The photoreceptor cell in the pineal organ of the Japanese common newt

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Summary. The photoreceptor cells in the pineal organ of the Japanese common newt are similar to typical vertebrate photoreceptors. It is supposed that the cells are responsible for the entrainment of locomotor rhythms as a consequence of their response to light.

Hendrickson and Kelly[2] reported that most photoreceptor cells in the pineal organ of a newt, *Taricha torosa*, were disorganized in adult stages. However, many well-organized photoreceptor cells have now been observed in the pineal organ of adult Japanese common newts, *Cynops pyrrhogaster pyrrhogaster*.

Newts were anesthetized with 0.2% MS222 (Sankyo Co. Ltd.), the epidermis and cranium were removed and the region which included the pineal organ was fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in cacodylate buffer at pH 7.4, postfixed in 1.5% osmium tetroxide in cacodylate buffer, dehydrated in an ethanol series and propylene oxide, and embedded in Spurrs medium (TAAB). Specimens were sectioned with a Porter-Blum MT-1 ultramicrotome, and thin sections were stained with uranyl acetate and lead citrate and observed in an Hitachi H300 electron microscope.

The pineal organ is located just beneath the cranium, on the dorsal surface of the diencephalon. It consists of several lumina, lined with supporting cells amongst which are found a smaller population of cells which resemble photoreceptors of the lateral eyes of vertebrates (fig. 1). Each cell has an outer segment, an inner segment containing the nucleus, and a synaptic region (fig. 2). The outer segment protrudes into the lumen, and consists of a stack of discs whose organization resembles that of vertebrate cones. Some of the outer segment lamellae are disorganized to form whorl structures. The inner segment contains numerous mitochondria and is joined to the outer segment by a connecting cilium which consists of typical 9+0 centriolar

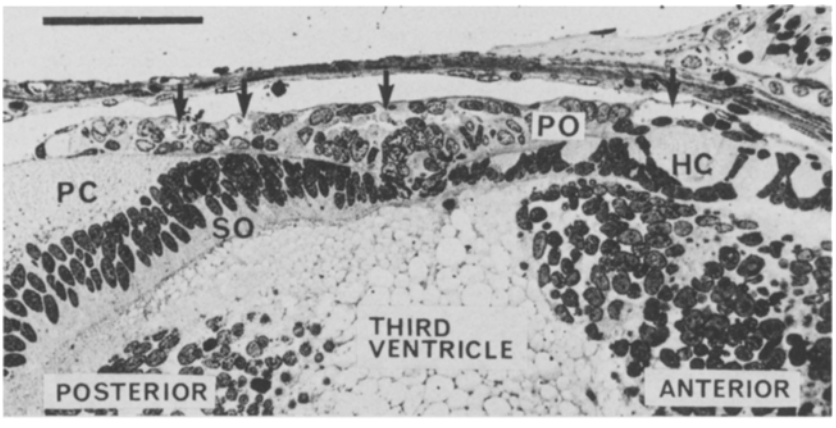

Figure 1. Light micrograph of a median sagittal section through the pineal region. The pineal organ (PO) can be seen dorsal to habenular commissure (HC), sub-commissural organ (SO) and posterior commissure (PC). Outer segments of photoreceptor cells protrude into lumina (arrows). Bar indicates 50 µm.

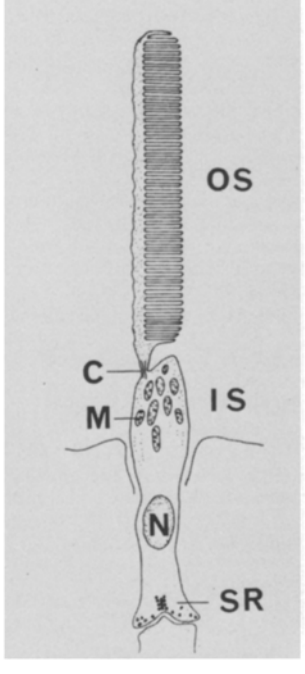

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Figure 2. Schematic representation of the photoreceptor cell in the pineal organ of the Japanese common newt. OS, outer segment; IS, inner segment; C, connecting cilium; M, mitochondria; N, nucleus; SR, synaptic ribbon.

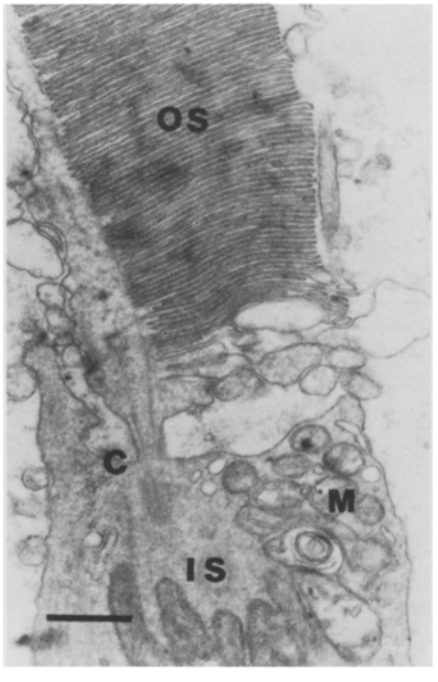

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Figure 3. Electron micrograph of a longitudinal section of the photreceptor cell. The outer segment (OS) consists of a series of membrane discs. The inner segment (IS) contains a accumulation of mitochondria (M). They are connected by the connecting cilium (C) which consists of a 9+0 centriolor apparatus. Bar indicates 0.5 µm.

apparatus (fig. 3). The synaptic terminals are filled with clear synaptic vesicles and characterized by synaptic ribbons (fig. 4). The pineal photoreceptors do not form a distinct, stratified, structure as is typical of vertebrate lateral retinae, and the number of photoreceptors in a single lumen is small compared to the large population of supporting cells.

The pineal organ is known to function as a photoreceptor in a variety of amphibians[3,4], and has been reported to be a major influence on circadian rhythms in birds[5-8]. The locomotor activity of the Japanese common newt under a 12:12 light-dark schedule shows a circadian rhythm which cannot be entrained after ablation of the pineal organ. In constant darkness (12:12 dark-dark) the rhythms persist. The locomotor activity rhythm is entrained by light-dark cycles in newts whose lateral eyes have been enucleated but whose pineal organs are intact. This indicates that the cone-like cells are functional photoreceptors, and that the pineal organ does have the function of extraocular perception of light. It seems that one role of the pineal organ in the newt is to effect entrainment and/or oscillation of the circadian locomotor rhythms.

Further studies are in progress to analyze the mechanisms of the synapse of the photoreceptor, and of the neuronal network of the pineal organ.

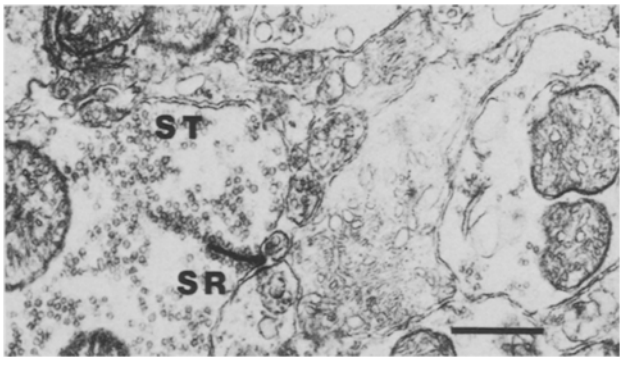

Figure 4. Electron micrograph showing synapse. The synaptic terminals (ST) are filled with clear synaptic vesicle. The synaptic ribbons (SR) are surrounded by vesicles. Bar indicates 0.5 μm.

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Fluid fluxes in the ferret trachea[1]

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Summary. A net reabsorption of fluid was observed in isolated ferret trachea under control conditions. Cholinergic stimulation resulted in net secretion of fluid while atropine blocked this response without any effect on the basal process of fluid reabsorption.

Mechanisms regulating water and ion movements across mammalian tracheal epithelia are essential to maintain effective mucociliary transport[2]. While ion transport across this tissue has been well-studied[3-5], data on the fluid movements which accompany these ionic fluxes are limited[6,7]. This report describes in vitro studies of fluid fluxes across the ferret tracheal epithelium.

Materials and methods. Male ferrets *(Mustela putorius furo)* weighing between 800 and 1000 g were used in these experiments. The ferret was sacrificed by exsanguination and a tracheal segment was excised and rinsed in a modified Krebs-Henseleit solution. The trachea was trimmed of connective tissue and then mounted in a plexiglass perfusion chamber. The chamber was then filled with the modified Krebs-Henseleit solution containing inulin. The pH of the solution was maintained between 7.35 and 7.40 by continuous bubbling with 95% O_2 and 5% CO_2. This produced a partial pressure of oxygen in the bath and lumen greater than 100 torr. The temperature of the bath was held at 37 °C by means of a circulating water bath. Stationary perfusion of the tracheal lumen, using a modification of the split oil column technique, similar to that employed by Gertz et al.[8] was used in these studies. The perfusate bubble occupied approximately 2.0 cm of the excised tracheal segment. Water fluxes were measured by determining changes in the concentration of inulin in the perfusate. Preliminary studies demonstrated that inulin did not cross the ferret tracheal epithelium. Inulin concentration was measured by a spectrophotometric assay adapted from the fluorometric technique developed by Vurek and Pegram[9].

The net water movement at each interval was calculated by the equation:

$$\frac{\text{BIA} - \text{SIA}}{\text{BIA}} \times \text{Vp} = \text{Net fluid secreted or reabsorbed}$$

where BIA was the baseline inulin absorbance level, SIA was the sample absorbance level, and Vp was the volume of the perfusate. At each interval, BIA was adjusted not only for the volumes removed as samples but also for calculated water fluxes from previous intervals. The flux or flow rate per trachea was expressed as μl/h × trachea. The viability of the preparation was checked at the end of each experiment by exposing the luminal surface to trypan blue to see whether or not the epithelial cells took up the dye.

Results. Over the 2-h baseline period in the 7 tracheas a net reabsorption of fluid at a flux of 30 ± 3 μl/h × trachea ($\bar{x} \pm SE$) was observed. Cholinergic stimulation by addition of 10^{-6} M carbamylcholine to the submucosal bathing solution in these same tracheas resulted in a net secretion of