Cromhatic and Luminance Contrast Sensitivities in Asymptomatic Carriers from a Large Brazilian Pedigree of 11778 Leber Hereditary Optic Neuropathy

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Purpose. To determine whether asymptomatic 11778 LHON carriers demonstrated impairments in (1) chromatic red/green (R/G) and blue/yellow (B/Y) contrast sensitivity functions (CSF) and in (2) luminance contrast sensitivity functions in the spatial CSF (SCSF) and temporal CSF (TCSF) domains.

Methods. Twenty-five carriers (8 male, 17 female; 34.1 ± 15.1 years of age) of homoplasmic 11778 LHON from the same well-described family and 30 age-matched controls (17 male, 13 female; 29.2 ± 7.1 years of age) were tested in one eye, randomly selected. Of the 25 eyes tested, 18 had normal fundus, 5 had swelling and microangiopathy, and 2 had temporal pallor. The R/G and B/Y CSFs were obtained after equiluminance correction with bichromatic sinusoidal gratings at 0.3, 0.7, and 2 cycles per degree (cpd); the SCSFs were obtained with achromatic gratings at 0.3, 2, 6, and 12 cpd; and the TCSFs were obtained at 2, 10, 20, and 35 Hz with sinusoidal modulation of a 2.7° field with a superimposed spatial Gabor function.

Results. Differences between carriers and controls were statistically significant for all spatial frequencies of chromatic and luminance SCSFs, but not for the TCSFs. R/G equiluminance settings of carriers differed from those of controls (P < 0.001), requiring higher luminance in the green; B/Y equiluminance settings were not statistically different in carriers and controls. Fundus findings did not correlate with CS results.

Conclusions. Luminance and chromatic spatial CS losses that affected all tested spatial frequencies, are reported in LHON asymptomatic carriers with the mtDNA 11778 mutation. No losses were found in the temporal CSF. An intriguing finding is that the blue system is substantially spared in this LHON family. These represent subclinical visual impairments in otherwise asymptomatic LHON carriers. (Invest Ophthalmol Vis Sci. 2005;46:4809–4814) DOI:10.1167/iovs.05-0455

Leber hereditary optic neuropathy (LHON) is a maternally inherited mitochondrial disorder that causes sudden and permanent bilateral loss of central vision in young adults, with profound loss of visual acuity and dyschromatopsia.1–5 These abrupt symptoms may be preceded by alterations in visual function.6 A few studies of visual function in asymptomatic LHON carriers show that they may have visual losses. Nikoskelainen et al.6 reported that before the onset of acute LHON, a progression of color vision loss was observable. Abnormalities in color vision, fundus changes, and VEP alterations were described in patients who later became affected.7,8 The observation of visual losses in asymptomatic carriers of LHON has also been confirmed in a large Brazilian pedigree in which the carriers were compared with spouses or male descendants within the same family. LHON carriers showed abnormalities in color vision and in the VEP (Gualtieri M, et al. IOVS 2004;45:ARVO EAbstract 4351; Quiros PA, et al. IOVS 2003;44: ARVO EAbstract 940; Quiros PA, et al. IOVS 2004;45:ARVO EAbstract 4356) and in fundus alterations.10

As in other mitochondrial optic neuropathies, LHON primarily affects the papillomacular bundle (PMB).11 Postmortem histopathologic analysis of two LHON patients showed a 95% to 99% loss of retinal ganglion cells (RGCs) resulting in severe thinning of the ganglion cell layer. Morphologic evidence of ongoing retinal ganglion cell death indicates that the degenerative process continues throughout the entire life of the affected patient.

Given that most PMB fibers subserve color and high spatial frequency contrast sensitivities, the earliest changes detectable may be subtle impairments in these functions. To test this hypothesis, we measured chromatic contrast sensitivities of the color opponent systems and achromatic temporal and spatial contrast sensitivities.

Methods

Participants

This study followed the tenets of the Declaration of Helsinki. Informed consent was obtained from the subjects after explanation of the nature of the study. We examined 25 asymptomatic carriers (8 male, 17 female; 34.1 ± 15.1 years of age) of homoplasmic 11778 LHON mutation from a previously identified large Brazilian pedigree2 and 30 age-matched controls (17 male, 13 female; 29.2 ± 7.1 years of age, with best-corrected visual acuity [VA] of 20/20 or better). Inclusion criteria were clear ocular media, absence of known neurologic disorders, and informed consent. Exclusion criteria were history of glau-
TABLE 1. Demographic and Ophthalmologic Data of LHON Carriers

<table>
<thead>
<tr>
<th>Patient/Eye</th>
<th>Gender</th>
<th>Age (y)</th>
<th>(logMAR)</th>
<th>Snellen</th>
<th>Fundus</th>
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<tr>
<td>MAMR od</td>
<td>F</td>
<td>15</td>
<td>0</td>
<td>20/20</td>
<td>N</td>
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<td>JTZ os</td>
<td>M</td>
<td>15</td>
<td>0.2</td>
<td>20/30</td>
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<tr>
<td>LAT os</td>
<td>F</td>
<td>16</td>
<td>0.2</td>
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<td>YGM os</td>
<td>F</td>
<td>16</td>
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<td>20/20</td>
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<td>CTF os</td>
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<td>0</td>
<td>20/20</td>
<td>N</td>
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<td>MAL od</td>
<td>F</td>
<td>22</td>
<td>0</td>
<td>20/20</td>
<td>N</td>
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<tr>
<td>VMQ od</td>
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<td>25</td>
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<td>20/25</td>
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</tr>
<tr>
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<td>N</td>
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<tr>
<td>EJL os</td>
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<tr>
<td>EP od</td>
<td>M</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<td>0.2</td>
<td>20/30</td>
<td>N</td>
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<td>NAL os</td>
<td>F</td>
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<tr>
<td>GIM od</td>
<td>F</td>
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<td>0.2</td>
<td>20/30</td>
<td>N</td>
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<td>PT os</td>
<td>F</td>
<td>18</td>
<td>0.1</td>
<td>20/25</td>
<td>A (SM)</td>
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<tr>
<td>GMP od</td>
<td>F</td>
<td>18</td>
<td>0.2</td>
<td>20/30</td>
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<tr>
<td>FMG od</td>
<td>F</td>
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<td>0.1</td>
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<td>JPS os</td>
<td>F</td>
<td>36</td>
<td>0.1</td>
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<td>A (SM)</td>
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<td>MOM od</td>
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<td>M</td>
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<td>20/20</td>
<td>A (TP)</td>
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<tr>
<td>GCS od</td>
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<td>0</td>
<td>20/20</td>
<td>A (TP)</td>
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N, normal; A, abnormal; SM, swelling + microangiopathy; TP, temporal pallor.

The vision of asymptomatic LHON carriers has not been analyzed in great detail. Visual losses and other alterations might have been overlooked because of a lack of obvious symptoms or clinical signs. The present results constitute the first analysis of contrast sensitivities in the spatial—chromatic and achro-

**RESULTS**

Mean spatial contrast sensitivity in response to achromatic gratings peaked at 3 cpd in both groups. CS at the peak spatial frequency ranged from 74 to 232 in the control group and from 5 to 121 in the asymptomatic LHON carriers. Mean CS was significantly lower for carriers than it was for controls at all spatial frequencies tested (P = 0.005). The average loss was 2.8, 3.5, 3.9, 3.2, and 3.0 dB, respectively, for the frequencies 0.5, 3, 6, 12, and 20 cpd.

Figure 1A shows a uniform reduction in achromatic luminance contrast sensitivity throughout all tested frequencies. Individual data of carriers and controls are plotted in Figure 1B. The R/G equiluminance settings of carriers differed from those of controls (P < 0.001), requiring higher luminance in the green axis (Fig. 2A). However, the B/Y equiluminance settings of carriers and controls were not statistically different (Fig. 2B).

Mean CS for chromatic equiluminance gratings was lower in the LHON carrier group than in the control group for R/G (P < 0.005) and B/Y (P = 0.01) gratings for all spatial frequencies studied (Figs. 3 and 4). For the R/G gratings, the average losses were 3.4, 3.6, and 3.8 dB, and for the Y/B gratings the average losses were 2.5, 5.7, and 2.6 dB, respectively, for the frequencies 0.5, 3, 0.7, and 2.0 cpd.

No statistical difference was found between carriers and controls for temporal CS (Fig. 5). There was no correlation of CS results with age (Spearman R; P > 0.05) and no statistical difference between the CS results of carriers with and without fundus abnormalities (Table 1) in any of the conditions measured—spatial luminance and chromatic or temporal CS (Mann-Whitney U Test; P > 0.05).

**DISCUSSION**

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**Procedure**

Visual acuity was measured at 4 meters using an ETDRS chart (tumbling E). CS functions were measured using a computerized system (PSYCHO for Windows version 2.36 and a VSG 2/4 graphics board, with 14-bit resolution; Cambridge Research Systems, Rochester, Kent, UK). Stimuli were presented on a 19° video monitor (Trinitron GFD-420; Sony, Tokyo, Japan) at a resolution of 800 × 600 pixels, with a refresh rate of 100 Hz noninterlaced.

Spatial contrast sensitivity stimuli were sinusoidal horizontal gratings with a visual angle of 4 × 4°, presented at an average luminance of 34.3 cd/m². Red-green chromatic contrast sensitivity was obtained using a bichromatic grating of 4 × 4° of visual angle with counterphase modulated red and green sinusoidal waves. An equivalent stimulus was used for the blue-yellow chromatic contrast sensitivity. These stimuli were presented at 0.5, 0.7, and 2.0 cpd. The chromaticities of the stimuli in xy coordinates of the 1931 CIE color space were: D6500: 0.296, 0.31; green: 0.225, 0.333; red: 0.356, 0.275; blue: 0.254, 0.2; yellow: 0.386, 0.53. Both axes were orthogonal and corresponded to L-M isolating and S-isolating stimuli. Equiluminance adjustments for red-green and blue-yellow stimuli were obtained from each subject using a heterochromatic flicker photometry procedure at 20 Hz before chromatic contrast sensitivity measurements. Luminance spatial contrast sensitivity was measured at 0.5, 3, 6, 12, and 20 cpd. A spectrophotometer (CS1000; Minolta, Whitesett, NC) was used to calibrate luminance and chromaticity of the stimuli.

The temporal contrast sensitivity stimulus used a 2.7° field at an average luminance of 34.3 cd/m², with a superimposed spatial Gabor function of 1 SD at 1° and sinusoidal temporal modulation of luminance at 2, 10, 20, and 33 Hz.

CS thresholds were obtained by varying contrast with the method-of-adjustments procedure provided by the software used (PSYCHO, Cambridge Research Systems). Three threshold determinations were made for each spatial frequency as the mean of three ascending and descending trials. All patients were tested monocularly in a darkened room. The nonparametric Mann-Whitney sum of ranks test was used to analyze data obtained in the visual tests.

coma, maculopathy, neuropathy, or other ophthalmic abnormality that could diminish contrast sensitivity (CS). Fundoscopy had been previously performed by experienced neuro-ophthalmologists (AS, PQ, FS, AMDN) and revealed subclinical abnormalities that included microangiopathy and nerve fiber swelling.10 A randomly selected eye of each of the 25 carriers was tested; 18 of these had normal fundus, 5 had swelling and microangiopathy (SM), and 2 had temporal pallor (TP).

Best-corrected visual acuities of LHON carriers were 20/30 or better in 24 of the 26 participants and 20/40 in the other two. Table 1 presents demographic data of these asymptomatic LHON carriers.

In a seminal report,5 we described this 360-member pedigree with maternal recurrence of LHON, 265 of whom were examined personally in an international field investigation of more than 20 physicians and scientists who traveled to Colatina (Espirito Santo state), in a rural ally in an international field investigation of more than 20 physicians and scientists who traveled to Colatina (Espírito Santo state), in a rural

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Asymptomatic carriers of the homoplasmic 11778 LHON mutation examined here showed subclinical losses in spatial luminance and chromatic CSFs but not in the temporal CSF. Chromatic losses were more pronounced for the R/G than for the B/Y system. In addition, R/G equiluminance adjustments were made by asymptomatic LHON carriers who required higher intensities in the green than did controls, whereas B/Y equiluminance adjustments did not differ from those of controls. This is in line with our findings from color vision tests of much larger losses in the protan and deutan axes than in the tritan axis in carriers (Quiros PA, et al. IOVS 2004;45:ARVO E-Abstract 4336) and in affected LHON patients (Gualtieri M, et al. IOVS 2004;45:ARVO E-Abstract 4331). Lower CS for all spatial stimuli—achromatic, red-green, and blue-yellow—corresponds to what would be expected in an abnormality that affects central vision.

The activity of the L- and M-cones, processed in opposition by the midget ganglion cells, originates the parvocellular pathway, which mediates R/G in opposition; the koniocellular system is activated by the S-cones and is responsible for the yellow-blue channel.13 L- and M-cone inputs, processed additively by the parasol cells, originate in the magnocellular pathway, which mediates achromatic spatial and temporal functions.13 The parvocellular pathway also responds to variations in luminance at high spatial frequencies and slow temporal stimuli (up to 1 Hz).14 Chromatic and luminance spatial CS functions examined here were affected in asymptomatic LHON carriers, whereas the temporal CSs, likely mediated by the magnocellular system at the frequencies tested, was not extensively affected.

The interpretation of our findings on visual function in asymptomatic LHON carriers requires a good understanding of the pathophysiologic mechanism through which optic neuropathy develops. One favored hypothesis is that a wave of cellular death, possibly apoptotic, affects the retinal ganglion cells with a preference for the smaller caliber fibers of the PMB.15 Complex I dysfunction generated by the different LHON mutations may induce decreased adenosine triphosphate synthesis16 or increased production of reactive oxygen species.17 These biochemical alterations of the mitochondrial homeostasis may result in impairment of axoplasmic organelle transport and in decreased turnover of myelin metabolism. Thus, both the prelaminar unmyelinated and energy-dependent portion of the optic nerve and the postlaminar myelinated tract are believed to carry this chronic biochemical defect. In this context, given the evidence that the smaller caliber fibers of the PMB, mostly...
belonging to the parvocellular component, are affected preferentially and earlier in LHON,\textsuperscript{18} it is also conceivable that subclinical dysfunction of vision may be found in responses mediated by this cellular system.\textsuperscript{19} It is believed that the acute phase—when a synchronous signaling for cell death propagates, affecting first the PMB—is triggered when a threshold is crossed and the system loses its compensatory capability. The exact sequence of the molecular events that precipitate the beginning of cell death and axonal loss is unknown.\textsuperscript{19}

The different types of ganglion cells are represented throughout the entire retina, and their dimensions increase from the center to the periphery.\textsuperscript{20} In LHON, there may be selective preservation of the larger sizes of the small bistratified ganglion cells and of other newly discovered S-cone opponent ganglion cell types and parasol ganglion cells. Another factor that may contribute to the sparing of the S pathway is that the S-cones are absent in the central part of the fovea, within a diameter of 0.3° to 0.4°.\textsuperscript{21–23} The B/Y opponent ganglion cells \( (S_{on}/L_{off}/M_{off}) \) match the S-cone density.\textsuperscript{24} Their densest region occurs at 1° eccentricity.\textsuperscript{24,25}

The chromatic channels subserve the psychophysical responses to chromatic equiluminance stimuli, which are mediated by the parvocellular and koniocellular pathways. These stimuli silence the luminance channel, thought to be represented by the magnocellular pathway.\textsuperscript{26} In the present study, the absence of significant differences between the temporal CS of LHON carriers and of controls implies that the magnocellular system of LHON carriers might be relatively unaffected. However, the fact that the luminance spatial CS is shifted to higher thresholds also suggests that for stationary patterns, other mechanisms must underlie contrast sensitivity. In fact, Shapley and Hawken\textsuperscript{26} propose that the luminance mechanism might process high temporal frequency and low- and mid-resolution spatial frequencies to different achromatic patterns.

Although the present data show no statistical differences in the temporal CS of carriers and controls, there was large variability in these data, with very low CS in some carriers. This consideration, and the fact that magnocellular neurons are present throughout the entire retina, including the center of the fovea,\textsuperscript{20} make it difficult to rule out an involvement of the magnocellular pathway in LHON.

Spatial luminance CS losses were uniform throughout the spatial frequency range tested, thus reflecting losses in responses mediated by magnocellular, parvocellular, and koniocellular pathways. The finding that CS in R/G and B/Y was similarly affected was surprising. We had expected the latter to be less affected because other measures involving the B/Y system of the LHON carriers showed it to be less affected than those involving the R/G system. In fact, color discrimination measured in the same population (Cambridge Colour Test)

**Figure 3.** (A) Mean contrast sensitivities and standard errors for equiluminance red-green gratings for asymptomatic LHON carriers (circles) and controls (triangles). Differences were statistically significant at all spatial frequencies \((P < 0.005)\). (B) Individual red-green contrast sensitivities of asymptomatic LHON carriers (open circles) and controls (filled triangles). Solid line passes through means of contrast sensitivities of controls and dashed lines through means of contrast sensitivities of asymptomatic LHON carriers.

**Figure 4.** (A) Mean contrast sensitivities and standard errors for equiluminance blue-yellow gratings for asymptomatic LHON carriers (filled circles) and controls (filled triangles). Differences in contrast sensitivity between asymptomatic LHON carriers and controls were statistically significant at all spatial frequencies \((P < 0.005)\). (B) Individual blue/yellow contrast sensitivities of asymptomatic LHON carriers (open circles) and controls (filled triangles). Solid line passes through means of contrast sensitivities of controls and dashed lines through means of contrast sensitivities of asymptomatic LHON carriers.
revealed a much smaller loss in the blue system (Quiros PA, et al. IOVS 2004;45:ARVO E-Abstract 4336). In addition, equiluminance adjustments were not different from those of controls for B/Y gratings. This discrepancy between findings of equiluminance adjustment and contrast sensitivity is in line with the results found by Dobkins et al., who examined covariance mechanisms for R/G equiluminance, luminance contrast sensitivity, and R/G contrast sensitivity and found evidence of separate mechanisms for these functions.

In VEP recordings in patients with multiple sclerosis, Sar-tucci et al. also found that CS to equiluminance B/Y and R/G gratings was reduced similarly to that in controls. To explain a similar magnitude of CS losses, they suggested that there must be selective cortical amplification of the B/Y signals through which the cortical signals for R/G and B/Y stimuli are similar, even though there is a larger number of R/G than B/Y opponent ganglion cells in the retina and a larger ratio of ERG to R/G stimuli (Porciatti V, et al. IOVS 1999;40:ARVO Abstract 68). Other authors found similar losses in the two chromatically opponent systems in recordings from patients with Parkinson’s disease or multiple sclerosis.

In conclusion, we report for the first time that chromatic R/G and B/Y and achromatic spatial contrast sensitivities are reduced in asymptomatic LHON carriers. The losses were nearly constant across spatial frequencies and were of similar magnitude for all three measures. It is not possible to know at present whether these alterations are or are not predictive of the development into the acute phase of the disease. Longitudinal studies are necessary to know whether these visual losses are a mild consequence of carrying the 11778 mtDNA mutation or whether they bear a relationship to the acute phase.

**Acknowledgments**

The authors thank Luiz Carlos de Lima Silveira for discussions of this manuscript. They also thank José de Oliveira Gil (IEE-USP) for calibration of the stimuli and the Instituto de Psicologia da USP for transport of the equipment and the investigators to the field. The authors are also greatly indebted to Milton Moraes for providing space and infrastructure in his ophthalmology clinic in Colatina, Brazil, to conduct these studies.

**References**


