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Original Article

Evaluation of the safety of conventional lighting replacement by artificial daylight

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ABSTRACT

Background: Short morning exposure to high illuminance visible electromagnetic radiations termed as artificial daylight is beneficial for the mental health of people living in geographical areas with important seasonal changes in daylight illuminance. However, the commercial success of high illuminance light sources has raised the question of the safety of long hour exposure.
Methods: We have investigated the effect of the replacement of natural daylight by artificial daylight in Swiss mice raised under natural lighting conditions. Mice were monitored for neurotoxicity and general health changes. They were submitted to a battery of conventional tests for mood, motor and cognitive functions' assessment on exposure day (ED) 14 and ED20. Following sacrifice on ED21 due to marked signs of neurotoxicity, the expression of markers of inflammation and apoptosis was assessed in the entorhinal cortex and neurons were estimated in the hippocampal formation.
Results: Signs of severe cognitive and motor impairments, mood disorders, and hepatotoxicity were observed in animals exposed to artificial daylight on ED20, unlike on ED14 and unlike groups exposed to natural daylight or conventional lighting. Activated microglia and astrocytes were observed in the entorhinal cortex, as well as dead and dying neurons. Neuronal counts revealed massive neuronal loss in the hippocampal formation.
Conclusions: These results suggest that long hour exposure to high illuminance visible electromagnetic radiations induced severe alterations in brain function and general health in mice partly mediated by damages to the neocortex-entorhinal cortex-hippocampus axis. These findings raise caution over long hour use of high illuminance artificial light.

1. Introduction

Light exposure is a powerful environmental cue for the regulation of circadian rhythms in mammals. Important changes in daytime duration and daylight illuminance associate with geographical and seasonal changes, particularly Arctic and Antarctic latitudes, and winter. These changes result in alterations of the ocular diurnal rhythms, sleep-wake cycle, and other biological rhythms, including the neuroendocrine and immune system, accompanied by serious health problems like seasonal affective disorder (SAD), with a higher frequency of occurrence in women [1–5]. Typically, SAD patients display symptoms of major depressive disorders, including psychomotor retardation, agitation, and energy loss, anhedonia, indecisiveness, decreased interest and concentration, feelings of worthlessness, and suicidal ideation [6–9]. Laboratory rodents are also affected by seasonal changes, justify-

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ing their use in mechanistic studies of SAD and related conditions [10–12].

Artificial light positive properties at specific illuminance and exposure time allowed the development of phototherapy for the treatment of mood and biological disorders associated with seasonal changes. Daily exposure (morning, up to 2-h) to artificial daylight (bright white artificial light >8000 lx) improved well-being, sleep, daytime psychomotor vigilance performance, cortisol and melatonin levels, and SAD patients' condition [13–18]. Furthermore, strategic exposure to artificial daylight during daytime improved SAD-like primary and secondary features of Parkinson's disease [19], but also learning effectiveness and other cognitive abilities in people affected by seasonal changes [20,3,21], without eliciting any major safety concerns [22,23].

Together with other benefits of bright light and the low cost of the technology, the aforementioned positive effects of phototherapy contribute to the commercial success of daylight-grade artificial light sources. Surprisingly, considering notably emerging evidence supporting adverse effects of related electromagnetic radiations such as UVA [24,25], no report is available on the potential adverse reactions to long exposure to artificial daylight. In the present study, we assessed the impact of continuous artificial daylight exposure during daytime on the physiology, mood, cognitive functions, and number of cells in the brain of mice.

2. Methods

2.1. Animals and procedures

2.1.1. Animals and light exposure

Swiss female mice (n = 24) were raised under natural daylight, from birth to the age of 8 months, in the animal facility of the College of Pharmacy, Qassim University (Buraydah, Saudi Arabia). Then, these animals (36.87 ± 3.83 g weight) were randomly divided in three groups (n = 8) housed in transparent Plexiglas cages (70 cm × 70 cm, height 60 cm) in different rooms. A cage was in a room exposed to natural daylight (22.000 lx average luminance at cage floor), while the other two were in rooms either exposed to conventional lighting (500 lx at cage floor) or to artificial daylight (21.736 lx at cage floor) during the daytime. Lights were switched on and off by an automated system, based on dawn and dusk. Experiments in live animals were performed in Buraydah, Central Saudi Arabia, during the winter, thus civilian dawn was at 6:24 a.m. (local time) and dusk at 6:11 p.m. (daytime length: ~11h47 min). During the dark phase (night), rooms were kept under dim red light (3 lx at cage floor). Light intensity was monitored using a photometer (9152B, Pasco Scientific, Roseville, CA). In each room the temperature was constant (23.5 °C) and animals had *ad libitum* access to food and water.

The experiment and all procedures in live animals were approved by both the Research Center and the Ethical Committee of the College of Applied Medical Sciences, Qassim University, and performed according to EC Directive 2010/63/EU on the protection of animals in scientific experiments.

2.1.2. Experimental procedures

Animals exposed to natural daylight, conventional lighting, or artificial daylight were monitored daily, to detect signs of systemic and central nervous system toxicity such as shaggy fur, cachexia, vocalization when handled, and porphyrin deposits around the eye ('red tears'). Negative geotaxis and various reflexes were asserted (mainly posture, pinna, righting, contact righting, and corneal reflexes). Animal behavior in cages was recorded with a computerized system equipped with infrared cameras and analyzed to detect important changes in social behavior. The body weight was determined every three days.

On exposure days (ED) 14 and 20, mice were submitted to a battery of behavioral tests aimed at assessing changes in the mood, cognition, and motor function. Tests were performed between 11 a.m. (Zeitgeber 5, *i.e.* 5 h after light phase onset) and 2 p.m. (ZT 8), in rooms where animals were housed. The whole testing procedure required 30 min per animal. The performance of each animal was video recorded and scored offline. Skin temperature was determined in both ears at the beginning and the end of the battery of tests, using a non-contact infrared thermometer.

The experiment in live animals was stopped on ED21 due to increases in the aforementioned signs of toxicity, in order to comply with animal research ethical standards. Animals were sacrificed under deep gas anesthesia between ZT 5 and ZT 8. Blood was collected by cardiac puncture and brains dissected out and fixed. Blood was processed for liver function test. Brains' left hemispheres were processed for histopathological studies and stereological estimation of hippocampal neurons at the Department of Histology and Embryology, Ondokuz Mayis University (Samsun, Turkey). Instead, the right hemispheres were processed for immunohistochemical labeling of resident cells and markers of inflammation and apoptosis in the entorhinal cortex, at the Department of Anatomy, King Abdulaziz University (Jeddah, Saudi Arabia).

2.1.3. Artificial daylight exposure

Artificial daylight was delivered by a linear source lamp (LSL) system designed and optimized to produce isothermal, regular, and homogeneous electromagnetic radiation by Qassim University's Department of Physics [26]. The system was made of a focusing mirror and eight cool white fluorescent tubes (60 cm length, 3.3 cm diameter, 1800 lm) in the same horizontal plane. The distance between the LSL system and the cage floor was 60 cm.

2.2. Behavioral tests

The following tests were performed sequentially:

2.2.1. Footprint test

The footprint test was performed for gait and balance assessment. Mice with inked paws were allowed to walk freely along an enclosed box (70 cm long, 7 cm wide, and 20 cm high plexiglas walls) with a clean sheet of paper placed on the floor. After three consecutive tests, only one valid trial was considered per animal to exclude habituation phase-associated abnormal patterns [27].

2.2.2. Elevated plus maze

The EPM consisted of two open arms (30 cm × 7 cm, no wall), two closed arms (30 cm × 7 cm, with 20 cm high Plexiglas walls), and a common central platform (7 cm × 7 cm). The entire apparatus was elevated to 70 cm above floor level. Each mouse was plogical parameters scored included entries, time spent, and distance traveledlaced on the central platform of the maze, facing an open arm, and the behavior was recorded for 5 min. Etho in each arm. An entry occurred when all four limbs were within an arm. Head dips over the edge of open arms, rearing, grooming, sniffing, and freezing episodes were also counted.

2.2.3. Open field test

Exploratory behavior was examined in an open field arena in Plexiglas (40.6 cm × 40.6 cm, height 38.1 cm). The arena's floor was divided into a central (20.2 cm × 20.2 cm) and a peripheral zone (remaining 10.2 cm surrounding the central zone). The test was started by placing a mouse in the arena, facing the wall. Animal's activity in the chamber was recorded for 10 min using a camera mounted on side (approximately 50 cm from the floor) that

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