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LIGHT-INDUCED RETINAL DAMAGE USING DIFFERENT LIGHT SOURCES, PROTOCOLS AND RAT STRAINS REVEALS LED PHOTOTOXICITY

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Abstract—To save energy, the European directives from the Eco-design of Energy Using Products (2005/32/CE) have recommended the replacement of incandescent lamps by more economic devices such as Light Emitting Diodes (LEDs). However, the emission spectrum of these devices is enriched in blue radiations, known to be potentially dangerous to the retina. Recent studies showed that light exposure contributes to the onset of early stages of age-related macular degeneration (AMD). Here, we investigate, in albinos and pigmented rats, the effects of different exposure protocols. Twenty-four hours exposure at high luminance was compared to a cyclic (dark/light) exposure at domestic levels for 1 week and 1 month, using different LEDs (Cold-white, blue and green), as well as fluorocompact bulbs and fluorescent tubes. The data suggest that the blue component of the white-LED may cause retinal toxicity at occupational domestic illuminance and not only in extreme experimental conditions, as previously reported. It is important to note that the current regulations and standards have been established on the basis of acute light exposure and do not take into account the effects of repeated exposure.
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Key words: retina, Light Emitting Diodes, phototoxicity, pigmented rats, chronic light exposure.

INTRODUCTION

Artificial light consumes near to 20% of the world electricity production. To save energy, the European directives from the Eco-design of Energy Using Products (2005/32/CE) have recommended the replacement of incandescent lamps by more economic devices such as Light Emitting Diodes (LED). By 2019, LED will be the major domestic and public light source. LEDs emit mono chromic lights, and the less expensive and currently used method to produce white light from LED is to combine a blue LED with yellow phosphore coverage. The resulting spectrum is enriched in blue radiations, known to be potentially dangerous to the retina (Algvere et al., 2006). The other concerns are the high luminance level and the visual discomfort due to the punctual character of the emitting surfaces.

The role of sunlight exposure in the development and/or aggravation of retinal diseases and particularly age-related macular degeneration (AMD), which is associated with oxidative stress and inflammation, has been disputed for years (Ardeljan and Chan, 2013; Pinazo-Duran et al., 2014; McHarg et al., 2015). Indeed, cumulative light exposure, and particularly retinal exposure is difficult to estimate (Sliney, 2005). However, recently, based on large population studies, light exposure has been clearly recognized as a contributing factor in the appearance of the early stages of AMD (Klein et al., 2007; Sui et al., 2013).

In this context, light exposure must be considered as part of the environmental factors that can influence multiple physiologic processes and potentially impact pathologic retinal aging. The massive conversion from incandescent lights to LED incorporating devices in domestic lighting should be examined in more depth as recommended by the governmental agencies (ANSES report,(Saisine 2008SA0408) French Agency for Food, Environmental and Occupational Health and Safety).

Risk evaluation is based on epidemiologic studies, experimental results and exposure scenarios. But, while extreme acute exposures to high luminance lighting systems are frequently used in various models of light-induced retinal degeneration, few studies have evaluated the effects of different light sources in conditions close to domestic use (Peng et al., 2012; Shang et al., 2014).

In this study, we investigate, in albinos and pigmented rats, the effects of different exposure protocols. Twenty-four hours exposure at high luminance was compared to a chronic cyclic (dark/light) exposure at domestic levels for 1 week and 1 month, using different LEDs (Cold-white, blue and green), as well as fluorocompact bulbs (CFL) and Cold Cathode fluorescent lamps (CCFL) (fluorescent tubes).

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Abbreviations: AMD, age-related macular degeneration; BRB, blood retinal barrier; CCFL, Cold Cathode fluorescent lamps; CFL, fluorocompact lamp; ERG, electroretinogram; LE, Long Evans; LED, Light Emitting Diode; ONL, outer nuclear layer; PBS, phosphate-buffered saline; PNA, peanut agglutinin; W, Wistar.

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EXPERIMENTAL PROCEDURES

Animals

8-week-old albino Wistar (W) and pigmented Long Evans (LE) rats (Janvier laboratory, Le Genest St Isle, France) were used in these experiments. At least four rats were used per exposure condition and per time point. Rats were maintained on a 12-h/12-h light–dark (LD) cycle at 22 °C at a luminance below 250 lux, for 21 days before light-exposure experiments. All experimental procedures were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in Ophthalmic and Vision Research. Experimental procedures were submitted and approved by the local ethics committee European Council Charles Darwin, University Paris Descartes (Authorization N° – 05, Ce5/2012/019, A75-580).

Light sources

We used two types of lighting devices. For exposure to white LED, commercial cold white LED panel generating 2300 lumens during 24 h was used. The LED panel was placed above 8 transparent cages, placed on white surfaces, leaving enough space for air circulation and constant temperature maintenance at 21 °C. The illuminance measured at the rats' eyes position was 6000 lux (Photometre DT-8809A, CEM, China).

For long-term exposure, specific devices were built and characterized by Statice, France (Fig. 1A). Metallic boxes contained rows of LED with a diffuser in order to improve the directional uniformity of the radiation and avoid punctate sources. Alternatively, CCFL or CFL were uniformly distributed around the metal cages. Each cage was placed in a metallic device that was then placed in a ventilated cupboard allowing for a constant 21 °C temperature control (Fig. 1A). The light intensity was controllable and the distribution of light in the cage was homogenous whatever the rat position. Different types of LEDs were used: cold-white LED (pure white 6300 K), blue LED (royal blue 455–465 nm), and green LED (520–35 nm) (Z-power LED, Seoul Semiconductor, Korea). Exposure intensity was spectrophotometrically measured by Statice.

Exposure protocols

Acute exposure: LE and W rats were maintained in a cyclic light/dark (250 lux, 12 h/12 h) environment for 21 days. The day before light exposure, rats were dark-adapted for 16 h. The next day, pupils were dilated with 1% atropine (Alcon, Norvartis, Rueil Malmaison, France) under dim light, and rats were isolated in separate cages containing enough food for one day. After 24 h of exposure, rats were placed again in a cyclic light/dark (250 lux, 12 h/12 h) environment for 7 days and sacrificed for histology and immunofluorescence analysis. Control rats were submitted to the same pre conditioning protocol but not exposed to light. Different types of light sources and light intensities were used as detailed in Fig. 1B. For cold-white LED, different light intensities were tested from 6000 lux, to 1500, 1000 and 500 lux. Blue and green LEDs were used at 500 lux which is the domestic classic light intensity. CFL was used at 6000 lux and 500 lux, CCFL at 6000 lux. Illuminance was measured at the level of the rat eye.

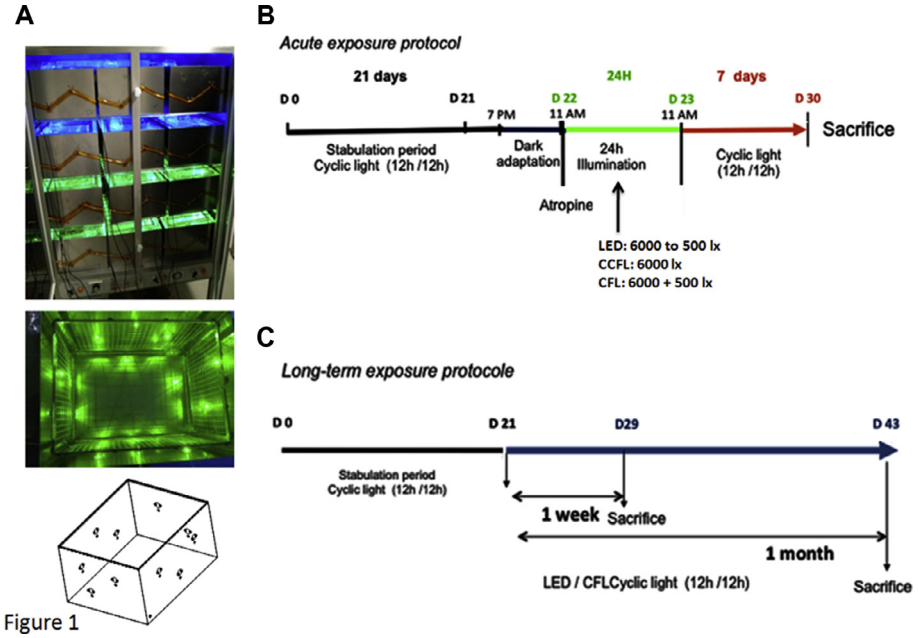

Figure 1

Fig. 1. LED device and exposure protocols. (A) LED containing device: all the walls of the animal's compartment were equipped with LEDs. (B) Acute exposure protocol: rats were kept in the normal cyclic light of the animal facilities for 3 weeks. Before exposure to LEDs on the device seen on panel A, they were dark-adapted and their pupils were dilated with atropin before LED exposure (6000, 1500, 1000 or 500 lx). After 24 h of exposure they were returned to the animal facility for 7 days and then sacrificed. (C) Long term exposure protocol: After the same stabulation period than before, the rats were exposed in the LED device, cyclically (12 h dark/ 12 h light, 500 lx) for one week or 1 month and then sacrificed.

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