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The Traffic of Particles in the Axonic Process of Vertebrate Cone-Type Photoreceptor Cells

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ABSTRACT—Differential-interference-contrast microscopy with video enhancement displayed the movement of particles for the first time in the isolated axonic process of cone-type photoreceptor cells of *Rana catesbiana*. This movement was observed under visible light which visual pigments could absorb. The number of retrograde moving particles in an arbitrary area on the axonic process was twice that of those moving in the anterograde direction. The mean velocities were 1.03 ± 0.55 μm/sec for anterograde particles and 0.41 ± 0.30 μm/sec for retrograde particles, which are of the same order as those found in isolated neurons.

INTRODUCTION

Photoreceptor cells in the vertebrate retina are primary sensory cells which communicate with the sensory neurons system *via* synapses. There is much light-dependent activity at the synapse, particularly an increased release of neurotransmitter from the synaptic ribbon in the absence of light [3]. It is predictable that photoreceptor cells require axonal transport for synaptic activity like other neurons. The axonal transport of usual neurons is manifested in the traffic of particles moving in both directions along the neuronal axon. This can be observed directly as moving particles (mitochondria and lysosomes) by differential-interference-contrast microscopy with electronic image processing [1, 2]. The axonal transport in photoreceptor cells has never been demonstrated experimentally so we have investigated this phenomenon in cone-type photoreceptor cells by differential-interference-contrast microscopy with video enhancement.

MATERIALS AND METHODS

Bullfrogs (*Rana catesbiana*) were kept in the dark overnight before use. Eyes were enucleated under dim red light, and the lens and anterior chamber were removed by surgery with a razor blade. Half of a resulting eye-cup was placed vitreous surface downwards on filter paper and the sclera and pigment epithelium were removed, leaving the photoreceptors exposed. After adding a few drops of oxygenated frog Ringer's solution to the retina [4], the tip of a glass micro-pipette was placed on the surface and gentle suction was applied to obtain intact cells. The resulting solution containing retinal cells was dropped onto a coverglass, which was attached with adhesive tape to the underside of a 0.5 mm thick stainless steel spacer. The upper side of this spacer was then covered with another coverglass to form a viewing chamber. Retinal cells were observed with an Allen video-enhanced contrast-differential interference contrast system, using an inverted microscope (Zeiss Axiovert, Germany). An oil-immersion planapochromat $\times 63$ objective and a $\times 4$ zoom lens were used. Images were obtained with a Newvicon video camera (Hamamatsu Photonics, Japan) and contrast was enhanced with a DVS-1000 computerized image processing system (Hamamatsu Photonics, Japan) [2, 5]. The microscope light source was a Halogen lamp passed through low-cut high-pass (>420 nm) and heat absorption filters. The irradiation power was about 0.1 μW and the field diaphragm of the condenser was used to limit the irradiation area to 100 μm in diameter, resulting in an irradiation rate of 10^{15} photons/cm^2. The resolution limit of particle size was approximately 0.1 μm in our video-images. Time resolution was 30 frames/sec.

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RESULTS AND DISCUSSION

We obtained many photoreceptor cells with intact outer segments, inner segments and cell bodies. However, the rod cell does not have a long axonic process, so no particle movement could be observed, and the contents of the rod inner segment were obscure, probably because of their high density. Some cone cells retained their long axonic process, but most had lost their pedicles (i.e. synaptic region). In some cone cells complete with their pedicles, we succeeded in observing particle movement. The thickness of the cell is an important factor to observe by our microscopic system. A thinner axonic process is better to get clear picture of the particles. We could not observe axonal transport in all intact cone cells, because some sustained damage and others

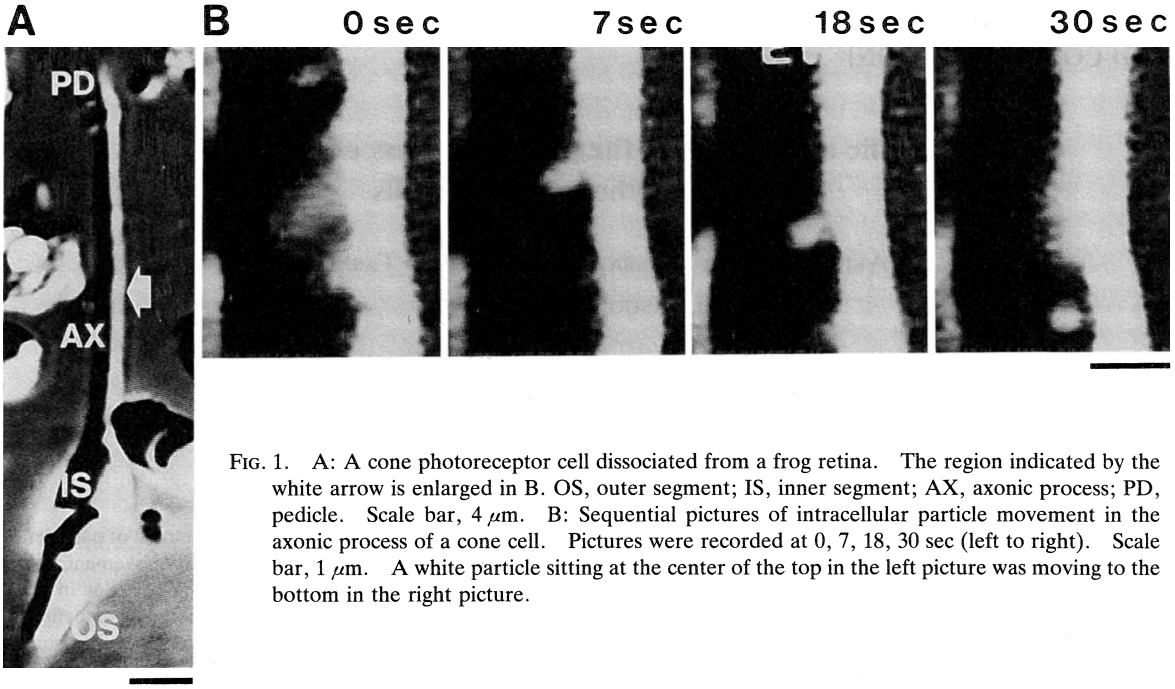

FIG. 1. A: A cone photoreceptor cell dissociated from a frog retina. The region indicated by the white arrow is enlarged in B. OS, outer segment; IS, inner segment; AX, axonic process; PD, pedicle. Scale bar, 4 μm. B: Sequential pictures of intracellular particle movement in the axonic process of a cone cell. Pictures were recorded at 0, 7, 18, 30 sec (left to right). Scale bar, 1 μm. A white particle sitting at the center of the top in the left picture was moving to the bottom in the right picture.

had axonic processes which were too thick to allow observation by our system.

Figure 1 shows an example of retrograde movement of a particle along the axonic process of a cone cell. In this cell, 10 particles were observed moving by retrograde transport in the same observation area over a 3-minute period. The mean velocity was 0.41 ± 0.30 μm/sec. Five particles moving by anterograde transport were also observed in the same area, with a mean velocity of 1.03 ± 0.55 μm/sec. The velocities of particle movement in both directions were similar to those reported for neurons, such as lobster axons and mouse dorsal root ganglion cells [6~10]. Transport in these cone-type photoreceptor cells was observed using a light source within the wavelength range of frog visual pigments. Photoreceptor cells under such conditions are expected to exhibit metabolic characteristics similar to a resting nerve cell, because the release of neurotransmitter is suppressed by light [3]. It is therefore reasonable to expect that the transport velocity in irradiated cone cells would likewise correspond to that of nerve cells.

In studies of axonal transport in other systems, the observed particles were ascribed to mitochondria and lysosomes, as confirmed by electromicroscopic [11] and fluorescent studies [12]. We have yet to confirm the identity of the particles observed in cone cells.

After more than 1 hour of continuous observation with the same cone cell, moving particles had stopped, presumably because of the damage from the light and/or heat of the light source. At that time, the shape of the cone cell was retained, but rod outer segments in the same field appeared damaged (as deduced from their changed shape). This suggests that the phenomenon of particle traffic is a property of living cone cells, rather than some physical effect.

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