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Asymmetry of focal macular photopic negative responses (PhNRs) in monkeys

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ABSTRACT

The photopic negative response (PhNR) is a slow, negative-going wave of the photopic electroretinogram (ERG) that appears after the b-wave. Recent studies have shown that the PhNR originates from the spiking activities of inner retinal neurons including the ganglion cells and their axons. The aim of this study was to determine whether there is any asymmetry in the amplitude of the PhNR elicited from the upper and lower macular areas, and between the nasal and temporal macular areas in rhesus monkeys. To accomplish this, we recorded focal macular PhNRs that were elicited by red hemi-circular stimuli presented on a blue background. We show that the PhNR from the upper macular area was significantly larger than that of the lower macular area, and the PhNR of the nasal macula was significantly larger than that of the temporal macula. These asymmetries were present in the focal PhNR elicited by both brief and long duration stimuli, and the asymmetries were completely eliminated by an intravitreal injection of tetrodotoxin (TTX). These results suggest that the upper–lower and nasal-temporal asymmetries of PhNR in the primate retina are mainly caused by TTX-sensitive spiking activities of inner retinal neurons.

1. Introduction

The photopic negative response (PhNR) is a slow, negative-going wave of the photopic electroretinogram (ERG) that appears after the b-wave. Studies by Frishman and colleagues have demonstrated that the PhNR originates from the spiking activity of inner retinal neurons including the retinal ganglion cells and their axons (Rangaswamy et al., 2007; Viswanathan et al., 1999, 2000).The PhNR has been used in clinical studies to evaluate the inner retinal function objectively in several diseases, including glaucoma (Colotto et al., 2000; Drasdo et al., 2001; Machida et al., 2008; Viswanathan et al., 2001), optic nerve diseases (Gotoh et al., 2004; Miyata et al., 2007; Rangaswamy et al., 2004), and retinal vascular diseases (Chen et al., 2006; Kizawa et al., 2006; Machida et al., 2004). In these studies, the PhNRs were elicited mainly by full-field stimuli, and there have been only a few studies where the PhNR were elicited from localized retinal areas (Clotto et al., 2000; Fortune et al., 2003; Viswanathan et al., 2000). In addition, there have been only two studies of the focal PhNR with simultaneous fundus monitoring (Kondo et al., 2008; Machida et al., 2008).

We have recently developed a new recording system of focal PhNR (Kondo et al., 2008), which was modified from Miyake et al., 1988. In this system, the examiner can monitor the position of the stimulus spot on the fundus precisely during the recordings. In addition, a red stimulus spot was used on a blue background illumination, because a recent study showed that this color combination was most effective in eliciting large PhNRs especially for weak to moderate stimulus intensities (Rangaswamy et al., 2007). With this system, we found that the amplitude of the PhNR of the focal ERG was relatively large in the macular area (Kondo et al., 2008). However, we did not examine whether there were any regional variations or asymmetry in the amplitude of the PhNR in the macular area of monkeys. We believe that when the focal macular PhNRs are recorded from normal and diseased retinas, it is important to know whether there are any regional variations or asymmetries in the focal macular PhNR.

Thus, the purpose of this study was to determine whether the focal PhNRs recorded from the upper and lower macular areas, and nasal and temporal macular areas using a hemi-circular stimulus were symmetrical. We show that there were distinct asymmetries of the PhNR amplitude in both the vertical and horizontal directions in monkeys. We examined how these asymmetries of the focal PhNR change after the spiking activities of the inner retinal neurons are blocked by an intravitreal injection of tetrodotoxin (TTX) in monkeys.

2. Methods

2.1. Animals

Five eyes of five rhesus monkeys (*Macaca mulata*) were studied. The animals were sedated with an intramuscular injection of

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ketamine hydrochloride (7 mg/kg initial dose; 5–10 mg/kg per h maintenance dose) and xylazine (0.6 mg/kg). The respiration and heart rate were monitored, and hydration was maintained with slow infusion of lactated Ringer solution. The cornea was anesthetized with topical 1% tetracaine, and the pupils dilated with topical 0.5% tropicamide, 0.5% phenylephrine HCl, and 1% atropine. All experimental and animal care procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by the Institutional Animal Care Committee of the Nagoya University.

2.2. Stimulus and observation system

Our system for recording focal PhNRs has been described in detail (Kondo et al., 2008). Briefly, an infrared fundus camera was modified to observe the fundus and stimulate the retina. Light emitting diodes (LEDs) were incorporated into the camera to be used for the stimulus and background illuminations. The infrared television fundus camera (Kowa VX-10, Tokyo, Japan) was modified to obtain a Maxwellian stimulating system. The image from this fundus camera was fed to a television monitor with a 45° view of the posterior pole of the eye. The position of the stimulus spot on the fundus could be moved by the examiner with a joystick, and the position was monitored on the television monitor (Fig. 1, upper trace).

A red LED ($\lambda_{max} = 627$ nm; LXK2-PD12-S00, Philips Lumileds, San Jose, CA, USA) was used as the stimulus source, and a blue LED ($\lambda_{max} = 450$ nm; L450, Epitex, Kyoto, Japan) was used for the background illumination that covered a retinal area of 45°. A hemi-circular red stimulus (15° in diameter) was used (Fig. 1, lower trace).

The luminance of blue background was fixed at 100 scot cd/m^2, which is known to be high enough to suppress the rod photoreceptors. The luminance of the red stimulus spot was 55 phot cd/m^2, and the stimulus durations were 10 and 150 ms. We have already shown that the responses recorded with this system were focal when the luminance of the red stimulus spot was ≤55 phot cd/m^2 and presented on a steady blue background of 100 scot cd/m^2 (Kondo et al., 2008). The strength of the brief flashes of 10 ms was 0.55 phot cd-s/m^2 in energy units. The stimulus repetition rate was fixed at 2 Hz.

The luminances of the stimulus and background were measured at the position of corneal surface, and then converted to the value at the retinal surface. These luminances were measured with a photometer (Model IL 1700; International Light, Newburyport, MA, USA).

2.3. Recording and analyses

ERGs were picked-up with a Burian-Allen bipolar contact lens electrode (Hansen Ophthalmic Development Labs, Iowa City, USA), and the ground electrode was attached to the ipsilateral ear. The responses were amplified, and the band pass filters were set at 0.5 and 1000 Hz. The ERGs were digitized at 5 kHz, and 100– 300 responses were averaged for each response (MEB-9100, Neuropack, Nihon Kohden, Tokyo, Japan).

The amplitude of the PhNR was measured from the baseline to the bottom of the negative trough after the b-wave for the brief flashes of 10 ms, or was measured from the positive peak of the b-wave to the negative trough after the b-wave for the long duration

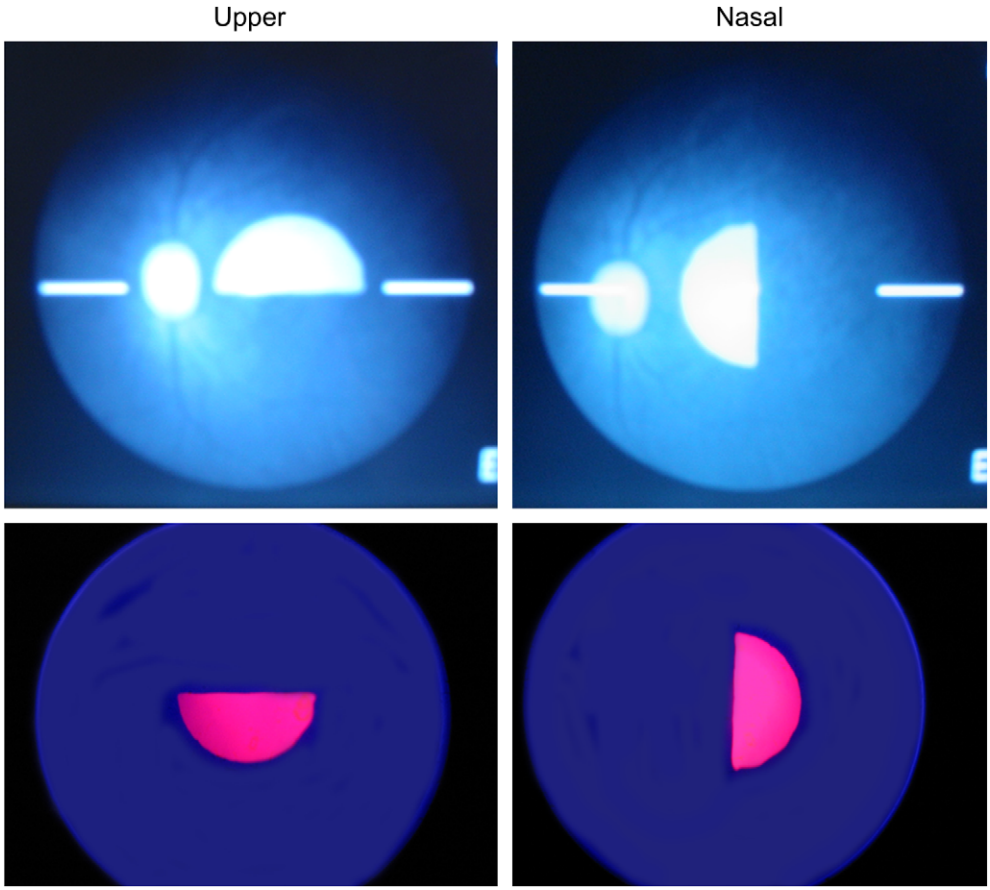

Fig. 1. Stimulus configuration for stimulating localized areas of the macula. Upper trace: Infrared fundus image of the monkey retina. The 15° hemi-circular stimulus is positioned on the upper (left) and nasal macula (right) of a rhesus monkey. Lower trace: Image of the red stimulus spot on the blue background. This image was photographed by a digital camera at the position of monkey's eye.