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Biophysical mechanism of transient retinal phototropism in rod photoreceptors

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Abstract

Oblique light stimulation evoked transient retinal phototropism (TRP) has been recently detected in frog and mouse retinas. High resolution microscopy of freshly isolated retinas indicated that the TRP is predominated by rod photoreceptors. Comparative confocal microscopy and optical coherence tomography (OCT) revealed that the TRP predominantly occurred from the photoreceptor outer segment (OS). However, biophysical mechanism of rod OS change is still unknown. In this study, frog retinal slices, which open a cross section of retinal photoreceptor and other functional layers, were used to test the effect of light stimulation on rod OS. Near infrared light microscopy was employed to monitor photoreceptor changes in retinal slices stimulated by a rectangular-shaped visible light flash. Rapid rod OS length change was observed after the stimulation delivery. The magnitude and direction of the rod OS change varied with the position of the rods within the stimulated area. In the center of stimulated region the length of the rod OS shrunk, while in the peripheral region the rod OS tip swung towards center region in the plane perpendicular to the incident stimulus light. Our experimental result and theoretical analysis suggest that the observed TRP may reflect unbalanced disc-shape change due to localized pigment bleaching. Further investigation is required to understand biochemical mechanism of the observed rod OS kinetics. Better study of the TRP may provide a noninvasive biomarker to enable early detection of age-related macular degeneration (AMD) and other diseases that are known to produce retinal photoreceptor dysfunctions.

Keywords

Retina; rod photoreceptor; phototransduction; time-lapse microscopy

1. INTRODUCTION

Located at the back of the eye, the retina is a complex neural system that consists of multiple types of neural cells for light capturing and early visual information processing.¹ Retinal rod and cone photoreceptors are the first-order neurons which are responsible for converting

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light energy into biochemical and bioelectrical activities. Earlier studies showed that the retina exhibited differential sensitivities to light entering the eye from different portions of the pupil.² The sensitivity to the light entering from the center of the pupil was higher than that of the light entering from the periphery of the pupil.³ This phenomenon is described as the Stiles-Crawford Effect (SCE). In general, it is believed that physical properties, such as the shape and orientation of retinal photoreceptors, govern the SCE. Retinal photoreceptors are physically oriented towards the center of the pupil⁴ and that architecture makes them most sensitive to the light entering from the center of the pupil. It is known that the SCE is predominantly observed in cone photoreceptors, which are responsible for photopic vision. However, why it is absent in rod photoreceptors, which are responsible for scotopic vision, is still a mystery.⁵ Recently, oblique light stimulation evoked transient retinal phototropism (TRP) has been detected in amphibian (frog) and mammalian (mouse) retinas.⁶ High resolution microscopy of freshly isolated retinas indicated that TRP is predominated by rod photoreceptors.⁶ The rod dominated TRP may provide quick compensation for light inefficiency, and thus explain why the rod system lacks a SCE, which was determined predominately with psychophysical methods.^{2,5} Comparative confocal microscopy and optical coherence tomography (OCT) revealed that the TRP was predominantly elicited from the rod outer segment (OS).⁷ However, the biophysical mechanism of rod OS dynamics is still unknown.

In this work, we have investigated the conformational changes of rod OSs correlated with localized visible stimulation. Frog retinal slices, which display a cross section of retinal photoreceptors and other functional layers, were used to test the effect of light stimulation on rod OSs. High spatiotemporal resolution near infrared (NIR) light microscopy was employed to monitor photoreceptor changes in retinal slices stimulated by visible light flashes.

2. METHODS

2.1 Sample preparation

Retinal samples were prepared in a dark room with dim red light illumination. After four hours of dark adaptation, both eyes were enucleated from euthanized frogs. The eyeball was hemisected along the equator with fine scissors and the lens and anterior structures were removed from the retina. The retina was separated from the retinal pigment epithelium (RPE). The isolated retina was then cut into slices with 150 μm thickness. The entire sample preparation procedure was performed in Ringer's solution containing 110.0 mM NaCl, 2.5 mM KCl, 1.6 mM MgCl_2 , 1.0 mM CaCl_2 , 22.0 mM NaHCO_3 , and 10.0 mM D-glucose. Retinal slices were immersed in a chamber filled with Ringer's solution and placed under a NIR light microscope for optical imaging. Retinal slices were illuminated from the side, which comprised a cross section of retinal photoreceptors and other functional layers. All experiments were performed following the protocols approved by the Animal Care Committee (ACC) at the University of Illinois at Chicago.

2.2 Experimental setup

A NIR light microscope (BX531WI, Olympus, Japan) with a 40× NA 0.8 water immersion objective lens was used for this study. The same experimental setup used for intrinsic optical signal (IOS)⁸ and TRP⁶ studies in freshly isolated retinas was used in this study. The NIR light for optical imaging was produced by a halogen lamp with a band-pass filter (wavelength band: 775–1,000 nm). The light stimulus was provided via a fiber-coupled white light emitting diode (LED) with a central wavelength of 550 nm (wavelength range: 450–650 nm). The retinal slices were stimulated by a rectangle shaped light stimulus pattern controlled by an adjustable slit. In this study, the NIR time-lapse microscopy images have been recorded from frog retinal slices stimulated by visible light. Retina slices were stimulated by a light flash of 1,000 ms duration. The stimulus intensity was $\sim 1.0 \times 10^{10}$ photon/ $\mu\text{m}^2 \cdot \text{s}$. All illustrated images in this article were captured at the speed of 20 frames/s, with frame resolution of 640×480 pixels. The entire experiment for each retinal preparation was completed within 2 hours after animal euthanasia.

2.3 Data processing

The movement of the photoreceptor OS was calculated based on the center of mass algorithm. A small rectangular window was selected on the tip of the rod OS and the centroid of the selected window was calculated for each video frame in sequence. If the background of the image is completely black (i.e. zero intensity) then the centroid movement in the vertical direction is exactly half of the physical movement of the photoreceptor i.e. if the photoreceptor is shrunk by 2 pixels in the vertical direction the centroid is moved 1 pixel in the vertical direction. However, the centroid movement varies if the background intensity varies. The background of the microscopy images was nonzero. In such case, the centroid method does not directly provide actual value of photoreceptor movement. This problem was resolved by calibrating each photoreceptor before centroid calculation. The calibration factor was calculated by shifting a specified window with known pixel number in the vertical direction. At each position of the window a centroid was calculated. The calculated centroid movement in the vertical direction was plotted against the known magnitude of the window shift in the vertical direction. The relation between these two variables was almost linear. A best fit line was plotted and the slope of the line was calculated. The slope of the line provides the relation between the photoreceptor movement and the centroid movement when the background was nonzero.

3. RESULTS

Figure 1 illustrates stimulus-evoked OS changes of individual rod photoreceptors. Rapid rod OS length and orientation changes were observed after the stimulation delivery. The magnitude and direction of rod OS changes varied with the photoreceptor position within the stimulation area. In the center of the stimulation region, the length of the OS shrunk, while in the peripheral region, the OS swung towards the center of the stimulation area in the plane perpendicular to the incident stimulus light. We calculated the movement magnitude of the rod tip using the method described in Section 2.3. The results showed that at the left-edge (rod 1) of the stimulus pattern, the rod OS tip movement was predominated by a 0.19 μm shift to the right (Fig. 1b); at the center (rod 2) of the stimulus pattern, the rod OS tip

movement was predominated by a $0.07\ \mu\text{m}$ shrinkage; i.e. OS length reduction (Fig. 1c); while at the right-edge (rod 3) of the stimulus pattern, the rod OS tip movement was predominated by a $0.32\ \mu\text{m}$ shift to the left (Fig. 1d).

Optical flow is a well-established method for calculating target movements between two images. It can accurately identify movements of fine details in sequential images, which makes the method suitable for tracking small movements of retinal cells.⁷ To verify the photoreceptor displacement, we adopted optical flow MATLAB software developed by Sun et al.⁹ Stimulus-evoked photoreceptor movement was quantified in magnitude and direction maps by comparing the images before and after the stimulation. Figure 2 showed optical flow mapping of transient morphological changes of all stimulated photoreceptors simultaneously. In order to automatically segment active areas and inactive areas in a displacement magnitude map, Otsu's thresholding method¹⁰ was used. The active area is the location with detectable photoreceptor displacement, while the inactive area is the location without detectable displacement. Segmented active areas were color coded in the direction map while inactive areas were coded as black. Figure 2b showed the magnitude map of rod OS changes. The direction map (Fig. 2d) showed two different colors at two different sides of the stimulation area, indicating motion in opposite directions; i.e., towards the direction of the center of the stimulation. The direction map revealed that photoreceptors located in the left of the stimulation center moved toward the 0° direction (right direction); whereas photoreceptors located in the right of the stimulation center moved towards 180° (left direction). The photoreceptors located in the center of the stimulation area moved by 90° , indicating shrinkage. Figure 2c showed a histogram of the movement directions.

To verify the reliability of the observed rod OS changes, we repeated the measurements with 6 retinal slices under identical experimental condition. The average transverse movement (tip shift) and shrinkage (length reduction) of 6 left-edge, 6 center, and 6 right-edge rods are shown in Fig. 3. Our results revealed that the peripheral rod OS tip shifted toward the center of the stimulus pattern; while the center rod OS length reduced unambiguously. As shown in Fig. 3, the average shrinkage and shift of the photoreceptors located at the center of the stimulation area were $0.18 \pm 0.07\ \mu\text{m}$ and $0.05 \pm 0.03\ \mu\text{m}$, respectively. Similarly, the average shrinkage and shift at the left peripheral region of the stimulation area were $0.24 \pm 0.24\ \mu\text{m}$ and $0.52 \pm 0.37\ \mu\text{m}$, respectively, and the average shrinkage and shift at the right peripheral region of the stimulation area were $0.24 \pm 0.27\ \mu\text{m}$ and $0.50 \pm 0.33\ \mu\text{m}$, respectively. The average shift of the photoreceptors located at the center of the stimulation area was smaller than the average shrinkage; similarly, the average shrinkage of the photoreceptors located in the peripheral regions was also smaller than the average shifts. Nevertheless, the photoreceptors are connected together, and therefore the measured shift and shrinkage might be influenced by neighboring photoreceptors.

4. DISCUSSION

Generally, rods are consisted of four distinct regions: synaptic region, cell body, inner segment and OS.¹¹ The OS consists of a dense stack of membrane discs enveloped by the plasma membrane where the phototransduction occurs. The discs contain a photopigment molecule, called rhodopsin, which is made up of a protein called opsin and a molecule called

retinal (derivative of vitamin A).¹¹ Phototransduction takes place in the photoreceptor OS, especially in the membranous discs. Therefore, arrangement of the discs is essential for effective phototransduction.¹ This study indicated that the arrangement of the discs in the rod OS alters when stimulated by visible light. Although we have no direct evidence regarding the mechanism of disc arrangement upon stimulation, shrinkage of the rod OS when the rod is fully inside the stimulation region and movement of the rod OS towards the direction of the center of the stimulation when a portion of the rod is stimulated indicates misalignment of the disc structure. Among vertebrate species, the packing density of photopigment molecules on the discs is uniform^{12,13}; therefore, when the discs are stimulated partially, perhaps only one side of the pigment molecules are bleached. Unlike cone discs, rod discs are not attached to the OS membrane. Rather, they are physically separated from the plasma membrane and are freely floating inside the OS membrane.^{1,14} When the free floating discs are stimulated partially from the side, only the chemical state of those photopigments that are stimulated by the light is changed, causing disc misalignment that results in rod OS movement towards the direction of the light. The unbalanced bleaching of the rod disc membrane is perhaps one of the factors that lead to TRP in the rod system. In contrast to rods, the discs membrane in cones remain attached to the OS membrane; therefore, TRP is absent in the cone system. The rods are homogeneously exposed when they are fully inside the stimulation region that leads to balanced bleaching, causing equal conformational changes of the disc membranes that result in rod OS shrinkage. A similar model has been proposed by Asai et al.¹⁵

Several researchers have shown great interest in investigating whether the conformational changes in rhodopsin is due to illumination leading to conformational changes in the disc membrane. Studies have reported swollen disc membranes and shrinkage with respect to the osmotic pressure of the bathing medium¹⁶, rod OS volume changes due to the illumination¹⁷, permeability changes in the photoreceptor disc membranes due to the illumination¹⁸, bleaching that causes the rhodopsin to sink into the lipid core¹⁹, conformational changes, such as turbidity, viscosity and light scattering intensity of the disc membrane upon its bleaching¹⁵, intradiscal space expansion and contraction with respect to the osmotic pressure of impermanent substances¹⁴ and birefringence of the rod OS changes after bleaching.²⁰ It has been reported that the illumination generates changes in the outer enveloping plasma membrane that lead to reduction in the dark current.¹⁸ Although disc membranes in rods are isolated and freely floating in the outer enveloping membrane, it is highly possible that disc membranes undergo conformational changes in such a way that the diffusion of ions during phototransduction would be compensated.

5. CONCLUSION

The rods at the center of the stimulation region shrunk as they were exposed to visible light homogeneously, while those in the periphery of the stimulation region shifted as only a part of the photoreceptor was exposed to light. Since the rod OS has a spring-like structure composed of dense stacks of discs along the longitudinal direction, the unbalanced bleaching of the photopigment on the disc membranes leads to shifts towards the center of the stimulation. The movement of rod OSs upon stimulation may elucidate the mechanism underlying TRP in rods. The stimulus-evoked rod OS changes may also contribute to the

IOS signals observed in both isolated retinas and intact animals.^{21–26} Further investigation is required to understand the biochemical mechanism of the observed rod OS kinetics. Better study of the TRP may provide a noninvasive biomarker to enable early detection of age-related macular degeneration (AMD) and other diseases that are known to produce retinal photoreceptor dysfunctions.

Acknowledgments

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Figure 1.

(a) Illustrations representative frog retinal slices. The yellow window indicates the stimulation area. Stimulus-evoked OS changes in rod photoreceptors at the left-edge (rod 1), center (rod 2), and right-edge (rod 3) are shown in (b), (c) and (d), respectively. At the center of the stimulus pattern, the rod OS tip movement was predominated by shrinkage, i.e. OS length reduction. At the left and right-edges of the stimulus pattern, the rod OS tip movement was predominated by a shift toward the center of the stimulus.

Figure 2.

(a) Microscopy image of a retinal slice. Yellow window illustrated the rectangular stimulation area. (b) Rod OS magnitude map derived from the microscopy video. (c) Histogram of rod OS directions. (d) Rod OS direction map. Inactive area mapped by black background. Active/inactive areas are separated according to the displacement magnitude using Otsu's thresholding method. The active area is the location with detectable photoreceptor displacement, while the inactive area is the location without detectable displacement.

Figure 3.

Averaged stimulus-evoked OS changes from 6 rod photoreceptors at the left-edge, center, and right-edge of the stimulus pattern, respectively. The OS tip movement was predominated by a shrinkage response at the center of the stimulus pattern, and by a shift change at the peripheral region of the stimulus pattern.