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Artificial blue light exposure induces anxiety-like behaviour, alters recognition memory and modifies hippocampal morphology in adolescent rats

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Abstract

Background: The constant artificial blue light exposure from electronic gadgets at night raises concerns about its impact on mood and brain functions. This study is designed to evaluate the effect of chronic exposure to artificial blue light from a light-emitting diode (LED) on emotionality, locomotion, novel object recognition memory and hippocampal cytoarchitecture in adolescent rats. **Materials and methods**: Male albino Wistar rats were exposed to artificial blue light (450 – 495 nm, and 100 Lux) for 14 days from a LED light source. Later, their emotionality, general locomotor behaviours and novel object recognition memory were tested by a computerised behavioural assessment system. After the behavioural assessments animals were euthanized to study the hippocampal cytoarchitecture by Nissl and Golgi-Cox staining. **Results**: In the open field test, latency to enter the centre zone was significantly increased in blue light exposed animals compared to controls. Total entries to the centre zone and percentage of time spent in the centre zone were slightly reduced in blue light exposed animals. The mean speed of animals in the centre, total zone transitions, and total distance travelled were not different between the two groups. In the elevated plus maze test, entries to the open arm were significantly reduced in blue light exposed animals. Time spent and distance travelled on the open arm were slightly reduced in blue light exposed animals. Entries to the closed arm and time spent in the closed arm were slightly increased but, the distance travelled in the closed arm was significantly reduced in the blue light exposed group. The mean speed of blue light exposed animals on the open arm, closed arm and centre were comparable in the two groups. Rearing duration and total zone transitions were slightly reduced but the total distance travelled was significantly reduced in the light exposed group. Novel object recognition was altered in the light exposed group as indicated by their negative discrimination index values. Hippocampal cornu ammonis-1 (CA1), and cornu ammonis-3 (CA3) regions demonstrated pyknotic cells and CA1 apical dendritic spine density was reduced in blue light exposed rats. **Conclusion**: Artificial blue light exposure induced anxiety-like behaviours, and significantly altered novel object recognition memory, but only mildly affected the general locomotor behaviours in adolescent rats. Moreover, it induced hippocampal cellular pyknosis and reduced CA1 apical dendritic spine density in blue light exposed adolescent rats.

Keywords Artificial blue light · Mobile phone · Anxiety · Novel object recognition memory · Hippocampus

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Introduction

We are amidst a modern digital age, where electronic devices and screens flood our existence. These devices have become the focal engagement point for a predominant demographic—teenagers and young adults. Most of these electronic gadget screens are made using LEDs which have a peak emission in the blue light range of 400–490 nm. The younger population's constant interaction with mobiles and computers has raised concerns for years about the negative effects of these devices on their development and behaviour.

Blue light can be found everywhere – in the natural light emitted by the sun, or in the artificial light emitted by LED lamps or screens (smartphones, tablets, computers, televisions). Blue light regulates our biorhythm or biological clock. The body uses the natural blue light from the sun to distinguish between day and night and to regulate our sleep–wake cycle. In the electromagnetic spectrum, blue light lies in the wavelength range between 380 and 500 nm. It therefore falls within the visible part of the spectrum; to which we are exposed every day. The perception of blue light stimulates and controls the production of the sleep hormone melatonin [3]. The body uses natural blue light from the sun to distinguish between day and night and to regulate our sleep–wake cycle. Although these facts are known, the effect of artificial blue light emitting from smartphones, tablets, computers, and televisions (490 nm with 100 Lux) and prolonged exposure to this during the night phase have not been investigated in detail. Its effects on mood and other brain functions and also on brain morphology have not been comprehensively studied. It has been reported that exposure to artificial light at night (ALAN) may cause negative health effects, such as cancer [22, 23], obesity circadian phase disruption, and sleep disorders [7].

Physiologically, mammals work in a sleep–wake cycle that is coordinated by the circadian rhythm. Circadian clocks are molecular clocks located in cells all over the body controlled mainly by the Suprachiasmatic Nucleus (SCN) of the hypothalamus. The internal clock functions autonomously, working independently even without external triggers or stimuli, yet it can synchronize with light–dark cycles through a mechanism known as photoentrainment. A change in the timing of the light–dark cycle, for example, light exposure at night time will result in a phase shift of circadian rhythms, and the connection between circadian rhythm disruption and mood alteration in humans is well established. Melatonin, a hormone that helps in sleep is secreted when light is absent. On average, the maximum levels of plasma melatonin in adults occur between 02:00 and 04:00 hours, which is important for optimising sleep timing with respect to the circadian clock. Blue light exposure during this time could potentially alter the release of this hormone, increase alertness, and reset the body's internal clock to a later time. Due to this, sleep disorders occur more frequently and people have a tougher time maintaining (rapid eye movement) REM sleep cycles and wake up feeling fatigued, tired, and moody [2, 43]. Hypertension, cardiovascular disease, and some types of cancers are associated with the inappropriate release of melatonin [37].

Blue light with a high-intensity wavelength (> 400 nm) has been shown to have deleterious effects on the eyes. Longer exposure time leads to worsening visual fatigue and near-sightedness. Symptoms such as diplopia, lack of focus, and poor concentration can affect productivity and efficiency [44, 45]. Some studies have suggested that there is a possible association between ALAN exposure and behavioural and physiological changes, (especially in mood and mental health), and the night time light exposure can lead to depressive behaviours in both nocturnal and diurnal animals [17, 41]. Other studies suggest that ALAN exposure during early development increases anxiety-like responses in adulthood in mice [4, 9].

There have been studies done on organisms such as *Drosophila* where researchers found that maintaining the flies in a 12 h cycle of blue LED and 12 h of darkness significantly reduced longevity compared with flies maintained in constant darkness or white light with blue wavelengths blocked. Exposure of adult flies to 12 h of blue light per day accelerated ageing phenotypes causing damage to retinal cells, brain neurodegeneration, and impaired locomotion. Blue light induces the expression of stress-responsive genes in old flies but not in young ones, suggesting that cumulative light exposure acts as a stressor during ageing [30, 39]. In our tech-driven world where screens are pervasive, understanding the impact of significant blue light exposure on our mental well-being and cognition is crucial. Therefore, exploring in depth the effects of blue light on mental health and mood behaviours is essential in navigating our modern, digitally immersive lifestyles. The current study evaluated the effect of chronic exposure to artificial blue light on emotionality, locomotion, novel object recognition memory and hippocampal cytoarchitecture in adolescent rats.

Materials and methods

Animals:

Two-month-old male albino Wistar rats were used (150–200 g in weight) for the study. They were housed in polypropylene cages—41 cm × 28cm × 14 cm (3 rats per cage). They were kept in a day-night cycle (12:12 h) in an air-conditioned room with food (*ad libitum*) and water supply. The Institutional Research and Ethics Committee approved the procedures used in the study (RAKMHSU-REC-093-2019-UG-M). All animals were handled with utmost care and in a humane manner throughout the experiment and all measures were taken to use the least number of animals to generate adequate data.

Experimental groups & design

In this experimental study, 20 rats were used, and they were randomly divided into two groups.
Group 1 (Control, n = 10): They remained in the home cage during the entire experimental period.
Group 2 (Blue LED Light exposed, n = 10): They were exposed to artificial blue LED light (450 – 495 nm and 100 Lux) for 12 h daily in the night phase of a 12-h light and 12-h dark cycle for 14 days from an LED source.
The control group was kept in their home cages for the entirety of the study (2 weeks) and the experimental group was put in transparent cages for the duration of blue light exposure only. Both animal groups were exposed to the experimenter's presence and handling every day for 2 weeks so that their behaviour during testing would not be affected by anxiety due to the presence of strangers.
On the 15th day, the animal's emotionality, locomotor behaviour, and novel object recognition memory were evaluated using open field, elevated plus maze, and novel object recognition tests. All behavioural assessment was done using a computerized video tracking system (SMART- Panlab, Version 3.0-Super Pack, Barcelona, Spain).

Blue light Exposure

The LED blue light source- RGB LED1000W colouring strobe (China) was used as the blue light source for the light exposure (450 – 495 nm). The blue light source was directed toward the animals from above (~ 50 cm) as depicted in Fig. 1. During blue light exposure, the stroboscopic mode was turned off and the light intensity was set at 100 Lux (similar to the light intensity emitted from a mobile phone during the night in a dark room). This was determined by a handheld flux meter (G9408-LOG MINI LIGHT METER, Gazelle). Animals were exposed to blue light between 8:00 PM to 8:00 AM (12 h/day) for 14 days. The position of the light source and cages remained the same during the entire duration of the experiment. During blue light exposure, the test group animals were kept in transparent cages

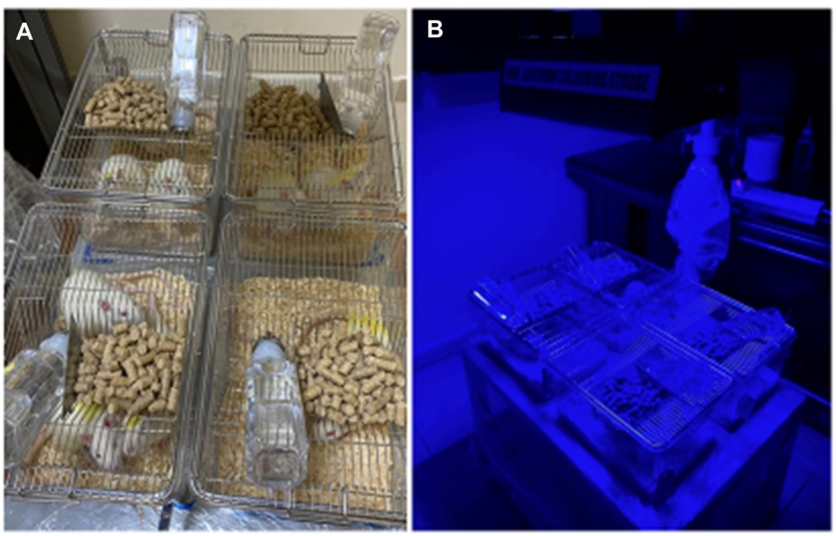

Fig. 1 Artificial blue LED light exposure set-up. Animals were under normal light exposure (A) and blue LED light exposure (B). The intensity of the blue LED source was set at 100 Lux

(36 cm × 23 cm × 16 cm) and placed in a room where there was no other light source present except the blue LED. The control animals were also placed in similar-sized cages and kept near their home cages for 12 h/day in the animal holding room of the animal house facility. Both group animals were returned to their home cages after 12 h and they were put back to the animal holding room until the next exposure session. This experimental pattern was followed every day for 14 days. Every day, the same experimenters handled animals, fed them, and this was continued until the entire duration of the experiment.

Behavioural Assessment

Open field test

A black square box made of material that is non-absorbent for odours (Square arena, LE800SC, Panlab, Barcelona, Spain) measuring 90 (W) × 90 (D) × 40 (H) cm was used for this purpose. It was placed on the floor to imitate an open field (OF) and was used to observe and analyze the behaviour of the rats. The Box was divided into 9 imaginary zones to make it easier to count the zone transitions as well as the amount of time spent in each zone. The duration of this test was 5 min for each animal where it was allowed to explore freely in the open field without any distractions. A video camera connected to a PC installed with SMART software (Version 3.0-Super Pack) recorded animals' behaviour. The general locomotor parameters such as distance travelled, zone transitions and mean speed were recorded. Anxiety-related parameters such as entrance latency to the centre zone, entries to the centre zone, and time spent in the centre zone were recorded and analysed.

Elevated plus maze test

An elevated plus-shaped maze (LE840, Panlab, Barcelona, Spain) in which two of its opposite arms were closed (closed by black walls) and the other two opposite arms were open with a transparent railing—to prevent the rats from falling. The elevated plus maze (EPM) measured, 100 (W) × 100 (D) × 100 (H) cm. It has two arms measuring 45 cm and a center region measuring 10 cm on all four sides which connects to four arms of the apparatus. The wall height of the apparatus was 35 cm and the entire apparatus was elevated 65 cm from the floor. The duration of this test was 5 min for each rat where the animal was allowed to explore freely between arms without a distraction. The animal was first placed into the centre of the maze that connects the four arms and the test was started. The rat's activity was observed and recorded via a video camera connected to a PC installed with SMART software (Version 3.0-Super Pack). Both anxiety-related and general locomotor parameters were recorded and analysed.

Object recognition memory test

A black square arena (LE800SC, Panlab, Barcelona, Spain) measuring 90 (W) × 90 (D) × 40 (H) cm was used for this purpose. It was divided into four quarters measuring 45 (W) × 45 (D) × 40 (H) cm by placing two dividers. One of the quarters was used as an experiment square arena for the novel object recognition test (NORT). This test procedure consisted of three phases: habituation, familiarization, and test phase. Each phase lasted for 3-min duration. During habituation, each rat was placed into the square arena facing the wall. Each rat was allowed to freely explore the arena and at the end of 3 min, they were returned to their home cages. The arena was cleaned using 70% ethanol after each trial to minimize the olfactory cues. Twenty-four hours after the habituation phase the familiar object test was conducted which also lasted 3 min. For doing this test, two similar objects were placed in each top corner of the square arena. In the SMART software, these areas were marked as zones 1 and 2 (familiar objects 1 and 2). Each of these rectangle zones measured 12 cm in length and 8 cm in width. In the SMART software animal head was selected as the animal track point and the zone transition criterion was animal head moves from one zone to another during the investigation of objects by animals. Once, the apparatus and the software were ready each animal was placed into the square arena facing the bottom wall. Time spent investigating each object was recorded and analyzed. Twenty-four hours after the familiarization test, the novel object recognition test (NORT), phase was conducted. The familiar object from the right top corner was removed and a novel object was placed instead. Like the familiarization phase, each animal was placed into the arena facing the wall and allowed to explore and investigate both objects for 3 min.

SMART software set-up remained the same during this session too. Time spent investigating familiar and novel object was recorded and analyzed. The arena was cleaned using 70% ethanol after each trial to minimize the olfactory cues. The discrimination index was calculated by the following formula; (time spent investigating the novel object—time spent investigating the familiar object) ÷ (time spent investigating the novel object + time spent investigating the familiar object). A positive value in the discrimination index represents more time investigating the novel object, a negative value represents less time spent and zero represents equal time spent investigating both objects.

Nissl staining and analysis of cell survival in the hippocampus

Coronal brain sections of 5 µm thickness were processed for Nissl staining (cresyl violet staining) as per the earlier published report [27]. Qualitative analysis of these sections was carried out using a bright field microscope. Photomicrographs of hippocampal CA1 and CA3 regions were obtained using a bright-field dual-headed microscope (Zeiss Primostar 3, Germany) connected to a Axiocam 208 colour camera (Zeiss, Germany), which is attached to a PC installed with ZEN 3.0 (blue edition, Zeiss, Germany) imaging software.

Golgi-Cox staining and dendritic spine count

Golgi-Cox staining was performed by following the earlier published reports [28, 29]. Spine density was determined at 30 µm length on secondary dendritic branches of hippocampal CA1 neurons. [24]. Secondary dendrites were imaged at 1000 × magnification using a bright-field dual-headed microscope (Zeiss Primostar 3, Germany) connected to Axiocam 208 colour camera (Zeiss, Germany). These images were opened in ImageJ software and dendritic spines along the 30 µm line were marked using the "Multi-point" tool and manually counted.

Statistical Analysis

The data is expressed as Mean ± SEM. The significant difference between groups was determined by performing the Student 't' test. One-way analysis of variance test, followed by Tukey's post hoc test was used for analyzing the familiarization data of the novel object recognition test. A 'p' value ≤ 0.05 was considered as statistically significant. GraphPad Prism software (San Diego, CA, USA) was used for data analysis.

Results

Open field test

The latency to enter the centre zone was significantly increased in blue light exposed animals (Fig. 2A; $F_{8,8} = 1.997$; $p = 0.03$) compared to controls indicating increased emotionality-like behaviour in these animals. Total entries to the centre zone (Fig. 2B; $F_{9,9} = 1.242$; $p = 0.25$), and percentage of time spent in the centre zone (Fig. 2C; $F_{9,9} = 5.987$; $p = 0.07$) were also reduced in blue light-exposed animals compared to controls but this difference was not statistically significant. The mean speed of animals in the centre (Fig. 2D), total zone transitions (Fig. 2E), and total distance travelled (Fig. 2F), by both the control and the blue light exposed animals were not significantly different. Although these parameters were reduced in blue light exposed animals this difference was not statistically significant.
Figure 2G, represents the animal video tracking of the control animal during the open field test. As evident from the image, the control group animals (Fig. 2G) explored the open field freely, making numerous zone transitions and crossing through the centre of the open field including making diagonal movements. The test group animals (Fig. 2H), however, preferred to stay close to the walls of the apparatus and spend more time in the corners – a phenomenon known as Thigmotaxis. Moreover, two test group animals preferred to stay in the bottom left corner of the apparatus and did not explore the open field as intended. The test group also made relatively fewer entries into the centre zone and displayed caution to move into the centre field suggesting altered emotionality in these animals.

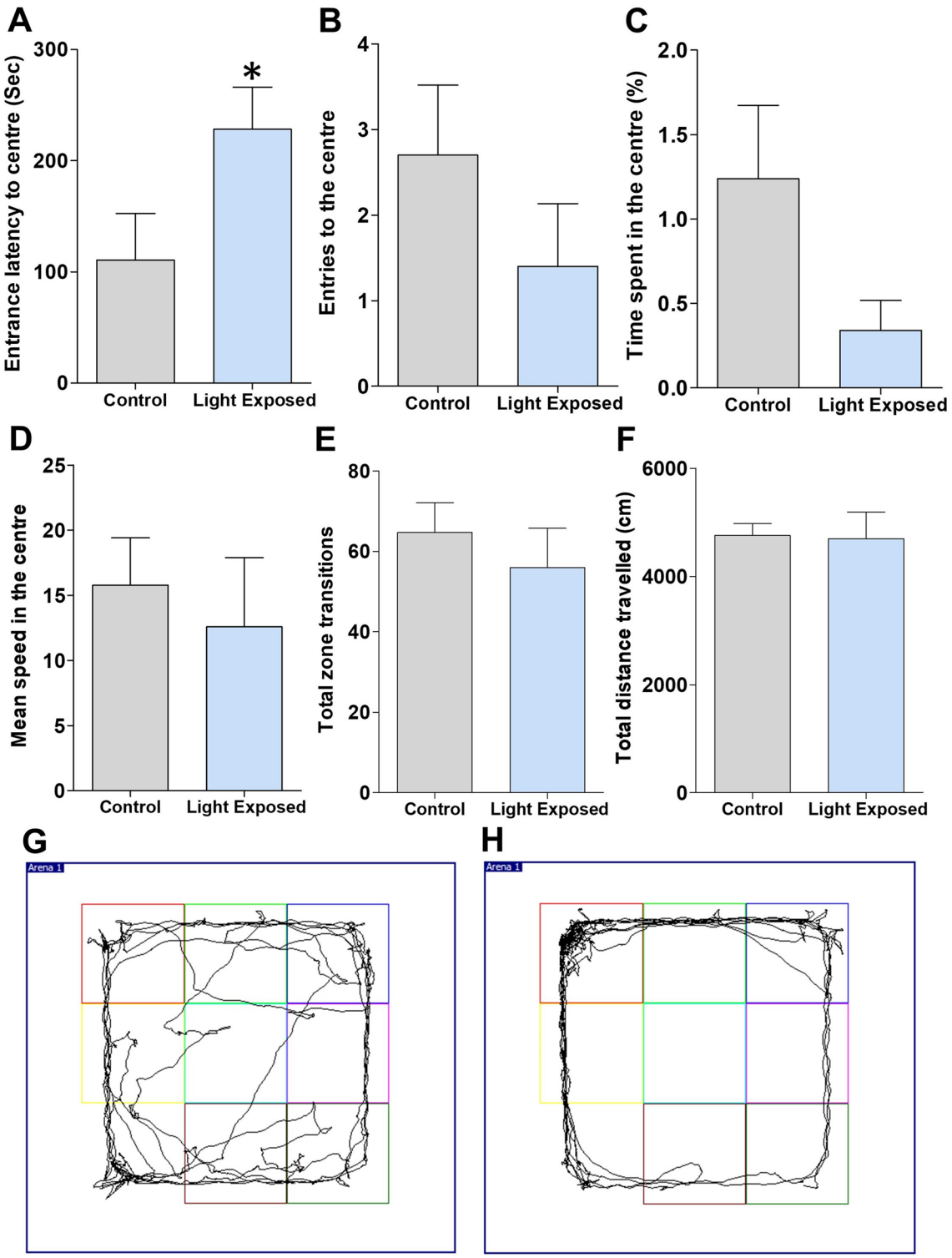

Fig. 2 Effect of artificial blue light exposure on animal behaviour in the open field test. Entrance latency to centre (A), entries to the centre (B), time spent in the centre (C), mean speed in the centre (D), total zone transitions (E), total distance travelled (F), and representative animal tracking of control (G) and blue light exposed animals (H) during the open field test. Note; The control group animals have well explored the open field including the centre square. (G). However, the test group animals (H) made significantly fewer entries to the centre zone of the open field. Note; n = 10, $*p \leq 0.05$

Elevated plus maze test

The control animals made significant number of entries to the open arm but blue light exposure altered this behaviour as demonstrated by their fewer open arm entries. This difference was statistically significant (Fig. 3A; $F_{6,9} = 10.69$; $p = 0.044$). Both time spent (Fig. 3B) and distance travelled (Fig. 3C) on the open arm were found to be reduced in blue light exposed animals but these were not statistically significant. Entries to the closed arm were reduced (Fig. 3D), but time spent in the closed arm (Fig. 3E) was increased in the blue light exposed group compared to the control (Fig. 3E). Nonetheless, these changes were not statistically significant. In contrast, the distance travelled in the closed arm was significantly reduced in the blue light exposed group (Fig. 3F; $F_{9,9} = 1.42$; $p = 0.001$). Light-exposed animals have made fewer entries to centre (Fig. 3G), spent less time in there (Fig. 3H) and travelled less distance on the centre (Fig. 3I) compared to the control animals. However, these differences were not found to be statistically significant.
The mean speed of blue light exposed animals on the open arm (Fig. 4A), closed arm (Fig. 4B) and the centre (Fig. 4) were comparable to that of the control group. Rearing duration (Fig. 4D) and total zone transitions (Fig. 4E) were reduced but not significantly in the blue light exposed group compared to the control group. However, the total distance travelled was significantly reduced in the blue light exposed group compared to the control group (Fig. 4F; $F_{9,9} = 2.013$; $p = 0.050$). Figure 4G, represents the video tracking of the animal path during the EPM test and it depicts that control rats were exploring the entire apparatus very well as indicated by their path both in the closed and open arms of the EPM (Fig. 4G). However, the blue light exposed animals did not explore the open arm very well (Fig. 4H) and they were stationary for most of the time of the experiment similar to freezing type of behaviour demonstrated by animals with elevated anxiety-like behaviour. As a result, the total distance travelled on the apparatus was also reduced in blue light exposed animals (Figs. 4F, H).

Novel object recognition test

Time spent on objects 1 & 2 on the familiarization test was comparable in both control and test groups (Fig. 5A). Statistical analysis did not show any significance in any of the situations tested such as time spent on object 1 by the control group, time spent on object 2 by control animals, time spent on object 1 by the test group, and time spent on object 2 by test group animals (Fig. 5A). On the contrary, during NORT, the blue light exposed group did not explore the novel object well compared to the control rats. As we can see, the discrimination index of the blue light exposed rats was negative compared to a positive value in control rats (Fig. 5B; $F_{9,9} = 1.732$; $p = 0.03$).
Figure 5C represent the video tracking of the animal path during the NORT where we can see that the control animal attempted to approach the novel object (green zone) several times compared to the familiar object (blue zone). As a result, the time spent in the green zone was is also more in these animals (Fig. 5B, C). In contrast to this behaviour, the blue light exposed animals did not explore the novel object well (Fig. 5B, D). Although this was the group pattern very few animals in the test group explored the novel object well but others behaved differently than expected such as exploring the familiar object more compared to the novel object. What this means is that when exposed to a familiar object alongside a novel object, rats normally frequently investigate and spend more time exploring the novel than the familiar object. This apparent 'unconditioned preference' for a novel object is considered an indication that a representation of the familiar object exists in memory which is altered in blue light exposed rats.

Nissl staining and analysis of cell survival in the hippocampus

Analysis of Nissl-stained sections from control and blue light exposed groups revealed apparent cell pyknosis (indicated by red arrowheads) in the CA1 region of the blue light exposed group (Figs. 6 C, D) compared to the control group (Figs. 6A, B). High magnification images of the CA1 regions demonstrated several darkly stained neurons in the blue light exposed groups (Fig. 6D) in comparison to the control group (Fig. 6B). Darkly stained neurons were also present (indicated by red arrowheads) in the CA3 region in blue light exposed rats (Fig. 6G, H) but, their numbers were not many compared to control rats (Fig. 6 E, F). However, as evident in Fig. 6H, the CA3 region was seen dispersed in the blue light exposed group and the cell band was observed to be thin with a lesser number of surviving cells compared to the CA3 region of the control rat (Fig. 6F).

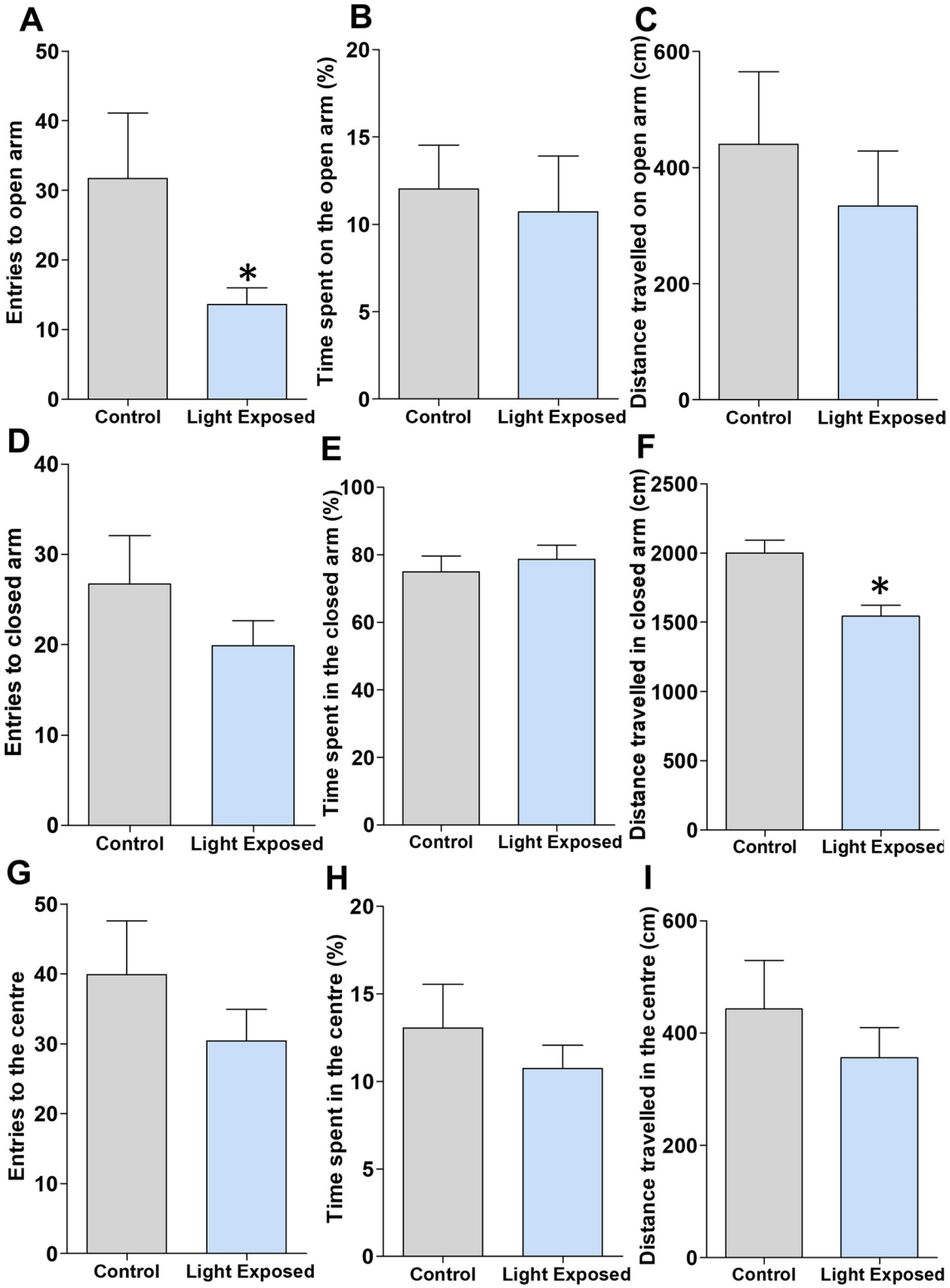

Fig. 3 Effect of artificial blue light exposure on animal behaviour in the elevated plus maze test. Entries to open arm (A), % time spent on the open arm (B), distance travelled on the open arm (C), entries to closed arm (D), % time spent in the closed arm (E), distance travelled in the closed arm (F), entries to the centre (G), time spent in the centre (H), distance travelled in the centre (I). Note; n = 10, $*p \leq 0.05$

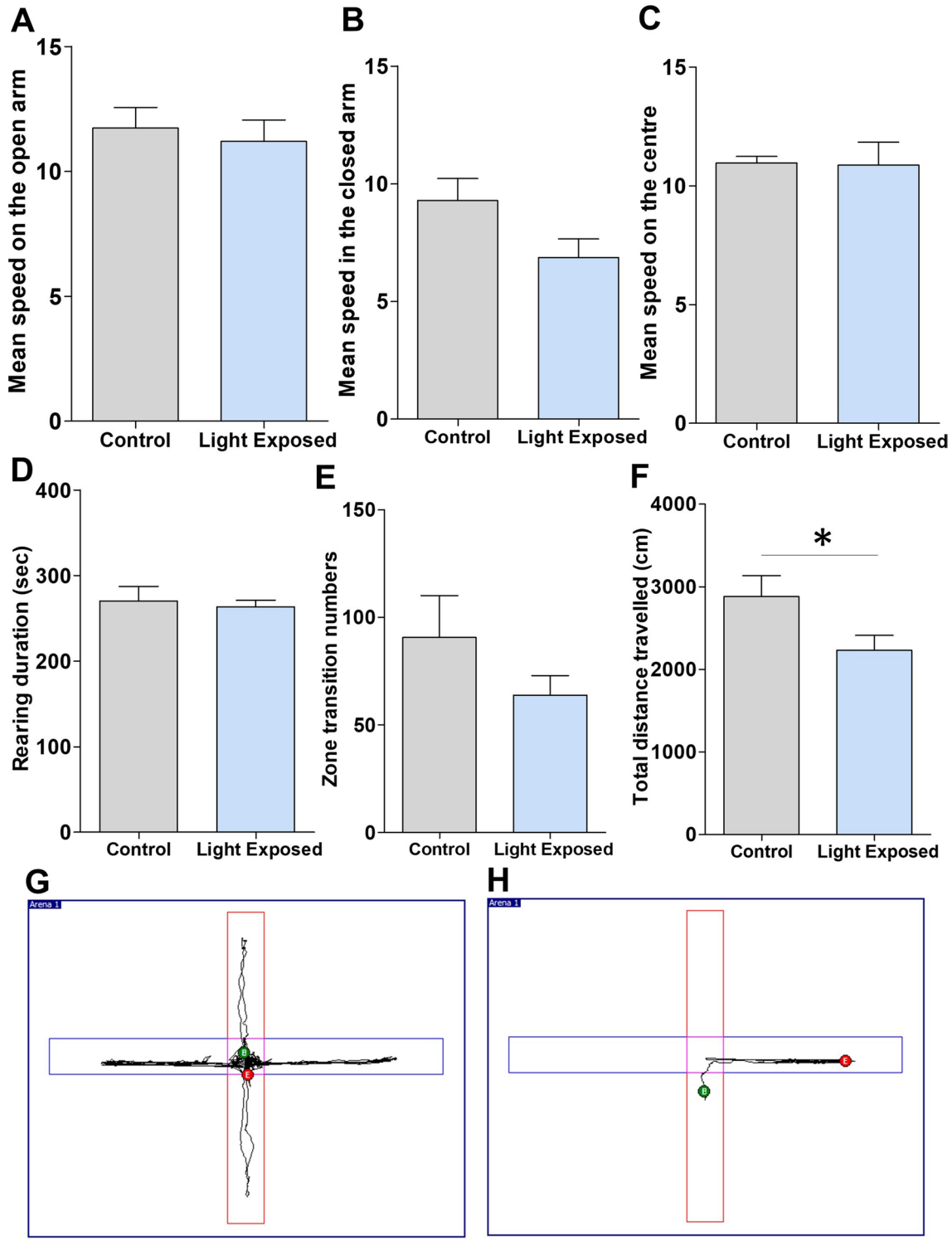

Fig. 4 Effect of artificial blue light exposure on animal behaviour during elevated plus maze test. Mean speed on the open arm (A), closed arm (B), and centre (C), rearing duration (D), zone transitions (E), total distance travelled (F) and representative video tracking of control (G), and blue light exposed animals (H) during EPM test. Note; The control group animal explored all the arms of the apparatus freely, while the test group preferred to remain in closed arms and their exploration of the open arm was reduced indicating increased emotionality. Note; n = 10, $*p \leq 0.05$

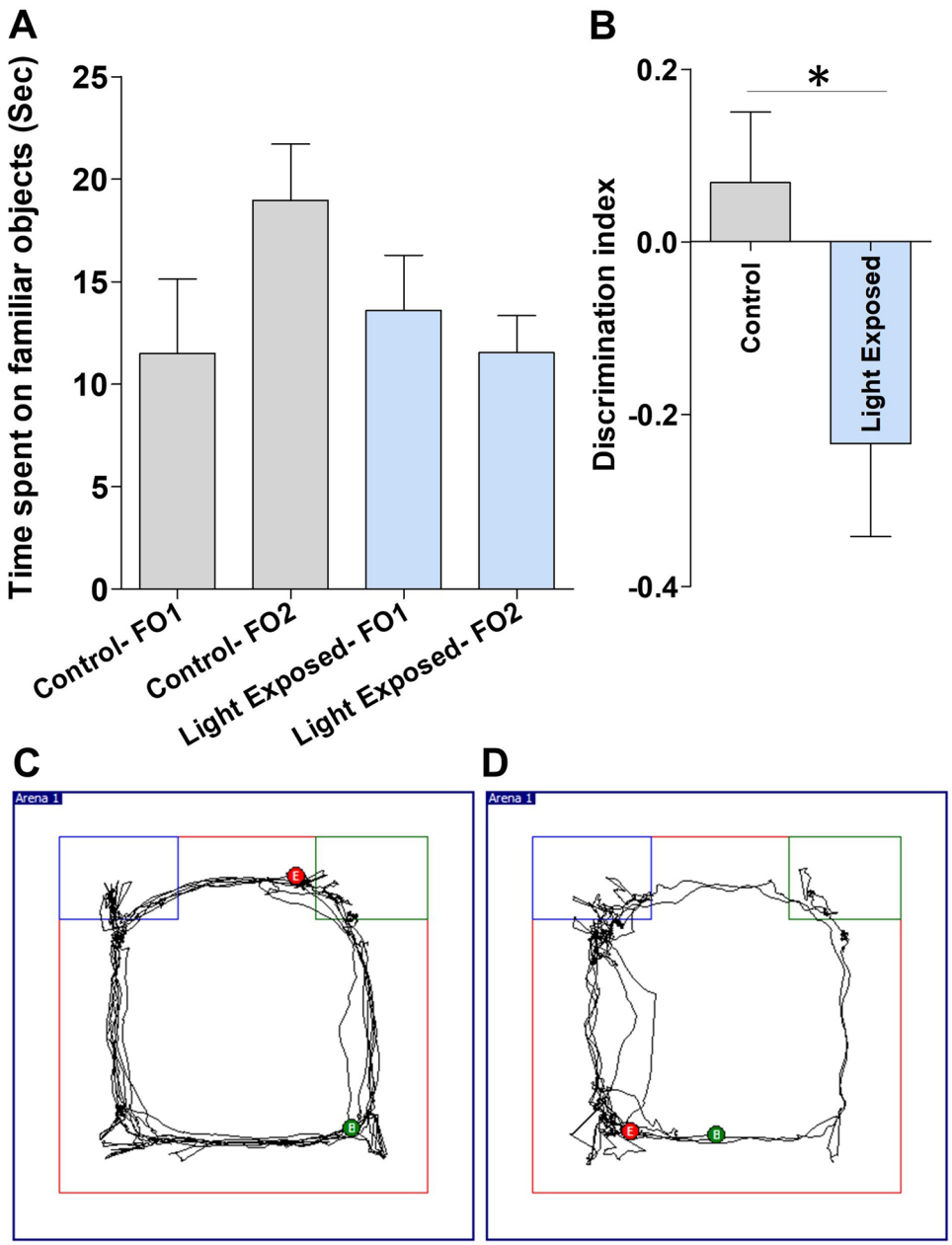

Fig. 5 Effect of artificial blue light exposure on novel object recognition test (NORT). Time spent on the familiar object (A), discrimination index (B), and representative animal video tracking of control (C) and blue light exposed (D) animals during the NOR test phase. Note; Both groups of animals have equally explored and investigated familiar objects 1 and 2 during the familiarization phase. However, blue light exposed animals did not investigate the novel object well compared to the control group which was indicated by their negative discriminative index value. n = 10; $^{*}p \leq 0.05$

Golgi-Cox staining and dendritic spine count

Hippocampal CA1 neuronal dendritic spine quantification revealed that the total dendritic spine density (indicated by yellow arrow) in the secondary dendrites of CA1 neurons in blue light exposed animals was significantly reduced (Fig. 7B, C) compared to the control animals (Fig. 7A). Statistical analysis demonstrated a significant reduction in the CA1 apical secondary dendritic spine density in the blue light exposed rats (Figs. 7B, C) when compared to control rats (Fig. 7A). In the control animals, the dendritic spines were plenty and their density in any 10 µm region among the total 30 µm length counted (marked by the green square bracket) was significantly high (Fig. 7A; green arrowheads) compared to blue light exposed rats. However, in the blue light exposed animals, the dendritic spine density in any 10 µm region among the total 30 µm length counted (marked by the yellow bracket) for each animal was sparse (indicated by yellow arrowheads) and less (Fig. 7C) compared to the control rats.

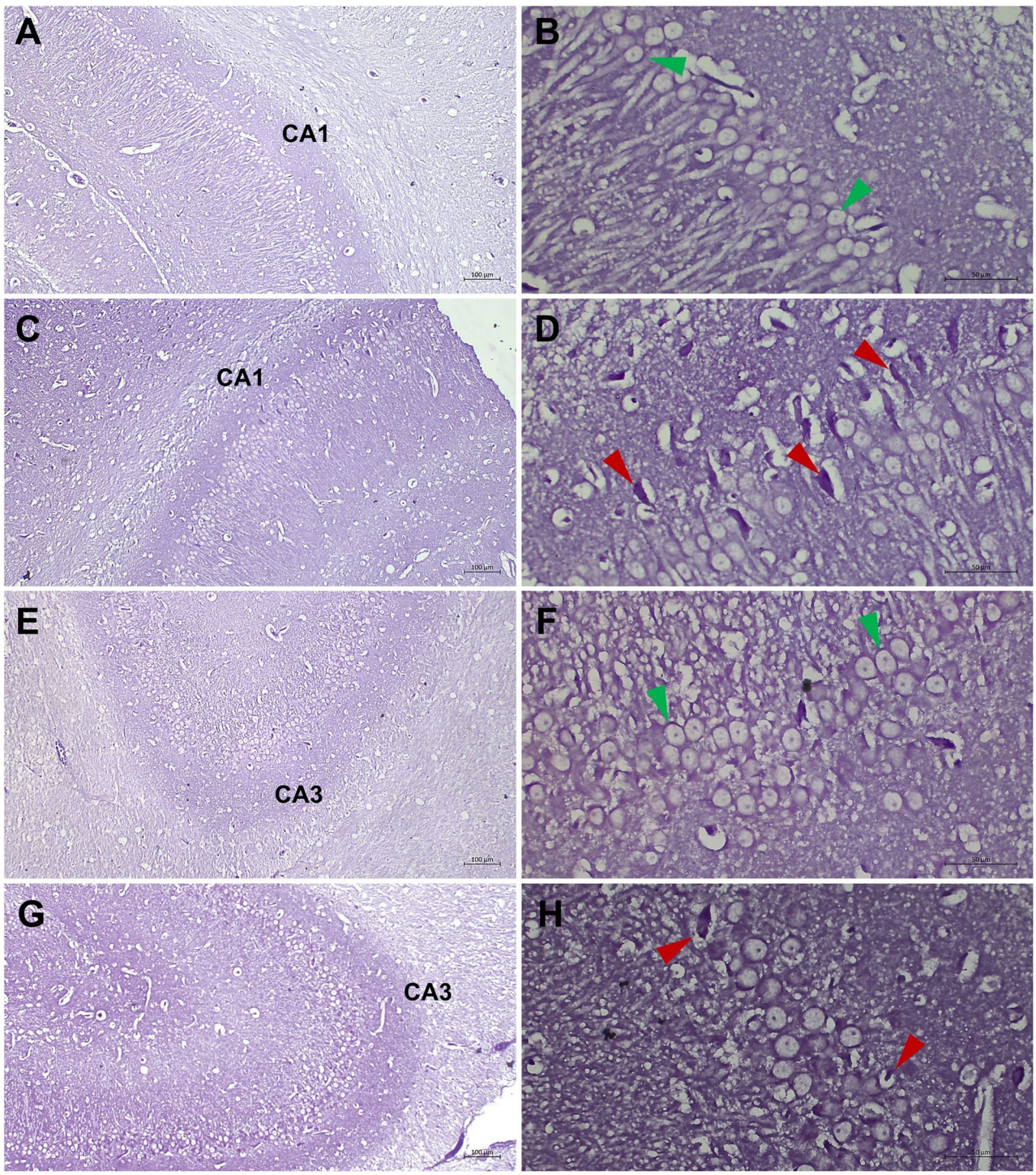

Fig. 6 Effect of artificial blue light exposure on hippocampal cytoarchitecture. Representative photomicrographs of Nissl stained CA1 subfield (A-D) from control (A, B), blue light exposed rats (C, D); and CA3 subfield (E–H) from control (E, F) and blue light exposed rats (G, H). Note; Green arrowheads indicate normal neurons and red arrowheads indicate pyknotic neurons; CA1- Cornu Ammonis- 1, CA3- Cornu Ammonis- 3; Magnification 100 × and Scale bar 100 µm in panels A, C, E, G; Magnification 400 × and Scale bar 50 µm in panels B, D, F, H

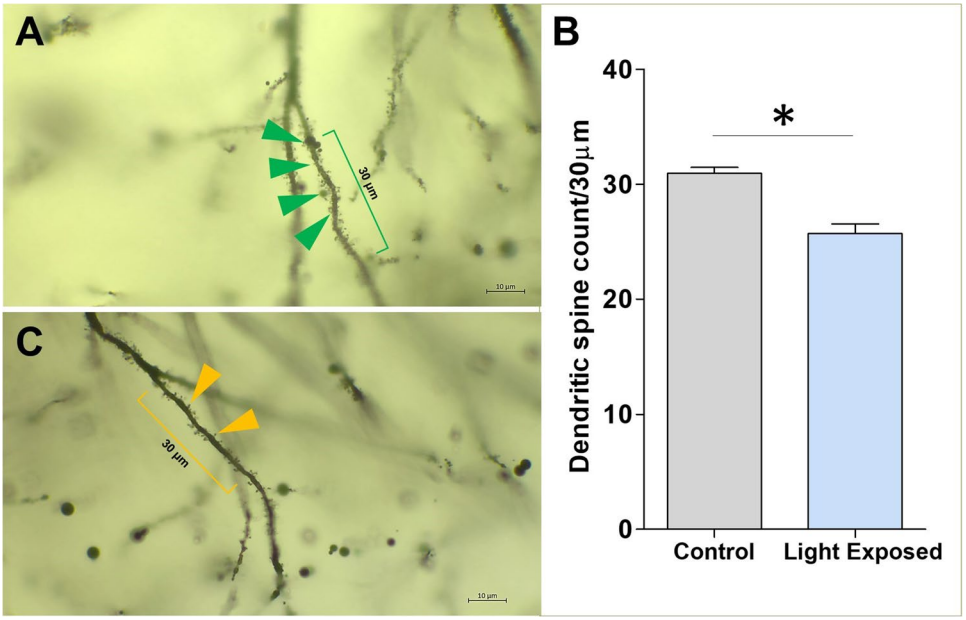

Fig. 7 Effect of artificial blue light exposure on hippocampal CA1 dendritic spine density. Representative photomicrographs of Golgi-Cox stained CA1 apical secondary dendrites from control (A), blue light exposed animals (C), and comparison of dendritic spine density in control and blue light exposed animals (B). Note; Green arrowheads indicate densely protruded dendritic spines on the secondary dendrites and yellow arrowheads indicate sparsely protruded dendritic spines on the secondary dendrites; CA1- Cornu Ammonis- 1, Magnification 1000 × and Scale bar 10 µm in panels A, C. *$p < 0.05$

Discussion

The data obtained with this research demonstrates that the emotionality of the blue light exposed rats was elevated when they were exposed to a novel arena. This was evident in both the OF and EPM tests. In the OF test, the centre zone exploration was significantly reduced in blue light exposed animals. Moreover, their activity was confined to a few corner squares which is a deviation from the normal exploration behaviour shown by the control animals. In the EPM test, the entries to the open arm and distance traveled in the open arm were significantly reduced in blue light exposed animals indicating anxiety-like behaviour. Both OF and EPM evaluate two different aspects of the anxiogenic behaviour in animals and it can be seen that blue light exposure induced a change in normal behaviour in both these paradigms.

The reasons for the above changes in the behaviour of animals could be many. It could be due to a change in the neurotransmitter levels that control anxiety-like behaviour [12], or changes in the biochemical indexes in specific brain regions controlling anxiety-like behaviour [1], or cellular changes in the brain regions that are responsible for coordinating and regulating these behaviours [20]. The histological analysis results point to this latter possibility. As demonstrated in the results, hippocampal cell loss and dendritic spine remodeling in blue light exposed animals could be one of the possibilities for the altered behaviour in blue light exposed rats. Although not evaluating the presence of necrotic or apoptotic markers in the hippocampus is a limitation of the current study the histological evidence points to a permanent change or remodelling is occurring in this brain region. Further studies are required to determine the molecular mechanism leading to cell loss under blue light exposure in this brain region responsible for regulating emotionality and memory.

The activation of the HPA axis and related changes could be another possible reason. The adrenal cortical cells in the zona fasciculata synthesize glucocorticoids (cortisol) in response to adrenocorticotropin hormone (ACTH) from the pituitary gland. Cortisol has a protective role during stressful conditions. Activation of the hypothalamic–pituitary–adrenal (HPA) axis is an essential part of stress adaptation and survival. Alteration in this axis and possibly suppression of cortisol secretion due to chronic blue light exposure could be another possible reason for the altered behaviour in rats. However, further biochemical, histochemical, or molecular studies are required to categorically confirm these possibilities.

Several preclinical studies showed that melatonin, a monoaminergic neurotransmitter released from the pineal gland, can ameliorate neurodegenerative pathologies and restore cognitive impairments [36]. Melatonin has also been shown to improve symptoms of anxiety, hence supplementing with melatonin for anxiety can improve sleep quality, regulate circadian rhythm, and ease negative feelings associated with anxiousness [21]. Changes in melatonin levels due to continuous exposure to blue light in the test group could be another possibility for the altered behaviour in rats. Several reports corroborate the view that blue light elicited a relatively greater suppression of melatonin than red and white light [21,

11, 16, 38]. Studies in humans have shown that blue light exposure when compared to other lights such as green revealed greater responses to 'blue' light for acute suppression of melatonin secretion, increase of body temperature, heart rate, and the reduction of subjective sleepiness with an increase in alertness [5, 8, 25].

Another finding of the current study is the minor alteration of the locomotor behaviour of rats. Although total zone transitions were decreased in the test group in the OF test, the total distance travelled was equal in both the control and test groups indicating that there is no significant motor deficit caused by blue light exposure. On the other hand, the distance travelled by the test group animals was reduced in the open arm, centre, and also in closed arm. This was an unexpected finding because the closed arm offers more protection. The test group exhibited a lesser number of entries into each arm as well as into the centre. This locomotor behavioural change could be due to either a lack of motivation for exploration due to the increased emotionality or due to a motor behaviour alteration. The reason for this minor alteration needs further investigation. It is reported that exposure of *Drosophila melanogaster* to 12 h of blue light per day accelerated aging phenotypes causing damage to retinal cells, brain neurodegeneration, and impaired locomotion [30].

Another important finding of the current study is the altered object recognition memory in blue light exposed rats. When exposed to a familiar object alongside a novel object, rodents investigate and spend more time exploring the novel than the familiar object. This apparent 'unconditioned preference' for a novel object indicates that a representation of the familiar object exists in memory. This concept forms the basis of the object recognition task in the study of memory functions in rodents [14, 15]. The blue light exposure significantly altered this unconditioned preference to investigate novel objects more compared to familiar objects. They behaved as if there was a poor representation of the familiar object in their memory. Hippocampal integrity is one factor that is important for object recognition memory. Evidence suggests that the functional integrity of the hippocampal activity is required in novel object recognition consolidation [10, 18, 35]. In addition, the catecholaminergic system has been of vital interest due to its role in several aspects of recognition memory. Dopamine is essential in recognition memory since the dopaminergic neuronal activity is modified by novel and salient stimuli [42, 32]. Changes in hippocampal functional integrity and dopaminergic system could be attributed to the altered object recognition memory in blue light exposed animals. There are reports that the exposure of rats or mice to constant light for several months was associated with a significant reduction in the number of dopaminergic neurons [33, 34]. In addition, transcranial blue light may impact human brain activity [40]. Taken together, these researches suggest the possible detrimental effects of blue light exposure on the brain and behaviour of both rodents and humans.

The deleterious effect of blue light exposure could be attributed to the excessive formation of reactive oxygen species (ROS). Several studies report that blue-light exposure results in the generation of ROS in the retina of mice [31], flies [6] and even in skin cells [26]. A study on *C. elegans* demonstrated that light exposure shortens their lifespan, increases ROS, and induces an unfolded protein response [13]. Studies also demonstrate that blue light exposure induced the expression of selected stress-response genes in *C. elegans* and in the photoreceptors of the retina of *Drosophila* [19]. Blue light exposure-induced alteration of oxidant stress and antioxidant defense status could be another possibility. As established already increased oxidative stress could potentially affect memory formation and retrieval. Blue light exposure induced decrease in melatonin secretion leading to altered behaviour in rats is another possibility. Analysis of melatonin and neurotransmitter such as dopamine could have been a useful parameter to categorically prove the biological effects of blue light and not doing these assays is another limitation of this study. Further biochemical and molecular studies are necessary to categorically determine the role of altered dopaminergic system, melatonin secretion, and ROS in modifying the behaviour and hippocampal cytoarchitecture in rats under chronic artificial blue light exposure.

Conclusion

Long-term artificial blue light exposure increased emotionality, significantly altered the novel object recognition memory, and mildly affected general locomotor behaviours in rats. It induced hippocampal cellular pyknosis and reduced cornu ammonis-1 (CA1) apical dendritic spine density in blue light exposed rats. However, care should be taken while extrapolating this data to humans. Nonetheless, the data from the current research is a warning that chronic blue light exposure could be detrimental to certain types of brain functions or behaviours. In a world where technology is an integral part of our daily lives, and our dependence on electronic devices increases, it is important to keep note of the negative effects of overuse of these gadgets to prioritize our mental and physical health.

Abbreviations LED: Light-emitting diode; ALAN: Artificial light at night; SCN: Suprachiasmatic Nucleus; REM: Rapid eye movement; RGB: Red green blue; NORT: Novel object recognition test; OF: Open field; EPM: Elevated plus maze; ACTH: Adrenocorticotropic hormone; HPA: Hypothalamic-pituitary-adrenal; ROS: Reactive oxygen species

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Authors' contributions S.N.N. conceived of the presented idea, designed, directed the project, analyzed and interpreted the results, and edited the manuscript.; I.A., R.A.B.E., and H.I.S., performed various experiments and wrote the initial draft of the manuscript; Y.M.B. performed Nissl staining, analysed slides, performed dendritic quantification, interpreted results and contributed to editing the manuscript. B.C.P.S., edited and approved the final manuscript. All authors have contributed to the final version of the manuscript and approved it.

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Declarations

Ethics approval and consent to participate The Institutional Research Ethics Committee approved all procedures used in the study (RAKMHSU-REC-093-2019-UG-M).

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