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The influence of intrinsically-photosensitive retinal ganglion cells on the spectral sensitivity and response dynamics of the human pupillary light reflex

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ABSTRACT

Historically, it was assumed that the light-evoked neural signals driving the human pupillary light reflex (PLR) originated exclusively from rod and cone photoreceptors. However, a novel melanopsin-containing photoreceptive cell class has recently been discovered in the mammalian retina. These intrinsically-photosensitive retinal ganglion cells (ipRGCs) project to the pretectum, the retinorecipient area of the brain responsible for the PLR. This study was therefore designed to examine the relative contribution of rod, cone and the melanopsin photoresponses of ipRGCs to the human PLR. We establish that the melanopsin photoresponse of ipRGCs contributes significantly to the maintenance of half maximal pupilloconstriction in response to light stimuli of 30 s or longer, even at low photopic irradiances. Furthermore, we show that the melanopsin photoresponse contributes significantly to three-quarter maximal pupilloconstriction in response to light stimuli as short as 2 s. We also demonstrate that cone photoresponses driving pupilloconstriction adapt considerably and contribute little after 30 s, but rod photoresponses adapt less and contribute significantly to the maintenance of pupilloconstriction in response to steady-state light stimuli at irradiance levels which are below the threshold of the melanopsin photoresponse.

1. Introduction

The pupillary light reflex (PLR) is a well studied neurological reflex characterized by a reduction in pupil diameter in response to an increase in retinal illumination. The PLR is an important clinical metric of retinal, midbrain and autonomic function (Girkin, 2003; Kawasaki, 2005) as well as being a major determinate of retinal image quality (Campbell & Gregory, 1960; Hirata, Yamaji, Sakai, & Usui, 2003; McDougal & Gamlin, 2008). Although it is well accepted that the major afferent influence on pupil diameter is environmental light levels, the nature of the light signal and the receptors responsible for its origin have historically been the subject of much disagreement (e.g. Alpern & Campbell, 1962; Loewenfeld & Lowenstein, 1993; ten Doesschate & Alpern, 1965). Such disagreements are now more understandable given the recent discovery of the retinal photopigment, melanopsin (Provencio et al., 2000), expressed by a novel class of retinal ganglion cells, which have been shown to contribute to the human PLR (Gamlin et al., 2007; Mure et al., 2009; Young & Kimura, 2008).

1.1. Intrinsically-photosensitive retinal ganglion cells

Recently, in mice and non-human primates, a class of retinal ganglion cells has been reported that express melanopsin (Dacey et al., 2005; Gooley, Lu, Chou, Scammell, & Saper, 2001; Hattar, Liao, Takao, Berson, & Yau, 2002), and are intrinsically photosensitive (Berson, Dunn, & Takao, 2002; Dacey et al., 2005). In addition to their intrinsic photosignal, these cells receive rod and cone inputs (Dacey et al., 2005; Jusuf, Lee, Hannibal, & Grunert, 2007). These cells have been termed intrinsically-photosensitive retinal ganglion cells (ipRGCs). The three primary projections of ipRGCs are the pretectum, the midbrain region associated with the PLR, the suprachiasmatic nucleus (SCN), the area of the brain responsible for circadian rhythms, and the intergeniculate leaflet (Dacey et al., 2005; Hattar et al., 2002, 2006). Although ipRGCs receive rod and cone inputs, their unique intrinsic photosensitivity ensures that they encode photic information differently from all other retinal ganglion cell types. In response to a pulse of light, these cells show a characteristic transient burst of firing at stimulus onset, which rapidly decays to a plateau of sustained firing that often extends well past stimulus offset (Berson et al., 2002; Dacey et al., 2005; Tu et al., 2005; Wong, Dunn, Graham, & Berson, 2007). It has been suggested that the initial burst of firing at stimulus onset is mediated by photoreceptors of the outer retina, while the sustained firing is driven by the melanopsin mediated intrinsic

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response (Dacey et al., 2005). In addition, more recent studies have provided evidence that outer retinal photoreceptors also contribute to sustained firing during long duration light stimuli (Drouyer, Rieux, Hut, & Cooper, 2007; Wong et al., 2007).

1.2. The role of ipRGCs in the mammalian pupillary light reflex

Initial studies investigating the influence of ipRGCs on the PLR utilized the mouse model, which allowed for the genetic manipulation of the different photoresponses involved in the reflex. It was shown that the PLR was present in rodless/coneless (rd/rd cl) mice, although the latency to maximal constriction was increased, and the irradiance needed to produce an equivalent constriction was higher than in wild type mice (Lucas, Douglas, & Foster, 2001). A subsequent study investigating the PLR in melanopsin knockout mice (opn4 −/−) found the PLR to be aberrant at high irradiances in these animals (Lucas et al., 2003).

Studies involving human and non-human primates have demonstrated a role for ipRGCs in the primate PLR. Gamlin et al. (2007) found that when outer retinal photoreceptive signals were blocked pharmacologically, the PLR persisted in macaques, and that the spectral sensitivity of the residual response was closely matched by the spectral sensitivity of melanopsin, which is maximally sensitive to 483 nm light. In addition, this study found that in both humans and macaques, the melanopsin photoresponse of ipRGCs is responsible for the post-illumination pupillary constriction which is seen following a period of high intensity light stimuli (Alpern & Ohba, 1972; Newsome, 1971).

Studies conducted prior to the discovery of melanopsin, also suggest that ipRGCs contribute to the human PLR. It has been shown that the pupils of rod achromats continues to respond to light increments well over levels commonly accepted to saturate rod photoreceptors (Alpern, Falls, & Lee, 1960), thus implying that an additional photopigment is involved in the pupillary responses of these individuals. Additionally, spectral sensitivity measurements of pupillary constriction to steady-state illumination have shown short wavelength sensitivity that is not well matched by either rod or S-cone contributions (Bouma, 1962; Laurens, 1923). Historical investigations of the response dynamics of the PLR are also suggestive of a role for ipRGCs in the behavior of the pupil in response to light increments. Several investigators have proposed models of pupillary dynamics which utilize both a transient and sustained component to the PLR (Kohn & Clynes, 1969; Privitera & Stark, 2006; Young, Han, & Wu, 1993). These transient/sustained dynamics are very similar to the cellular response of ipRGCs.

Two recent studies have investigated the influence of melanopsin on the human PLR. A brief report by Young and Kimura (2008), which reanalyzed previous data, reported the relative contribution of short and long wavelength light to the sustained component of the PLR, and suggested that melanopsin plays a role in the response. However, since this study did not examine the complete spectral sensitivity of the PLR, a more rigorous investigation is required to confirm the influence of melanopsin on the human PLR. In addition, Young and Kimura (2008) examined light-driven pupillary responses of 10 s or less, while the full contribution of the melanopsin photoresponse to steady-state pupil constriction is expected to develop with longer stimulus durations. A study by Mure et al. (2009) utilized the spectral sensitivity of the human PLR to investigate the possibility that melanopsin acts as a bistable photopigment similar to that of invertebrate opsins. Although spectral sensitivity data was generated for multiple stimulus durations, the bulk of their analysis was directed at ascertaining the existence of melanopsin bistability. Therefore, the present study was undertaken to more fully describe the relative contributions of rod, cone, and melanopsin light responses to the spectral sensitivity and response dynamics of the pupil during long light stimuli, and to compare these responses to those obtained with briefer stimuli.

2. Methods

2.1. Subjects

Six subjects participated in at least one of the three different experimental conditions of this study. All subjects had normal corrected visual acuity and normal color vision as measured by the Nagel anomaloscope, Farnsworth D-15, and HRR plate test. Subjects A, B, D, and F were males ages 33, 29, 51, and 27 years, respectively. Subjects C and E were females ages 33 and 40 years, respectively. Subjects D and E required approximately +2 diopters of visual correction. Three subjects (A, B, and F) participated in experiment 1. Five subjects (A–E) participated in experiment 2. Three subjects (A–C) participated in experiment 3. All experimental procedures were approved by the UAB Institutional Review Board, and were undertaken with the understanding and written consent of each subject.

2.2. Recording procedures

During experimental sessions, both of the subject's eyes were visualized under infrared illumination via video camera. Pupil diameters were measured in both eyes using ISCAN RK406 pupillometer systems, which were calibrated with apertures of known diameters placed at the plane of the subject's eyes. The positions of the right eye, left eye, and pupil diameters were sampled at 500 Hz. All samples were stored on computer disk for later analysis.

2.3. Behavioral task

Measurement of the subject's consensual PLR in response to monochromatic light stimuli was determined during the following behavioral task. Prior to each experimental session, the subject's right eye was dilated with topical 1% tropicamide in order to keep the pupillary response in an open-loop condition. Throughout an experimental session, the subjects' right eye was precisely aligned with the optical system and a 2° black cross was visible to the subject's right eye at all times. At the onset of a behavior trial, a target generated on a computer monitor (2° white cross, 1 cd/m^2) was presented to the subject's left eyes at optical infinity via a badal lens system (see Bennett, Rabbetts, & Bennett, 1998). The subject was instructed to fuse both targets and during this time a baseline measurement of pupil diameter was recorded. Throughout the behavioral task, the subject could visualize both targets, and was instructed to minimize and report any blurring or disassociation of the two targets which would indicate an undesirable change in accommodation or vergence angle. In this way, changes in accommodation and vergence angle, which could act as confounding influences in these experiments, were minimized.

Approximately twelve seconds after the onset of the white cross, a monochromatic light stimulus subtending 36° was presented in Maxwellian view to the right eye for approximately 4, 12, 34, or 110 s depending on the duration condition being assessed. The monochromatic light stimulus (9.5–15 log quanta/cm^2/s) was generated with 10 narrow band pass interference filters (8–10 nm full width at half maximum, Andover Corp.) between 450 nm and 650 nm. The spectral transmission through each of the interference filters used was shown to be reduced by at least 3 log units within a 15 nm deviation from the wavelength of peak transmission, as measured by a PR-680 spectroradiometer (Photo Research, Inc). The exact timing of the stimulus onset and offset