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Pineal Photoreceptor and Ganglion Cells in River Lamprey, *Lampetra japonica* -Two Types of Pineal Ganglion Cell-

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Abstract: Pineal organs of poikilotherm vertebrates transform the environmental light information into a humoral message and a neuronal activity. The former is melatonin, and the latter is modulation of the impulse in ganglion cells. The ganglion cells are physiologically classified into luminosity (achromatic) type and chromatic one, as the neural activity is modulated in two ways. We attempted to classify the pineal ganglion cells with morphological characteristics by means of the three-dimensional reconstruction method. In the pineal ganglion cells of river lamprey, there are two different features, oval and spherical. For comparison of their projection region in the brain, the tracing investigation was also carried out. The application of the neural tracer near mesencephalic tegmentum showed that only oval-shaped ganglion cells were labeled in the pineal organ. These results suggest that the oval-shaped ganglion cell is functionally different from the spherical one.

Key words: pineal organ, photoreceptor, ganglion cell, lamprey, three-dimensional structure

INTRODUCTION

The pineal organ of vertebrates is well known as one component of the circadian system, and photic information is the main regulatory factor for the pineal activities. Mammalian pineals receive input from the retina via the suprachiasmaitc nuclei and the sympathetic nervous system [1]. On the other hand, the pineal organs of lower vertebrates possessing photoreceptors can receive the environmental photic information by themselves. They transform the photic information into a humoral message, melatonin, and a neural activity.

In regard to the latter, the information from the photoreceptor cells is conveyed to the central nervous system by modulating the impulse frequency of the ganglion cells. The discharges of the ganglion cells are modulated in two response types. One is the luminosity-type, sometimes called achromatic-type [2,3]. In luminosity type, the spike frequency decreases in spikes response to light stimuli of all wavelengths. The other is the chromatic type. In chromatic type, ganglion cells are inhibited by UV and shorter wavelengths and excited by green and longer wavelengths. Namely, pineal ganglion cells are classified into two types by their physiological characteristics. However, there are a few studies for morphological characteristics of the pineal ganglion cells, though pineal neurons, including the ganglion cell and the inter-neuron, were well investigated with the histological techniques [4]. In the present study, we investigated the presence of the different type of ganglion cells by use of three-dimensional reconstruction technique, and also the projection region of the ganglion cells.

The lamprey used in this study possesses one of the best-differentiated pineal eyes. Structural evidence concerning the photoreceptive capacity in the pineal organ of lampreys has been provided by light- and electron-microscopy [5, 6]. Electrophysiological investigations proved that the lamprey pineal organ is capable of transducing a light signal into an electrical response [7, 8, 9]. Furthermore, it has been shown that the pineal organ of lamprey is involved in regulation of circadian rhythmicity [10] and that melatonin is rhythmically secreted [11, 12].

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MATERIALS AND METHODS

Animals

River lampreys, *Lampetra japonica*, were commercially obtained from Ebetsu fishery. The animals were kept in an aquarium at about 10 °C under 12-h light/ 12-h dark cycle before the experiments.

Neural tracer

After decapitating, the intact brains were carefully dissected out and placed in a plastic dish filled with an oxygenated lamprey Ringer 's solution.

1) For three-dimensional structure; the pineal tract was transected 2-3 mm away from the pineal organ using microscissors, and biocytin (Sigma, USA) was applied to the cut end. After 20 min, biocytin was rinsed away with the Ringer's solution 3 times and the brain was incubated in the oxygenated Ringer's solution for 6 hr at 4 °C.
2) For injection of the tracer to the brain; the biocytin solved with the Ringer's solution was injected near mesencephalic tegmentum using the microinjector (IM-30, Narishige, Japan). After injection, the brain was incubated in the oxygenated Ringer's solution for overnight at 4 °C.

Following incubation, the brains were fixed in Zamboni's fixative for, at least, 1 day, rinsed with 0.1 M phosphate buffer (PB) three times, cryoprotected in PB containing 30 % sucrose and then sectioned using a Bright cryostat at -20°C. These sections (30 μm thick) were mounted on gelatin-coated microscope slides. To visualize neurobiotin, the sections were incubated for 5 hr at the room temperature with streptoavidin conjugated Alexa 594 (Molecular Prove, USA) and rinsed in phosphate buffered saline.

Photomicrographs were taken with fluorescence microscope (Power BX, Olympus, Japan), and confocal laser scanning microscope (TCS-NT, Leica, Germany).

Reconstruction

The method of the reconstruction refers to the previous study [13]. In brief, serial confocal images were taken by serial optical sectioning using the confocal laser scanning microscope. Three-dimensional images were reconstructed from these serial confocal images by means of a volume-rendering method, using reconstruction software (Voxel View, Vital Image, USA).

RESULTS

The pineal organ displays three major divisions: the end-vesicle, the atrium and the pineal stalk. In this study, the ganglion cells at the end-vesicle were investigated by means of the neural tracing method. Most of ganglion cells are located at the basal layer of the ventral side in the end-vesicle (Fig.1A). Some ganglion cell processed the dendrite-like and the axon-like processes and the former extended toward the peripheral region of the end-vesicle. A few photoreceptor cells were labeled with retrograde tracer. It is appeared that in the ganglion cells there are two different features; ellipsoidal and round (1B). We investigated the three-dimensional structures of the ganglion cells using the confocal laser microscope to confirm the structures. Figure 1C shows the reconstructed three-dimensional images of the ganglion cells. There are two different types of the pineal ganglion cells in shape; oval (or teary) shape and spherical. In both ganglion cells, two basal processes, axonal and dendritic processes, are found at their basal portion.

The tracer was injected near mesencephalic tegmentum using the glass capillary filled with biocytin. Labeled cells and terminals were found at the several areas of the brain. In the pineal organ, some cells at the end-vesicle are labeled by the tracer, and are only oval in shape.

DISCUSSION

The studies for the pineal organ have shown that it displays three principal characteristic features; photoreceptive function, melatonin production and endogenous circadian oscillator. As a consequence, the pineal organ is regarded as a transducer to transform photic information into bio-signals, which are humoral and neural signals. In the lamprey pineal organ, various evidences have been provided by ultrastructural, immunocytochemical, electrophysiological and biochemical investigations for the photoreceptive capacity [5, 6, 7, 8, 14]. Histological studies have demonstrated that there are at least five types of the photoreceptors in the lamprey pineal organ [15, 16, 17]. The ganglion cells, which convey the signal to the brain, are physiologically classified to chromatic and luminosity (achromatic) types. In the present study, two morphologically discriminate ganglion cells, oval- and spherical-shaped, were found in the lamprey pineal organ. For corresponding these two types of ganglion cells with luminosity type and chromatic one, electrophysiological studies combining with histological experiments are indispensable.

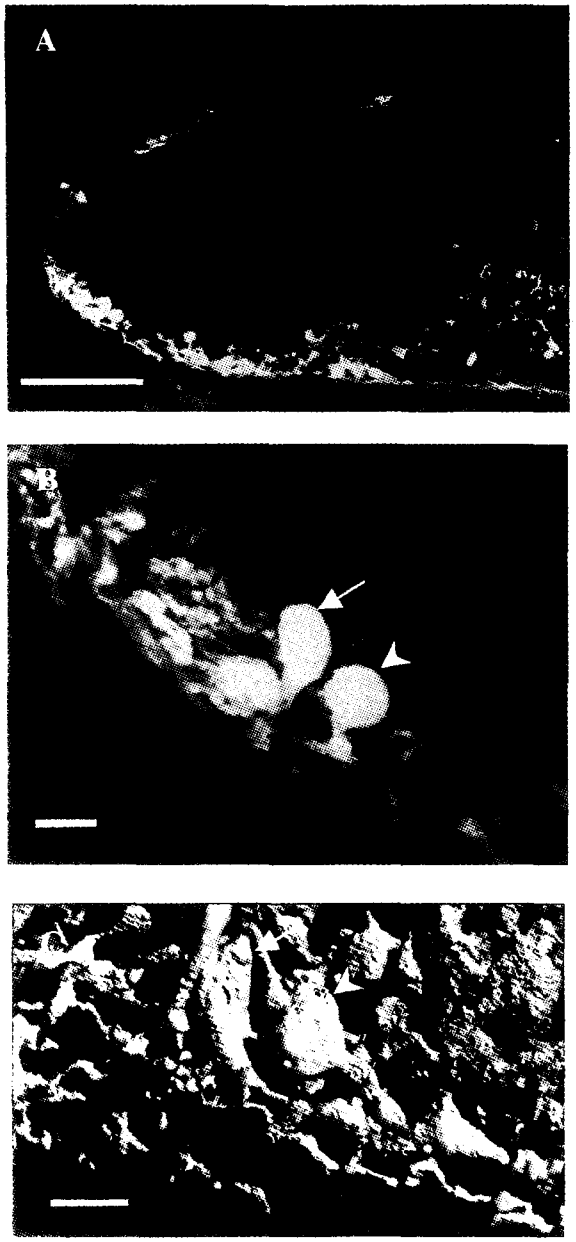

Fig.1 Pineal ganglion cells of the river lamprey.
A: Strongly labeled ganglion cells and nerve fibers in the ventral portion of the end-vesicle. Bar = 100μm. **B:** A superimposed image of the ganglion cells. Ellipsoidal (arrow) and round (arrowhead) ganglion cells are found in the basal region. Bar = 10 μm. **C:** 3-D image of the ganglion cells. There are oval (arrow) - and spherical (arrowhead) - shaped ganglion cells in the pineal organ. Bar = 10 μm.

Fig.2. Labeled ganglion cells (arrows) in the pineal organ by the neural tracer injected near mesencephalic tegmentum. All labeled ganglion cells in the end-vesicle are ellipsoidal in shape. Bar = 100 μm.

The investigation for the target cells of signals is important to demonstrate the function of the pineal organ. Experimental tracing studies in lampreys have indicated that the pineal organ has projections to the pretectum, optic tectum and mesencephalic tegmentum [18]. Since Pombal et al. [18] did not distinguish for the morphological characteristics of labeled ganglion cells, we examined the 3-D structure of labeled ganglion cells with application of biocytin to mesencephalic tegmentum. As a result, only oval-shaped ganglion cells were labeled in the pineal organ. We presume that the physiological characteristics may be different between the oval-shaped ganglion cells and the spherical ones. More cautious electrohysiological experiments are necessary to confirm this hypothesis. However, it is very difficult because there are a few labeled ganglion cells in the pineal organ.

In river lamprey, the pineal organ is essential for the locomotor activity rhythm under light-dark conditions, because the light-dark entrained locomotor activity rhythm persists after removal of the eyes, but not after pinealectomy[10]. The lamprey became as active as night during the daytime after pinealectomy. Besides, the melatonin rhythm is obvious in the lamprey pineal organ; low secretion during the daytime and high during the nighttime [11]. However, melatonin secretion rhythm depends on temperature, apparent at 20°C but not at 10°C [12]. In general, river lampreys were kept at about 10°C, and the locomotor activity was measured at about 10°C. These suggest that the pineal organ regulated the locomotor activity rhythm without the melatonin pathway. Consequently, neural information, another output from the pineal organ to the brain, may be important for the regulation of the locomotor activity.

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