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Pupil responses derived from outer and inner retinal photoreception are normal in patients with hereditary optic neuropathy

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ABSTRACT

We compared the pupil responses originating from outer versus inner retinal photoreception between patients with isolated hereditary optic neuropathy (HON, $n = 8$) and healthy controls ($n = 8$). Three different testing protocols were used. For the first two protocols, a response function of the maximal pupil contraction versus stimulus light intensity was generated and the intensity at which half of the maximal pupil contraction, the half-max intensity, was determined. For the third protocol, the pupil size after light offset, the re-dilation rate and re-dilation amplitude were calculated to assess the post-light stimulus response. Patients with HON had bilateral, symmetric optic atrophy and significant reduction of visual acuity and visual field compared to controls. There were no significant mean differences in the response curve and pupil response parameters that reflect mainly rod, cone or melanopsin activity between patients and controls. In patients, there was a significant correlation between the half-max intensity of the red light sequence and visual field loss. In conclusion, pupil responses derived from outer or inner retinal photoreception in HON patients having mild-to moderate visual dysfunction are not quantitatively different from age-matched controls. However, an association between the degree of visual field loss and the half-max intensity of the cone response suggests that more advanced stages of disease may lead to impaired pupil light reflexes.

Patients with optic nerve damage typically demonstrate deficits in visual function and in the pupil light reflex. The magnitude of the visual and pupillary deficits (Kardon et al., 1993; Johnson et al., 1988), while generally correspondent can sometimes be notably discordant. Such a visual-pupillary dissociation has been observed in patients with primary hereditary optic neuropathy. Patients with Leber hereditary optic neuropathy (LHON) were first described to have the unusual combination of profound visual loss with remarkably normal pupillary contractions to light stimulation (Nakanishi et al., 1994; Wakakura and Yokoe, 1995; Nikoskelainen et al., 1996; Bremner et al., 1999; Bose et al., 2005; Kawasaki et al., 2010; Moura et al., 2013). Subsequent investigation revealed a similar discordance in the visual and pupillary deficit in patients with dominant optic atrophy (DOA), the most common form of hereditary optic neuropathy (Bremner et al., 2001). These two disorders share a common pathogenetic mechanism of mitochondrial dysfunction as the cause of ganglion cell death and optic atrophy. A differential susceptibility among retinal ganglion cell types to metabolic insult and neurodegeneration caused by mitochondrial dysfunction is presumably the basis for the visual-pupillary dissociation observed in hereditary optic neuropathy (La Morgia et al., 2010).

A subset of retinal ganglion cells expressing the photopigment melanopsin primarily subserves non-visual light responses such as synchronization of circadian rhythm and regulation of the pupil size (Berson et al., 2002; Hattar et al., 2003; Gamlin et al., 2007). These intrinsically photosensitive retinal ganglion cells (ipRGCs) have giant-sized cell bodies, are sparse in number and their large-caliber axons form the principle, if not sole, conduit of afferent pupillomotor signal from the retina to the pretectal olivary nucleus of the dorsal midbrain. An immunohistochemical analysis of ipRGCs in retinas of patients with primary hereditary optic neuropathy revealed a relative resistance of these cells to neurodegeneration caused by mitochondrial dysfunction (La Morgia et al., 2010). Compared to control retinas in which ipRGCs

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comprised 1% of total retinal ganglion cells, the two patients with LHON showed a markedly exaggerated proportion of ipRGCs (49%) among the surviving retinal ganglion cells.

In this study, we sought to select and quantify two types of pupil responses, those deriving primarily from outer retinal photoreception (rods, cones) and those having predominant input from inner (melanopsin-mediated) retinal photoreception, in patients with isolated hereditary optic neuropathy. We used colored light pupillometry to preferentially bias contribution from a single photoreceptive element (rods, or cones, or melanopsin) to the afferent pupillomotor signal in ipRGCs. We compared pupil responses to light onset and following light offset between patients and controls, and also examined the correlation between the pupil response and visual function.

The study was conducted according to the tenets of the Declaration of Helsinki and received authorization from the local ethical review board for human research in Lausanne (Switzerland). All study participants provided oral and written informed consent for study participation. Patients were recruited from the neuro-ophthalmology unit of Hôpital Ophtalmique Jules-Gonin, University Eye Service (Lausanne, Switzerland). The eight patients reported herein are a subgroup of a larger study population who underwent testing to investigate how disease-related changes in the physiologic responses mediated by ipRGCs might influence complex cognitive and behavioral functions in humans. The results reported in this paper addressed the separate question of visual-pupillary dissociation, as described in the introductory paragraph.

A diagnosis of isolated hereditary optic neuropathy was based on the following clinical criteria: subnormal vision documented at childhood or young adulthood, evidence of stable or progressive visual dysfunction since then, bilateral and symmetric central visual loss, bilateral optic atrophy, a positive family history of subnormal vision and bilateral optic atrophy, and investigative tests including electroretinography and neuroimaging that were negative for any other cause of optic nerve damage. A defined molecular defect was not considered necessary for a clinical diagnosis of hereditary optic neuropathy but for patients in whom gene testing had been performed, the results were noted. No patient had hearing loss. No patient had diabetes. Eight healthy age-matched control subjects were recruited from flyers posted in the region of Lausanne (Switzerland). These subjects had a normal ophthalmologic examination with no previous or current history of ocular disease. For patients and controls, exclusionary criteria were: age under 19 years, diabetes, smoking, pregnancy, use of topical or systemic medication which could potentially affect pupillary function (e.g. antidepressants) and any prior history of ocular inflammation or trauma.

All study participants had a baseline ophthalmologic examination which included best-corrected visual acuity, color vision testing with Ishihara color plates, alternating light test to examine for a relative afferent pupillary defect and funduscopy. Visual fields were assessed using threshold automated perimetry of the central 30° (Octopus 101, Interzeag, Bern–Köniz, Switzerland). The macula and peripapillary retinal nerve fiber layer (RNFL) were examined by optical coherence tomography (OCT; Stratus 3000, Carl Zeiss, Meditec, Inc., Dublin, CA).

Computerized pupillography was performed under dark and light adapted conditions at approximately 4 h after the habitual wake time of each subject. A Color Dome Ganzfeld ERG apparatus (Diagnosys, Lowell, Massachusetts USA) was used to present a full-field 1 s or 30 s light stimulus at preselected spectral bandwidths of 635 ± 20 nm (red light) and 463 ± 26 nm (blue light) to both eyes simultaneously on undilated pupils. Light intensities used in this study ranged from −4.0 to 2.5 log cd/m^2 [0 log = 1 cd/m^2 = 12.6 log photons/cm^2/s (blue light); and 1 cd/m^2 = 12.8 log photons/cm^2/s (red light)]. The choice of this luminance was based on previously published protocols (Kawasaki et al., 2012a,b; Park et al., 2011), and luminance outputs were based on specifications provided by the manufacturer. A dual channel binocular pupillometer mounted on an eye frame (Arrington Research, Scottsdale, AZ USA), continuously recorded the pupil diameter of both eyes at 60 Hz for the duration of the stimulus light sequence of each test protocol. Details of this instrumentation have been reported previously (Kardon et al., 2009).

Three pupil testing protocols (each weighted to favor either rod, or cone, or melanopsin contribution to the ipRGC activity) were used. The rod-weighted and cone-weighted protocols (hereafter called rod and cone protocols) were modified versions of blue and red light stimulus sequences previously developed (Park et al., 2011; Kawasaki et al., 2012a,b). In this study, the rod protocol (blue light sequence) was performed first and was preceded by a 10 min (min) period of dark adaptation (=0 cd/m^2). Following 30 s of pupillary recording in total darkness, a sequence of seven blue light stimuli of 1 s duration each was presented, starting at intensity of −4.0 log luminance (cd/m^2) and increasing by 0.5 log-unit steps up to −1.0 log luminance (cd/m^2). The inter-stimulus dark interval increased from 3 s to 10 s in order to permit the pupil to return to baseline size before the next light stimulation.

The cone protocol (red light sequence) was preceded by 10 min of room light adaptation (92 lx). Following 30 s of pupillary recording in total darkness, a sequence of four red light stimuli of 1 s duration each was presented, starting at 1.0 log luminance (cd/m^2) and increasing by 0.5 log-unit steps until 2.5 log luminance (cd/m^2). The inter-stimulus dark interval was 20 s and permitted the pupil to return to baseline size before the next light stimulation.

The melanopsin-weighted protocol (hereafter called the melanopsin protocol) was preceded by 10 min of dim light adaption (<5 lux). Following 30 s of pupillary recording in total darkness, a 1 s bright red light then a 1 s bright blue light stimulus equiluminant for photopic sensitivity at 200 cd/m^2 (=14.9 log photons/cm^2/s for blue and 15.1 log photons/cm^2/s for red light; according to the manufacturer of the Ganzfield apparatus), was presented. The dark interval after red light was 30 s and the dark interval after blue light was 60 s.

The pupil data from the right and left eye recordings were analyzed on a spread sheet and a customized filter was applied to remove artifacts from blinking and eye movements (Microsoft Excel 2002, Visual Basic for Applications V. 6.5). Pupil tracings were then smoothed by a polynomial smoothing function (Savitzky-Golay; Origin Pro v.8.50 SRO). The baseline pupil size was defined from the averaged size during the first 30 s of recording in darkness. Actual pupil size was divided by baseline pupil size to convert all values to relative pupil size (RPS). Pupil contraction (expressed as a percentage) at any given time point was 1 minus relative pupil size times 100. For the rod and cone protocols, the maximal contraction for each light intensity (on a log-scale) was obtained for HON patients and controls and was plotted as a function of stimulus light intensity by using nonlinear curve fit analysis (Kawasaki et al., 2012a). From the fitted exponential curves, the light intensity at which a 50% pupil response occurred (half-max light intensity) was determined for each subject and averaged across the two subject groups.

The distinctive pupillographic feature of melanopsin activation is persistence of pupillary constriction after stimulus light termination. Therefore, for the melanopsin protocol, in addition to the immediate pupil constriction to light, we also analyzed the pupillary recovery in darkness following termination of a bright 200 cd/m^2 light stimulus. For the 1 s light stimulus, we calculated the minimum pupil size (MPS) to light onset as well as the post-stimulus pupil size (PSPS) at the 6th s as the mean RPS between