Intrinsic Optical Signal Imaging of Retinal Activation

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Abstract

Fast intrinsic optical signals (IOSs) correlated with stimulus-activated retinal responses are reviewed. Fast IOSs have a time course comparable to the stimulus-evoked electrophysiological kinetics of the retina, and thus promise a new methodology for high-resolution evaluation of the physiological health of the retina. However, practical application of fast IOSs for retinal study and diagnosis is challenging because of their low sensitivity and limited specificity. Using isolated amphibian retinas, a series of experiments to optimize and characterize fast IOSs has been conducted. Fast, high-resolution near-infrared light imaging disclosed both positive (increasing) and negative (decreasing) optical responses in adjacent retinal areas, which satisfied spatial resolution essential to the differentiation of IOSs from opposite polarities. At the subcellular (∼μm) level, fast IOSs often exceeded 5% ΔI/I, where ΔI is the dynamic optical change, and I is the background light intensity. Experiments with isolated frog retinas suggest that negative IOSs stem primarily from the photoreceptor layer, while positive IOSs come from inner retinal layers.

Key Words: electrophysiology, intrinsic optical signal, near infrared light imaging, neural activity, retinal diagnosis

Introduction

Many eye diseases, such as age-related macular degeneration (AMD), diabetic retinopathy (DR), and glaucoma, can produce pathological changes in photoreceptors or inner retinal neurons. Early detection of retinal changes correlated with these eye diseases can substantially both enhance prevention and help treat visual impairment. Electrophysiological measurements can provide objective evaluation of the retina. Recent investigations have demonstrated the potential of electrophysiological measurements such as electroretinography (ERG), for early detection of AMD, glaucoma, and DR. ERG measurements are simple, sensitive, and helpful for clinical applications. However, traditional full-field ERG involves the massed measurement of electrophysiological responses of the entire retina, and thus lacks the resolution for exploring spatial details of retinal neural functions. Focal ERG, pattern ERG, and multifocal ERG can facilitate the identification of localized retinal dysfunctions. However, the applications of focal, pattern, and multifocal ERG are still limited by low spatial resolution, and cannot provide direct information on retinal morphology. Optical methods such as fundus examination can offer high spatial resolution evaluation of diseases associated with abnormal structural changes in the retina, but structural and functional changes of the retina are not always correlated. Combined structural and functional evaluation of the retina will improve diagnosis and treatment evaluation.

Intrinsic optical signal (IOS) imaging promises concurrent structural and functional evaluation of the retina with high spatial resolution. Stimulus-evoked transient IOSs have been extensively used for spatiotemporal mapping of dynamic brain activities. Recently, several imaging techniques have been demonstrated for IOS imaging of stimulus-evoked retinal activation. Conventional fundus cameras were modified to detect IOSs in anesthetized animals, such as cats and macaques, as well as in awake humans. Time-domain and frequency-domain optical
coherence tomography (OCT) imagers have been used for depth-resolved recording of IOSs in isolated frog\textsuperscript{28} and rabbit\textsuperscript{30} retinas, and in living rat eye.\textsuperscript{29} Both flood-illumination\textsuperscript{31} and laser scanning\textsuperscript{32} adaptive optics ophthalmoscopes have been validated for in vivo imaging of IOSs in living humans. Although in vivo recordings of IOSs have been successfully demonstrated,\textsuperscript{27,29,31–33} practical application of IOS imaging for retinal diagnosis is presently challenging owing to its complexity and inconsistency in terms of the time courses and signal polarities of reported IOSs. In principle, both stimulus-evoked retinal neural activity and the corresponding hemodynamic and metabolic changes can produce transient IOSs associated with retinal stimulation. While IOSs associated with hemodynamic and metabolic changes\textsuperscript{37,38} can provide important information for a healthy assessment of the visual system, they are relatively slow and cannot directly track fast neural activities in the retina. Fast IOSs, which have time courses comparable to electrophysiological kinetics, are desirable for direct evaluation of the physiological health of photoreceptors and inner neurons.

Transient IOSs tightly correlated with phototransduction procedures have been detected in isolated states both in outer segments of photoreceptors\textsuperscript{34} and in isolated retinas.\textsuperscript{35,36} Recent development of adaptive optics imagers\textsuperscript{37,38} allows IOS imaging of phototransduction changes of individual photoreceptors in vivo. Fast IOSs correlated with retinal ON and OFF responses have been detected in eyecup slices.\textsuperscript{37} Fast IOSs closely associated with action potential and postsynaptic potential have also been observed in other neural tissues.\textsuperscript{39–42} However, in vivo imaging of fast IOSs, which have time courses comparable to ERG kinetics in the retina, is still technically challenging.\textsuperscript{32} Better understanding of the sources and mechanism of fast IOSs may provide insight into optimization of instrument designs and test protocols to pursue robust imaging of fast IOSs that tightly correlate with ERG responses. Direct, high spatiotemporal resolution mapping of fast retinal neural activities will provide improved retinal diagnosis and treatment evaluation of a variety of eye diseases such as AMD, DR, and glaucoma, which can cause retinal neural dysfunction.

Isolated retinas, free from the complications of hemodynamic changes and eye movements, can provide a simple preparation for understanding the sources and biophysical mechanism of fast IOSs, and for optimizing the design of retinal imaging instruments. A series of experiments was conducted with isolated amphibian (frog and salamander) retinas to optimize and characterize fast IOSs in the retina. Until recently, it was believed that fast IOSs associated with stimulus-evoked neural activity were inherently tiny signals with high background light intensity, and difficult for practical optical imaging. However, using optimized near-infrared (NIR; 800–1000 nm) light illumination and improved spatiotemporal resolution, we have recently demonstrated high performance IOS imaging of retinal neural activity in isolated amphibian (frog and salamander) retinas. A series of experiments was conducted to characterize the relationship between fast IOSs and retinal neural activation.

### NIR Light Imaging of Fast IOSs in the Retina

Several versions of NIR light imaging systems for IOS imaging of the retina were constructed. Details of experimental set-ups, preparation of isolated retinas, and data processing procedures have been described in previous reports.\textsuperscript{43–45} A conventional microscope can be readily modified for transmitted light microscopy of transient IOSs in isolated retinas (Fig. 1). Using a home-built functional OCT imager,\textsuperscript{28} reflected light detection of transient IOSs was demonstrated. The isolated retina was placed in a recording chamber filled with Ringer’s solution\textsuperscript{46} and illuminated continuously with a NIR light (800–1000 nm) for recording the dynamic IOSs. A white light flash (intensity, ∼1.0 × 10^7 [550 nm photons]/ms μm^−2) was used for stimulation. The experiments were performed following protocols approved by the Institutional Animal Care and Use committees of the Los Alamos National Laboratory\textsuperscript{28,45,47} and University of Alabama at Birmingham.\textsuperscript{49,50} Before the eyes were removed for the experiment, the frog was rapidly euthanized by decapitation and double pithing. The procedure was conducted in a dark room with dim red illumination. After removal of the intact eye, the globe below the equator was hemisected with fine scissors to remove the lens and anterior structures before removing the retina. After the retina was sliced radially, a small wedge of tissue was used to allow it to lie flat in the recording chamber. These experiments disclosed similar stimulus-evoked fast IOSs in frog and salamander retinas.\textsuperscript{35} In order to simplify the description and keep the data constant, all IOSs in this article are of frog (\textit{Rana pipiens}) retinas.

### Fast Photodiode Recordings of IOSs

A single-channel detector, a fast photodiode, was used in the early experiments to demonstrate and optimize the transmitted NIR light recording of retinal neural activities. Several coherent and incoherent light sources for recording intrinsic optical signals in stimulus-activated retinas were compared. The experiments indicated that the signal-to-noise ratio (SNR) of optical measurements using an incoherent light source, such as a NIR light-emitting diode (LED) or a halogen lamp with NIR filter, was at least three times better than optical measurements with coherent light sources, such as a NIR laser diode.\textsuperscript{45} With an incoherent NIR light source, clear IOSs from single-pass measurements could routinely be recorded.\textsuperscript{45,47} The magnitude peak of IOSs recorded by the fast photodiode was at the level of 10^4 ΔI/I (Fig. 2), where ΔI is the dynamic optical change, and I is the background light intensity. The small magnitude of the transient optical response agreed with previous photodiode measurements of IOSs in the retina\textsuperscript{36,37} and other neural tissue.\textsuperscript{38,40–42,48} Data showed that both a waves (negative peak) and b waves (positive peak) could be clearly recorded in simultaneous electrophysiological measurements, but a positive-going NIR-transmitted light change dominated the corresponding IOSs (Fig. 2).