Visual Neuroscience (2008), **25**, 487–491. Printed in the USA.

doi:10.1017/S0952523808080590

Irreversible color vision losses in patients with chronic mercury vapor intoxication

CLÁUDIA FEITOSA-SANTANA,[1,2] MIRELLA T.S. BARBONI,[1,2] NESTOR N. OIWA,[1,2] GALINA V. PARAMEI,[3] ANA LUISA A.C. SIMÕES,[2] MARCELO F. DA COSTA,[1,2] LUIZ CARLOS L. SILVEIRA,[4,5] AND DORA F. VENTURA[1,2]

[1]Núcleo de Neurociências e Comportamento, Universidade de São Paulo, São Paulo, Brazil
[2]Departamento de Psicologia Experimental, Instituto de Psicologia, Universidade de São Paulo, São Paulo, Brazil
[3]Department of Psychology, Liverpool Hope University, Liverpool, United Kingdom
[4]Núcleo de Medicina Tropical, Universidade Federal do Pará, Belém, Brazil
[5]Departamento de Fisiologia, Centro de Ciências Biológicas, Universidade Federal do Pará, Belém, Brazil

(RECEIVED October 10, 2007; ACCEPTED March 22, 2008)

Abstract

This longitudinal study addresses the reversibility of color vision losses in subjects who had been occupationally exposed to mercury vapor. Color discrimination was assessed in 20 Hg-exposed patients (mean age = 42.4 ± 6.5 years; 6 females and 14 males) with exposure to Hg vapor during 10.5 ± 5.3 years and away from the work place (relative to 2002) for 6.8 ± 4.2 years. During the Hg exposure or up to one year after ceasing it, mean urinary Hg concentration was 47 ± 35.4 μg/g creatinine. There was no information on Hg urinary concentration at the time of the first tests, in 2002 (Ventura et al., 2005), but at the time of the follow-up tests, in 2005, this value was 1.4 ± 1.4 μg/g creatinine for patients compared with 0.5 ± 0.5 μg/g creatinine for controls (different group from the one in Ventura et al. (2005)). Color vision was monocularly assessed using the Cambridge Colour Test (CCT). Hg-exposed patients had significantly worse color discrimination ($p < 0.02$) than controls, as evaluated by the size of MacAdam's color discrimination ellipses and color discrimination thresholds along protan, deutan, and tritan confusion axes. There were no significant differences between the results of the study in Ventura et al. (2005) and in the present follow-up measurements, in 2005, except for worsening of the tritan thresholds in the best eye in 2005. Both chromatic systems, blue-yellow and red-green, were affected in the first evaluation (Ventura et al., 2005) and remained impaired in the follow-up testing, in 2005. These findings indicate that following a long-term occupational exposure to Hg vapor, even several years away from the source of intoxication, color vision impairment remains irreversible.

Keywords: Cambridge Colour Test, Mercury toxicity, Chromatic discrimination, MacAdam's color discrimination ellipses, Neurotoxicology

Introduction

Human activity is responsible for one-third to two-thirds of the total Hg found in the environment. The atmosphere Hg level is very low and does not pose a health risk. However, steady release of industrial Hg in modern time resulted in three to six times more Hg in the atmosphere than two centuries ago. The amount of Hg found in soil at particularly hazardous waste sites can be 200 thousand times higher than the natural levels and the number of workers exposed to Hg is estimated to be in the order of several thousands (Cavalleri et al., 1995; ATSDR, 1999).

Color vision impairment has been observed in workers occupationally exposed to Hg vapor, particularly in fluorescent lamp industries (Ventura et al., 2004, 2005; Feitosa-Santana et al., 2007), production of thermometers, thermostats, and barometers (Cavalleri et al., 1995; Cavalleri & Gobba, 1998), chlorine and sodium hydroxide industries (Urban et al., 2003), manipulation of dental amalgam (Canto-Pereira et al., 2005), and gold mining (Silveira et al., 2003; Rodrigues et al., 2007).

Although the mechanisms for Hg-dependent visual losses are not yet understood, it is certain that the retina is implicated in the visual losses. Hg deposits can be found in the retina of non-human primates exposed to Hg vapor, in the optic nerve, retinal pigment epithelium, inner plexiform layer, vessel walls, and ganglion cells (Warfvinge & Bruun, 1996, 2000). In line with this, alterations in retinal function were reported by Ventura et al. (2004) who showed changes in the full field electroretinogram and in the multifocal electroretinogram in a group of former workers previously exposed chronically to Hg vapor in their work setting. These findings do not exclude additional

Address correspondence and reprint requests to: Cláudia Feitosa-Santana, Av. Prof. Mello Moraes, 1721, Bloco A, Sala D9, 05508-900 São Paulo, Brazil. E-mail: claudia@feitosa-santana.com

impairment in central nervous system structures, as implied in the losses found in the neuropsychological assessment of the same group of patients (Zachi et al., 2007).

Color vision has been evaluated using the Lanthony D-15 desaturated test (D-15d) (Lanthony, 1986), and error scores with this test were significantly higher in groups exposed to Hg vapor than for controls and were predominantly located along the tritan axis indicating an impairment of the blue-yellow system (Cavalleri et al., 1995; Urban et al., 2003; Feitosa-Santana et al., 2007).

However, when the Cambridge Colour Test (CCT) was used to evaluate Hg-exposed patients, both chromatic systems were affected, the blue-yellow and the red-green color opponent pathways (Silveira et al., 2003; Ventura et al., 2005; Rodrigues et al., 2007). The authors suggested that the difference with previous results could be explained by the CCT, a more sensitive test in detecting color vision impairment than the arrangement tests such as the FM-100, D-15, and D-15d.

The results were also different regarding the possible reversibility of color vision losses caused by exposure to Hg vapor. The results of Cavalleri and Gobba (1998) indicate that at least to some extent the color vision losses due to Hg vapor toxicity could be reversible. In their original study, Cavalleri et al. (1995) found color vision losses in a group of workers with 115 ± 61.5 μg Hg/g creatinine, a value much higher than the 35 μg Hg/g creatinine—limit established by the ACGIH (2001). One year later, the work conditions of safety had been significantly improved, the urinary concentration of exposed workers had restored to safe levels (10 μg Hg/g creatinine), and their color vision had returned to normal (Cavalleri & Gobba, 1998). In contrast, Feitosa-Santana et al. (2007), using the D-15d test, evaluated a group with long-term exposure to Hg vapor (10.6 ± 5.2 years) and found that even after the exposure had ceased for several years (6.4 ± 4.0 years) and the mean urinary concentration returned to within the safe levels (<5 μg Hg/g creatinine), color discrimination was still impaired. Their results strongly suggested that color vision losses are not reversible.

In the present study, the CCT was used for a longitudinal evaluation of workers occupationally exposed to Hg vapor to address the issue of reversibility of their color vision losses once they had been kept away from the intoxication source. Tests were performed originally in 2002 (Ventura et al., 2005) and compared with the reevaluation three years later, in 2005.

Materials and methods

Color vision was assessed in 20 patients (mean age 42.4 ± 6.5 years, range 33–54 years; 6 females), corresponding to a subgroup of the Hg-exposed subjects studied by Ventura et al. (2005). The patients were chronically exposed to Hg vapor for 10.5 ± 5.3 years (range 4–24.5) and away from exposure source (relative to 2002) for 6.8 ± 4.2 years (range 1–15). The mean urinary concentration was 47 ± 35.4 μg Hg/g creatinine (range 1.2–134.7) during exposure or up to one year after exposure. There was no information about Hg urinary concentration at the time of the initial tests (Ventura et al., 2005), but at the time of the follow-up tests, in 2005, it was 1.4 ± 1.4 μg Hg/g creatinine (range 0–4.3). The patients were sent by the Oscar Freire Institute, University of São Paulo (São Paulo, Brazil) (Ventura et al., 2005; Barboni et al., 2008; Feitosa-Santana et al., 2007). The control group (different from that in Ventura et al. (2005)) included 20 subjects (mean age 40.5 ± 6.3 years, range 31–55 years; 8 females) having mean urinary concentration 0.5 ± 0.5 μg Hg/g creatinine (range 0–1.7).

Inclusion criteria were visual acuity measured with the Snellen optotypes better than 20/30 and absence of ophthalmological pathologies or diseases that affect the visual system. Exclusion criteria were history of psychiatric disorders before the occupational exposure to Hg, other heavy metals exposition, or other hazardous chemical compounds, alcoholism, and smoking (more than five cigarettes per day). All subjects were tested monocularly: for the patients, both eyes; for the controls, one eye randomly chosen.

Informed consent was signed by all subjects. The procedures complied with the tenets of the Declaration of Helsinki and were approved by the Research Ethics Committees of the Institute of Psychology and the University Hospital of the University of São Paulo.

Tests were performed using the CCT version 2.0 software (Cambridge Research Systems, CRS, Rochester, UK) in a microcomputer XTC-600 (Dell Dimension, Winston-Salem, NC) equipped with a VSG 5 graphics card (Cambridge Research Systems, CRS, Rochester, U.K.) and a Sony FD Trinitron color monitor GDM-F500T9 (Sony Electronics Inc., Tokyo, Japan) with 100 Hz temporal resolution and 800 × 600 spatial resolution. Monitor luminance and chromatic calibrations were performed respectively with a Minolta CS1000 photometer. Visual stimuli consisted of a Landolt "C" target, composed of circles of a given chromaticity having a series of different randomly chosen sizes and luminances which were presented against a background of constant chromaticity. Tests were performed in a dark room with illumination provided only by the monitor with the visual stimuli. A subject was positioned 2.6 m away from the monitor providing 1° of visual angle for the gap in the letter "C." The gap appeared randomly in one of four orientations (up, down, left, and right), and the subject's task was to indicate the position of the opening by pressing a corresponding button of a response box.

The test started with presentation of a saturated color and proceeded to a less saturated color each time the subject gave a correct response. Conversely, an incorrect response or no response was followed by the presentation of colors with higher saturation. After 11 reversals (the first four were ignored and the result was calculated as the mean of the last seven), the staircase was terminated, and a color discrimination threshold computed. We adopted a time-response limit of 6 s for each trial, for both patients and controls.

The CCT offers two testing conditions: the Trivector test and the Ellipses test. The Trivector test measures color discrimination thresholds relative to a background chromaticity (CIE 1976: $u' = 0.1977$, $v' = 0.4698$) as excursions in $u'v'$ units along the protan, deutan, and tritan confusion axes. The Ellipses test measures color discrimination thresholds along a number of color space vectors. We measured one ellipse using an eight vector protocol and the same background chromaticity as in the Trivector test. The software compiled the responses, plotted the threshold for each vector, and fitted an ellipse through the thresholds centered on the background chromaticity, whose ellipse parameters were further used in the statistical analysis (Mollon & Reffin, 1989, 2000; Regan et al., 1998; Ventura et al., 2003).

Since the data were not normally distributed, we applied nonparametric tests—Mann-Whitney U test and Wilcoxon matched-pair test, with the level of significance of $p < 0.05$ (Statistica 6.0, StatSoft, Tulsa, OK). In addition, linear regression analysis was performed to investigate a possible relation between CCT results

Table 1. *Color discrimination thresholds in u'v' units (first line, mean ± SD; second line, Q1/median/Q3) for the controls in 2002 (Ventura et al., 2005) and in the present study, in 2005, and for the Hg-exposed patients in 2002 (Ventura et al., 2005) and in the present follow-up study, in 2005*

			Hg-exposed patients in 2002		Hg-exposed patients in 2005	
	2002 Controls	2005 Controls	Best eyes	Worst eyes	Best eyes	Worst eyes
Trivector						
Protan	56 ± 21 43/49/79	56 ± 22 42/46/80	81 ± 42 51/68/102	119 ± 81 72/80/154	84 ± 53 51/64/106	121 ± 76 64/102/138
Deutan	54 ± 18 45/54/71	55 ± 19 46/54/71	81 ± 47 55/62/80	117 ± 71 62/96/149	89 ± 55 44/77/122	124 ± 83 54/102/167
Tritan	112 ± 59 70/91/158	107 ± 48 69/88/159	120 ± 112 68/93/113	210 ± 187 103/142/228	142 ± 92 71/124/167	189 ± 114 93/151/267

and Hg urinary concentration, exposure length, and time away from exposure to Hg vapor.

Results

Table 1 shows color discrimination results obtained with the Trivector test for both controls and Hg-exposed patients. Hg-exposed patients were differently affected in their best and worst eyes (protan vector, $p < 0.022$; deutan vector, $p < 0.022$; Wilcoxon matched-pair test). Fig. 1 shows differences in the ellipses area between the best and worst eyes ($p < 0.006$; Wilcoxon matched-pair test).

Color discrimination thresholds were significantly higher in the 2005 Hg-exposed subjects in comparison with controls for both their worst eyes (protan vector, $p < 0.001$; deutan vector, $p = 0.005$; tritan vector, $p = 0.022$; ellipse area, $p < 0.001$), and best eyes (ellipse area, $p < 0.003$; Fig. 1) (Mann-Whitney U test). In the present study, there were no significant differences between patients' and control subjects' best eyes in the Trivector test for the protan ($p = 0.099$), deutan ($p = 0.057$), and tritan ($p = 0.361$) vectors (Mann-Whitney U test).

We calculated tolerance limits (percentile boundaries 95%) for the CCT parameters to assess the percentage of Hg-exposed patients' eyes whose thresholds exceeded the limits either in 2002 (Ventura et al., 2005) or in the follow-up tests, in 2005, in comparison to the present control group. These results are presented in the Table 2.

Table 2. *The percentage of Hg-exposed patients' eyes exceeding the CCT threshold limits (percentile boundaries 95%) in comparison to the control group in 2005*

	Hg-exposed patients in 2002		Hg-exposed patients in 2005	
	Best eyes	Worst eyes	Best eyes	Worst eyes
Trivector				
Protan	25%	40%	30%	60%
Deutan	20%	50%	35%	55%
Tritan	10%	30%	15%	45%
Ellipse 1				
Area	30%	55%	45%	65%

Regression analysis revealed no significant relationship between color discrimination and age ($p > 0.42$), exposure duration ($p > 0.26$), or mean urinary Hg concentration ($p > 0.16$).

Data presented in Ventura et al. (2005) and in the follow-up tests, in 2005, were related by using the Wilcoxon matched-pair test. Except for the best eye, tritan vector ($p = 0.03$), this analysis showed no significant differences for any color discrimination parameter after the three-year period. For the best eyes: protan vector, $p = 0.87$; deutan vector, $p = 0.30$; and ellipse area, $p = 0.20$. For the worst eye: protan vector, $p = 0.74$; deutan vector, $p = 0.94$; tritan vector, $p = 0.97$; and ellipse area, $p = 0.10$ (Fig. 1).

Discussion

The results of this study provide evidence that the color vision loss in the Hg-exposed patients was diffuse without any selectivity for a given cone axis, thus indicating that both chromatic systems were affected, blue-yellow and red-green (Fig. 1). This is in agreement with previous findings of Silveira et al. (2003), Ventura et al. (2005), and Rodrigues et al. (2007).

The asymmetry between the two eyes of Hg-exposed patients is a feature of acquired dyschromatopsia (Hart, 1987). Our results are in agreement with previous work in the same group of patients that has also revealed differences in visual sensitivity between the two patient's eyes (Ventura et al., 2005; Feitosa-Santana et al., 2007).

While Ventura et al. (2005) have found statistical differences for all parameters measured with the CCT for both the best and worst eyes, we have not found any significant difference between Hg-exposed patients' and control subjects' best eyes in the protan, deutan, and tritan vectors. This discrepancy between the two studies may have resulted from either a slightly different composition of the present control group in comparison to that measured by Ventura et al. (2005) and/or a difference in color discrimination thresholds measured by the test for the best eye. The possibility of the best eye sensitivity was statistically tested; the results indicate no color vision improvement, but even color vision worsening in Hg-exposed patients for the tritan thresholds ($p = 0.03$). These findings suggest, at least for this group, the irreversibility of color vision losses.

Table 2 shows that for all the color discrimination measurements the percentages obtained in the longitudinal study were higher in 2005 than in 2002 (Ventura et al., 2005). These findings suggest that there was no improvement in color discrimination, but

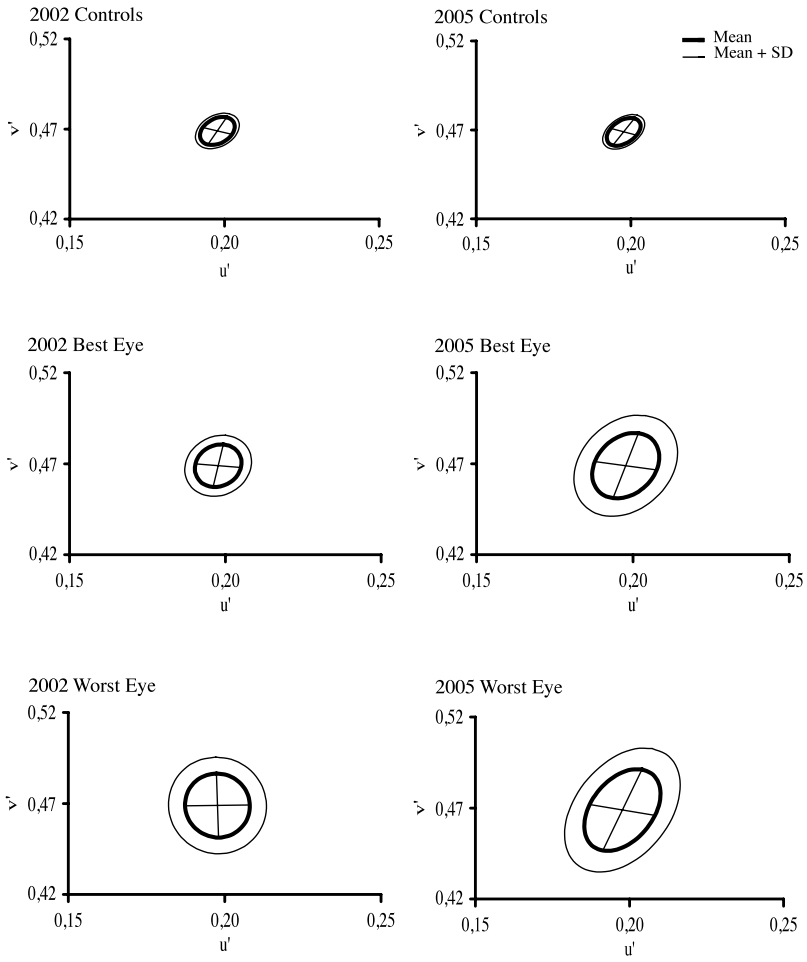

Fig. 1. MacAdam's ellipses presented within the CIE 1976 chromaticity diagram ($u'v'$ units) for the controls in 2002 (Ventura et al., 2005) and the controls in 2005, and for the best and worst eyes for the Hg-exposed patients in 2002 and in the follow-up study, in 2005. Legend: bold line—mean; regular line—mean plus one standard deviation.

even a worsening of color vision function between the two sets of tests (2002 and the follow-up in 2005).

The lack of any relationship between color discrimination thresholds and age exposure duration, or mean urinary Hg concentration, is in agreement with previous reports (Ventura et al., 2005; Feitosa-Santana et al., 2007).

The discrepancy between the present findings and those of Cavalleri and Gobba (1998) who reported reversibility in color discrimination after a period of one year away from exposure might be due to various confounding factors influencing an outcome of color vision assessment, such as the duration of exposure to Hg, urinary Hg concentration, color test protocol, and others (Paramei et al., 2004).

The results of the present study provide evidence that after cessation of exposure to Hg vapor for 6.8 ± 4.2 years (range 1–15 years), color discrimination impairment can still be found, thus pointing to the irreversibility of color vision losses in long-term occupational exposure to Hg vapor.

Acknowledgments

This study was supported by grants to DFV from FAPESP (Projeto Temático 02/12733-8), CNPq (523303/95-5), and to DFV and LCLS from CAPES/PROCAD (0019/01-1). This research group is also supported by the FINEP research grant IBN-Net "Rede Instituto Brasileiro de Neurociência" (01.06.0842.00). CFS, MTSB, and NNO hold FAPESP fellowships (05/53974-6, 05/57897-6, and 05/59668-4, respectively). LCLS and DFV are CNPq Research Fellows. The authors thank Marcilia Medrado-Faria for permission to recruit patients for this study.

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