Intrinsic Signal Imaging in Macaque Retina Reveals Different Types of Flash-Induced Light Reflectance Changes of Different Origins

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PURPOSE. Intrinsic signal imaging is a newly developed technique that can map the neural activity of tissues noninvasively. It has been used to map the functional organization of the retina by recording flash-induced light reflectance changes in the cone and rod photoreceptors. The purpose of this study was to investigate the properties of the intrinsic signals in the monkey’s retina. To accomplish this, the intrinsic signals and the electroretinograms (ERGs) evoked by the same stimuli were measured under different recording conditions.

METHODS. The fundus of macaque monkeys was observed with infrared light and recorded with a charge-coupled device (CCD) camera. The intrinsic signals were measured as retinal light reflectance changes induced by diffuse or focal flash stimuli. ERGs were recorded under the same stimulating conditions. The reflectance changes induced by different flash intensities, flash intervals, and background luminance were compared.

RESULTS. The intrinsic signals were categorized into different groups based on the location in the fundus. Fast signals (peak: ~100 ms) were recorded from the posterior retina including the fovea, and slow signals (peak: 5.0–6.0 seconds) were recorded from the optic disc and nonfoveal posterior retina. The threshold of the slow signal changes was comparable to that of the ERG b-wave, and the thresholds of the fast signals were higher than that of the ERG a- and b-waves.

CONCLUSIONS. The retinal intrinsic signals are composed of several components with different response properties and different sources. This recording technique may be useful for mapping the retinal function in eyes with various disorders. (Invest Ophthalmol Vis Sci. 2007;48:2903–2912) DOI:10.1167/iovs.06-1294

Assessing the functional properties of the retina objectively is essential for making a correct diagnosis and prognosis in various retinal disorders. Although recent advances in imaging techniques—for example, optical coherence tomography (OCT),1,2 have revealed the morphologic changes in retinal structures, the functional properties of the retina cannot be evaluated with these imaging techniques. Thus, the electroretinogram (ERG) is still the only practical method of assessing neural activities in the retina.

Intrinsic signal imaging is a well-established imaging technique that translates neural activity into a visual image. This method measures the stimulus-induced light reflectance changes in tissues and has recently been used to assess the cone- and rod-induced retinal responsiveness in macaque monkeys.3 It has also been used to examine the near-infrared reflectance changes in the human retina4–6 and optic nerve head.7 This noninvasive objective technique has good potential for development as a tool for the early detection of retinal dysfunction in cases of age-related macular degeneration, retinitis pigmentosa, and other retinal diseases.

However, before this tool can be brought into the clinic, a detailed knowledge of the properties and origin of the signals obtained by intrinsic signal imaging is necessary. Based on past investigations of intrinsic signal imaging in the cerebral cortex, the decrease in light reflectance (i.e., darkening after a visual stimulus) correlates strongly with local neural activity.8–11 In the retina, however, the source and the properties of the intrinsic signals appeared to be more complex than in the cerebral cortex due to its complex layered structure.

The purpose of this study was to investigate the basic properties of the retinal intrinsic signals. To accomplish this, we recorded the intrinsic signals of the macaque retina and ERGs under various recording conditions but using the same diffuse flash stimuli. In addition, we recorded the intrinsic signals evoked by focal flash stimuli. The results indicate that the intrinsic signal of the monkey’s retina is composed of different components that originate in different layers of the retina.

METHODS

The procedures used to record the intrinsic signals have been described in detail.3

The experiments were performed on two Rhesus monkeys (Macaca mulatta) and one Japanese monkey (Macaca fuscata). The results from monkeys 1 and 2 are shown in Figures 1 through 5, and those from monkey 3 in Figures 6 and 7. After an intramuscular injection of atropine sulfate (0.08 mg/kg), the monkeys were anesthetized with droperidol (0.25 mg/kg) and ketamine (5.0 mg/kg) and then paralyzed with vecuronium bromide (0.1–0.2 mg/kg per hour). They were artificially ventilated with a mixture of 70% N2O, 30% O2, and 1.0% to 1.5% of isoflurane. The EEGs, ECGs, expired CO2, and rectal temperature were monitored continuously throughout the experiments. Before the recordings, the pupils were fully dilated with topical tropicamide (0.5%) and phenylephrine hydrochloride (0.5%). The experimental protocol was approved by the Experimental Animal Committee of the Riken Institute, and all experimental procedures were performed in accordance with the guidelines of the Riken Institute and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.
Intrinsic Signal Imaging and Data Analysis

A modified digital fundus camera system (NM-1000; Nidek, Aichi, Japan) was used to observe and measure the light reflectance changes from the ocular fundus. The fundus images were recorded with a charge-coupled device (CCD) camera (PX-30BC; Primetech Engineering, Tokyo, Japan), and the images were digitized with an IBM-compatible computer equipped with a video frame grabber board (gray-level resolution, 10 bits; spatial resolution, 640 × 480; temporal resolution, one-thirtieth of a second; Corona II; Matrox, Quebec, Canada). The camera was focused on the macular vessels, and the area recorded covered 45°, which included the macula, the superior and inferior vascular arcades, and the optic disc. We mainly investigated three retinal sites: the fovea, the posterior retina between the macula and inferior temporal artery, and the optic disc (Fig. 1A).

The fundus was continuously monitored with light from a halogen lamp filtered through an infrared interference filter (840–900 nm). Visible light could not be used for fundus monitoring because the light reflectance changes induced by bleaching of the photopigments have a polarity opposite to that of the intrinsic signals, leading to incorrect mapping of the stimulus-evoked responses topographically.

Each recording trial consisted of 300 video frames collected at 30 frames per second for a total recording time of 10 seconds. To determine the time course of the flash-induced reflectance changes, we averaged the gray-scale values of 15 video frames collected in 0.5 second for each of the data points (Figs. 1, 6, and 7).

An unfiltered Xenon flash (duration: 1 ms) was given to the whole posterior pole of the ocular fundus or to a focal region of the posterior retina, 500 ms after the initiation of data acquisition. The maximum flash intensity (0 Log-unit intensity) measured at the cornea was 308.0 cd·s/m² (measured at 50.2 mm from the object lens, by a photometer: model II-1700; International Light Technologies Inc., Peabody, MA). The timing of the data acquisition and stimulus delivery was under computer control.

Changes in light reflectance from the ocular fundus after the stimulus, such as a darkening (a decrease in light reflectance), and a brightening (an increase in light reflectance), of the retina, were measured. Under infrared observation, the light reflectance of the whole posterior retina decreased (the fundus image became darker) after a flash stimulus (Fig. 1B). The optical signal was calculated as follows: (1) the gray-scale values of the image obtained after the stimulus, such as a darkening (a decrease in light reflectance), and a brightening (an increase in light reflectance), of the retina, were measured. Under infrared observation, the light reflectance of the whole posterior retina decreased (the fundus image became darker) after a flash stimulus (Fig. 1B). The optical signal was calculated as follows: (1) the gray-scale values of the image obtained after the

**FIGURE 1.** Fundus photograph and time courses of intrinsic signals after a flash stimulus. (A) Fundus photograph of normal retina showing the regions analyzed. (B) Time courses of two-dimensional images of ocular fundus showing the light reflectance changes during a 10-second trial without (top) and with (bottom) a flash stimulus. Left: fundus images taken at the beginning of a trial; right: light reflectance changes after a flash. Thirty consecutive video frames collected during 1 second were averaged for one poststimulus fundus image. Darkened regions indicate a decrease of light reflectance after the flash stimulus. (C) Plot of the time courses of light reflectance changes in a single trial after a diffuse flash stimulus in the three locations shown in (A). The time after the flash is shown on the abscissa. Arrowhead: point of delivery of the flash. Each point is the average of 15 video frames collected during 0.5 second of the light reflectance changes. Colored arrows: F signal at fovea, D signal at the optic disc and R1 and R2 signals in the nonfoveal posterior retina. The time course of the reflectance changes during the first 500 ms after a flash is shown in the bottom graph where each point is the average of two video frames during one-fifteenth of a second. (D) Time courses of light reflectance changes in a single trial after a diffuse flash, measured at the fovea and four different regions within 12° from the fovea in each quadrant. Amplitudes are indicated as values relative to the light reflectance changes at the end of each trial (1.0). The four regions tested in each quadrant are indicated as distances from fovea (3°, 6°, 9°, and 12°).
stimulus were divided, pixel by pixel, by those obtained during a 0.5-second period before the stimulus, and (2) this ratio was rescaled to 256 levels of gray-scale resolution to show the stimulus-induced reflectance changes.

A deterioration of the signal can be caused by movement artifacts: small involuntary eye movements, blood pressure pulsations, and respiratory-associated movements. Eye movements are the most serious artifacts because a different fundus position would be analyzed during the pre- and poststimulus periods. However, these artifacts can be minimized by giving sufficient amounts of muscle relaxants to block eye movements. Pulsations cause small movements of the retinal arteries and optic disc. However, the pulsation-derived reflectance changes are less than one tenth of the stimulus-induced reflectance changes and are almost negligible if 15 video frames are averaged during the 0.5 seconds (Fig. 1C).

Artifacts from respiratory movements produced large light reflectance changes, which were 20% to 40% of the stimulus-induced reflectance changes. This artifact is due to changes in blood flow or volume and periodic back-and-forth movement of the eye, synchronized with the respiration. In our recordings, the respiration-induced artifacts were significant, and the respirator had to be stopped during a recording period of 10 seconds. With the respirator stopped, we could record stable intrinsic signals whose quality was sufficient to map the retinal reflective changes in a single trial without either averaging or offline analysis with realignment of the images.6

To measure the time course of the signal changes, we recorded two trials under the same conditions and averaged the results. We found that each trial had to be recorded with at least a 20-minute interval to allow a recovery of the signal production from the previous stimulus. Thus, in experiments where the 11 stimulus intensities were recorded, up to 7 to 8 hours were necessary to record two trials under each condition. Because only two recordings were obtained under each recording condition, the type of statistics that could be used was limited. Unlike ERG recordings, the amplitudes of intrinsic signals are vulnerable to changes in the heart rate, blood pressure, and corneal reflectance. Because it is critical for quantitative comparisons to measure the responses under the same physiological conditions, averaging many trials was impractical for our experimental protocol.

Electroretinograms
A bipolar contact lens electrode (Mayo, Aichi, Japan) was used to record the ERGs. The ERGs were amplified × 10,000 and the band-pass filters were set at 0.3 to 500 Hz (Power Lab; AD Instrumments, Colorado Springs, CO). A 45° brief white xenon flash stimulus was delivered through the same observation optical system to stimulate the retina while the fundus was monitored with the infrared observation light. As the ERGs evoked by the same diffuse flash stimulus under different conditions and were averaged.

RESULTS

To analyze the flash-induced intrinsic signals and electrophysiological responses, we used the value of the initial peak of light reflectance change at the fovea (F: 15 × 15 pixels), the value at the flexural point of light reflectance change (R1), or the value at the end of the recording trial (R2) in the inferior retina (60 × 40 pixels), and the lowest value of light reflectance at the optic disc (D: ~70 × 50 pixels; Fig. 1C).
Stimulus Intensity

The intrinsic signal images and ERGs recorded after a diffuse flash are shown in Figures 2A, 3A, 4A, and 5A, and the intensities of the intrinsic signals and ERG amplitudes are shown in Figures 2B, 3B, 4B, and 5B.

Under dark-adapted conditions (Fig. 2), the amplitudes of a- and b-waves of the ERGs increased as the stimulus intensity increased. The intrinsic signals of D and R2 had the same threshold as that of the b-wave, and their amplitudes also increased as the intensity increased. The amplitudes of D and R2 reached a plateau at -6.0 log units and did not change significantly with higher intensities. R2 increased again with higher flash intensities over -0.3 log unit in monkey (M)1 and -0.7 log unit in M2.

The threshold of R1 was higher than that of D, R2, and the ERG a-wave. The amplitude of R1 increased gradually with increasing flash intensities. The amplitude of F also increased with increasing flash intensities, but its threshold was higher than that of any of the other intrinsic signals.

Under light-adapted conditions (30 cd/m²), the amplitudes of the a- and b-waves increased progressively with increasing flash intensities, but that of the b-wave decreased with intensities higher than -3.0 log units, due to the photopic hill phenomenon (Fig. 3). The thresholds of D and R2 of the intrinsic signal images were higher than those in the dark-adapted condition by 2.1 log units in M1 and 2.8 log units in M2. The thresholds of the D and R2 signals and ERG a- and b-waves were the same in M1.

The threshold of R1 was the same in both dark- and light-adapted conditions. In both conditions, the threshold of F was the same in M1 but was 0.7 log unit lower than that in M2 in the dark-adapted condition. What was striking was that the amplitude of R2 was smaller with brighter flashes in M2 under light-adapted conditions and the light reflectance change became approximately zero at -0.3-log-unit intensity. There was a tendency for the amplitude of the R2 signal to decrease with intensities that were 1.0 to 2.0 log units higher than the threshold of the R1 signal.

Effect of Changes in Flash Intervals

After a bleaching with a bright flash, the amplitudes of the a- and b-waves were reduced and the amplitudes increased with increasing time in the dark (Fig. 4). The recovery of the ERG amplitudes appeared slower than that in other studies because our flash intensity was 1.7 log units more intense than that of the ISCEV (International Society for Clinical Electrophysiology of Vision) standard flash.

For the intrinsic signals, only the F signal had a pattern similar to that of the ERGs (i.e., the amplitude increased gradually with longer intervals in the dark after the preceding flash). D, R1, and R2 had peaks at 3 to 5 minutes after the preceding flash, and the amplitudes decreased at 10-minute intervals. These findings indicate that the source of the intrinsic signals of the optic disc and the nonfoveal posterior retina are different from that of the fovea.

Effect of Background Luminance

The amplitudes of the a- and b-waves decreased progressively as the background illumination increased (Fig. 5). For the intrinsic signals, only the D signal to decrease with intensities that were 1.0 to 2.0 log units higher than the threshold of the R1 signal.

- **Figure 2.** Intrinsic signal images and ERGs after a diffuse stimulus in dark-adapted conditions. (A) Fundus images of the intrinsic signals (left) and ERGs (right) after a diffuse flash in the dark-adapted condition with stimulus intensities from -8.8 to 0 log units. Intrinsic signal images from a single trial averaged from 5.0 to 8.0 seconds after the flash. The darkened region in fundus images indicates light reflectance decrease after the flash. The ERGs recorded from monkeys M1 and M2 are shown. The relative log flash intensity responses to the maximum flash are indicated. D: optic disc, F: fovea. (B) Amplitudes of R1, R2, F, and D of the intrinsic signals in response to increasing flash intensities are shown as light reflectance changes for M1 and M2. Right: the amplitudes of the ERG a- and b-waves in response to the same stimulus series. Note that negative values of light reflectance changes are plotted to indicate the strength of intrinsic signals.
means that the posterior retina appeared brighter after a flash at the later phase of a recording trial with bright background illumination.

Responses at Optic Disc

We have shown that the thresholds of the intrinsic signals at the optic disc were comparable to the threshold of the ERG b-waves and that even a dim stimulus can evoke a strong signal at the optic disc (Fig. 2). To determine the contribution of blood-related changes to the intrinsic signals at the optic disc, we measured the responses from different regions within the optic disc (Fig. 6A): (1) the central region where the central retinal artery and vein run perpendicular to the imaging plane (Center), (2) over the superior branch of the central retinal artery (Artery), (3) over the superior branch of the central retinal vein (Vein), (4) temporal and nasal regions where large

![Intrinsic signals and ERGs after a diffuse stimulation under light-adapted conditions.](A) Fundus images of the intrinsic signals (left) and ERGs (right) after different stimulus intensities (~6.7–0 log-unit intensity). Representative signal images for a single trial averaged from 5.0 to 8.0 seconds after a flash are shown. (B) Amplitudes of R1, R2, F, and D of the intrinsic signals and the a- and b-waves of the ERGs in response to various flash intensities are as described in Figure 2.

![Intrinsic signals and ERGs after a diffuse stimulus recorded at different times after bleaching.](A) Fundus images of intrinsic signals (left) and ERGs (right) evoked by a diffuse flash (~0.3 log unit) at different intervals (0.5–60 minutes) after a bleaching flash at the same intensity. Representative images from a single trial averaged from 5.0 to 8.0 seconds after a flash are shown. (B) Amplitudes of R1, R2, F, and D of the intrinsic signals and a- and b-waves of the ERGs at different flash intervals. Amplitudes are relative to the maximum for each signal component.
vessels are not present (Temporal and Nasal), and (5) the entire optic disc (D). A diffuse flash of −0.7 log unit intensity was used for stimulation, and 17 consecutive trials with 3-minute intervals were averaged.

The light reflectance changes were especially large in the central region where the central retinal artery and vein pass through the optic nerve (three times larger than that in the whole region; Fig. 6B). The light reflectance changes over the superior branch of the central retinal artery and vein were 1.2 times larger than that of the whole region. Although the size of the intrinsic signals varied in different regions within the margins of the optic disc, the time course at each region seemed to be almost the same (Fig. 6C).

**Focal Stimulation**

The recording of the focal macular ERG is a technique used to measure the electrical responses in the macula by focally stimulating the macular region.24,25 Focal flash stimuli can be given to the posterior retina with our recording system, however, our system is not set up to deliver a background illumination to suppress the rod responses and cannot measure the electrical activity in the stimulated region. We have stimulated focal regions of the posterior retina, and compared the time course of the intrinsic signals in both the stimulated and nonstimulated regions in dark-adapted conditions.

First, the macular area including the fovea was focally stimulated with an 8.8° circular stimulus (Figs. 7A, 7B). The light...
reflectance in the stimulated region decreased (Fig. 7B; F) and the region that darkened exactly matched the location of the stimulus (Fig. 7A). In contrast, the region without stimulation became brighter at the later phase of a recording trial (Fig. 7B; I). When two quadrants of the posterior retina were stimulated with the macula spared, the intrinsic signal showed exactly the same darkening pattern as the shape of the stimulus (Fig. 7C). The stimulated posterior retina (I) showed a negative $R_1$ and negative $R_2$ signal (i.e., a darkening; Fig. 7D). The nonstimulated area of the posterior pole (S) was brighter (positive $R_2$), which is usually not observed under other recording conditions. The fovea (F), where the stimulus was masked, did not show any light reflectance changes after the flash.

**DISCUSSION**

The origin of the intrinsic signals in the cerebral cortices has been extensively investigated; however, most of the studies have dealt with the deoxygenation of hemoglobin. The standard hypothesis is that the intrinsic signals in the cerebral cortex arise from light reflectance changes due to the many metabolic changes after neural activation. For example, the intrinsic signal measured at 570 nm is dominated by changes in the blood volume in the capillaries: That at 600 to 650 nm is dominated by the changes in the deoxygenation level of hemoglobin, and that in the infrared region is dominated by changes in tissue light scattering. Although different metabolic changes are highlighted at different wavelengths, the optical responses obtained at these wavelengths had nearly the same spatial pattern of activation as that of the activated neurons. Whatever wavelength was chosen for the measurement of reflectance, the most critical premise for evaluating the intrinsic signal has been that it is the darkening (i.e., a decrease in light reflectance), that correlates with the local neural activity. This is more or less true of the intrinsic signal images in the retina; however, the spatial distribution of the signals appeared to be more complicated when the retina is focally stimulated.

Our goal was to find out what each signal component represented by comparing the intrinsic signals with the ERGs recorded under the same conditions. Although the spatially localized responses of the intrinsic signals cannot be directly compared with the responses in full-field ERGs, this comparison may provide us with some keys to determine the possible mechanisms of the production of the intrinsic signals, because the neuronal mechanisms of the production of the ERGs have been well investigated.

It is important to understand that, in principle, the intrinsic signals are not necessarily produced by photoreceptors: There may be differences in the site of the photoreceptor and the site for producing the signals. The light reflectance changes reflect the summation of the stimulus-evoked metabolic changes happening in the 10 retinal layers, each of which may produce signals with different characteristics. The same difficulty arises when the origin of the different components of the ERGs is investigated. Thus, the type of activated photoreceptors and
the location where the signal is produced should be carefully separated.

D Signals

The threshold of the D signal was comparable to that of the ERG b-wave in the dark-adapted condition (Fig. 2). The threshold was higher by 2.0 to 3.0 log units under light-adapted conditions (Fig. 3), and the amplitude was greatly decreased in the presence of background illumination (Fig. 5). These results indicate that the D signal evoked by a dim flash (weaker than −5.0 or −6.0 log units) under dark-adapted conditions reflects the activation of rod photoreceptors, and those evoked by stronger flash reflects the activation of both cone and rod photoreceptors. The D signal evoked under light-adapted conditions reflects mainly the activation of the cone photoreceptors.

The time course of the D signal is slow and is probably produced by a flash-induced blood volume or flow increase. The decrease in light reflectance is due to the increased light scattering of the red blood cells. Previous studies have shown that flashing lights can increase the blood flow at the optic disc of humans and cats. An increase in blood volume is known to produce optically measurable changes in blood volume or flow increase. A contribution by the blood vessels is not present, the light reflectance changes showed a time course similar to that in the central region (Fig. 6C). The threshold for the D signal is derived from the blood volume or flow changes in the capillaries. As for the question of whether the blood volume or flow contributes more significantly to this signal, we do not have any evidence to conclude which has the greater role and recommend that the mechanism of blood-induced signal, we do not have any evidence to conclude which has the greater role and recommend that the mechanism of blood-induced signal be thoroughly investigated.

Neural activity in the optic nerve causes shrinkage of the extracellular space due to cellular swelling, and this was detected optically by intrinsic signal imaging in rats. We believe that part of the scattering changes may be due to swelling of the axons of the ganglion cells or of the glial cells. Its contribution to the whole intrinsic signal, however, may be masked by the relatively large reflectance changes due to changes in blood volume or flow. A contribution by the changes in deoxygenated hemoglobin concentration in the capillaries to the intrinsic signal may also exist, although it is believed to be negligible compared with that of tissue light scattering under infrared light observation.

R2 Signals

The properties of the R2 signal were similar to those of the D signal, except that R2 became very small and in some cases became positive under light-adapted conditions (Figs. 3B, 5B). The R2 signal is probably a complex of different components and origins because the posterior retina is a complex layered structure, and its signal properties cannot be explained simply by the blood volume or flow changes in the capillaries.

We suggest that the inner retina may be the main contributor to the D and R2 signals because this type of slow signal was not observed at the fovea, which lacks the inner retinal layers including the blood vessels. Our data did not allow us to determine which type of cells contribute the most to the flash-evoked responses observed at the optic disc or the posterior retina.

We attempted to keep the systemic condition of the anesthetized monkeys as constant as possible during the data acquisition. In some trials, however, the heart rate became unstable and rapid changes occurred during consecutive recordings under the same stimulus conditions. For example, the heart rate increased from 120 per minute to 140 per minute during two consecutive trials in one monkey. Although such data obtained under unstable conditions were discarded, we did note that it was always the amplitudes of D and R2 signals that were affected by the changes in the heart rate. In contrast, the amplitudes of F and R1 signals were much less affected by changes in heart rate (data not shown). This observation suggests that the D and R2 signals are related to blood-induced changes more than are the F and R1 signals.

It was interesting that the amplitudes of D and R2 signals were largest with 3- to 5-minute flash intervals (Fig. 4B). This finding is very different from the results of ERGs. It is possible that the mechanism by which neural activity is converted to the vascular response (i.e., neurovascular coupling) is most effectively activated when the stimuli are given repeatedly at intermediate intervals. This possibility should be investigated more extensively.

F Signals

The F signal, which is the average of light reflectance changes within the central 300 μm in diameter was faster than the D and R2 signals and reaches its peak within 100 to 200 ms (Fig. 1C). The threshold for the F signal was much higher than any other signals and was the same in both dark- and light-adapted conditions. The characteristic anatomic structure of the fovea (viz., the absence of rod photoreceptors, capillaries and other inner retinal layers), indicates that the F signal reflects the activation of cone photoreceptors under any recording conditions.

The light-scattering changes due to the microstructural changes after activation of the cone photoreceptors are probably the source of the F signals because the foveal avascular region is free of capillaries and not subject to the changes in hemoglobin concentration or blood volume after neural activation. Recent functional OCT studies using blood-free slice preparations showed that the reflectance in the photoreceptor layer is strongly changed by neural activation followed by microscopic morphologic changes.

R1 Signals

The amplitude of the R1 signal increases with an increase in stimulus intensity under both dark- and light-adapted conditions as did the F signal. The threshold of R1 lies between the threshold for the optic disc and fovea and was the same under both dark- and light-adapted conditions. This leads us to think that cone photoreceptors mainly contribute to the R1 signal, because bleaching of rods in the bright condition did not change the R1 threshold. It is difficult to assume, however, that rod and cone photoreceptors play different roles in light reflectance changes.

The property of the R1 signal is complicated in another way. The abrupt darkening after a flash may well be explained by the photoreceptor responses like the F signal, but the results in Figure 4B strongly suggest that R1 share the same signal origin with D and R2 signals: The amplitude of R1 signal did not increase with longer interstimulus intervals, but attained a maximum with 3- to 5-minute intervals as with the D and R2 signals. We suggest that the R1 signal is produced not
only by photoreceptors but also by other inner or middle layer structures, although our data do not provide any evidence for the exact origin.

A summary of the various properties in four signal components is shown in Table 1.

**Focal Stimulation**

Focal stimulation of the retina is one way to evaluate local neural activity in a dysfunctional retina and has been applied clinically with the focal macular ERG. The intrinsic signals measured with focal stimuli showed that this technique can also be used to study local responses. The focally stimulated region showed a decrease in the light reflectance after the stimulus, and this darkened region exactly matched the location of the focal stimulus (Figs. 7A, 7C). It was striking that the nonstimulated posterior pole showed a slow light reflectance increase after a fast light reflectance decrease (Fig. 7B, I). In another case, the nonstimulated posterior pole showed only a light reflectance increase (Fig. 7D; S).

The brightening observed in the nonstimulated region in late phase (Fig. 7B; I, Fig. 7D; S) may be explained by (1) some type of horizontal interaction by, for example, horizontal cells, through which stimulated neurons could affect the reflectivity of the neurons outside the stimulated region, or (2) the spatial interaction in the intrinsic signals between the stimulated and nonstimulated regions via an inhomogeneous distribution of capillary blood flow. These explanations, however, do not account for the strong and homogeneous brightening over the whole posterior region triggered by a small focal stimulus. It is possible that the properties of the signals, such as polarity and threshold, are different in different retinal layers, and the difference in signal time course between stimulated and nonstimulated regions would reflect the difference of layers that mainly contribute to the light reflectance changes.

Recently, OCT imaging of neural activity has been demonstrated in the feline visual cortex, isolated frog and rabbit retina, and intact rat retina. Functional OCT studies in slice preparations have revealed the complex nature of flash-evoked changes in the reflectances from various intraretinal layers: a decrease of near infrared scattering in the photoreceptor layer and an increase in the ganglion cell layer, or a decrease in the photoreceptor inner segment and increase in the internal plexiform layer and photoreceptor outer segment. Srinivasan et al. first reported the results of functional OCT signals in the intact retina and demonstrated the flash-evoked reflectance increase in the photoreceptor outer segments. In these studies, the increase in light reflectance after a flash was mainly observed in the photoreceptor layer, whereas the decrease was mainly observed in monkey and human retinas. This difference in signal polarity may be attributable to the difference in the methods used to measure the reflectances. In addition, other factors, such as the use of sectioned preparations that lack the RPE layer and blood supply, differences among species, and differences in the recording region in the retina, should also be considered.

The light-scattering changes after a flash observed in functional OCT is thought to be derived from the structural changes in the outer segment discs, membrane hyperpolarization, cell swelling, and changes in the composition of the interphotoreceptor matrix. These sources can also explain the rapid light reflectance changes observed in our study. As suggested by our results and those of functional OCT studies of retinal sections, the characteristics of the light reflectance changes after a flash are different in different layers and different retinal locations and may be far more complex than the conventional idea of intrinsic signals mainly investigated in the cerebral cortex. Interpretation of the retinal intrinsic signal is thus difficult, and maximum care should be taken in choosing the proper recording conditions and which signal is most closely correlated with the neural activities of the retina.

In conclusion, our results showed that the intrinsic signals in the retina are composed of several components of different origins, although the precise cellular mechanisms of signal production were not determined. The sensitivity of intrinsic signal images was high enough to detect weak neural activity in the retina (e.g., the signal in the posterior retina and the optic disc were as sensitive as the ERG b-wave in the dark-adapted condition). Moreover, the distribution of intrinsic signals reflects not only the cellular distribution in the retina but the current level of the activities. Although the source of the signal was much more complex than initially thought, by carefully selecting the proper recording condition, this imaging technique may have a potential to estimate the neural responses of different origins and obtain more useful information about various types of retinal disorders with different etiologies than the conventional electrophysiological examinations such as full-field ERGs, focal macular ERGs, and multifocal ERGs.

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**References**


**Table 1. Properties of Four Components in Retinal Intrinsic Signals**

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<tr>
<th>Properties of Four Components in Retinal Intrinsic Signals</th>
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<th>R2</th>
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<td>Contribution of blood-related light reflectance changes</td>
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<tr>
<td>Contribution of inner or middle layer</td>
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<tr>
<td>Contribution of outer layer</td>
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