermore, parts of the tail skin were generally lost during sample preparation. Therefore, to determine the number of superficial neuromasts on the body, we counted the number of neuromasts that appeared only on the main body trunk and excluded the tail portion behind the hindlimbs. The number of superficial neuromasts on one side of the body was similar between larval and adult salamanders, averaging 49.2 for larvae, 46 for adult females, and 42.25 for adult males (P=0.080, Table 1). Metamorphosed salamanders had on average of 44 (n=2, range=42–46) neuromasts.

The DL on the surface of the body extended from the middle part of the main body trunk to the rear end of the hindlimbs and was positioned above the ML. This line consisted of 4–10 neuromasts (Fig. 3). The number of neuromasts in the line was not different between larval and adult salamanders (P=0.444, Table 1). The orientation of the sensory hair cells of the superficial neuromasts on the body was consistent with the orientation of the neuromasts and the neuromast lines (Fig. 3). The orientation of the dorsal neuromasts was roughly perpendicular to the rostrocaudal body axis (Fig. 3). The ML, where most neuromasts were embedded, was connected rostrally to the caudal end of the POP on the head and extended caudally to the tip of the tail (Fig. 3). The line was parallel to the rostrocaudal body axis. In the ML, adult males had an average of 14.75 neuromasts, whereas larvae and adult females had an average of 18.8 and 17 neuromasts, respectively. The difference between...
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The number of neuromasts in the VL ranged from 18 to 30, and no differences were found between larval and adult salamanders (*P*=0.828; Table 1). The VL, which lies parallel to the rostrocaudal body axis, extended ventrally from just behind the forelimbs to the front of the hindlimbs. The number of neuromasts in the VL ranged from 18 to 30, and no differences were found between larval and adult salamanders (*P*=0.828; Table 1).

**Pit organ**

**Morphology**

The pit organ was circular and smaller than the superficial neuromast, but was larger than the ampullary organ (Table 1). The ratio of the longest and shortest diameter of the pit organ was similar among larval and female and male adult salamanders: 1.14±0.01 for larvae, 1.16±0.02 for adult females, and 1.15±0.03 for adult males (*P*=0.726). The size of the pit organ was not different among larval and female and male adult salamanders (*P*=0.093, Table 1): 80.46±11.09 μm for larvae, 76.84±6.62 μm for adult females, and 112.72±9.15 μm for adult males. The pit organ of metamorphosed salamanders was similar in size to that in larval salamanders: 79.95±1.86 μm. The pit organ consisted of hair cells, supporting cells, and mantle cells (Fig. 1C, D). The elongated mantle cells were more distinct in adult salamanders, due to the position of the mantle cells, which were sunken below the epidermal surface (Fig. 6A, B), than in larval salamanders (Fig. 5B, C). We also observed several basal cells under the supporting cell layer (Fig. 1D).

The orientation of the sensory hair cells of pit organs was not distinct in larval salamanders (Fig. 5). In adult salamanders, the sensory hair cells of some pit organs were directionally oriented (Fig. 6D, F), but those of other pit organs were not (Fig. 6C, E). Two individuals (one male and one female) out of four had pit organs in which the sensory hair cells were directionally oriented, whereas the sensory hair cells of the other two individuals were not. If the sensory hair cells exhibited directionality, this was consistent with the directionality of the pit organ and the pit line. In the pit organs that exhibited directionality, the microvilli of several supporting cells bundled into a group and resembled a bud-

**Fig. 4.** SEM images of neuromasts from larval (A, C, E) and adult (B, D, F) *Hynobius leechii*. Abbreviations: Cu, cupula; su, supporting cell; mc, mantle cell; k, kinocilium; st, stereocilia; m, microvilli. Bar in E=3 μm; bars in other panels=10 μm.
ding flower (Fig. 6D, F), whereas in the pit organs that did not exhibit directionality, the microvilli of the supporting cells did not form a group (Fig. 6C) or formed only a very weak group (Fig. 6E). The supporting cell microvilli in some pit organs of larval salamanders were longer than those of other supporting cells, regardless of the length of the kinocilia (Fig. 5C, D).

We were often able to identify the stereocilia on the sensory maculae of larval pit organs (Fig. 5A, D), but these stereocilia were not clearly visible in adult salamanders due to the elongated microvilli of the supporting cells (Fig. 6C–F). In superficial neuromasts, the stereocilia surrounding each kinocilium were always clearly distinct (Fig. 4D, E).

Adult salamanders had more kinocilia on the sensory hair cells of the pit organ than did larval salamanders: 5.19±0.90 (n=21 cells from five individuals) for larvae and 14.38±3.31 (n=8 cells from two males and one female) for adult salamanders (Mann-Whitney U test, z=2.938, P=0.003). We did not find the cupula in larval salamanders, even though we detected relatively long (>25 μm) kinocilia on the sensory hair cells of the pit organs (Fig. 5A, B). However, in one adult male specimen we confirmed the presence of the cupula in two pit organs in which the sensory hair cells exhibited directionality (Fig. 6B). The two cupulae were longer (70.62 and 87.60 μm) than those of the superficial neuromasts (49.10±7.05 μm, n=3) in the same individual. In these pit organs, although the cupulae encircled most parts of the kinocilia, we observed the long, bare stalks of the kinocilia, which were not embedded in the cupula and not surrounded by the microvilli of the supporting cells (Fig. 6D, F). The bare stalks in the pit organs were significantly longer than those in the superficial neuromasts: 6.82±0.33 μm (28 kinocilia from six cells) for the pit organs and 2.49±0.48 μm (19 kinocilia from three cells) for the superficial neuromasts (Mann-Whitney U test, Z=5.307, P<0.01). This difference was often important for discriminating pit organs from superficial neuromasts in certain adult individuals under the SEM.

**Distribution**

The number of pit organs per side of the body was different among larval and female and male adult salamanders (H₂=7.92, P=0.019, Table 1): 18 (range=13–25) for larvae, 10 (range=6–13) for adult females, and 11.25 (range=10–13) for adult males. In particular, adult females had fewer organs than had larval salamanders (P<0.05). Metamorphosed salamanders had on average 13 (n=2, range=12–14) pit organs.

Pit organs were found only on the surface of the head, in four different receptor lines in different areas (Fig. 2B). The pit organs were arranged in a single row in all lines. The anterior pit line (AP) lay just above the SO, between the rostral and caudal ends of the orbit. The AP contained one to six pit organs, and their orientation was parallel to the rostrocaudal body axis. The middle cheek pit line (MC) was located at the center of the cheek, just below the rostral end of the POP. We found zero to two pit organs in the MC. The middle pit line (MP) was positioned at the rostral end of the main PO and contained three to eight pit organs. The MP was perpendicular to the rostrocaudal body axis. The gular (G) pit line was located at the center of the gular, just below the rostral end of the POP. We found zero to two pit organs in the GC. The middle pit line (MP) was positioned at the rostral end of the main PO and contained three to eight pit organs. The MP was perpendicular to the rostrocaudal body axis. The gular (G) pit line contained the highest number of pit organs: 10 were observed in a larval salamander. This line was easily discriminated from other pit lines or superficial neuromast lines by its position at the most caudal point of the lower jaw. The G lay caudally below the MA and was positioned roughly perpendicular to the rostrocaudal body axis. In adult salamanders, the number of pit organs in the pit lines was...
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We found no pit organs in the MC of most adults, and we often found only a single pit organ in the AP. However, we always found more than two pit organs in both the MP and G.

**Ampullary organ**

**Morphology**

The ampullary organ was found to be a circular receptor that consisted of hair cells, supporting cells, and mantle cells (Fig. 1E, F). The hair cells were located near the supporting cells. Elongated mantle cells of the ampullary organ encircled the supporting and hair cells more completely than did mantle cells of the neuromast and pit organ. The ratio between the longest and shortest diameter of the ampullary organ was similar among larval and female and male adult salamanders: 1.11±0.01 for larvae, 1.16±0.04 for adult females, and 1.13±0.04 for adult males (P=0.897). The ampullary organs of adult males and females were larger than those of larval salamanders: 43.44±2.83 μm for larvae, 66.70±2.95 μm for adult females, and 77.65±5.04 μm for adult males (H=9.76, P=0.008; Table 1). The size of the ampullary organs of metamorphosed salamanders was similar to that of larval salamanders; it was 40.08±1.60 μm (Table 1). The degree to which ampullary organs were sunken below the epidermal surface differed based on their location. The ampullary organs were more sunken below the epidermis in the rostral area (Figs. 2A, 7A), whereas they were approximately level with the epidermal surface at the center and rear of the head (Fig. 7C). This difference was more obvious in larval than in adult salamanders.

Under the SEM, mantle cells in the ampullary organ were not clearly present in larval salamanders (Fig. 7A, C); however, they were evident in adult salamanders, because the mantle cells were further developed and formed a distinctive boundary with the epidermal surface (Fig. 7B, D, F). In sunken ampullary organs, some parts of the organ were roofed with mantle cells, but most parts were still visible from above (Fig. 7A). The morphology of the microvilli on the sensory hair cells of the ampullary organ was similar between larval and adult salamanders (Fig. 7C, D).

![Fig. 6. SEM images of two morphological types of pit organ in adult Hynobius leechii: (A, C, E) one type; (B, D, F) the other type. Abbreviations: Cu, cupula; su, supporting cell; mc, mantle cell; k, kinocilium; st, stereocilia. Bars=10 μm.](image-url)
We found kinocilia on the sensory hair cells of the ampullary organ (Fig. 7B, C, E), and the number of kinocilia was similar between larval and adult salamanders: 2.75±0.63 (n=16 cells from five individuals) for larvae and 1.4±0.87 (n=5 cells from two individuals) for adult salamanders (Mann-Whitney U test, \( P = 0.354 \)). The kinocilia were about 1.5 \( \mu \)m long. Hair cells on the ampullary organ were not directionally oriented. We observed no cupulae on the sensory hair cells of either larval or adult ampullary organs.

**Distribution**

The ampullary organ was only found on the head, where it was distributed at or within the superficial neuromast and pit organ lines (Fig. 2B). In larval salamanders, many ampullary organs were found in the MA and POP (Fig. 2B). We did not find a group of ampullary organs near the external gill. We often found two ampullary organs that were joined together or a single organ that had two sensory maculae (Fig. 7F). The number of ampullary organs per side of the body differed significantly between larval and female and male adult salamanders: 86 for larvae, 41.5 for adult females, and 64 for adult males (\( H_2=7.62, P=0.022 \); Table 1). In particular, there were fewer ampullary organs in adult female salamanders than in larval salamanders (\( P<0.05 \)). A metamorphosed salamander had 69 ampullary organs.

**DISCUSSION**

We have described the morphological characteristics of the lateral line receptors (the superficial neuromast, the pit organ, and the ampullary organ) of *H. leechii*, a species that externally fertilizes its eggs. This detailed description of the pit organ in urodeles is only the second study of its kind. This study, comparing the receptors of larval and adult salamanders was also the first conducted for salamanders with external fertilization. In addition, we report the presence of kinocilia on the hair cells of the ampullary organ and describe two different morphologies of the pit organ in adult salamanders. Upon the transition from a larval to an adult salamander, the number of lateral line receptors generally decreased, whereas the size of the receptors increased.

We could discern three types of lateral line receptor in...
larvae, metamorphosed salamanders one week after metamerism, and adult females and males, but could not find the receptors in metamorphosed salamanders one month after metamorphosis, possibly as a result of acclimation to life on land (Fritzsch, 1990). Since breeding adult male and female salamanders have well-developed lateral line receptors, it is unlikely that lateral line receptors permanently degenerate during terrestrial life in metamorphosed salamanders. Rather, it is likely that the receptors dedifferentiated, starting with post-larval terrestrial life, and that a thick epidermal layer covered the receptors (Russell, 1976; Fritzsch and Wahrnschaff, 1983; Fritzsch et al., 1987). Additional histological studies of the skin of metamorphosed and non-breeding adult salamanders are thus necessary to discriminate whether terrestrial forms of *H. leechii* possess dedifferentiated receptors under the epidermis or, alternatively, lose their receptors during metamorphosis.

*Hynobius leechii* was found to possess four basic superficial neuromast lines: the SO, IO, OR, and MA, as is the case for most urodeles (Northcutt, 1989, 1992). The presence of additional four neuromast lines (A, POP, JU, and PO), as described in the axolotl (Northcutt, 1992), was clearly demonstrated in larval *H. leechii* salamanders. In addition, the identification of three different neuromast lines on the surface of the body was similar to previous results from other urodele studies (Northcutt, 1992). Lateral line receptors of *H. leechii* did not develop stitches, a result that is consistent with previous findings (Lannoo, 1987; Fritzsch and Neary, 1995). In particular, the G, which is considered to be an SO in the Chinese giant salamander (*Hong* et al., 1995), was determined to be a G, based on the distinctive morphology of the pit organ under the SEM. This result is consistent with those of axolotl studies (Northcutt, 1992; Northcutt et al., 1994). In adult salamanders, the pit line becomes obscure due to the reduced number of receptors in most of the lines. In addition, we found that some lateral line receptors were sunken beneath the epidermal surface; this morphology was first reported in urodeles (Fritzsch, 1989). If a receptor type was sunken in an individual, some cells of the other receptors types were also found under the epidermis; this may have been caused by health conditions or by the length of the period that the individual stayed in the water. Further studies might reveal whether or not such a sunken morphology is normal and what conditions contribute to it.

We found the pit organ in four different regions on the surface of the head of *H. leechii*. Unlike the pit line of the axolotl (*Northcutt and Bleckmann, 1993*), each pit line was composed of a single row of two to 10 receptors. Since the study of the Chinese giant salamander did not clearly describe the morphology of the pit organ (*Hong et al., 1995*), our detailed description of the pit organ in *H. leechii* is the first for externally fertilizing salamanders. In adult salamanders, some pit lines, but not the G, were indistinct due to the reduced number of receptors in these lines. Several stereocilia supported kinocilia on the sensory hair cells of the pit organ, and this result is consistent with those of the axolotl study (*Northcutt and Bleckmann, 1993*). Meanwhile, unlike the results of the axolotl study (*Northcutt and Bleckmann, 1993*), we detected no cupulae in larval salamanders. It is not clear whether the larval pit organ develops a cupula, considering that kinocilia >20 μm long were still present without cupulae in larval salamanders.

Specifically, we found two different morphologies of pit organ in adult salamanders. The first morphology was similar to that of the pit organ of larval salamanders in which sensory hair cells did not exhibit directionality and the number of kinocilia was around 10. This morphology is also similar to the pit organ described in the axolotl study (*Northcutt and Bleckmann, 1983*). The second type of pit organ displayed strongly directional sensory hair cells, and the number of kinocilia was >20, similar to the case of superficial neuromasts. We found the second type of pit organ in all different pit lines in one male and one female among the four individuals examined, suggesting that the second type of pit organ may exist at high frequency. This type of pit organ has not previously been reported for urodeles. It will be interesting to determine the percentage of individuals possessing the second type of pit organ and whether physiological properties and functions differ between the two different morphological types of pit organ.

Both larval and adult salamanders have ampullary organs. We found kinocilia on the sensory hair cells of the ampullary organ, as did previous studies of caecilian and cave salamanders (*Hetherington and Wake, 1979; Fritzsch and Wahrnschaff, 1983; Istenic and Bulog, 1984*). In *H. leechii*, the sinking of the organ below the epidermal surface was not obvious, unlike in the Chinese giant salamander (*Hong et al., 1995*). From above, we could always observe >80% of the sensory hair cells of the ampullary organ. In adult salamanders, the number of ampullary organs decreased, but their size increased. Development of the mantle cell in adults was more distinct. Some of these differences in the ampullary organ between larval and adult salamanders have been noted previously (Fritzsch and Bolz, 1986). Urodele adults that have ampullary organs often eat and breed under dim light or in the dark (*Istenic and Bulog, 1984*). Considering that *H. leechii* usually breeds at night under dead leaves or rocks (*Park and Park, 2000*), the ampullary organ may play an important role in mating. In addition, electrical sensing by larval *H. leechii* could be helpful in decreasing the possibility of cannibalism by detecting approaching potential cannibalistic larvae (*Park et al., 2005*) through changes in the surrounding electromagnetic field.

The reproductive function of the mechanosensory lateral line system in vertebrates has not been well explored. Previous electrophysiological studies on the axolotl (*Munz et al., 1982*) and a recent abstract (*Jeon et al., 2005*) showed that *H. leechii* males and females respond to water vibrations generated by another male, meaning that the lateral line system could function to detect reproductive signals in this species. Morphological studies of the lateral line system could facilitate studies about the functional significance of this system in the mating of salamander species with external fertilization.

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