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Vestibular Ganglion Cells in Developing Chick Embryo

Hiroshi Yamashita, Toru Sekitani, Shiro Endo, Hiroaki Shimogori and Kenji Okami

Department of Otolaryngology, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan
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Abstract The morphologic development of the vestibular ganglion and nerve and otic vesicle in the embryos of White Leghorn chicken was studied in chick embryos from 3.5 days of age up to one day after hatching. In 3.5-day-old chick embryos, the seventh and eighth cranial nerve ganglia appeared as a single facial-acoustic ganglion mass near the otocyst. In 4-day-old chick embryos, a facial-acoustic ganglion mass was divided into the two parts, the primitive stato-acoustic ganglion medially and the primitive geniculate ganglion laterally. The vestibular and spiral ganglia from the stato-acoustic ganglion were completely separated in 6-day-old chick embryos. Penetration of vestibular nerve fibers into the sensory area of the otocyst took place in chick embryos incubated during 6 to 8 days, since the macula statica and crista ampullaris appeared in 6-7-day-old and in 7-8-day-old chick embryos, respectively. In 12-day-old chick embryos, vestibular ganglion cells were arranged regularly in the direction proceeding from the otocyst to the brain, but cell size was variable as evident from bipolar and small round cells present in the ganglion.

Key Words : Vestibular ganglion cells, Embryonic development, Morphology, Chick embryo

Introduction

The embryological investigations of the inner ear have been well documented, but the vestibular ganglion and the vestibular nerve fibers have not been studied embryologically except in a few reports of Anniko (1) and Van De Water (2). The chick embryo develops in a short period of time and it is easy to obtain homogeneous sample according to the stage classification set up to specify each developmental stage. In chick embryos, each organ matures during the embryological phase (3) and is thus ideal for embryological study. In the following are presented some light-microscopic and scanning electron microscopic findings on the vestibular ganglion and vestibular nerve of chick embryos at various stages of development.

Materials and Methods

1) Animals

A total of 125 developing chick embryos from 3 days of incubation until 1 day after hatching were used in this study.

Fresh hatchery eggs of Babcock layer, White Leghorn hens, were obtained within two days after laying. To avoid natural embryogenesis before incubation, the eggs were preserved at 18～20℃. They were placed in an incubator adjusted to an optimal incubation temperature of 38℃ and humidity of 60%. (incubator : Showa Furanki Co. P-300)

2) Light microscopy

Ear regions of chick embryos at each incubation stage were removed under a stereoscopic

microscope, fixed in 10% neutral buffered fromalin and embedded in paraffin. Sections of 5-6 μm thickness were stained with Hematoxylin -eosin stain (H.E.) and Klüver-Barrera stain (K. B.), and the otocyst, vestibular nerve, and vestibular ganglion were examined with light and differential interference microscopes.

3) Scanning electron microscopy (SEM)

The otocyst or portion corresponding to the inner ear was dissected, fixed in a mixture of 2. 5% glutaraladehyde and 2% paraformaldehyde and postfixated with 1% osmic acid. After dehydration in an ethanol series, the tissues were

Table 1 Development of vestibular ganglion

incubation day	mitosis	size major minor	shape	nucleus : cytoplasm	meylo-genesis	Nissl's substances	palisade arrangement
3.5		5 ~ 7 × 3 ~5	round~	5 : 1			
4		5 ~ 8 × 4 ~ 7	elliptic	4 : 1			
5		9 ~ 5 × 4 ~ 7		4 : 1			
6		10~ 6 × 7 ~ 5		3 : 1			
7		10~ 6 × 7 ~ 5		3 : 1			
8		15~ 8 ×10~ 5		2 : 1			
9		16~ 8 ×12~ 6	elliptic~	1 : 1			
10		20~10×14~ 6	spindle	1 : 1			
11		20~10×14~ 6		1 : 1			

(μm)

Table 2. Development of otocyst and vestibular nerve

incubation day	endo-lymphatic duct	saccule	utricle	semicircular duct	ampulla of s.d.	sensory cells	nerve fiber
3.5							
4							
5							
6							
7							
8							

dried with a critical point dryer, and coated with a layer of platinum, and the vestibular ganglion was examined under a scanning electron microscope (JSM-T300).

Results

1) Light microscopic findings of the otocyst, vestibular ganglion cells and vestibular nerve (Tables 1 and 2).

A facial-acoustic ganglion mass (4) was observed on the rostral side of the otocyst in 3.5-day-old chick embryos. The wall of the otosyst protruded into the lumen at a point in contact with the ganglion mass (Fig.1). The ganglion mass was composed of cells with a little cytoplasm showing mitosis. The otocystic wall consisted of pseudo-stratified epithelium containing 4-5 nuclear layers. The epithelial cells had a round or oval nucleus. In 4-day-old chick embryos, the facial-acoustic ganglion mass separated into primitive stato-acoustic (2) and geniculate ganglia and proceeded toward the brain (Fig.2). In 5-day-old chick embryos, the utricle, saccule, and semicircular duct could be identified. The stato-acoustic ganglion moved further toward the brain and started dividing into 2 masses, the vestibular and spiral ganglia. In 6-day-old chick embryos, both the vestibular and spiral ganglia were completely separated (Fig.3). At this time, ampullae of the semicircular ducts were noted. In the macula of the utricle and saccule, sensory cells were found and otoconia were observed on their surfaces. In 7-day-old chick embryos, axonal and dendritic nerve fibers extending from the vestibular ganglion cells were well observed and a bundle of dendritic nerve fibers had already reached the sensory epithelia of the utricle and saccule (Fig.4). The ampullar epithelium was connected to a bundle of vestibular nerve fibers in 7-8-day-old embryos (Fig.5). In 9-day-old chick embryos, myelogenesis took place in the vestibular nerve and Nissl bodies appeared in the cytoplasm of the ganglion cells (Fig.6,7).

2) Scanning electron microscopy of vestibular ganglion cells

Vestibular ganglion cells in 12-day-old chick embryos were bipolar in shape (Fig.8). In 14-day-old embryos, satellite cells in the vestibular ganglion could be identified (Fig. 9). In 16-day-old embryos, most of the vestibular ganglion cells clearly showed bipolar shape, having axonal and dendritic fibers on the both sides of the cell body (Fig. 10), and a small round cell having a nerve spike was observed (Fig.11). In 18-day-old chick embryos, well defined spindle-shaped bipolar cells were noted (Fig.12). They were mostly elliptic or spindle shaped, occasionally spherical, and measured 10～30μm in major diameter and 7～14μm in minor diameter.

Discussion

The facial-acoustic ganglion mass appeared near the otocyst in 3.5-day-old chick embryos and continued to divide into the geniculate, vestibular and spiral ganglia up to the 6-day-old chick embryos. Mitosis of vestibular ganglion cells was observed up to 4-day-old chick embryos, but not 5-day-old chick embryos whose vestibular and spiral ganglia were separated. Similarly, in mice, mitotic activity of vestibular ganglion cells was noted by the 12th gestational day (6), but not on the 14th gestational day when vestibular and spiral ganglia were separated (4). Sher (4) reported that in mice, both ganglia were separated at the time nerve fibers became connected to the sensory cells of the otocyst. Similar findings were obtained from chick embryos. Friedman (7) reported that nerve axons, nerve endings and other synaptic structures were absent within the neuroepithelium of embryos not older than 3-day-old to 5-day-old chick embryos. In the present study, the vestibular nerve fibers connected to the sensory cells of the otocyst in 6～8-day-old chick embryos. Anniko (1) reported that Schwann cells enclosed vestibular ganglion cells and their peripheral nerve fibers in mice on the 15th-16th gestational day. Peusner (5) found myelogenesis of the vestibular nucleus to start in 8～9-day-old chick embryos. We showed that myelogenesis of vestibular nerve fibers and ganglion cells occurred in 9-day-old chick embryos. Landolt (8) observed vestibular

ganglion cells in a pigeon to greatly vary in size, by scanning electron microscopy. Essentially the same was noted only in the developmental stage of embryos of this study. Knowlton (9) described that major morphogenetic change was complete in 7-day-old chick embryos, this being in agreement with our finding that the primordium of each organ of the inner ear including the vestibular ganglia was most evident in 7-day-old chick embryos and that the formation and development of the vestibular sensory organs was closely related to the maturity of vestibular ganglion cells.

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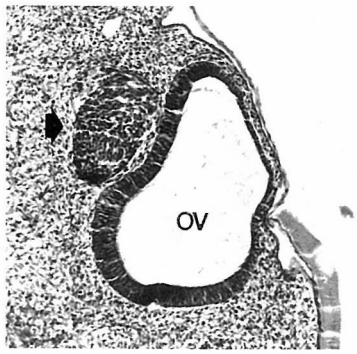

Fig 1 : 3.5-day-old chick embryo.
Arrow indicates to the facial-acoustic ganglion. H.E.×40
ov : otic vesicle

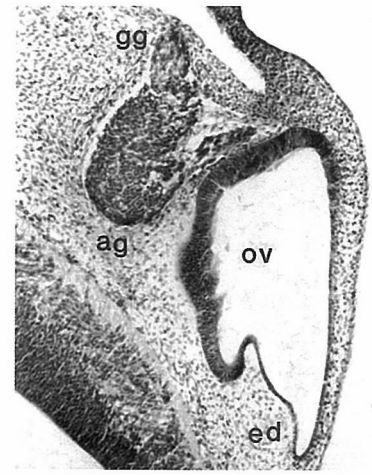

Fig 2 : 4-day-old chick embryo. A stato-acoustic ganglion has moved to the medial side. H.E.×40 ag : stato-acoustic ganglion gg : geniculate ganglion ed : endolymphatic duct

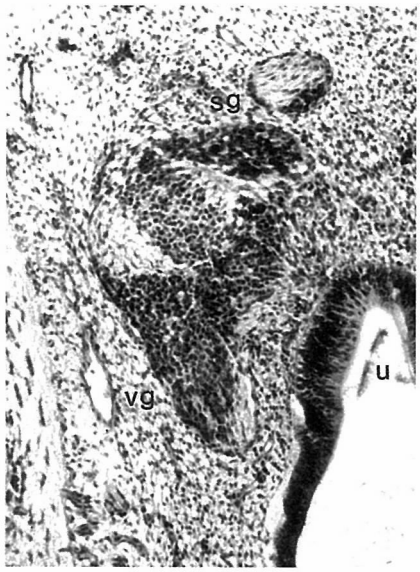

Fig 3 : 6-day-old chick embryo. A stato-acoustic ganglion has devided into 2 parts, anlages of vestibular and spiral ganglia. Dense mesenchymal tissue is noted between them.
H.E.×50 vg : vestibular ganglion sg : spiral ganglion u : utricle

Fig 4 : 7-day-old chick embryo. A vestibular ganglion with nerve fibers located between the utricle and rhombencephalon.
K.B.×25 s : saccule u : utricle
lc : lateral semicircular canal vfp : peripheral fibers of vestibular nerve
vfc : central fibers of vestibular nerve

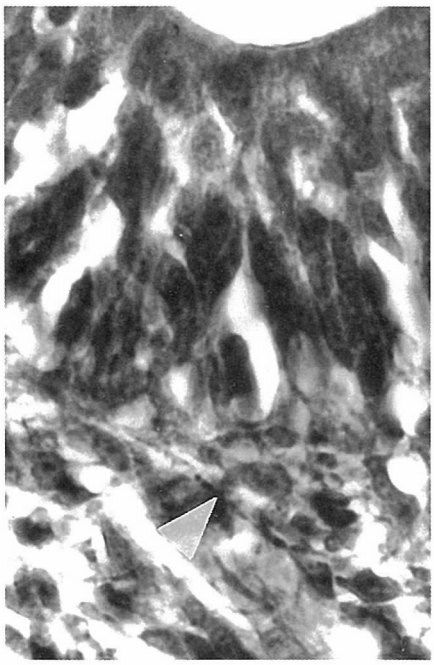

Fig 5 : 7-day-old chick embryo. A vestibular nerve fiber is connected to an ampullar epithelial cell (arrowhead). K.B.×500

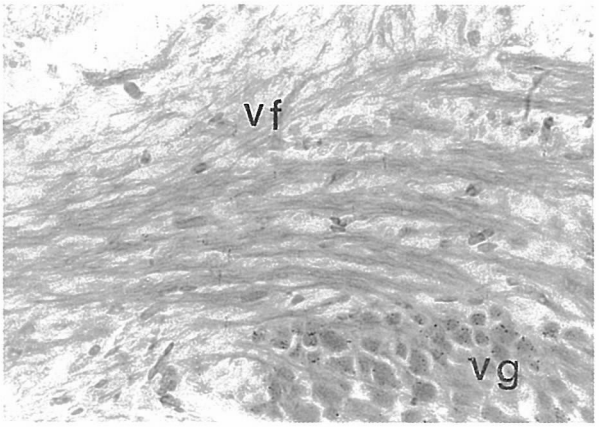

Fig 6 : 10-day-old chick embryo. Vestibular nerve fibers have myelin sheaths stained with luxol fast blue. LFB×160

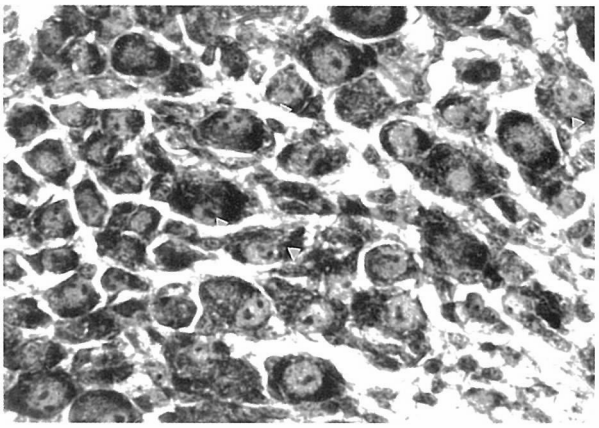

Fig 7 : 10-day-old chick embryo. Vestibular ganglion cells contain large numbers of Nissl bodies. Arrowheads point to satellite cells. K.B.×200

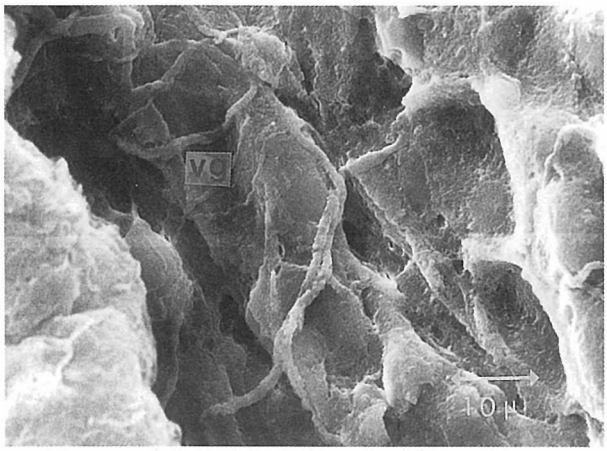

Fig 8 : 12-day-old chick embryo. A vestibular ganglion cell bipolar in shape. vg : vestibular ganglion

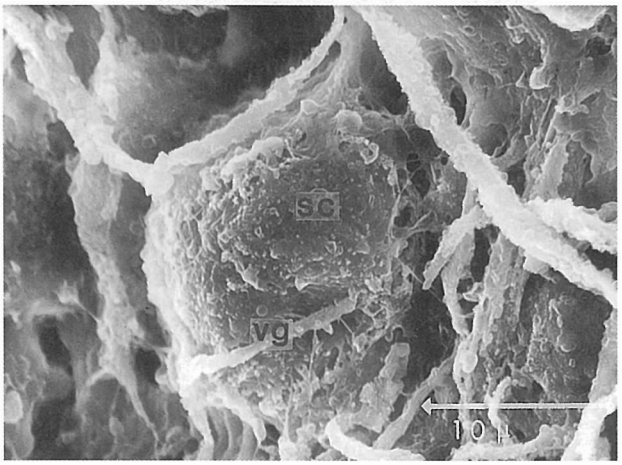

Fig 9 : 14-day-old chick embryo. A satellite cell can be seen on the vestibular ganglion cell. sc : satellite cell

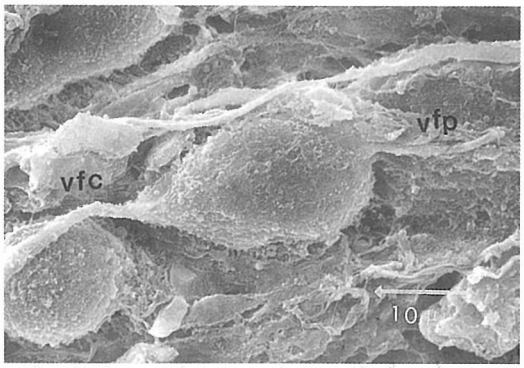

Fig 10 : 16-day-old chick embryo. Two vestibular ganglion cells, one small and round cell and the the large and spindle-shaped. vfp : peripheral fiber of vestibular nerve vfc : central fiber of vestibular nerve

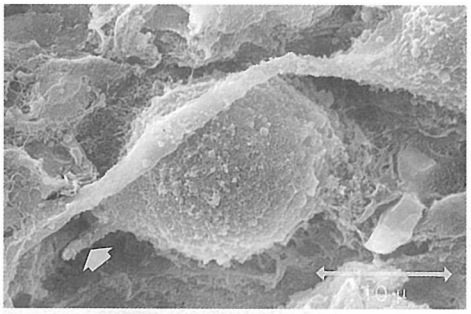

Fig 11 : 16-day-old chick embryo. A small round cell having a nerve spike (arrow).

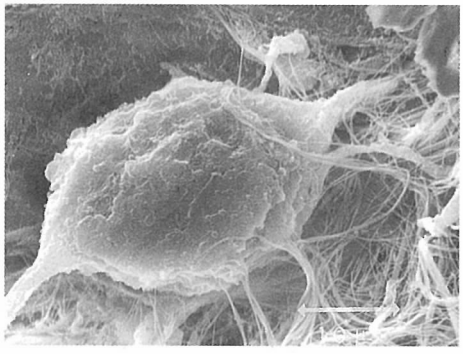

Fig 12 : 18-day-old chick embryo. A well defined bipolar vestibular ganglion cell spindle in shape.