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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

captured both vertical and horizontal activities. Mouse locomotor activity was tracked with an application developed in MATLAB computing environment (MathWorks, Natick, MA) using motion tracking in image sequences. Entries, time spent, and distance traveled in the central and peripheral zones were determined, as well as the time spent in the corners.

2.2.4. Y-maze spontaneous alternation test

The test was performed under reduced light (~100 lx), as recommended [28,29]. Y-maze consisted of three Plexiglas arms (length 30 cm, width 7 cm, height 20 cm) symmetrically placed (120° angle between arms). Each mouse was placed in the center of the maze and spontaneous activity was recorded for 5 min using a camera placed above the maze. The following were determined using MATLAB applications: the number and sequence of arm entries, the time used to perform the first three and the last three alternations, the total distance traveled, and the average speed (distance traveled per time unit). Direction changes occurring after sniffing the wall of another arm (hesitation during arm change) were also determined. An entry occurred when all four limbs were within the arm.

2.3. Liver function test

Levels of total protein, albumin, globulin, triglycerides and cholesterol, as well as the activities of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined in sera using commercially available kits (bio-Merieux Laboratory Reagents and Products, France), according to the manufacturer's instructions. The albumin/globulins ratio and the fraction of total protein represented by albumin and globulins were calculated.

2.4. Tissue processing and histopathological studies

Brains were sequentially fixed in 10% formalin for 8-h, abundantly rinsed in PBS, dried in increasing concentrations of ethanol, soaked in xylene, and embedded in paraffin using Thermon Shandon Citadel tissue processor (GMI Inc., Ramsey, MN, USA).

2.4.1. Histopathology and stereology

Sections of embedded tissues (20 μm-thick) were made throughout the entire left brain hemisphere in the coronal plane, with a rotary microtome (Leica RM2125RT) using steel blades. After a random selection of the first section, the rule of systematic random sampling with 1/5 ratio was applied to the section selection. Selected sections were mounted on slides, deparaffinized, rehydrated, stained with cresyl violet (Nissl staining), dehydrated, and then, mounted with Entellan using standard procedures. The morphology of neurons in the hippocampal formation was examined.

Stereological analyses were performed using optical fractionator method [30] and a computerized stereological workstation equipped with StereoInvestigator software (version 9.0, MicroBrieldField; Colchester; USA). Briefly, before starting the counting, areas (coordinates) of CA1, CA2, CA3, and dentate gyrus were determined on every section and for each animal using an atlas and a bright field microscope with motorized stage (microscope objective 4×). These areas of interest were scanned along the x- and y-axes. The x/y grid sizes, the step size, and the area of unbiased counting frame at microscope objective 100× were determined (*i.e.* the most appropriate step and frame to avoid counting CA1–CA3 and dentate gyrus neurons twice). Afterwards, pyramidal cells (respectively, granular cells) were counted in CA1–CA3 areas (respectively, the dentate gyrus) using the unbiased counting frame (microscope objective 100×). Then, total numbers of neurons in the dentate gyrus and CA1–CA3 were estimated with the optical fractionator technique (coefficient of error ≤0.05) applied using the relevant program of the stereological workstation.

2.4.2. Immunohistochemistry

Standard procedures were used, as previously described [31], on randomly selected sections (6 μm-thick) made on paraffin-embedded brain right hemispheres, in the coronal plane. Primary antibodies used were goat anti-NeuN (mature neuron marker, 1:1000 dilution), goat anti-GFAP (astrocyte marker, 1:100 dilution), goat anti-iba1/CD68 (microglial marker, 1:1000 dilution), goat anti-TNF-α (pro-inflammatory cytokine, 1:500 dilution), rabbit anti-Fas (apoptosis mediator, 1:500 dilution), rabbit anti-Fas ligand (death receptor, 1:500 dilution), and rabbit anti-caspase 3 (marker of apoptosis, 1:500 dilution) (Santa Cruz Biotechnology, CA). Biotinylated anti-goat IgG and biotinylated anti-rabbit IgG (1:200 dilution, Santa Cruz Biotechnology, CA) were the secondary antibodies. The chromogen substrate 3,3′-diaminobenzidine hydrochloride (DAB) was applied and counterstained with hematoxylin. Glass coverslips were placed using DPX mounting medium. Sections labeled were observed with a computerized light microscope including an Olympus BX53 microscope equipped with an Olympus DP73 camera (Olympus, Tokyo, Japan), under 4×, 20×, 40× and 100× objectives.

2.5. Data analysis

One way ANOVA for independent groups followed by LSD test was used to assess the statistical significance of inter-treatment group changes in body weight, body temperature, and behavioral test parameters. Repeated measures ANOVA followed by LSD test was used to assess the statistical significance of differences between ED14 and ED20 performances in behavioral tests. Differences with p-value lower than 0.05 were significant. Data are presented as mean ± SEM.

3. Results

3.1. Animal general condition

3.1.1. Neurological examination

From ED8 onward, artificial daylight–exposed animals were more agitated when handled than animals of other groups and expressed audible vocalizations. Besides, fights were more frequent in the cage of artificial daylight–exposed animals. Thus, three animals presented with bite injuries on the back on ED10, and almost all presented with such injuries on legs, feet, and the back on ED20. These animals also displayed shaggy and dirty furs, decreases in exploratory activity, regular freezing episodes, as well as slower visual placing and negative geotaxis responses. They also resisted more to the separation from grid during the grip strength evaluation.

However, no major locomotor impairment was observed in any group; grip strength (measured by the ability to hold tight to a grid) was also comparable between groups; and no marked change was observed in the reflexes assessed, in the lacrimation (the eyes were not dry), and in the salivation (no dry mouth).

3.1.2. Body weight and body temperature

The effect of artificial daylight exposure on the body weight is shown in Fig. 1A. Body weight increased linearly in, and was comparable between, natural daylight- and conventional lighting–exposed groups ($y = 1.02x + 36.1$, $R^2 = 0.97$ and $y = 1.17x + 35.6$, $R^2 = 0.98$, respectively). However, a comparable increase was observed in artificial daylight group only between ED 0 and ED 4 ($y = 1.75x + 34.88$, $R^2 = 0.99$). Afterwards, animals of this group displayed a transient decrease in body weight following a polynomial progression ($y = 0.58x^2 - 3.53x + 41.43$, $R^2 = 0.94$), with

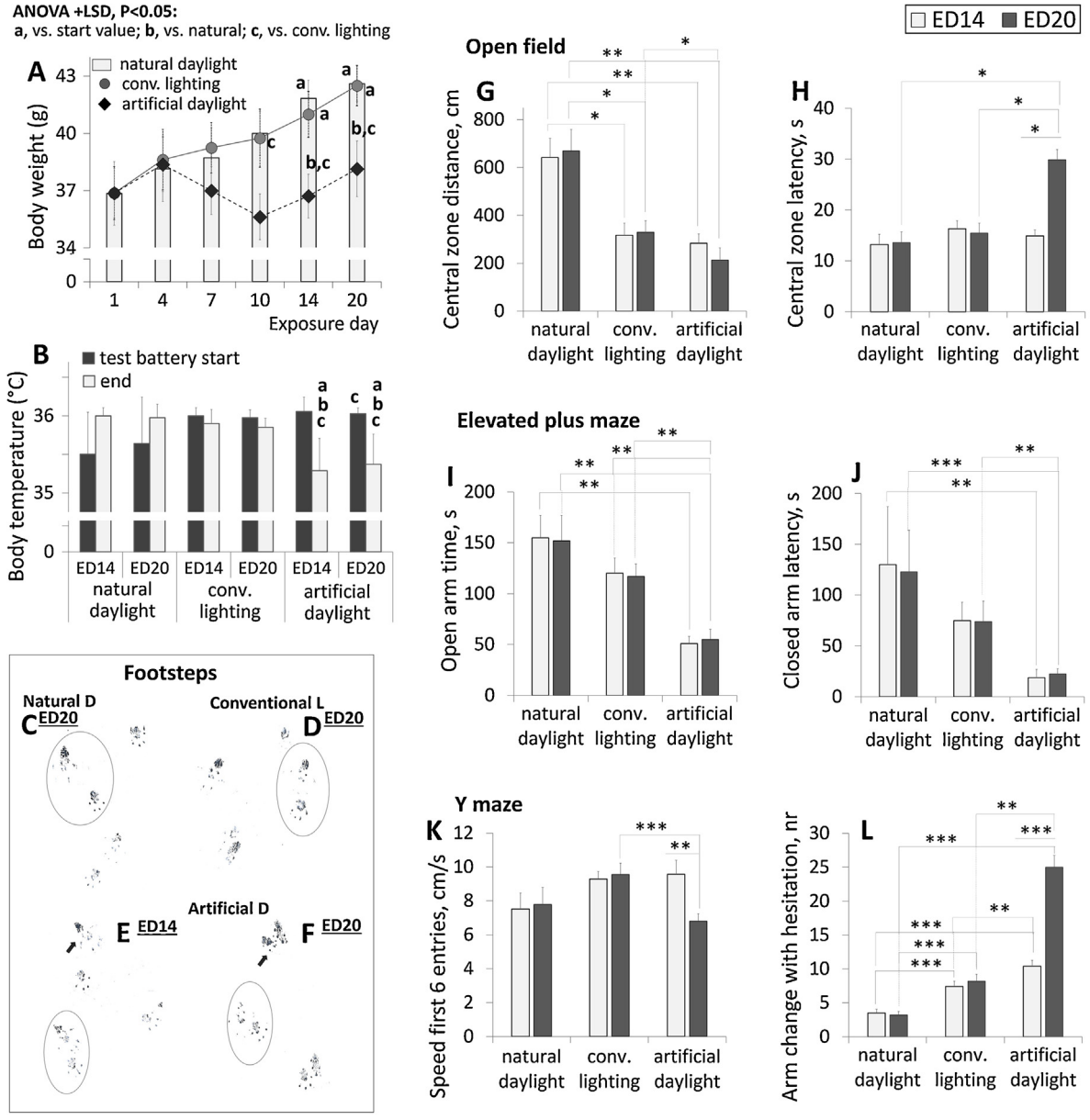

Fig. 1. Body weight and behavioral tests.
A. Body weight progression. Note the non-linear and lower increase in artificial daylight-exposed animals. B. Body temperature before and after test battery. Note the decrease in temperature at the end of tests in artificial daylight group on both exposure day (ED) 14 and 20. C–F. Footprints of representative cases of animals exposed to natural daylight (C) and conventional lighting (D) on ED 20, and animals exposed to artificial daylight on ED 14 (E) and 20 (F). Note the regular pattern of steps in natural daylight exposed (D), the slightly irregular steps in conventional lighting-exposed (E), and the decreased ground contact surface of hind paws (oval shapes) and incoordination of walking compared with both natural daylight and conventional lighting groups (arrows) in artificial daylight-exposed (F). G–L. ED 14 (light gray) and EG 20 (dark grey) behavioral tests' results. G, H. Distance traveled in the central zone of the open field arena (G) and central zone latency (H). Note the significant decrease in the distance traveled in the central zone and increase in the latency in artificial light-exposed group. I, J. Time spent in elevated plus maze open arms (I) and closed arm latency (J). Note the significant decreases compared to natural daylight-exposed group. K, L. Average speed at the first six entries in Y-maze arms (K) and number of arm changes made with hesitation (L). Note the decrease in speed and increase in arm changes with hesitation in artificial daylight-exposed group more marked on ED 20 than ED 14. Data are mean ± SEM. ANOVA + LSD test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

a restorative inflection point on ED10 (Fig. 1A). The average body weight of the group exposed to artificial daylight was significantly lower, compared to the other groups, from ED10 onward ($p = 0.034$) (Fig. 1A).

The effect of artificial daylight exposure on pre- and post-battery test's body (skin) temperature is shown in Fig. 1B. Body temperature before the battery of behavioral tests was slightly higher in conventional lighting- and artificial daylight–exposed groups compared to natural daylight–exposed. Body temperature at the end of the behavioral tests was significantly lower in artificial daylight group on both ED14 and ED20 compared with values before battery test ($p = 0.03$ on ED14, $p = 0.01$ on ED20) and with post-battery values of other groups (ED14: $p = 0.01$ *vs.* conventional lighting, $p = 0.033$ vs. natural daylight/ED20: $p = 0.02$ vs. conventional lighting, $p = 0.028$ *vs.* natural daylight) (Fig. 1B).

3.1.3. *Serum molecules reflecting hepatic function*

The effect of artificial daylight exposure on levels of serum proteins, lipids, and hepatic enzymes is shown in Table 1. Slight increases in total cholesterol and triglyceride levels were observed. Total protein, albumin, and globulin levels were higher in artificial daylight–exposed group, compared with natural daylight- and,

Table 1
Effect of light source/intensity on levels of serum proteins and molecules reflecting hepatic function.

	natural daylight	conv. lighting	artificial daylight	ANOVA: F; p values (F crit = 4.6)			Reported ranges[a]
				nat. D *vs.* Conv. L	nat. D *vs.* art. D	Conv. L *vs.* art. D	
Serum protein levels (g/L)							
Total protein	70.5 ± 1.2	74 ± 0.5↑	79.6 ± 0.5↑	8; 0.025*	52; 0.0001***	68;0.0001***	67 ± 9
Albumin	39.4 ± 1.9	41.3 ± 0.8	47.8 ± 1.4↑	0.9; 0.36	12.4; 0.008**	20; 0.002**	46.5 ± 7.8
% of total	55.9 ± 2.8	55.8 ± 2.8	60 ± 1.7↑	0.001; 1	1.65; 0.235	15.2; 0.005**	38 ± 9.8
Globulin	29.4 ± 1.3	24.5 ± 1.3	38.8 ± 1.5↑	2.4; 0.16	22; 0.0016**	20; 0.002**	29.5 ± 6.4
% of total	41.5 ± 6	33 ± 3.7	48.8 ± 1.7↑	4.5; 0.06	10; 0.012*	15; 0.005**	38 ± 9.8
A/G ratio	1.4 ± 0.26	1.8 ± 0.53	1.2 ± 0.06	0.14; 2.711	0.31; 1.13	0.047; 5.49*	1.7 ± 0.34
Hepatic enzymes and lipid levels (mg/dL)							
ALT (IU/L)	68.7 ± 39.2	71.5 ± 36.4	186.6 ± 63.1	0.91; 0.013	12.6; 0.008**	12.5;0.007**	60 ± 13.2
AST (IU/L)	29 ± 13.5	39.6 ± 7.6	67.4 ± 27	0.16; 2.315	8.11; 0.021*	0.05; 5.96	38 ± 21.9
T. cholesterol	125 ± 60	121 ± 60	118 ± 80	0.1; 0.81	0.001; 0.97	0.04; 0.85	110 ± 29
Triglycerides	231 ± 63	225 ± 63	248 ± 31	0.01; 0.9	0.06; 0.81	0.5; 0.51	150 ± 42

[a] Ranges reported by recent studies performed under normal laboratory lighting in mice [33,20,37,3]. T. cholesterol: total cholesterol. ANOVA +LSD test: *P < 0.05; **P < 0.01; ***P < 0.001. Data are mean ± SEM.

in a lesser extent, conventional lighting–exposed. The levels of these proteins were also higher than physiological values [32–34] (Table 1). Albumin/globulins ratio in artificial daylight group was significantly lower (respectively, slightly lower) compared to conventional lighting (respectively, natural daylight).

Serum levels of the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were higher in artificial daylight–exposed animals compared to natural daylight- and conventional lighting–exposed. Levels of AST and ALT in artificial light group were higher than physiological values reported in the literature [32–34] (Table 1).

3.2. Behavioral tests

3.2.1. Footprints' analysis

Representative cases of animals exposed to natural daylight, conventional lighting, and artificial daylight are shown in Fig. 1C–F. Artificial daylight–exposed animals displayed static gait alterations marked by a decreased size of contact (more obvious in hind paws) and an incoordination of walking (gait ataxia) on ED20 (Fig. 1F) compared with their walking performance on ED14 (Fig. 1E), and also compared with both natural daylight- (Fig. 1C) and conventional lighting–exposed (Fig. 1B). Notably, some animals exposed to conventional lighting presented slightly irregular step patterns compared with animals exposed to natural daylight.

3.2.2. Open field

Open field test results are shown in Fig. 1G, H and Table 2. The distance traveled in the open field arena was significantly decreased in artificial daylight–exposed group on both ED14 (p = 0.002) and ED20 (p = 0.0059) compared with natural daylight–exposed (Table 2). The distance traveled in the central zone of the arena was significantly lower in conventional lighting- and artificial daylight–exposed compared with natural daylight–exposed on both ED14 (p = 0.014 and p = 0.009, respectively) and ED20 (p = 0.03 and p = 0.009, respectively). The distance traveled in the central zone by artificial daylight–exposed was also significantly lower than conventional lighting–exposed (p = 0.048) (Fig. 1G). Time to the first entry in the central zone (central zone latency) was significantly higher in artificial daylight–exposed group on ED20 compared with ED14 (p = 0.042), but compared also to the other groups (p = 0.014 *vs.* natural daylight– and p = 0.03 *vs* conventional lighting–exposed) (Fig. 1H). The time spent in corners was significantly higher in artificial daylight–exposed compared with the other groups on ED14 (p = 0.0013 vs. natural daylight, p = 0.0034 *vs* conventional lighting) and ED20 (p = 0.005 *vs.* natural daylight, p = 0.039 *vs* conventional lighting) (Table 2). Central zone entries and relative time, rearing (on hind legs and against wall), and grooming latency were significantly decreased in artificial daylight group, while freezing and sniffing episodes were increased (Table 2).

3.2.3. Elevated plus maze

The results of EPM test are shown in Fig. 1I, J and Table 2. The time spent in maze open arms was significantly decreased in artificial daylight–exposed group compared with conventional lighting–exposed (p = 0.001 on ED14 and p = 0.0012 on ED20) and natural daylight–exposed (p = 0.0051 on ED14 and p = 0.0029 on ED20) (Fig. 1I). Closed arm latency was significantly decreased in artificial daylight–exposed group compared with conventional lighting– (p = 0.052 on ED14 and p = 0.037 on ED20) and natural daylight–exposed (p = 0.0051 on ED14 and p = 0.0023 on ED20). Closed arm latency of conventional lighting–exposed was also shorter compared with natural daylight–exposed (p = 0.037 on ED20) (Fig. 1J).

Rearing against wall frequency was significantly increased in conventional lighting- (p = 0.018) and in artificial daylight–exposed (p = 0.002) on ED14 compared with natural daylight. Instead, on ED20 a decrease in this parameter was observed in artificial daylight–exposed compared with ED14 (p = 0.0001), but also conventional lighting– (p = 0.025) and natural daylight–exposed (p = 0.007) (Table 2). As also shown in Table 2, open arm entries, central platform time, and head dips over the open arms, and freezing episode frequency were significantly decreased in artificial daylight–exposed group compared to natural daylight, and in a lesser extent, conventional lighting. On the other hand, fecal boli and episodes of rearing, undirected sniffing, and grooming attempts (grooming episodes shorter than 3 s) were increased (Table 2).

3.2.4. Y-maze

Y-maze test results are shown in Fig. 1K, L and Table 2. The average speed in the first six entries was decreased in artificial daylight–exposed group on ED20 compared with ED14 (p = 0.008) and to conventional lighting–exposed (p = 0.001), but not natural daylight–exposed (Fig. 1K). A similar scenario was observed with the speed difference between the first six and the last six entries (Table 2). The frequency of episodes of arm change with hesitation (sniffing of an arm entrance followed by the choice of another arm) was significantly higher in artificial daylight–exposed group on ED20 compared with ED14 (p = 0.003) and to other groups (p = 0.0003 *vs.* conventional lighting–exposed, p = 0.0001 *vs.* natural daylight–exposed) (Fig. 1L). Arm change with hesitation episodes was more common in conventional lighting–exposed than in natural daylight–exposed (p = 0.01) (Fig. 1L).

Table 2
Effect of light exposure on animal performance in behavioral tests.

	Natural daylight		Conventional lighting		Artificial daylight	
	ED14	ED20	ED14	ED20	ED14	ED20
Open field						
Total distance, cm	2930 ± 250	2561 ± 200	2072 ± 197 $^{b\downarrow}$	2159 ± 257	1801 ± 190 $^{b\downarrow}$	1607 ± 217 $^{b\downarrow}$
Central zone time, % total	13.4 ± 2.1	12.7 ± 2.2	18 ± 3	18.5 ± 3.4	11.2 ± 2.1$^{c\downarrow}$	5.9 ± 1.4$^{a,b,c\downarrow}$
Central zone entries, nr	22 ± 1.8	21.8 ± 2	15.1 ± 2.6$^{b\downarrow}$	16.1 ± 2.3b$^{\downarrow}$	13.7 ± 1.7$^{b\downarrow}$	11.9 ± 2.9$^{b\downarrow}$
Time spent in corners, s	119.1 ± 26	111.6 ± 28	156.8 ± 22	174.3 ± 29	257.3 ± 26$^{a,b\uparrow}$	246.9 ± 31 $^{a,b\uparrow}$
Rearing on hind legs, nr	26.2 ± 5.7	25.6 ± 6.3	32.6 ± 6.3	35.7 ± 6.8	38.1 ± 13.8	18.4 ± 5.5$^{c\downarrow}$
Rearing against wall, nr	41.1 ± 3	39.8 ± 2.5	51 ± 5.5	55.3 ± 4.9$^{b\uparrow}$	51.3 ± 8.3	40.7 ± 6$^{c\downarrow}$
Undirected sniffing, nr	24 ± 3	21.4 ± 1.6	34.9 ± 4.4$^{b\uparrow}$	38.4 ± 5.3$^{b\uparrow}$	51.3 ± 8.7$^{b\uparrow}$	62.6 ± 3.3$^{b,c\uparrow}$
Wall sniffing, nr	34.1 ± 3.1	34.6 ± 2.9	48.1 ± 4$^{b\uparrow}$	52.7 ± 4$^{b\uparrow}$	47.4 ± 6.4$^{b\uparrow}$	77.3 ± 5.9$^{a,b,c\uparrow}$
Floor sniffing, nr	13.1 ± 1.3	12.2 ± 1.1	20.8 ± 2.3$^{b\uparrow}$	23.1 ± 3$^{b\uparrow}$	41.9 ± 3.1$^{b,c\uparrow}$	67.3 ± 4.5$^{a,b,c\uparrow}$
Grooming, nr	5.1 ± 1.4	5.8 ± 1.4	7.3 ± 1.5	7.3 ± 1.6	6.6 ± 1.5	5.4 ± 1.2
Grooming latency, s	191.8 ± 44	193 ± 38.5	85.8 ± 23$^{b\downarrow}$	92 ± 23.4$^{b\downarrow}$	84.1 ± 4.3$^{b\downarrow}$	99.9 ± 15$^{b\downarrow}$
Freezing episodes, nr	1.5 ± 1	1.4 ± 0.7	1.6 ± 0.5	1.3 ± 0.5	4.1 ± 1.5	4.1 ± 0.9$^{b,c\uparrow}$
Elevated plus maze						
Distance, cm	411 ± 60	420 ± 65	544 ± 61	527 ± 58	469 ± 45	390 ± 55
Total arm entries, number	13.1 ± 2.8	13.4 ± 3.1	15.3 ± 2.7	15.1 ± 1.7	13.1 ± 1.9	7.9 ± 2.0$^{a,c\downarrow}$
Open arm entries, % total	51.6 ± 8.9	50.6 ± 9.9	51.8 ± 5.4	50.9 ± 5.6	17 ± 2.7$^{b,c\downarrow}$	19.5 ± 4.9$^{b,c\downarrow}$
Central platform time, s	21.9 ± 2.4	22.2 ± 2.6	32.3 ± 5.3	25.9 ± 5.1	31.1 ± 5.2$^{c\downarrow}$	18.4 ± 4.3$^{c\downarrow}$
Head dips over open arm, nr	18.1 ± 2.5	18.6 ± 2.7	37.3 ± 5$^{b\uparrow}$	34.3 ± 6.4$^{b\uparrow}$	15.3 ± 1.9$^{c\downarrow}$	13.9 ± 2.7$^{c\downarrow}$
Rearing against wall, nr	5.7 ± 2.0	6.2 ± 2.0	13.5 ± 2.1$^{b\uparrow}$	14.4 ± 1.9$^{b\uparrow}$	17.4 ± 2.0 $^{b\uparrow}$	7.1 ± 1.2$^{a,c\downarrow}$
Rearing, nr	3.5 ± 1.7	4 ± 1.8	14.4 ± 4$^{b\uparrow}$	14.1 ± 3.1$^{b\uparrow}$	14.4 ± 3.4$^{b\uparrow}$	11.3 ± 2.6$^{a,c\downarrow b\uparrow}$
Sniffing, nr	18.2 ± 2.2	18.2 ± 2.5	40.9 ± 3.8$^{b\uparrow}$	44.6 ± 4.6$^{b\uparrow}$	63.4 ± 8.1$^{b,c\uparrow}$	62.3 ± 5.7$^{b,c\uparrow}$
Grooming attempts, nr	5.6 ± 1.0	5.8 ± 1.1	4.6 ± 1.1	5.8 ± 0.9	3.7 ± 0.6	6.6 ± 0.8$^{a\uparrow}$
Freezing, nr	2.7 ± 1.1	2.8 ± 1.1	2.1 ± 0.5	1.8 ± 0.7	3.6 ± 1.0	4.3 ± 0.6$^{c\uparrow}$
Fecal boli, nr	1.2 ± 0.3	0.8 ± 0.3	1.3 ± 0.5	1.9 ± 0.6	1.9 ± 0.9	2.3 ± 0.7$^{b\uparrow}$
Y maze						
Distance, cm	938 ± 82	818 ± 98	1330 ± 168$^{b\uparrow}$	1320 ± 188$^{b\uparrow}$	1349 ± 137$^{b\uparrow}$	1181 ± 93$^{b\uparrow}$
Alternation, nr	19.2 ± 1.4	18.8 ± 1.6	26.6 ± 2.7$^{b\uparrow}$	27.8 ± 3.7$^{b\uparrow}$	24.3 ± 3.1	18.6 ± 1.8$^{c\downarrow}$
X-Y-Z alternations, nr	10.5 ± 0.8	10 ± 0.6	15.4 ± 1.7$^{b\uparrow}$	17.2 ± 2.4$^{b\uparrow}$	15.0 ± 2.5	10.9 ± 1.4$^{c\downarrow}$
Speed first entries – last, cm/s	3.6 ± 1.1	3.5 ± 1.2	6.1 ± 0.4	6.0 ± 0.7	6.6 ± 0.9	4.0 ± 0.5$^{a,c\downarrow}$

ED14, ED20: exposure days 14 and 20. **a,b,c:** ANOVA +LSD test, *vs.* respective exposure day 14 (**a**), *vs.* natural daylight (**b**), and vs. conventional lighting (**c**). Data are mean ± SEM.

The distance traveled in the Y-maze was significantly shorter in natural daylight–exposed than in other groups on both ED14 (p = 0.012 *vs.* conventional lighting, p = 0.001 vs. artificial daylight) and ED20 (p = 0.01 vs. conventional lighting, p = 0.004 *vs.* artificial daylight) (Table 2). On the same hand, the frequency of consecutive visits of three different arms was higher in conventional lighting–exposed group on ED14 (p = 0.008) and ED20 (p = 0.005) compared with natural –exposed, but on ED20 artificial daylight–exposed displayed a lower frequency compared with conventional lighting–exposed (p = 0.03). Similar, arm alternation frequency was also lower in natural daylight–exposed, except on ED20 where a drop was observed in artificial daylight–exposed (p = 0.02 *vs.* conventional lighting).

3.3. Brain histopathological studies

3.3.1. Neuronal morphology and counts

The analysis of Nissl-stained neurons in the hippocampal formation revealed marked neuronal loss in artificial daylight–exposed animals. This loss was particularly marked in the dentate gyrus. Fig. 2A–C shows dentate gyrus granule cells of representative cases of animals exposed to natural daylight, conventional lighting, and artificial daylight. While dentate gyrus granule cells had normal shapes in natural daylight- (Fig. 2A) and conventional lighting–exposed (Fig. 2B) animals, these neurons were condensed in artificial daylight–exposed (Fig. 2C).

Results of neuronal counts in CA1, CA2, and CA3, and in the dentate gyrus are shown in Fig. 2D, E. Absolute counts of pyramidal neurons in CA1, CA2, and CA3 revealed significant decreases in artificial daylight–exposed (P < 0.001), and in a lesser extent, in conventional lighting–exposed animals compared with natural daylight–exposed (Fig. 2D). Similar results were obtained in counts of dentate gyrus granule cells (Fig. 2D). Relative values of neuronal counts (percent of natural daylight counts) suggested that the dentate gyrus was more affected than other structures of the hippocampal formation in artificial daylight–exposed compared to conventional lighting–exposed (Fig. 2E). Relative counts also revealed a decrease (∼15%) in pyramidal neurons in conventional lighting–exposed animals (but not in granule cells) compared with natural daylight–exposed (Fig. 2E).

3.3.2. Expressions of inflammation, apoptosis, and brain resident cell markers

The expressions of markers labeled in the entorhinal cortex of representative cases of animals exposed to natural daylight, conventional lighting, and artificial daylight are shown in Fig. 3. The markers of activated microglia (iba-1) (Fig. 3A–C), astrocytes (GFAP) (Fig. 3D–F), and neurons (NeuN) (Fig. 3G–I) were overexpressed in most artificial daylight–exposed animals, compared to the other groups. In addition, the first displayed enlarged astrocytes, microglia in activated macrophage shape, and dead (or dying) neurons (Fig. 3A–I).

Also unlike the other groups, animals of the artificial daylight–exposed group displayed increased expressions of: (*i*) the pro-inflammatory cytokine TNF-α (Fig. 3J–L); (*ii*) the receptor mediating cell-cell interaction-induced cell death associated with marked inflammation FAS (Fig. 3M–O) and its ligand (Fig. 3P–R); and (*iii*) the marker of apoptosis caspase 3 (Fig. 3S–U).

4. Discussion

The results of the present study suggest systemic and central nervous system functional alterations in mice exposed exclusively to artificial daylight during daytime for 20 days. Notably, significant decreases in the frequency of rearing against wall, *i.e.* in attempts to escape, in the open field and the elevated plus maze

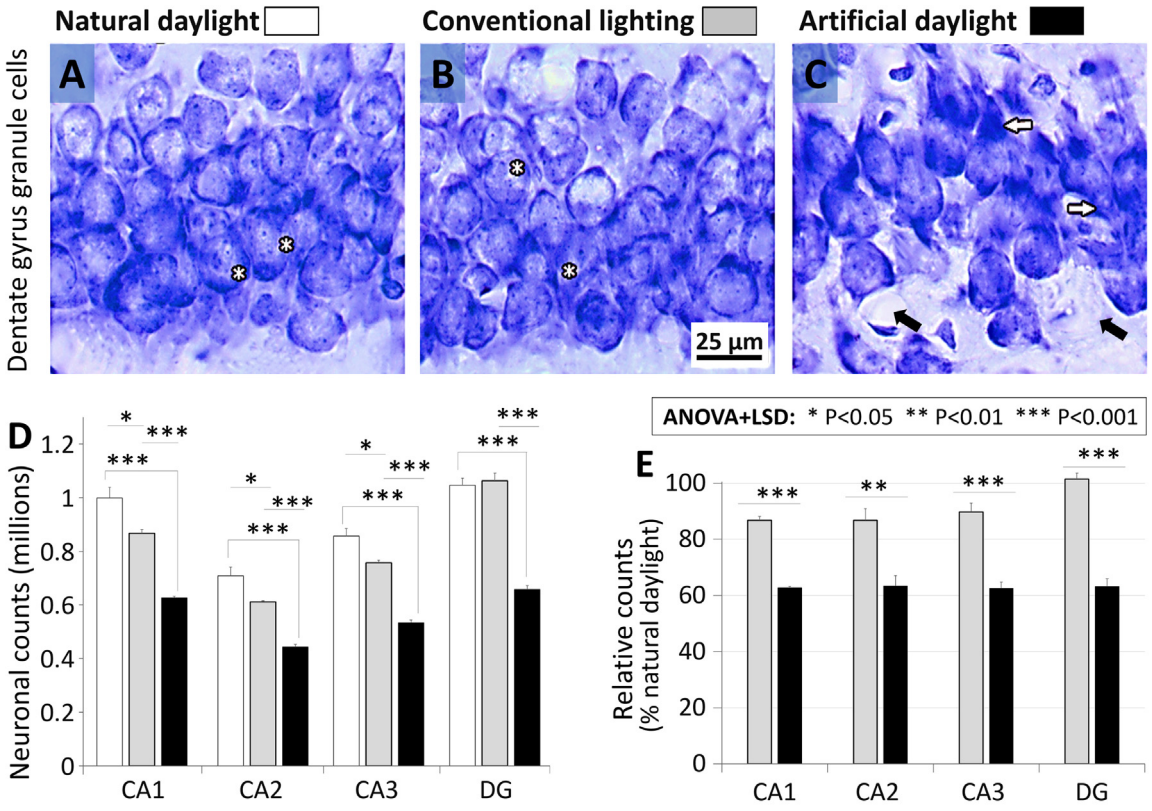

Fig. 2. Hippocampal neuron observation and counts.
A–C. Micrographs of Nissl-stained dentate gyrus neurons of representative cases of animals exposed to natural daylight (A), conventional lighting (B), and artificial daylight (C). Note the dead (black arrows) and the condensed cells (white arrows) in arficial daylight-exposed (C) and the normal shapes of granular cells in the other groups (asterisks) (A, B). D, E. Hippocampal neuron counts. Absolute (D) and relative (E) cell counts in the CA1–CA3 (pyramidal cells) and dentate gyrus (DG, granular cells) of animals exposed to natural daylight, conventional lighting, or artificial daylight during daytime for 20 days. Note the marked decreases in neuronal population in artificial daylight-exposed group. Data are mean ± SEM.

(EPM), as well as drastic decreases in the speed at first entries in Y-maze arms were observed in artificial daylight group on ED20, unlike ED14 and unlike natural daylight and conventional lighting groups. These observations indicate that animals exposed to artificial daylight developed coping deficit for the aversive situations presented by the ethological tests [35,36]. Other indicators of depression were observed in artificial daylight–exposed animals in the third week, including: shaggy and dirty fur, decreased grooming, increased aggressiveness, regular freezing episodes, decreased exploratory activity, and body weight loss.

The performance of artificial daylight–exposed animals in the behavioral tests performed on ED20 also suggested other signs of cognitive and motor impairment. Anxiety was suggested by increases in sniffing episodes and by post-testing hypothermia, a hallmark of mild chronic stress exposure [37,38]. In addition, increased latency to first entry in the anxiogenic areas of the EPM (open arms) and of the open field arena (central zone) were observed, together with decreases in the distance traveled in these areas. Moreover, thigmotaxis, a stable and robust indicator of discomfort manifested by the tendency to remain close to the walls (secure area) [39,40] was markedly increased in artificial daylight–exposed, as indicated by increases in the number of activities close to walls in all the behavioral tests performed. Besides, a shift in behavioral baseline was indicated by increases in EPM central platform time [41]. Prefrontal cortex dysfunction was indicated by decreased spontaneous alternations in the Y-maze [42,43]. Spatial memory impairment was indicated by increases in episodes of arm change with hesitation and decreases in consecutive explorations of three different arms in the Y maze [42,44,45]. Furthermore, the incoordination of walking and decreased ground contact surface of hind paws observed in artificial daylight-exposed, compared with the other groups, indicated a motor dysfunction with central nervous system involvement.

We investigated the affection of a major sensorimotor gating structure, the entorhinal cortex, as a potential mechanism for the cognitive deficits induced by the exposure to artificial daylight. Immunohistochemical labeling of resident cells in the entorhinal cortex of artificial daylight–exposed animals revealed the presence of enlarged astrocytes and brain macrophages, suggesting that astrocytes and microglia were activated. On the same hand, the pro-inflammatory cytokine TNF-α was overexpressed, and many resident cells were positive for the inflammation-induced death receptor Fas and its ligand. Neuronal marker NeuN was overexpressed, indicating that neurons were injured, and many neurons were positive for the marker of apoptosis caspase 3. These observations suggested the presence of a marked inflammation in the entorhinal cortex associated with neuronal loss. Using stereological techniques, we estimated the number of Nissl-stained neurons in the hippocampal formation, a major functional target of the entorhinal cortex. Losses in dentate gyrus granule cells and as well as CA1–CA3 pyramidal neurons of hippocampus were observed, and cell numbers were significantly decreased. These findings suggest that neuronal loss in the neocortex-entorhinal cortex-hippocampus axis resulted at least partly from detrimental neuroinflammation, and were among the drivers of brain functional alterations observed in this study following replacement of natural daylight with artificial daylight.

Furthermore, liver function test, performed to assess general health status, suggested an affection of the hepatic function in artificial daylight–exposed animals. Marked increases in serum levels

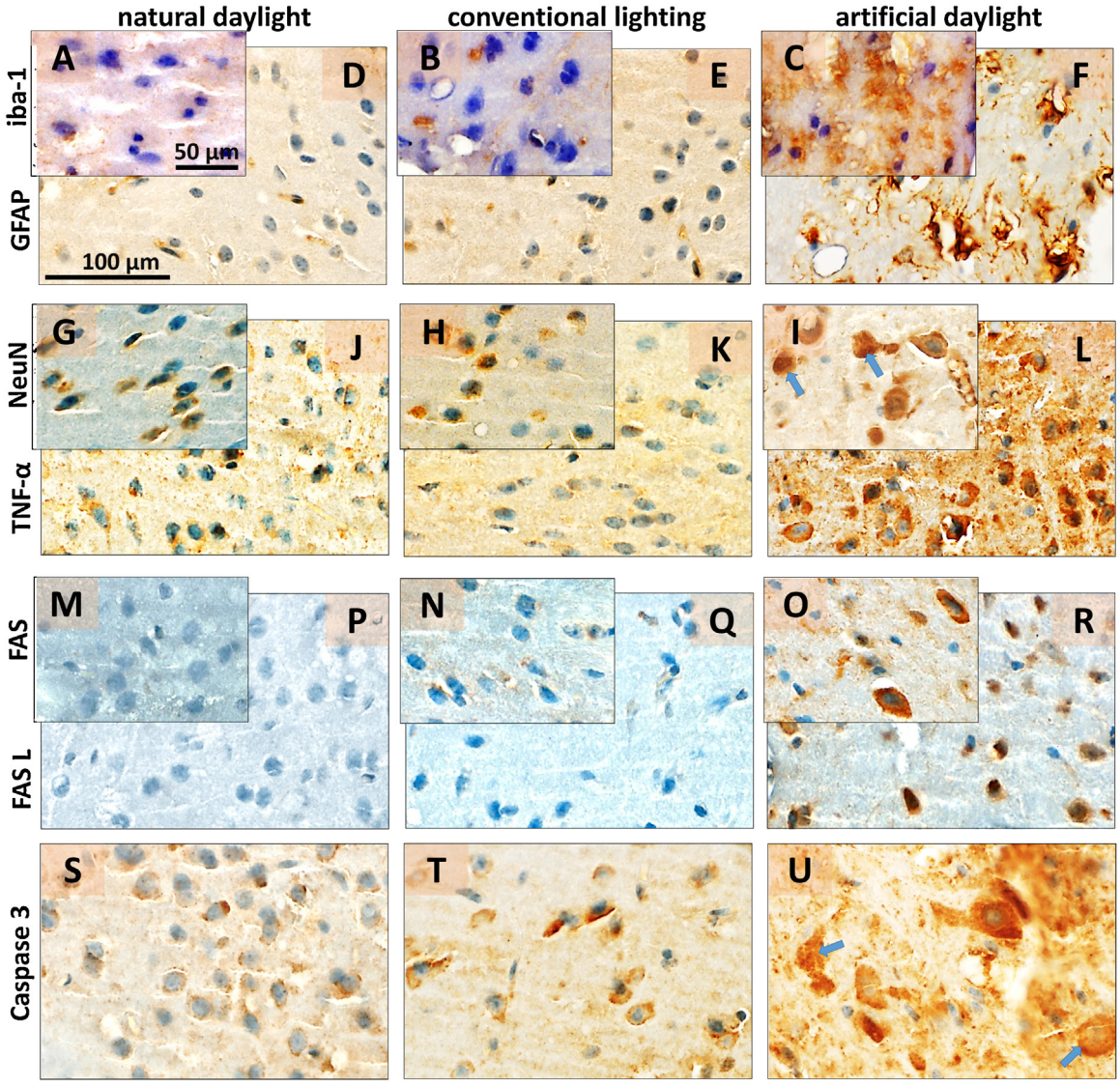

Fig. 3. Entorhinal cortex immunolabeling.
Representative cases of natural daylight, conventional lighting, and artificial daylight-exposed animals. A–I. Brain resident cells: microglia (iba-1) (A–C), astrocytes (GFAP) (D–F), and neurons (NeuN) (G–I). J–U. Markers of inflammation and apoptosis: TNF-α (J–L), Fas (M–O), Fas ligand (P–R), and caspase 3 (S–U). Note the dying neurons in the artificial daylight-exposed (arrows in I, U), the increased expressions of TNF-α (L), Fas (O), Fas ligand (R), and caspase 3 (U), as well as the marked activation of microglia (C) and astrocytes (F).

of the enzymes AST and ALT were observed, as well as decreased albumin/globulins ratio, and abnormally high levels of total proteins, albumin, and globulins. Considering that these alterations are well-established indicators of high-grade hepatotoxicity [46–48], it appears that artificial daylight would have resulted in animal death if the exposure had continued. This finding suggests that liver-to-brain signaling may also have accounted among the causative or aggravating factors of the cognitive deficits and mood alterations observed in the present study [49–51].

Altogether, our results suggest that long hour exposure to artificial daylight may have tremendous negative effects on mouse brain. Interestingly, an early study in humans reported mild cognitive impairment following daytime exposure to bright artificial light [52]. Thus, artificial daylight exposure for long hours may be detrimental for mental health in humans as well. Alarmingly, mouse exposure to conventional lighting in our study induced a mild cognitive deficit and motor impairment. Although this observation should be verified in humans, it raises caution over keeping children indoor under conventional lighting during the daytime. For instance, teaching institutions should probably avoid using conventional lighting when natural daylight is available.

5. Conclusions

The present study addressed the consequences of replacing natural daylight by either artificial daylight or conventional lighting for general health and cognition in mice. Mice presented with signs of hepatotoxicity, as well as increasing signs of neurotoxicity, anxiety, depression, cognitive alterations, and motor impairment that became severe in the third week of exposure. The involvement of the neocortex-entorhinal cortex-hippocampus axis was suggested by immunohistochemical studies that revealed neuroinflammation and neuronal loss in the entorhinal cortex, but also by massive neuronal loss in the hippocampal formation confirmed by stereological cell estimation. Mild cognitive and motor impairments were also observed in animals exposed to conventional lighting. These findings suggest that the replacement of natural daylight by artificial daylight, and in a lesser extent conventional lighting, had detri-

mental effects on the brain function and general health in mice. Our findings raise concern over the extended use of artificial light during the daytime, and call for studies assessing the intensity and exposure time safe for humans, particularly for children, the most sensible group in the human population.

Conflicts of interest statement

Authors declare no conflict of interest.

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