Cone Activation Delaying Recovery Time of Rod Sensitivity after Flash Exposure

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SUGITA, Y., ITABASHI, R. and TASAKI, K. Cone Activation Delaying Recovery Time of Rod Sensitivity after Flash Exposure. Tohoku J. exp. Med., 1988, 156 (4), 305-310 —— The recovery time of rods to the fully dark adapted state after flash exposure was psychophysically and electrophysiologically measured. The recovery time increased abruptly once cones were activated. This abrupt increment was not found for the receptor component (P ‡V) of the frog electroretinogram. It was suggested that the cone activation results in inhibitory influence upon the recovery process of the rod sensitivity. —— rods; cones; rod-cone interaction; inhibition; dark adaptation

The primary concern in the vision research is to investigate the effect of light upon the visual system. In the duplex retina which has both rods and cones, for example human retina, the light of weaker intensity activates only rods and an observer cannot perceive the color of the light. The more intense light can activate both rods and cones and the observer can perceive the color of the light. However, it had been always proved to be difficult to show the duplex system of the human retina objectively, i.e., by studying relationship between the stimulus intensity and the amplitude of the b-wave of the human electroretinogram (ERG) (see, for example, Armington 1974). Recently, Tasaki et al. (1988) showed two different components in the intensity-amplitude relation of the b-wave of the human ERG. An observer could perceive the color of the green test flash only at the intensities in the upper component. Therefore, they concluded that the lower component reflects the rod activity alone while the upper both rods and cones, and that the duplex system of the human retina can be investigated by objective methods.

On the other hand, both the change of adaptive state and the recovery time to the original adaptive state have been implicitly postulated to be increased with increasing stimulus intensity. However, the relationship between the stimulus intensity and the recovery time over wide range of the intensity has not been examined yet. The purpose of the present study was to investigate this relation-
ship, expecting to show some aspects of the duplex system of the vertebrate retina.

**Methods**

**Human study**

Data are represented only from R.I., a relatively experienced psychophysical observer with normal vision. Similar results were obtained from two other observers. The ERG was recorded with a contact lens electrode (type ERG-ED2, Kowa, Tokyo) wetted with methylcellulose solution, and the potential was amplified (time constant: 0.5 sec) and displayed on a storage oscilloscope. The amplitude of the b-wave was measured on the oscilloscope display. For illuminating the retina evenly, three green light-emitting diodes (LEDs; Stanley Electric, Tokyo: type BG5534S, wavelength 555 nm, halfband width 30 nm) were mounted to the contact lens. A test- and a discrimination flash were presented by these LEDs. The LED is a proven source of light, the intensity and duration of which can be accurately controlled (Tasaki et al. 1988). The duration of the test- and the discrimination stimulus was 100 msec. The maximal intensity (indicated as “0” log-units in the text and figures) at the surface of the LED was 33 μW.

The subject’s pupil was dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride. After 40 min dark adaptation, the subject was lain supine to keep a stable baseline. The cornea was anesthetized with 0.4% procaine hydrochloride and the contact lens electrode was inserted under dim red light. Light threshold was first measured. The intensity of the discrimination flash was fixed at this threshold intensity. The duration between the test flash onset and the first perception of the discrimination flash was measured as the recovery time. The intensity-amplitude relation of the ERG b-wave and intensity-recovery time relation were then examined simultaneously.

**Frog study**

Five bull frogs (Rana catesbiana) weighing 180–200 g had been maintained under 20°C 12:12 LD cycle. They were lightly anesthetized with urethane (5 mg/kg) and immobilized with suxamethonium chloride (5 mg/kg). The cornea, lens and vitreous humor were removed and the retinal surface was exposed. The eyecup was filled with Ringer solution (NaCl 6.5 g, KCl 0.14 g, NaHCO₃ 0.2 g, NaH₂PO₄ 0.01 g, 1 l water). One silver-silver chloride electrode was placed inside of the eyecup and another was attached to the cornea of the other eye and used as an indifferent electrode. The light source was a green LED placed 2 cm from the retinal surface.

After 2 hr dark adaptation, both the intensity-amplitude relation and the intensity-recovery time relation of the b-wave were examined. Then, Ringer solution containing additional 5 mM sodium aspartate was filled inside of the eyecup to isolate the P III component, the receptor component of the ERG. Both the intensity-amplitude relation and the intensity-recovery time relation of the P III component were examined. The intensity of the discrimination flash was fixed at −5.5 log-units for the b-wave and −2.45 log-units for the P III. To minimize measurement error, the duration between the test flash onset and the moment when the amplitude to the discrimination flash recovered to 80% of the original amplitude measured at the beginning.

**Results**

**Human study**

In stimulus intensities lower than −1.5 log-units, an observer could detect the difference of the intensities but not the color of the green test flash (555 nm), suggesting that the flash activated only rods. However, at the intensities above −1.5 log-units, the observer could perceive the color of the flash, suggesting
that both rods and cones were activated. Fig. 1 (upper) illustrates the \( b \)-wave amplitudes as a function of log stimulus luminance of the green light. It should be noted that, in the intensity-amplitude function, the discontinuity is found at the intensity of \(-1.5\) log-units, and that the function is apparently represented by two different components divided by the discontinuity. The color of the green light flash was perceived only at the intensities above the discontinuity. Therefore, the upper component in the function can be considered to reflect the cone activity and the lower the rod activity. The functional difference between rods and cones could be demonstrated not only by subjective observation but also by objective investigation of the relationship between the light intensity and the amplitude of the \( b \)-wave of the human ERG.

Up to \(-1.5\) log-units of stimulus intensity, the recovery time was increased as the stimulus intensity was increased. However, the recovery time was increased abruptly when the stimulus intensity was above \(-1.5\) log-units. The relationship between the light intensity and the recovery time required for the \( b \)-wave is

![Diagram showing intensity-amplitude relation of the human ERG \( b \)-wave for green light (upper). Measurements were made with increasing stimulus intensities. Note that a break is found at the intensity of about \(-1.5\) log-units. The lower graph represents the intensity-recovery time relation. Note that a break is again found at the intensity of about \(-1.5\) log-units.](image)
shown in Fig. 1 (lower). The intensity-recovery time function is also presented by two components divided by the discontinuity. Of special interest is that the discontinuity in the intensity-recovery time function is found at the same intensity as that in the intensity-amplitude function. Obviously rods needed much longer recovery time than expected from the data of Rushton (1965) when cones were activated.

**Frog study**

Results in the frog study are quite similar to those in the human study. An example of the intensity-amplitude function of the ERG b-wave obtained from one frog is shown in Fig. 2 (upper). Here again, apparent discontinuity can be found and the function is represented by two components. Furthermore, in the intensity-recovery time function, the discontinuity is also found at the same intensity as that in the intensity-amplitude function.

The intensity-amplitude function of the P III component obtained from the

![Fig. 2. Intensity-amplitude relation (upper) and intensity-recovery time relation (lower) of the frog ERG b-wave. Measurements were made with increasing stimulus intensities. Note that a break is found in both relations at the intensity of about -1.75 log-units.](image-url)
Fig. 3. Intensity-amplitude relation (upper) and intensity-recovery time relation (lower) of the frog ERG P III component. Measurements were made with increasing stimulus intensities. Note that a break is found only in the intensity-amplitude relation.

same frog is also represented by two different components. Furthermore, the discontinuity is found at the same intensity as that for the b-wave (Fig. 3, upper). However, there is no apparent discontinuity in the intensity-recovery time function of the P III component (Fig. 3, lower). It should be noted that the recovery time required for the P III component was much shorter than that for the b-wave.

DISCUSSION

Granit et al. (1939), comparing the electrical retinal response with the quantity of photopigment simultaneously, showed that the rise in sensitivity lags behind the increase in the photopigment concentration. In the present study, the recovery time required for the photo-receptor component of the ERG was found to be much shorter than that for the b-wave. Therefore, the dark adaptation processes seems to be mediated by at least two different mechanisms.

Cornsweet (1970) calculated the data of Campbell and Rushton (1955) and showed that the relationship between the proportion of bleached pigment and the
intensity of adapting light is represented by a single sigmoid curve. Rushton (1965) showed that the time course of rhodopsin regeneration in the living eye is represented by an exponentially proportional curve with a time constant of 7.5 min. Furthermore, in the present study, the abrupt increment was not found for the receptor component of the ERG. Therefore, the abrupt increment of the recovery time in the present study should be mediated by the mechanism other than the kinetics of photopigment. Since the abrupt increment is observed only when the adapting flash is sufficiently intense to stimulate cones, the cone activation might result in inhibitory influence upon the dark adaptation of the rod system.

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References