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Pupillary correlates of light-evoked melanopsin activity in humans

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Abstract

We investigated whether cones are the only photosensitive process mediating the photopic pupillary light reflex. New analyses were performed on previously published recordings, focusing on those evoked by the onset of photopically equated short- and long-wavelength stimuli. Comparisons between responses revealed contraction differences that slowly grew to a peak and gradually declined. The late contraction was associated with short wavelengths and appeared mostly at the higher stimulus intensities. We conclude that cones are not the only photoreception process mediating the photopic ON-reflex and infer that melanopsin is another. Melanopsin contributes to the steady-state pupil size in daylight illumination.

Keywords: Cones; Melanopsin; Photopic pupillary light reflex; Late contraction

1. Introduction

When the eye is exposed to new light levels, the pupil constricts transiently and then settles toward a steady-state diameter. Historically, early investigators agreed that the transient pupillary contractions were mediated by rods and by cones in night- and day-illumination, respectively (Alpern & Campbell, 1962; Bouma, 1965). But they disagreed about the rod's contribution to the photopic steady-state pupil. The steady-state pupil was found to be insensitive to the direction from which light struck the retina (Bouma, 1965; Spring & Stiles, 1948) and the directional insensitivity persisted at illuminations above rod-saturating levels (Bouma, 1965). These findings implied either that rods did not ordinarily saturate or that there existed a directionally insensitive photopic photoreceptor which had yet to be discovered.

Recently, a new photoreception process was discovered in the vertebrate retina (e.g., Foster & Hankin, 2002). The basis for the new process is a light-sensitive pigment *melanopsin* that is embedded in a minority class of retinal ganglion cells. Light shone on such ganglion cells produced membrane depolarization and spike discharges, even in the absence of rod or cone inputs. This photoreception system operated predominately at light levels well above photopic threshold and was presumably insensitive to the direction from which light struck the retina. Its action spectrum is close (but not identical) to that of rods.

Melanopsin photoreception has been implicated in primate and human retinal processing (Brainard et al., 2001; Dacey et al., 2005; Hankins & Lucas, 2002; Hannibal et al., 2004; Provencio et al., 2000; Thapan, Arendt, & Skene, 2001). In primates, melanopsin photoreception is associated with giant retinal ganglion cells that apparently also receive input from both the rod and cone pathways. Electrophysiological recordings show that these ganglion cells are strongly activated by rods and cones and displayed a rare S-OFF, (L + M)-ON type of color-opponent receptive field (Dacey et al., 2005).

Two lines of evidence suggested that melanopsin-associated ganglion cells contribute to the pupillary light reflex in primates (Gamlin et al., 2007). First, the pupillary light reflex was shown to persist after all rod and cone signals

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to the ganglion cells were pharmacologically blocked, implying that the pupil was driven by a non-cone, non-rod photoreception process. Second, the action spectrum for the pupillary contractions closely matched the absorption spectrum of melanopsin.

Evidence also suggested that melanopsin contributes to the pupillary light reflex in humans. Gamlin and colleagues developed a novel method of study based on the relatively faster temporal characteristics of the rod- and cone-in comparison to the melanopsin-generated retinal ganglion cell activities (Dacey et al., 2005). When bright lights are turned off, the rod- and cone-generated activities dissipate rapidly, thereby creating a period in darkness when only the slowly dissipating melanopsin-associated activity would remain. That this occurs was evidenced by the presence of a sustained pupillary contraction with an action spectrum that closely matched that of the melanopsin absorption spectrum (Fig. 4 in Gamlin et al., 2007).

The objective of the present study was to provide both an independent test of the hypothesis that melanopsin contributes to the human pupil and an alternative method of assessing its contribution. We propose to examine the effects of melanopsin photoreception on the pupil in response to the light onset. The *light-onset* method was inspired by an observation made earlier in rabbits (Knapp, 1985). Recording the pupillary light reflex in rabbit eyes that were treated with APB, Knapp observed the emergence of a long-latency contraction (Fig. 1, gray curves in the printed article, red curves in the web version). At the time, the mechanism underlying the late contraction was unknown but it now seems plausible that it originated from the melanopsin pathway. Knapp's observation suggested that the onset of light could generate a melanopsin-induced effect on the pupil and that the effect could be differentiated from those of rods and cones by its very late time-to-peak.

The study of late contractions in the human pupillary light reflex, however, could be complicated by task-related pupillary effects. Task-related effects are common in situations where an observer is instructed to prepare for or to execute a motor response (Richer & Beatty, 1985). The anticipated task-related effects in our pupillary recordings would be associated with instructions such as to always fixate prior to the stimulus presentation or to refrain from blinking until after the trial is completed. Task-related effects are particularly obvious in studies using a repeated-measure design. The effects begin prior to the stimulus onset or are present in the recordings made with no stimulus or near threshold stimuli (e.g., Young, Kimura, & Delucia, 1995). The effects are manifested as slow "drifts" in the pupil diameter. An upward drift (pupil widening) could obscure the presence of a slow contraction. A downward drift (pupil narrowing) could mimic

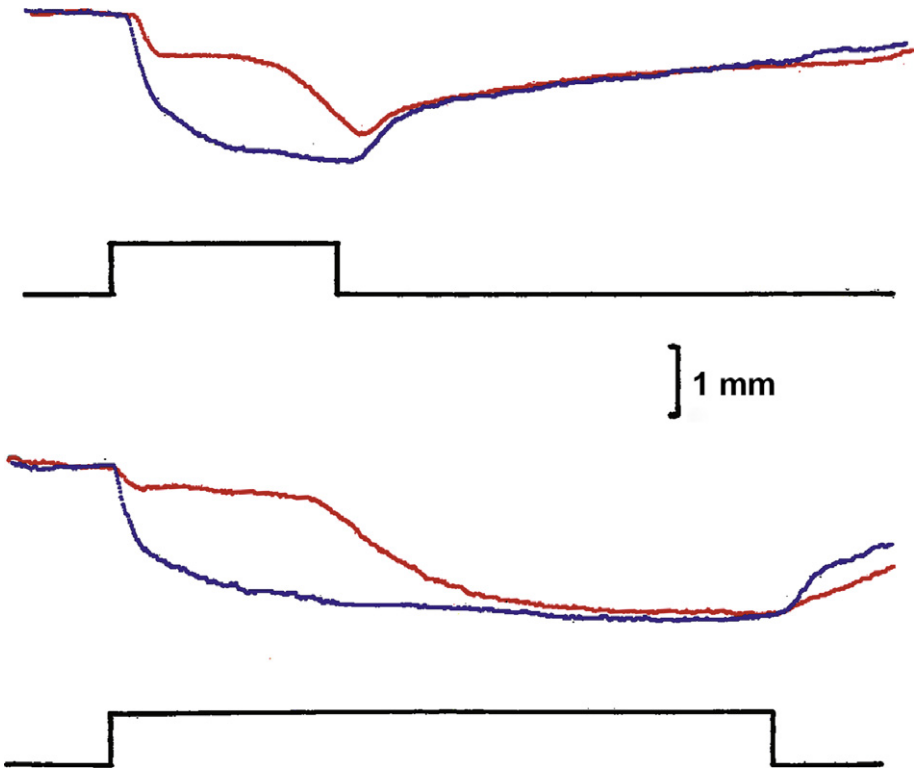

Fig. 1. Time-averaged waveforms of a rabbit's pupillary light reflex under normal (black) and APB-treatment (gray) conditions. Pupillary contraction in mm is represented by a downward deflection. Upper and lower response waveforms were evoked by stimulus of 5- and 15-s durations, respectively. Pupillary responses in the APB condition exhibited two contractions. The first began near or at the same time as the normal contraction. The second (i.e., *late contraction*) typically appeared after a 3-s latency. Figure was adapted from Knapp (1985). Gray and black lines in this and subsequent figures are colored red and blue, respectively, in the web version of this paper.