Histological Studies on the Pineal Organ of 15 Species of Teleosts*

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Since the work of DODT¹⁾, the photosensitivity of pineal organ of fish has been detected electrophysiologically in some fishes²⁻⁵⁾. Furthermore, some structural evidences for the photosensory function are presented by the electron microscopic studies on piscine pineal organs.⁶⁻¹⁰⁾ However, only a few species of fishes were used in these studies. On the other hand, some observations had been made on the pineal window of deep sea fishes and migratory ones such as tunas and scombrids, taking phototaxis and color change of fish into account¹¹⁻¹⁴⁾. But no detailed histological informations are available on the pineal organ in various species of fishes.

The present report deals with histological investigations on the pineal organ of 15 species of teleosts, as the first step in the studies on the pineal organ of fishes.

Materials and Methods

The species used in the present examination are shown in Table 1. Using living fishes, firstly macroscopic observations were made on the pineal window which is found in the dorsal head skin between the lateral eyes. Then fishes were decapitated and entire heads were fixed in 10% neutral formalin, ZENKER-formol, BOUIN's or ELFTMAN's fluids for histological investigation. After fixing for 24-48 hours, the specimens were decalcified by means of electric decalcification, dehydrated in alcohol, and embedded in paraffin.

Table 1. List of fishes investigated.

Scientific name	Common name	Scientific name	Common name
<i>Salmo gairdnerii irideus</i>	rainbow trout	<i>Oryzias latipes</i>	medaka
<i>Salvelinus fontinalis</i>	brook trout	<i>Lebistes reticulatus</i>	guppy
<i>Plecoglossus altivelis</i>	ayu	<i>Mugil cephalus</i>	grey mullet
<i>Diaphus coeruleus</i>	hadakaiwashi	<i>Lateolabrax japonicus</i>	Japanese seabass
<i>Zacco temminckii</i>	kawamutsu	<i>Chrysophrys major</i>	porgy
<i>Carassius auratus</i>	wild goldfish	<i>Acanthogobius flavimanus</i>	Japanese goby
<i>Cyprinus carpio</i>	carp	<i>Choerodon azurio</i>	Ira
<i>Pelteobagrus undiceps</i>	bald goby		

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Serial sections of 6–10 μ were made and stained with MAYER's acid haemalum-eosin, GOMORI's chrome haematoxylin-phloxine, HEIDENHAIN's iron haematoxylin-light green or MALLORY's triple method. Some sections were stained also with 1% thionine, KLÜVER-BARRERA's luxol fast blue-cresyl fast violet or BODIAN-OTSUKA's method¹⁵⁾.

Results

The pineal organ of fishes consists of two parts; an anterior vesicular part (pineal vesicle) and a narrow posterior portion (pineal stalk). In many species of fishes, the pineal lumen found in the pineal vesicle is opened into the third ventricle of diencephalon (Fig. 1).

Fig. 1. Diagrammatic figure of sagittal section through the pineal region of teleostean fish. b: bony cranium, c: chondrocranium, d: dermis ds: dorsal sac, e: epidermis, hc: habenular commissure, m: muscle, ol: olfactory lobe, op: optic lobe, pc: posterior commissure, pl: pineal lumen, ps: pineal stalk, pv: pineal vesicle, so: subcommissural organ, tv: transverse velum.

Fig. 2. Pineal window of rainbow trout (semi-diagrammatic figure) pv: pineal vesicle observed through the window.

Pineal window: In some species of fishes the head skin above the pineal organ is translucent and lacking in melanophores. This translucent region of head skin was termed the "pineal window" by RIVAS¹³⁾ (Fig. 2). Through this window the pineal vesicle is visible as orange portion. This orange color is due to the rich vascularization of pineal organ. From the present investigation, the presence of pineal window was ascertained in the following species of fishes; *Salmo gairdnerii irideus*, *Salvelinus fontinalis*, *Plecoglossus altivelis*, *Diaphus coeruleus*, *Zacco temmincki*, *Carassius auratus*, *Cyprinus carpio* and *Mugil cephalus* (Plate I, 1). On the other hand, pineal window could not be detected in *Pelteobagrus undiceps*, *Oryzias latipes*, *Lebistes reticulatus*, *Lateolabrax japonicus*, *Chrysophrys major*, *Acanthogobius flavimanus* and *Choerodon azurio* (Plate I, 2).

Pineal epithelium: Among the fish species investigated, remarkable differences were observed in the histological structure of pseudostratified epithelium of pineal organ.

Fig. 3. Diagrammatic figures showing 6 types in the pineal epithelium of teleosts investigated in the present report. 1: flat type, 2: fold type, 3: convoluted type, 4: small space type, 5: compact type, 6: intermediate type.

From the present observations, the authors classified roughly the pineal organ of fishes into six types (Fig. 3). 1) Goldfish, *C. auratus* and carp, *C. carpio* have sac-like pineal organs. Primitive flat epithelium and a wide central lumen are observed in these organs (Plate I, 3). The authors applied the term "flat type" to such pineal organs. 2) In ayu, *P. altivelis*, the pineal epithelium increases in thickness and infolding is observed in some places (Plate I, 4). The authors applied the term "fold type" to such a pineal organ. 3) Thickness and infolding of pineal epithelium are remarkable and pineal lumen is detectable only as very narrow space (Plate I, 5) in the following species of fishes; *Salmo gairdnerii irideus*, *Salvelinus fontinalis*, *Diaphus coeruleus*, *Zacco temmincki* and *Acanthogobius flavimanus*. The term "convoluted type" was given to such a type of pineal epithelium. 4) In *Oryzias latipes* and *Lebistes reticulatus*, the pineal lumen diminishes in size and only remnant small spaces are found in some places of epithelium (Plate I, 6). Consequently, pineal lumen opening into the third ventricle cannot be found. The term "small space type" was given to this type of pineal epithelium. 5) In *Pelteobagrus unidiceps* and *Chrysophrys major*, the pineal organs are compact in structure and pineal lumen or small spaces could not be detected (Plate I, 7). The authors applied the term "compact type" to these pineal epithelia. 6) As for the pineal organs of *Mugil cephalus*, *Lateolabrax japonicus* and *Choerodon azurio*, a wide lumen and flat epithelium with infolding in some places are observed in the pineal vesicle. On the contrary, pineal stalk is formed of convoluted epithelium and narrow space lumen within the epithelium (Plate I, 8). The term "intermediate type" was applied to this type of pineal organ.

Photoreceptor cells: Many cytoplasmic processes protruding into the pineal lumen were detected along the epithelial lumen in the pineal organ of *Carassius auratus*, *Cyprinus carpio*, *Mugil cephalus* and *Choerodon azurio* (Plate II, 9–12). These processes seem to correspond to the inner and outer segments of the pineal photoreceptor cells which are

detectable under electron microscope. Accordingly, the cells with these processes seem to be pineal photoreceptor cells. In *C. auratus*, these processes are long and thick, and detectable in both regions of pineal vesicle and stalk (Plate II, 9). In *C. azurio* many small round processes are found along the lumen of pineal epithelium (Plate II, 10). These processes protruding into the pineal lumen were found often in the pineal organs of flat and intermediate types. On the contrary, these processes were detected occasionally in the organs of fold and convoluted types (Plate II, 13 and 14). Furthermore, detection and identification of these processes were difficult in the pineal organs of small space and compact types.

Basal cytoplasmic processes were detected also in these photoreceptor cells. These processes were stainable with HEIDENHAIN's iron haematoxylin (Plate II, 15 and 16). This indicates that the pineal receptor cells may be primary sensory cells.

Ganglion cells: From the present investigation, ganglion cells were detected in the following species of fishes; *Salmo gairdnerii irideus*, *Salvelinus fontinalis*, *Diaphus coeruleus*, *Cyprinus carpio*, *Pelteobagrus undiceps*, *Mugil cephalus*, *Lateolabrax japonicus*, *Chrysophrys major* and *Acanthogobius flavimanus* (Plate III, 17-22). However, in the other species of fishes, ganglion cells were not distinguishable. In many species of fishes, ganglion cells were located in the central region of the pineal epithelium (Plate III, 17-22). This central region was stained with eosin in faint color in the preparation of MAYER's acid haemalum-eosin stain, but detailed structure was obscure under light microscope (Plate III, 23). From the electron microscopic observation, it was confirmed that this region is "neuropile"⁽¹⁰⁾. Neuropiles are composed of numerous basal processes of the receptor cells, dendrites of the ganglion cells and fine processes of the neuroglia cells⁽⁶⁾. Compared with other kind of cells in pineal organ, the ganglion cell is a little larger in size and has a large nucleus and a clear nucleolus. Axon and dendrites were observed in the ganglion cells stained with 1% thionine (Plate III, 18 and 20). However, the ganglion cell in piscine pineal organ is not a typical one, because of scant amount NISSL substances within the cell. The axons of ganglion cells seem to form pineal tract and to penetrate into the pineal stalk. This pineal tract was recognized in the preparation stained with BODIAN-OTSUKA's method⁽¹⁵⁾ (Plate III, 24). However, the final destination of the pineal tract could not be clarified in the present investigation.

In addition to the photoreceptor and ganglion cells, other kinds of cells, supporting, ependymal and neuroglia cells, and present in pineal epithelium of fish. Histology of these cells will be clarified in our further investigation.

Discussion

In the fish having pineal window, it is conceivable that the environmental light can

reach pineal organ through the window. From the present investigation, however, 7 species of fishes were confirmed as the fishes lacking pineal window. In these fishes the region corresponding topographically to pineal window is made up of pigmented skin, developed muscle and calcified cranium. HOLMGREN¹¹⁾ and VILTER¹²⁾ described the pineal window in several species of deep sea fishes belonging to Clupeida and Myctophida. RASQUIN¹⁴⁾ found also the pineal window in some species of Cyprinida, Cyprinodontida and Percida. However, they did not made histological observations on the piscine pineal organs, in relation with photosensory function.

HOLMGREN¹⁷⁾ reported in brook trout, *Salvelinus fontinalis*, that the infolding of pineal epithelium was modified to fold during the growth of fish. HAFEEZ and FORD¹⁸⁾ obtained a similar result in sockeye salmon, *Oncorhynchus nerka*. According to KELLY¹⁹⁾, furthermore, the pineal epithelium of the newt, *Taricha torosa* is flat type in the early stage after hatching. Afterward, the epithelium increases in thickness and finally transform into compact type in late adult stage. As described above, remarkable histological variations were observed in piscine pineal organs and six types were distinguished. From the present observations and the reports mentioned above, the authors present a series of diagrammatic figures (Fig. 3). Namely, the pineal epithelium of a fish species in advanced phylogenetic position may be primitive flat type in early stage of growth. As the age advances, however, pineal epithelium may be transformed finally into the most developed compact type through fold, convoluted and small space types. In some species of fishes, pineal epithelium may differentiate toward intermediate type. In many other species, pineal epithelium may remain at various stages of development such as fold type, convoluted one and so on.

Although the identification of photoreceptor cells is difficult under light microscope, the cytoplasmic processes into the pineal lumen seem to correspond to inner and outer segments of the photoreceptor cells¹⁰⁾. As pointed out above, these processes are found very often in flat or intermediate type of pineal organ with a wide lumen. These processes were difficult to observe in the pineal organ of fold or convoluted type, and also could not be detected in compact or small space type. On the basis of these observations, it is suggested that the pineal photoreceptor cells may be modified to other type of cells with different function. To clarify this, further investigations are being carried out.

Spike discharges characteristic to ganglion cells were recorded by HANYU and NIWA^{3,4)} from the pineal vesicles of three species of teleosts, *Salmo gairdnerii irideus*, *Plecoglossus altivelis* and *Mugil cephalus*. In the present investigation, ganglion cells were detected in the pineal organ of 9 species of teleosts, even though they were not typical ones.

The present histological investigation provides some support to the following assumption-

tion. The environmental light penetrates through the pineal window and stimulates the processes of the photoreceptor cells. Excitation or inhibition of the photoreceptor cells is transmitted to the ganglion cells and then the information may reach the central nervous system by way of the pineal tract. However, further detailed studies are necessary before such a conclusion can be drawn.

Summary

Histological observations were made on the pineal organ of 15 species of teleosts and the following results were obtained.

1. The presence of pineal window was ascertained in 8 species of fishes.
2. Histologically, piscine pineal organs were classified into 6 types, i.e. flat, fold, convoluted, small space, compact and intermediate types.
3. The presence of cytoplasmic processes into the pineal lumen was confirmed in the pineal epithelium of flat, fold, convoluted and intermediate types.
4. Ganglion cells were detected in the pineal organ of 9 species of fishes, even though they were not typical ones.

With reference to the photosensory function, discussion was made on the histological structures of the pineal organ of fishes.

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Explanation of Plate I, II and III.

Plate I. Sagittal sections through the pineal region of various teleost species. 1: *Salmo gairdnerii irideus* with the pineal window, $\times 200$. 2: *Acanthogobius flavimanus* without the pineal window, $\times 200$. 3-8 show various types of pineal epithelium. 3: flat type (*Carassius auratus*), $\times 80$. 4: fold type (*Plecoglossus altivelis*), $\times 200$. Arrows show folds of pineal epithelium. 5: convoluted type (*S. gairdnerii irideus*), $\times 200$. 6: small space type (*Oryzias latipes*), $\times 200$. 7: compact type (*Pelteobagrus undiceps*), $\times 80$. 8: intermediate type (*Mugil cephalus*), $\times 200$. BOUIN's fixation (1-8). MAYER's acid haemalum-eosin (1-4, 6 and 7) and thionine stain (5 and 8).

Plate II. Arrows show cytoplasmic processes protruding into the pineal lumen (9-14), or basal processes of receptor cells (16). 9: *C. auratus*, $\times 800$. 10: *Choerodon azurio*, $\times 800$. 11 and 12: *M. cephalus*, $\times 800$. 13: *Diaphus coeruleus*, $\times 800$. 14-16: *S. gairdnerii irideus*, $\times 300$ (15) and $\times 800$ (14 and 16). BOUIN's fixation (9-16). H-E (9 and 14), thionine (10-12), KLÜVER-BARRERA's luxol fast blue-cresyl fast violet (13) and HEIDENHAIN's iron haematoxylin-light green stain (15 and 16).

Plate III. The ganglion cells (17-22), neuropile (23) and pineal tract (24) are shown in the Plate. 17 and 18: *S. gairdnerii irideus*, $\times 800$ (17) and $\times 2000$ (18). 19 and 20: *D. coeruleus*, $\times 2000$. 21: *M. cephalus*, $\times 800$. 22: *A. flavimanus*, $\times 800$. 23 and 24: *P. altivelis*, $\times 800$. BOUIN's fixation (17-23) and neutral formalin fixation (24). Thionine stain (17, 18, 20 and 21), KLÜVER-BARRERA's method (19), H-E (22 and 23) and BODIAN-OTSUKA's method (24).

Abbreviations used in Plate I, II and III. b: bony cranium, c: chondrocranium, d: dermis, ds: dorsal sac, e: epidermis, f: fold, g: ganglion cell, m: muscle, n: neuropile, pl: pineal lumen, ps: pineal stalk, pt: pineal tract, pv: pineal vesicle, s: small space.

Plate 1.

Plate 2.

Plate 3.