Evaluating Neural Activity of Retinal Ganglion Cells by Flash-Evoked Intrinsic Signal Imaging in Macaque Retina

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Purpose. Intrinsic signal imaging (ISI) detects light-induced microstructural or metabolic changes in retinal tissues. Thus, activities of the rod and cone systems could be mapped topographically. However, no direct evidence indicates the cellular origin of the signals. The purpose of this study was to determine whether and how retinal ganglion cells (RGCs) contribute to ISI.

Methods. In anesthetized macaque monkeys, the properties of intrinsic signals were investigated by simultaneous measurement of the retina and the primary visual cortex (V1) with different wavelengths of observation light, measurement of the flash-induced blood flow changes by laser Doppler flowmetry, and intravitreal injection of tetrodotoxin (TTX).

Results. Slow components of ISI correspond well to the flash-induced blood flow increase. Intrinsic signals of the posterior retina are partially decreased, and the signal of the optic disc is completely abolished by intravitreal injection of TTX at a concentration that should reduce the neural activities of RGCs. The intrinsic signal at the fovea did not change significantly after TTX injection.

Conclusions. Photoreceptors in the outer retina and RGCs in the inner retina are major contributors to the intrinsic signals, and the activity of the RGCs can be mapped by using fast and slow components of the signal in the posterior retina. The functional organization of the RGC layer has not been objectively mapped; results presented here indicate that the ISI has the potential to do this. (*Invest Ophthalmol Vis Sci.* 2008;49: 4655–4663) DOI:10.1167/iovs.08-1936

With the advancements in techniques, functional imaging of neural activities in the animal retina has become feasible by intrinsic signal imaging (ISI),[1–5] functional magnetic resonance imaging (fMRI),[6,7] and functional optical coherence tomography (fOCT).[8,9] Recently, the fast phototransduction process in single photoreceptor cells (flash-induced scintillation) in the living human eye could be observed with a high-speed flood-illumination retina camera equipped with adaptive optics.[10,11] These imaging techniques, though technically early for clinical application, may perhaps be used as diagnostic tools to detect various functional disorders in the human retina at the early stages, before subjective and anatomic disorders become permanent. For example, in the eyes of adults with glaucoma, one of the leading causes of blindness in the world,[12] the function of some retinal ganglion cells (RGCs) is altered, and the visual field is lost corresponding to the extent of the RGC dysfunction. However, it is well known that numerous RGCs have already lost their function before visual field loss can be detected by psychophysical examination.[13–15] Moreover, the activity of RGCs is not reflected in the conventional electrophysiological examinations, such as electroretinography (ERG). At present, there is no clinically established way to map objectively the dysfunctional area of RGCs over the retina. ISI is a well-established imaging technique recently used to translate neural activities elicited by photic or electrical pulses to visible changes in the appearance of the retina.[1–5] ISI has an advantage over fOCT and fMRI in that the response distribution of cone- and rod-induced retinal activities over the entire posterior ocular fundus can be topographically mapped with fine spatial resolution.[1,2] This is important because identification of the affected site is essential for the diagnosis and the treatment of diseases. The ISI, however, does not have spatial resolution in depth because it measures the light reflectance changes passing through all the retinal layers.

In our previous studies on the retina of macaque monkeys, we categorized the flash-induced intrinsic signals into fast and slow components: the fast signals peaked at 100 to 200 ms and were observed in the posterior retina including the fovea, and the slow signals peaked usually at more than 6 seconds and were observed at the optic disc and nonfoveal posterior retina.[1–3] Based on the response properties of the intrinsic signals together with the electroretinograms evoked by the same stimuli under different recording conditions, we propose that the slow components of retinal intrinsic signals reflect the activity of the inner or middle layer of the retina, though the cellular origin of this component has not been investigated in detail.

The purposes of this study were to investigate the source of each component of the intrinsic signals by simultaneous measurement of the ISI of the retina and the primary visual cortex (V1) with different wavelengths of observation light, to measure blood flow changes in the ocular fundus after a flash stimulus by laser Doppler flowmetry, and to measure ISI after intravitreal injection of tetrodotoxin citrate (TTX; Wako Pure Chemical Industries, Ltd., Osaka, Japan). Our results demonstrate that the time course of the intrinsic signal at the optic disc did not differ with the wavelength of the observation light, that the time course of the blood flow changes after a flash stimulus was approximately the same as the slow component of the intrinsic signals at the posterior retina and the optic disc,

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and that TTX injection resulted in abolition of the slow component of the intrinsic signals at the posterior retina and the optic disc and in partial decrease of the fast component at the posterior retina though the signal at the fovea did not change. These results suggest that the slow components of the flash-induced intrinsic signals observed at the posterior retina and the optic disc reflect blood flow increases and that part of the fast component at the posterior retina reflected the local light-scattering changes. Both changes resulted, in part, from the activation of RGCs in the inner retina.

METHODS

Experiments were performed on three rhesus monkeys (*Macaca mulatta*; M1, M2, M3) and one Japanese monkey (*Macaca fuscata*; M4). M1 was used for experiment 1, M2 and M3 were used for experiment 3, and M4 was used for experiment 2. The experimental protocol was approved by the Experimental Animal Committee of the RIKEN Institute, and all experimental procedures were carried out in accordance with the guidelines of the RIKEN Institute and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

After intramuscular injection of atropine sulfate (0.08 mg/kg), the monkeys were anesthetized with droperidol (0.25 mg/kg) and ketamine (5 mg/kg) and then were paralyzed with vecuronium bromide (0.1–0.2 mg/kg/h). They were artificially ventilated with a mixture of 70% N_2O, 30% O_2, and 1% to 1.5% isoflurane. Electroencephalograms, electrocardiograms, expired CO_2, and rectal temperatures were monitored continuously throughout the experiments. Before recordings, the pupils were fully dilated with topical tropicamide (0.5%) and phenylephrine hydrochloride (0.5%).

Three experiments were performed. In experiment 1, intrinsic signals were measured from the optic disc and primary visual cortex (V1) simultaneously with different wavelengths of the observation light to clarify the contribution of blood oxygenation. In experiment 2, flash-induced blood flow changes of the ocular fundus were measured by laser Doppler flowmetry and compared with the time course of the flash-induced intrinsic signals measured with an infrared observation light. In experiment 3, flash-induced intrinsic signals of the ocular fundus were measured before and after intravitreal injections of TTX. The effect of TTX was confirmed by measuring the photopic negative response (PhNR) of ERG.

Intrinsic Signal Imaging and Data Analyses

Procedures used to record the intrinsic signals have been described in detail.[1,2] A modified digital fundus camera system (NM-1000; Nidek, Aichi, Japan) was used to observe and measure light reflectance changes from the ocular fundus after 30 minutes of dark adaptation. The fundus was continuously monitored with light from a halogen lamp filtered through one of three band-pass interference filters: green (570 ± 10 nm), red (630 ± 20 nm), and infrared (870 ± 30 nm). For experiments 2 and 3, only the infrared filter was used. Fundus images were recorded with a CCD camera (PX-30BC; Primetech Engineering, Tokyo, Japan; spatial resolution, 640 × 480; temporal resolution, 1/30 seconds), and the images were digitized with a personal computer equipped with a video frame grabber board (Corona II; Matrox, Quebec, Canada; gray level resolution, 10 bits). The fundus camera photographed a 45° area of the posterior pole that included the fovea, superior and inferior vascular arcades, and optic disc.

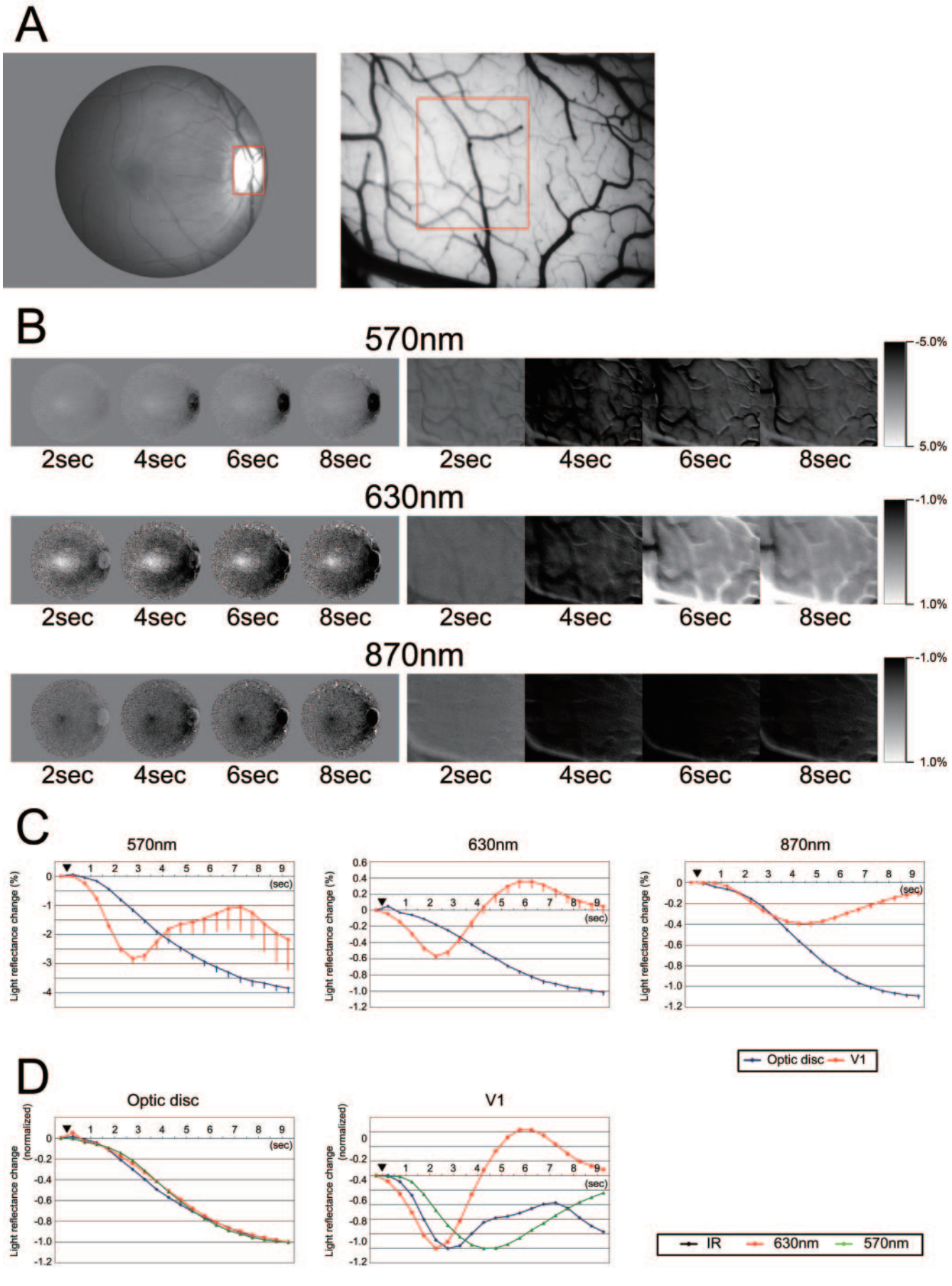
Signal intensities were measured at three retinal sites: the fovea (15 × 15 pixels, 1.75° in diameter), the posterior retina between the fovea and the inferior temporal artery (95 × 25 pixels), and the optic disc (40 × 60 pixels). To compare the intensities of the intrinsic signals in experiment 3, we used the value of the lowest peak of the light reflectance of the foveal response (Fig. 4A, Fovea), the averaged value during the initial 500 ms after the flash for the fast component of the posterior retina (Fig. 4A, R_{fast}), and the averaged value during the last 500 ms at the end of recording trials for the optic disc response (Fig. 4A, Optic disc). To extract the slow component of the posterior retina, we subtracted the value R_{fast} from those during the last 1500 ms at the end of recording trials (Fig. 4A, R_{slow}).

To measure the flash-evoked intrinsic signals in the cortex (experiment 1), a stainless steel chamber (17 mm in diameter) was mounted on the skull over the contralateral side of V1 in M1. The skull and the dura mater were removed inside the chamber, and the chamber was filled with silicone oil (ADATO-SIL-OL 1000; Bausch & Lomb GmbH, Heidelberg, Germany) and was tightly sealed with a glass coverslip to reduce the movement of the cortex. The cortical surface was illuminated by two fiber-optic light guides through the glass coverslip window, and light reflectance was recorded with the same type of a CCD camera used in retinal recording.[16,17] Light from a halogen lamp was filtered through three band-pass filters: green (570 nm), red (630 nm), and infrared (870 nm). The entire imaged area measured 8.8 × 6.6 mm and was imaged on 640 × 480 pixels. For the measurement of signal intensity, we averaged the light reflectance changes in the central region, which covered 3.05 × 3.63 mm (222 × 264 pixels; Fig. 1A, right). The camera was focused 500 μm below the cortical surface. A recording trial consisted of 300 (experiment 1), 360 (experiment 2), or 450 (experiment 3) video frames collected at a rate of 30 frames/s for a total recording time of 10, 12, or 15 seconds, respectively.

For stimulation, an unfiltered xenon flash (duration, 1 ms) was given to the entire posterior pole of the ocular fundus (500 ms in experiments 1 and 3 and 2 seconds in experiment 2 after the beginning of data acquisition). The flash luminance measured at the cornea was 56.1 cd · s/m^2, measured at 50.2 mm from the objective lens by a photoradiometer (IL-1700; International Light Technologies, Peabody, MA). Timing of the data acquisition and stimulus delivery was under computer control.

Changes in light reflectance following stimulus—darkening [decrease in light reflectance] or brightening [increase in light reflectance]—of the retina were measured in the ocular fundus. Under infrared observation, the light reflectance of the entire posterior retina decreased (fundus image became darker) after flash stimulus. The optical signal was calculated as follows: grayscale values of the image obtained after the stimulus were divided, pixel by pixel, by those obtained during the prestimulus period, and this ratio was rescaled to 256 levels of grayscale resolution to show the stimulus-induced reflectance changes. To determine the time course of the flash-induced reflectance changes, the grayscale values of 15 video frames collected in 0.5 second were averaged for each datum point. We tried to make the total recording time as short as possible to keep the physiological conditions, such as corneal transparency, heart rate, and blood pressure, stable and to prevent photographic damage of the neural tissue

FIGURE 1. Intrinsic signals measured simultaneously from the optic disc and V1 with different wavelengths of the observation light. (**A**) Images of the recorded regions in experiment 1: ocular fundus (*left*) and cortical area V1 (*right*). Areas used for data analysis are shown by the *rectangles*. (**B**) Time courses of two-dimensional images of the ocular fundus (*left*) and cortical area V1 (*right*) showing the light reflectance changes evoked by a flash stimulus recorded with different wavelengths of the observation light. Thirty consecutive video frames collected during 1 second were averaged for one poststimulus image. Darkened regions indicate a decrease of light reflectance after the flash stimulus. Note that foveal regions observed with 570 and 630 nm become brighter after the flash because of the strong bleaching of cone photopigments. (**C**) Plot of the time courses of flash-evoked light reflectance changes. The time after the flash is shown on the abscissa, and the delivery of the flash is indicated by the *arrowhead* (same in the following figures). Each point is the average of 15 video frames collected during 0.5 second of the light reflectance changes. Averages of 10 trials are shown with the SE of the means. (**D**) Plot of the time courses of flash-evoked light reflectance changes, presented as relative values to the maximum in the optic disc and V1.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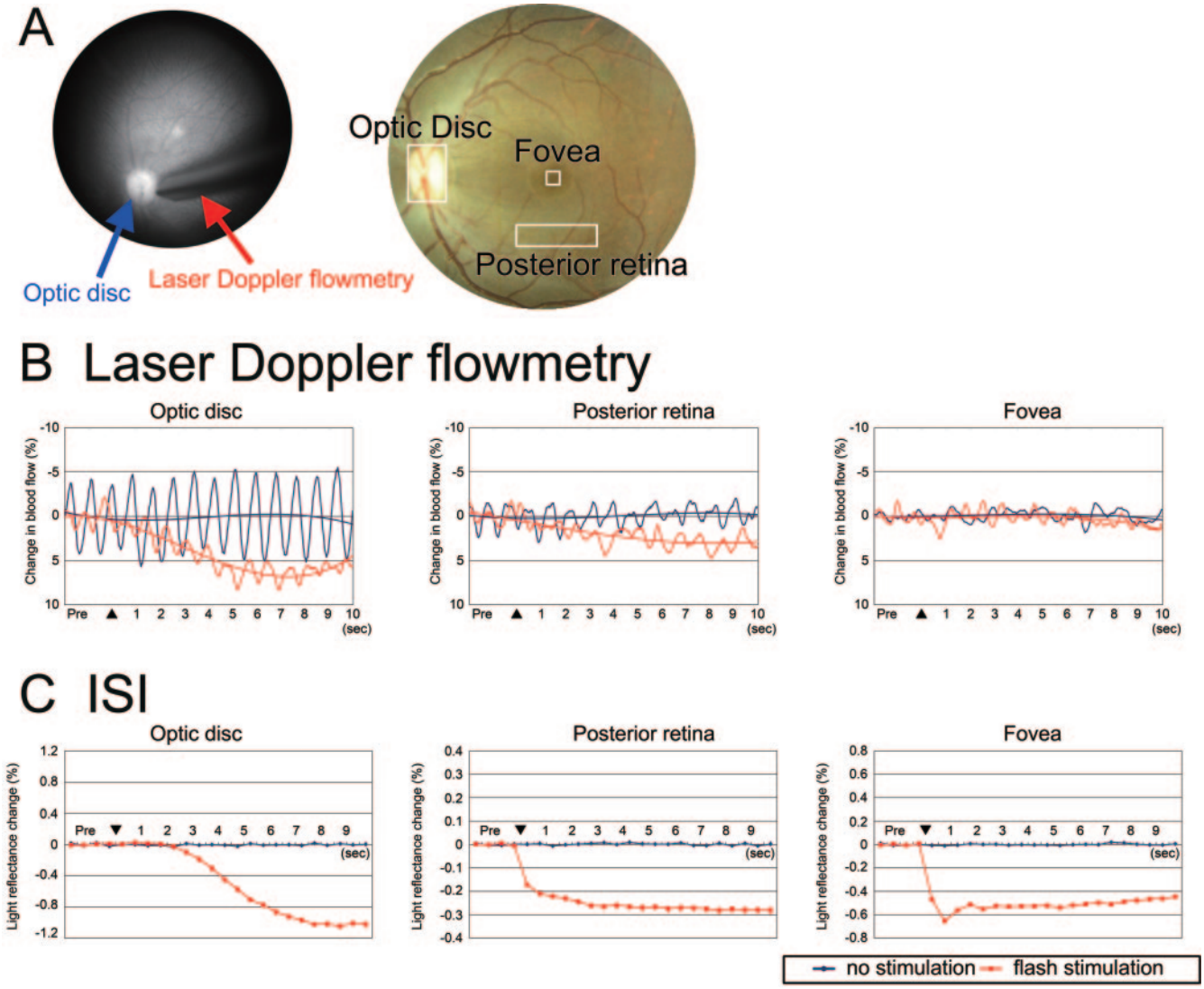

FIGURE 2. Flash-induced blood flow changes of the ocular fundus by a laser Doppler flowmetry. (**A**, *left*) Measurement of blood flow at the optic disc by laser Doppler flowmetry. The probe in the vitreous cavity and the location of the optic disc are indicated by arrows. *Right*: fundus photograph of normal retina showing the regions for the ISI analysis in experiment 2. (**B**) Plot of the time courses of blood flow changes with (*red*) or without (*blue*) flashes, measured with a laser Doppler flowmetry, from three locations indicated in (**A**). Averages of 20 trials are shown with polynomial trendlines (order 3). (**C**) Plot of the time courses of light reflectance changes with (*red*) or without (*blue*) flashes measured by the ISI, from three locations indicated in (**A**). Averages of 10 trials are shown with SE.

by the visible observation light. The data of 10, 10, and 15 consecutive trials were averaged in experiments 1, 2, and 3, respectively.

Measuring Blood Flow by Laser Doppler Flowmetry (Experiment 2)

Flash-induced blood flow changes were measured in the left eye of M4 by a laser Doppler flowmetry system (Periflux 5010; Perimed, Stockholm, Sweden; solid-state diode laser, 780 μm; power, <1 mW; probe, PF403; probe diameter, 450 μm; fiber separation, 250 μm; time constant, 0.03 s) under aseptic conditions. After local peritomy of the conjunctiva, the laser probe was inserted through a scleral port made at the 2 o'clock position 3 mm from the corneal limbus. The size of the sclerotomy was small enough to keep the intraocular pressure normal and constant during recording. The laser probe was fixed firmly by a manual micromanipulator placed in front of the monkey's face. For measurement of the blood flow changes at the optic disc and the perimacular region, the tip of the probe was placed less than 1 mm above the target (Fig. 2A) and was directed on the vessel-free regions to measure the averaged blood flow changes in capillaries. Blood flow was represented in arbitrary units (perfusion unit [PU]). Blood flow changes evoked by the same intensity of flash stimulus was calculated by dividing blood flow after the stimulus by the averaged blood flow during a 2-second period before the stimulus. Data of 20 consecutive trials were averaged.

TTX Injection (Experiment 3)

TTX dissolved in physiological saline (50 μL, 8 μM), was injected into the vitreous cavity of the left eye of M2 and M3 under anesthesia. Before injection, 50 μL aqueous humor was removed from the corneal limbus by a 27-gauge needle. TTX was then injected though the pars plana (3 mm posterior to the corneal limbus) into the geometric center of the vitreous by a 27-gauge needle at the superior temporal position. The same amount of saline was injected by the same procedure into the vitreous cavity of the fellow eye for control recordings. Slow intrinsic signals of the optic disc and the posterior retina are very sensitive,[2] and if small local regions in which RGC function has not been completely blocked remain, the blood flow increase of the optic disc may be strongly triggered for that local region. Thus, we had to

completely block RGC responses over the entire retina. Moreover, the intrinsic signals were susceptible to the physiological conditions, such as intraocular pressure, corneal curvature, and vitreous transparency, of the whole body and of the eyeball. To obtain sufficient pharmacologic blockade and recovery of the physiological condition of the eye, the recording was not conducted on the day of injection. Intrinsic signal imaging and electroretinographic recordings were made 1 week before TTX injection, 1 day after injection, and 4 weeks after injection.

Electroretinographic Recordings (Experiment 3)

Electroreginograms were recorded under light-adapted conditions on the same day of the ISI measurements. An LED contact lens electrode with a background illumination source (Mayo, Aichi, Japan) was inserted into one of the eyes. After 15 minutes of light adaptation (background, 25 cd/m^2), a white flash of intensity 3 cd · s/m^2, duration 3 ms, was given 15 times at 1-second intervals. Electroretinograms were amplified 10,000× and the band-pass filters were set at 0.3 to 500 Hz (PowerLab; AD Instruments, Colorado Springs, CO). The PhNR was measured from the baseline to the first negative trough after b-wave in the single-flash cone ERG (Fig. 3A).[18,19]

Results

Experiment 1

The time course of the intrinsic signals evoked by three wavelengths (570, 630, and 870 nm) of the observation light were compared at the optic disc and cortical area V1 (Fig. 1). Although the absolute reflectance changes were different (Fig. 1C), the time course of the changes at the optic disc was the same for the three wavelengths (Fig. 1D). Reflectance slowly decreased after the flash and reached its negative peak at the end of the recording period.[2] The time course at cortical area V1, on the other hand, differed for the different wavelengths (Figs. 1C, 1D). The onset of reflectance decrease was the earliest with 630 nm, followed by 570 nm and 870 nm. With 630 nm, the light reflectance change reached a negative peak 2 seconds after flash and was followed by an increase in the light reflectance that overshot the baseline reflectance. With 540 nm and 870 nm, the light reflectance change reached a negative peak at 3 and 4.5 seconds after flash, respectively, but the large overshoot increase was not observed. This pattern in the time course of the signals in cortical area V1 was the same as when a grating stimulus was used.[16,20]

Experiment 2

Flash-induced blood flow changes of the ocular fundus were measured by laser Doppler flowmetry and were compared with the intrinsic signals evoked by the same flash intensity. The retina was observed by infrared light (Fig. 2). Blood flow at the optic disc gradually increased after the flash and reached a peak 7.5 seconds after the flash (Fig. 2B). This time course was similar to that of the intrinsic signals (Fig. 2C). Similarly, the blood flow in the posterior retina gradually increased after the flash and reached a peak at 8 seconds (Fig. 2B). This time course, however, was different from that of the intrinsic signal. The time course of the blood flow at the posterior retina did not have the fast changes observed in the ISI (Fig. 2C).

We have reported[2] that the flash-evoked intrinsic signal in the posterior retina had two components, a fast light-reflectance decrease that peaked at 100 to 200 ms (Fig. 4A, R_{fast}) and a slow light-reflectance decrease that peaked at 6 seconds or later (Fig. 4A, R_{slow}).[2] Blood flow changes in the posterior retina seemed to match only the slow component of the ISI. In the foveal area, a flash-evoked blood flow change could not be observed by laser Doppler flowmetry, though large and fast light reflectance decreases were observed in the ISI (Figs. 2B, 2C).

Experiment 3

Flash-induced intrinsic signals of the ocular fundus were measured before and after intravitreal injection of TTX (Fig. 3). On the day of each recording, photopic electroretinograms were recorded to evaluate inner retinal activity by measuring the PhNR.[18,19] From all the components of the electroretinogram, only the PhNR amplitude was reduced after TTX injection, and it recovered to normal levels 4 weeks after injection (Fig. 3A).

Pseudocolor maps of the signal distribution, averaging 7 to 9 seconds after flash, in the posterior pole are shown in Figure 3A. One day after TTX injection, intrinsic signals at the optic disc and the posterior retina, but not the foveal region, were reduced. The signal at the fovea did not change. Four weeks after injection, responses in the whole posterior pole appeared to be the same as before injection.

The time course of the intrinsic signals before and after TTX injection is shown in Figure 3B. The response at the optic disc was abolished after TTX injection. The fast component was partially reduced at the posterior retina, and the slow component was completely abolished by TTX injection. The response at the fovea was not affected by TTX injection.

Amplitudes of the four components of the intrinsic signals (optic disc, R_{fast}, R_{slow}, Fovea) for 3 recording days in two monkeys were compared (Fig. 4). Statistical analyses was performed with the Mann–Whitney *U* test to compare group means, and the differences were considered significant when $P < 0.05$. No significant changes were observed in the control eyes either in the optic disc or in the posterior retina. Signals of the optic disc and the R_{slow} component were abolished 1 day after TTX injection. R_{fast} signals were significantly reduced 1 day after injection (59.9% and 78% compared with preinjection levels in M2 and M3, respectively). The intensity of the foveal response was not changed by TTX injection, but statistical analysis could not be used for the foveal responses because the foveal response required 30 minutes of dark adaptation, and only the initial trial after adaptation could be used for the comparison.[2]

Discussion

Experiment 1

We have measured the flash-evoked intrinsic signals simultaneously from the optic disc and cortical area V1. The three wavelengths, 570 nm, 630 nm, and 870 nm, of the observation light were selected because they could best determine the changes in blood volume, deoxygenated hemoglobin, and tissue light scattering, respectively. We found that the time course of the intrinsic signals in area V1 had a typical pattern, similar to that recorded with grating stimulus by other authors.[16,20] The reflectance pattern at the optic disc was the same at all wavelengths (Fig. 1D), suggesting that the slow signal observed at the optic disc was not related to the degree of oxygenation of the hemoglobin.

It is generally assumed that light-scattering changes represent microscopic morphologic changes elicited by neural activity, such as cell swelling associated with ion and water movements and changes in synaptic vesicle density associated with synaptic transmission. Light-scattering changes are believed to be free of changes in blood flow and oxygenation, and amplitudes are constant whichever wavelength is used as the observation light.[16]

The amplitude of the signal at the optic disc, however, was almost four times larger at 570 nm than at 630 nm or 870 nm,

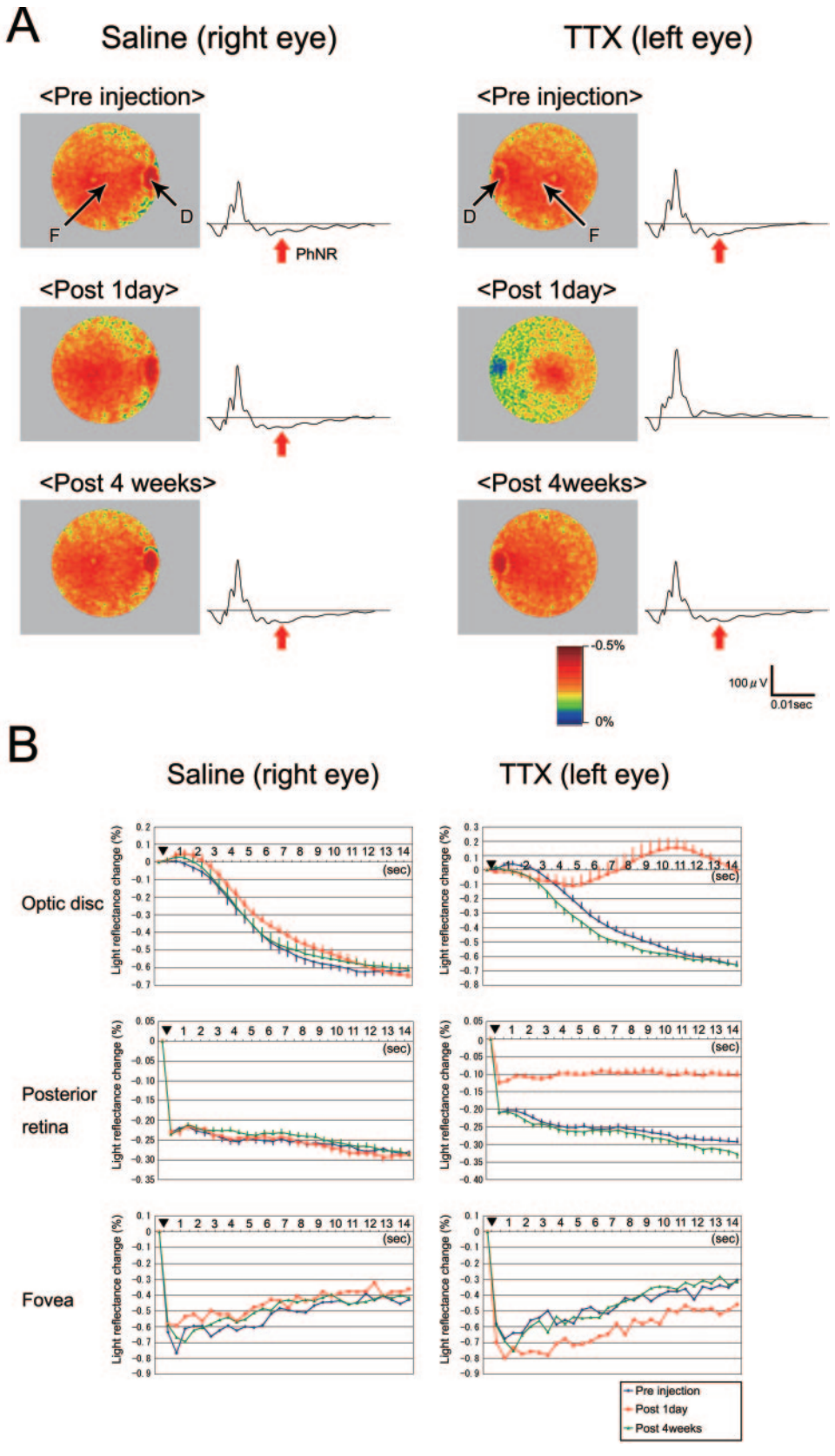

FIGURE 3. Intrinsic signals of the ocular fundus before and after the intravitreous injection of TTX. (**A**) Pseudocolor response topography of flash-evoked light reflectance changes with saline or TTX injection, measured before, 1 day after, and 4 weeks after injection. Each image was the average from 7 to 9 seconds after a flash. Electroretinographic responses recorded on the same day. *Arrows*: locations of optic disc (D) and fovea (F). *Red arrows*: PhNR. (**B**) Plot of the time courses of light reflectance changes with saline or TTX injection, measured from three locations indicated in Figure 2A. Results before, 1 day after, and 4 weeks after injection are shown by different colors. In the optic disc and posterior retina, the averages of 15 trials are shown with SE.

probably because the increase of the light-scattering signal at the optic disc reflected changes in the flow of red blood cells in the vessels, which were triggered by the neural activity in the inner retina. It is well known that hemoglobin absorbs more green light than red light. The ratio of blood-related light reflectance changes to the total tissue light reflectance changes can be larger when illuminated at 570 nm than at 630 nm or 870 nm; this was confirmed by the results of experiment 2.

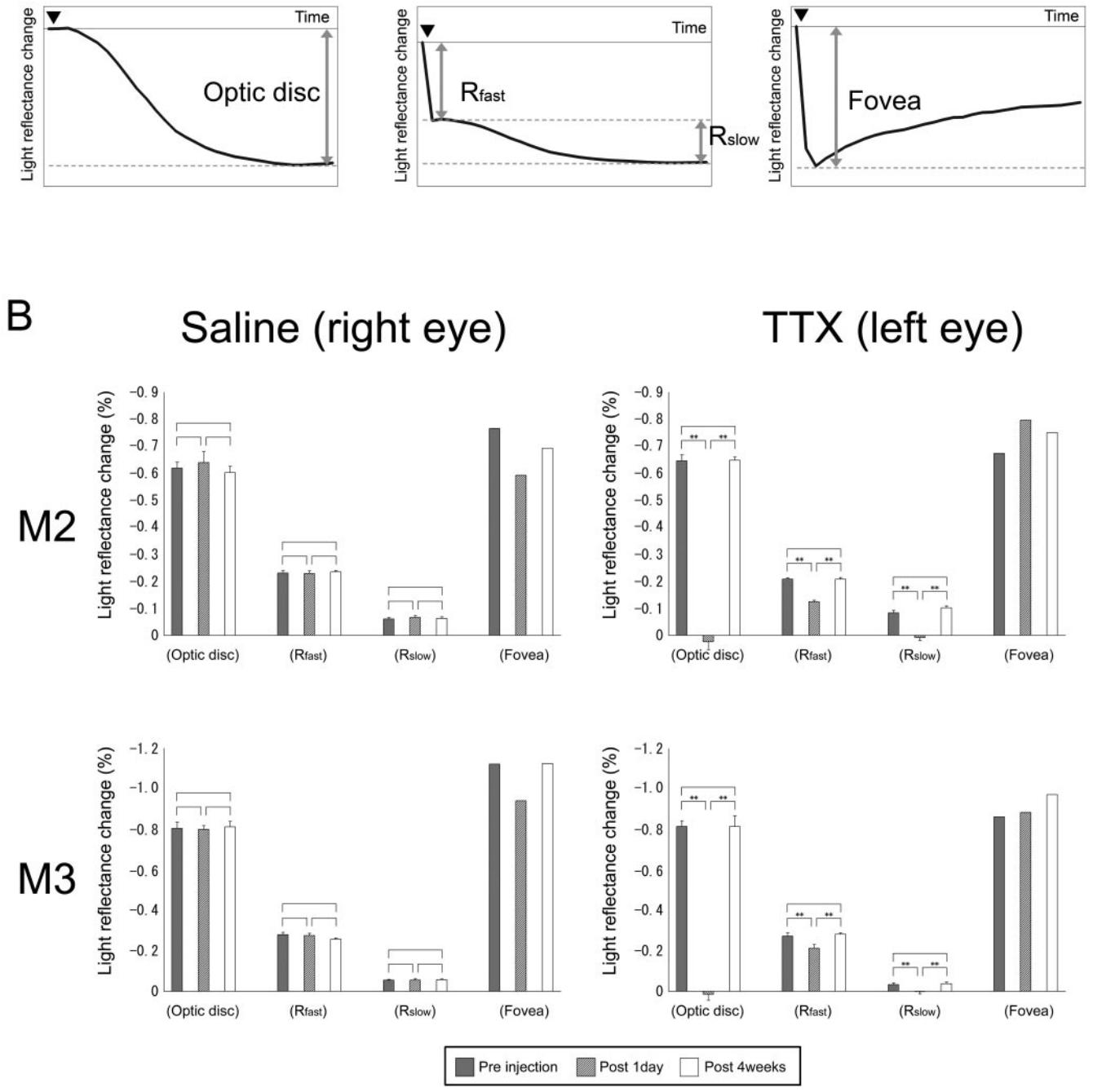

FIGURE 4. Change in four components of intrinsic signals after TTX injection. (**A**) Definition of the four components in flash-evoked intrinsic signals. (**B**) Changes in ISI signals after saline or TTX injection are shown for four signal components in M2 and M3. $^{**}P < 0.01$, statistically significant.

Experiment 2

Experiment 2 showed that the slow intrinsic signals at the optic disc and the posterior retina reflected blood flow increases after stimulation. The fast changes observed in the ISI at the fovea and the posterior retina could not be detected by a laser Doppler flowmetry. Although the fast intrinsic signals were relatively large, they were independent of blood flow changes. At the fovea, the blood flow changes are believed to reflect changes in the choroidal blood flow because of the absence of capillaries in the central 300 μm of the retina. Blood flow in this region was constant even after a flash stimulus.

Riva et al.[21,22] showed that blood flow on the optic nerve head measured by laser Doppler flowmetry increased after diffuse flicker stimulation in cats and humans.[21,22] The stimulus duration used in their studies was as long as 60 seconds, and the precise time course of the signals immediately after stimulus onset could not be determined. However, with extensive experiments, together with the data of patients with glau-

coma, they concluded that flash-evoked blood flow changes were induced by the activity of the RGCs.[21–24]

Experiment 3

The RCGs are the major retinal elements in retina that have spiking activity, and TTX can reduce the activity of RCGs by blocking the Na^+-dependent spikes.[25,26] The PhNR is known to be reduced in eyes with experimental glaucoma and after TTX injection and, thus, is highly dependent on the spiking activity of inner retinal neurons.[18,19] In our study, one of the eyes was injected with TTX, and the inner retinal activity was completely blocked. This suggested that the slow intrinsic signals observed at the posterior retina and the optic disc represented the blood flow increase after flash-induced spiking activity in the inner retina. Foveal signal was not affected by TTX at all because there are no RGCs in the foveal region.

Part of the fast component of the posterior retina represents the spiking activity of the inner retina and is attributed to light-scattering changes but is not related to blood flow. Another part of the fast intrinsic signals at the posterior retina that was not affected by TTX might have originated from activity from more distal neurons than the RGCs. The cellular origin of the non–blood-related fast light-scattering signals, however, may not be easily solved because each type of neural cells (photoreceptors, bipolar cells, Müller cells) may have its own independent characteristics for producing reflectance changes after neural activation. Recently, in vivo fOCT showed that the properties of the flash-evoked light-scattering changes were different in the outer and inner segments of the photoreceptors.[8,9]

Amacrine cells are also known to produce spikes, and the proportion of amacrine cells that contribute to the intrinsic signal is unknown. We should remember that the slow intrinsic signals in the posterior retina and the optic disc do not represent the activity of RCGs exclusively. Riva et al.[24] have shown that the blood flow increases at the optic disc after flickering flash stimulus is abnormally reduced in patients with ocular hypertension and early glaucoma. Considering that RGCs but not amacrine cells are the major neural elements affected by these disorders,[13–15,27–29] it is reasonable that the reduction of RGC activity in the posterior pole can be detected by the ISI.

Conclusions

Recently, a selective reduction in the PhNR of the electroretinogram has been reported in patients with glaucoma and optic nerve diseases.[18,19] Although the PhNR had been expected to be used to detect glaucoma at its early stage, it must be remembered that the electroretinogram is the mass response of the whole retina and that no spatial information, such as the location of scotoma in the Bjerrum area, can be obtained by this technique. Some attempts have been made to map the RGC function objectively by multifocal ERG.[30–32] However, it is still impossible to precisely discriminate the dysfunctional region of RGCs because of low spatial resolution.

The ISI, on the other hand, has high spatial resolution and is limited mainly by the resolution of the CCD camera and biological artifacts produced by the subjects. Our results showed that fast and slow signal components in the posterior retina were reduced by TTX. Theoretically, the fast signal component should have better spatial resolution because it is produced directly by the light-scattering changes immediately after neural activation of the inner retina. The slow signal, on the other hand, may not have such a good spatial resolution because it reflects the blood flow increases after neuronal activities, and the mechanisms underlying neurovascular coupling in the primate retina are still under investigation.

It is believed that stimulus-induced blood flow changes can be regulated by chemical mediators, such as nitrogen oxide, pH, and pCO_2,[33,34] but it is still unknown how close neural activity and blood flow increases correspond. Peppiatt et al. have recently reported that pericytes in the retinal capillaries can modulate the blood flow in response to changes in neural activity in isolated rat retinas. They proposed that a mechanism exists that finely regulates local blood flow at the capillary level.[35] If this is correct, then stimulus-evoked blood flow change may be used as a probe by which the activated region of the retina can be precisely mapped. This indicates that not only the fast but also the slow signals in the posterior retina may be used to map the functional status of the RGCs. Although correspondence between a region of low visual sensitivity and a reduction of the ISI signals should be confirmed in experimental animal models or in patients with local visual field defects, the ISI has the potential to be a diagnostic tool for mapping the functional status of the RGCs topographically.

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