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The pattern of retinal ganglion cell dysfunction in Leber hereditary optic neuropathy

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ABSTRACT

Leber inherited optic neuropathy (LHON) is characterized by subacute bilateral loss of central vision due to dysfunction and loss of retinal ganglion cells (RGCs). Comprehensive visual electrophysiological investigations (including pattern reversal visual evoked potentials, pattern electroretinography and the photopic negative response) performed on 13 patients with acute and chronic LHON indicate early impairment of RGC cell body function and severe axonal dysfunction. Temporal, spatial and chromatic psychophysical tests performed on 7 patients with acute LHON and 4 patients with chronic LHON suggest severe involvement or loss of the midget, parasol and bistratified RGCs associated with all three principal visual pathways.

1. Introduction

Leber hereditary optic neuropathy (LHON) (OMIM 535000) is a primary mitochondrial DNA (mtDNA) disorder that presents with bilateral subacute loss of central vision (Nikoskelainen et al., 1996; Yu-Wai-Man and Chinnery, 2013; Yu-Wai-Man et al., 2014). The majority of patients harbour one of three common mtDNA mutations (m.3460G > A in *MTND1*, m.11778G > A in *MTND4* and m.14484T > C in *MTND6*) that affect complex I subunits of the mitochondrial respiratory chain (Mackey et al., 1996). Despite the universal cellular role of mitochondria, retinal ganglion cells (RGCs) within the papillomacular bundle are particularly severely affected accounting for the characteristic dense central or caecocentral scotoma in this disorder. Although the underlying pathological process is still not fully defined, this tissue specificity has been ascribed to an increased vulnerability of RGCs to both disturbed mitochondrial energy metabolism and the increased formation of reactive oxygen species (Carelli et al., 2004a; Carelli et al., 2004b; Lin et al., 2012; Sadun et al., 2000; Levin, 2015; Sadun et al., 2015). LHON shows maternal inheritance, but there is variable disease penetrance and a marked sex bias with about 50% of male carriers losing vision during their lifetime compared with about 10% of female carriers (Mackey et al., 1996).

The histopathological observation of loss of the small calibre axons that constitute the papillomacular bundle was originally observed on histopathological sections of post mortem optic nerve samples obtained several decades after disease onset (Sadun et al., 1994; Kerrison et al., 1995; Sadun et al., 2000). More recently, in vivo studies involving high-resolution optical coherence tomography (OCT) have revealed a major loss of the temporal peripapillary nerve fiber (RNFL) and macular RGC layers within 3 months of disease onset. Interestingly, pathological thinning within the macular RGC layer was an early sign that was already apparent in the presymptomatic phase (Barboni et al., 2005; Barboni et al., 2010; Akiyama et al., 2013; Zhang et al., 2014; Mizoguchi et al., 2015; Balducci et al., 2015). Following disease conversion, swelling of the peripapillary RNFL spreads circumferentially from the inferotemporal segment of the optic disc to involve the remaining quadrants, before RNFL atrophy becomes established within 3–9 months after the onset of visual loss. Hyperemia and fluctuating

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mild swelling of the prepapillary RNFL can also be seen in unaffected LHON carriers without visual loss or progression to full disease conversion (Nikoskelainen et al., 1982).

The function of the papillomacular bundle may be assessed objectively using pattern reversal visual evoked potentials (PR-VEP) and pattern electroretinography (PERG). Reported PR-VEP abnormalities in LHON are consistently severe, but the utility of the PERG in LHON is more controversial and there are conflicting reports in the literature regarding the timing of responses and the sequence of losses (Nikoskelainen et al., 1977; Hrynchak and Spafford, 1994; Mashima et al., 1997; Sharkawi et al., 2012; Ziccardi et al., 2013; Ziccardi et al., 2015; Jarc-Vidmar et al., 2015). The full-field photopic negative response (PhNR) has been used to assess generalized RGC function in glaucoma and other acquired optic neuropathies (Machida, 2012; Morny et al., 2015), but there are no published studies of PhNR in LHON. The applicability of PhNR as a potential objective functional index of RGC function in LHON therefore warrants further investigation.

RGCs are classified into the three major subtypes of RGCs, namely midget, parasol and small bistratified ganglion cells, which are thought to contribute to the parvocellular, magnocellular and koniocellular pathways, respectively. These distinct RGC populations and their associated pathways can be tested by modifying standard psychophysical measures. In general, the processing of high spatial frequency information has been linked with the parvocellular pathway whereas high temporal frequency information is thought to be integrated by the magnocellular pathway. Red-green processing and blue-yellow processing have been linked with the parvocellular and koniocellular pathways, respectively. Parallel processing in the retina and visual pathways have been the subject of several recent comprehensive reviews (e.g., Dowling, 1987; Wässle and Boycott, 1991; Rodieck, 1998; Lennie and Movshon, 2005; Lee et al., 2010; Dacey et al., 2014).

LHON is thought to mainly affect midget RGCs, which have the smallest calibre axons and are the predominant subtype within the papillomacular bundle, mediating visual information including high spatial frequencies and red-green chromaticity (Sadun et al., 1994; Hrynchak and Spafford, 1994; Kerrison et al., 1995; Sadun et al., 2000). In contrast, another melanopsin-expressing RGC subtype appears relatively preserved and this peculiarity likely accounts for the frequently preserved pupillary light reflexes, even in severely affected LHON patients (Kawasaki et al., 2010; La Morgia et al., 2010). One previous report indicated mild impairment of the magnocellular pathway in unaffected LHON carriers (Gualtieri et al., 2008), but there are no robust data regarding the involvement of the parasol and small bistratified RCG subtypes in LHON.

In this study, we investigated the pattern of RGC dysfunction in a well-phenotyped cohort of LHON patients in both the acute and chronic phases of the disease by using a comprehensive visual electrophysiological and psychophysical assessment protocol. Our aim was, firstly, to characterise the electrophysiological responses to better define the phenotypic features of LHON and to establish the most appropriate methods for monitoring RGC function and disease progression objectively. Secondly, we used psychophysical tests of temporal, spatial and chromatic vision to investigate the relative involvement of distinct RGC populations in the pathophysiology of LHON.

2. Methods

2.1. Subjects

This was a prospective case study of 12 affected patients (A1–A12) and 9 unaffected carriers (U1–U9) harboring one of the three common mtDNA LHON mutations (Table 1). In addition, retrospective visual electrophysiological data for 5 affected LHON patients (A13-A17) were retrieved from the hospital database of Moorfields Eye Hospital, London, UK. In total, there were 4 affected female and 13 affected male patients. Affected LHON patients and unaffected LHON carriers in our cohort underwent an ophthalmological examination that included the following investigations as indicated in Table 1: best corrected visual acuity (BCVA) assessment using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart; slit lamp examination; automated Humphrey visual field perimetry (Program 30-2, Humphrey Visual Field Analyzer, Model 750, Humphrey Instruments, San Leonardo, CA); and optical coherence tomography (OCT) imaging of the macula and the optic nerve head (see details below).

The normal subjects for psychophysical tests were 15 individuals aged 17 to 78 years old at the time of testing with normal BCVA and normal color vision as assessed by standard color vision tests. Only 12 of the normal subjects had their L-cone temporal contrast sensitivities measured. Written informed consent was obtained from all subjects or their guardians. The study was approved by the local ethics committees at Moorfields Eye Hospital and University College London and it conformed to the standards of the Declaration of Helsinki.

2.2. Optical coherence tomography (OCT) imaging

The Spectralis™ platform (Heidelberg Engineering Ltd., Heidelberg, Germany) was used for SD-OCT imaging of the macula and the optic nerve head. Automated segmentation and thickness analyses were performed for perifoveal volumetric retinal B-scans using the Heidelberg Engineering segmentation tool, included in the Spectralis Glaucoma Module software (version 6.0). Of the 10 retinal layers that were automatically defined and manually confirmed, the following thickness values were recorded from the four sectors of the inner ring (between 1 and 3 mm diameter) of the nine macular ETDRS subfields as described elsewhere (Majander et al., 2016): (i) retina, (ii) retinal nerve fiber layer (RNFL), (iii) combined GCL and inner plexiform layer (IPL), (iv) inner nuclear layer (INL), (v) outer plexiform layer (OPL), (vi) combined OPL and outer nuclear layer (ONL), and (v) inner retina. The thickness of the outer retinal layers was calculated by subtracting the thickness of the inner retinal layers from the total retinal thickness. Normative data was generated from SD-OCT images of 48 healthy eyes of 48 subjects (Majander et al., 2016). For peripapillary RNFL measurement a 3.5-mm-diameter circular scan centered on the optic disc was used and the data for six sectors were collected.

2.3. Electrophysiology investigations

Twelve subjects underwent electrophysiological testing including pattern reversal and flash visual evoked potential (PVEP; FVEP) and pattern electroretinography (PERG), incorporating the standards of the International Society for Clinical Electrophysiology of Vision (ISCEV; Odom et al., 2010, Bach et al., 2013). Pattern ERGs were recorded to a 0.8- degree check size using both a standard checkerboard field (12 × 15°) and additionally to a large field (24 × 30°; LF PERG; Lenassi et al., 2012). The full-field photopic negative response (PhNR) was additionally recorded in 7 cases using diffuse red flash stimulation (640 nm) at 5 flash strengths (0.5, 1.0, 2.0, 5.0 and 10.0 $cd{\cdot}s{\cdot}m^{-2}$), superimposed on a blue background (450 nm; 2.25 $cd{\cdot}m^{-2}$). Gold foil electrodes were used and the results compared to normative data.

2.4. Psychophysical investigations

2.4.1. L- and S-cone critical flicker fusion and L-cone temporal contrast sensitivity

L- and S-cone temporal acuities (critical flicker fusion, CFF) and L-cone temporal contrast sensitivity functions (TCSFs) were measured using a Maxwellian-view optical system described in more detail elsewhere (Stockman et al., 2014a, 2005). Predominantly L-cone or S-cone stimuli were used for the CFF measurements. The L-cone stimulus was produced by flickering a 650-nm circular target of 4° visual angle in diameter superimposed in the center of a steady 481-nm circular