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Chronic Exposure to Artificial Light Spectra at night alter Neurobehaviour and Neurotransmitter levels in Albino Rats

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Abstract

Artificial light at night has been reported to have significant effects on the physiology and behaviour of animals by its impact on their circadian rhythm. This study investigated the effect of artificial light spectra at night on neurotransmitter activities and neurobehavioural changes in the albino rat. Blue (470 nm) and red (665 nm) lights were used; with ambient light and darkness serving as positive and negative controls, respectively. The rats were exposed to daylight from 6 am to 6 pm and 12 hours of artificial light (6 pm - 6 am) daily (light sources were 13 Watt compact florescent electric bulbs). Neurobehavioural outcomes were measured using the Open Field Test (OFT) and Morris Water Maze (MWM). Neurotransmitter activities (dopamine and serotonin) were determined using Atomic Absorption Spectrophotometry. Locomotion and exploration were significantly higher ($p < 0.05$) in rats exposed to blue and red light at days 60 and 90, respectively. Darkness elicited increased anxiety in the OFT compared to the ambient light. Rats exposed to red light and darkness for 90 days had significant memory deficits, while those exposed to blue light increased in learning and memory capability with increased duration of exposure. Dopamine and serotonin increased by 48.65 % and 17.99 %, respectively from day 60 to 90 in the rats under red light. Only serotonin increased under blue light. In conclusion, blue light seemed to enhance learning and memory in the rats while both red and blue lights stimulated dopaminergic and serotonergic activities post exposure. In conclusion, blue and red lights at night may be useful tools for the improvement of locomotory activity, enhancement of memory and amelioration of depression.

Keywords: Artificial Light; Albino Rats; Neurobehaviour; Dopamine; Serotonin

Introduction

Artificial light at night is presently a subject of concern due to its influence on body physiology and behaviour. This impact is as a result of its negative consequences on circadian rhythm which invariably results in health related issues such as depression [1]. Hathaway *et al*. [2] submitted that natural light has the highest levels of light needed for biological functions, but technological advancements and other anthropogenic activities have rapidly supplemented artificial light.

Light generally influences modulatory non-visual effects on a wide scope of biological activities [3] such as suppression of melatonin, regulation of sleep, synchronization of the circadian system, as well as improvements of alertness, cognition and behaviour [4]. The pathway for this effects is transduced to the brain photoreceptors known as melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGC), considered to play a crucial role in many of the non-visual biological effects of light in humans [5]. It is the only pathway through which light affect the non-visual functions; it receives inputs from rods and cones which exert a modification influence on their response to light ipRGC is the Vandewalle and Dijks [6].

Neurotransmitters transmit information from one neuron to another, which are made by amino acids. These transmitters, control major body functions including movement, emotional response, and the physical ability to experience pleasure and pain [7-9]. The most familiar neurotransmitters, which are thought to play a role in mood regulation, are serotonin, norepinephrine, dopamine, acetylcholine, and GABA [10]. Dopamine and serotonin (5-hydroxytryptamine or 5-HT) are neurotransmitters with conserved, important roles in the vertebrate nervous system [11]. Broadly, serotonin regulates mood while dopamine regulates movement [12] and it is also associated with happiness [13]. Dopamine is also involved in cognition, motivation, and movement [14]. Noting their importance, any factor affecting these neurotransmitters will automatically reflect in their downstream physiological and behavioural responses. This study therefore investigated the effect of artificial light spectra on neurobehaviour and neurotransmitter (serotonergic and dopaminergic) activity in the brain.

Materials and Methods

Breeding of research rats

Wistar albino rats, 24 females and 8 males, were procured from Institute for Medical Research and Training (IMRAT), Ibadan. The rats were transferred to the Zoo Park laboratory animal facility of the Federal University of Agriculture, Abeokuta (FUNAAB) where they were acclimatized for two weeks and fed ad libitum. At the end of two weeks, the males were introduced to the females with the mating ratio 3:1 (female: male). The females were examined to confirm pregnancy and non-pregnant rats were excluded from the research. As expected, delivery took place between day 21 and day 24 and sixty (60) day old male albino rats were used for this study with 15 rats per treatment, further divided into three replicates at 5 rats per replicate were used for this research.

Experimental cage

The experimental cage was made of wood, partitioned into cubicles of dimensions 60cm x 50cm x 40cm. Electric lamp sockets were fixed at the centre top of each cubicle to ensure uniform distribution of light within each cubicle.

Light treatments

Two monochromatic visible lights of the extreme wavelengths (Blue, 470 nm and Red, 665 nm) were used while ambient light and darkness served as positive and negative control. The light was generated from 13 watt compact florescent bulbs (Estar®, China) and was powered by solarinverter systems to ensure uninterrupted power supply. Light intensity was maintained between 300-350 lux within the cubicle using light meter.

Experimental design

The new born rats were raised by selected dams for the first 28 days after which they were weaned. A group of rats were kept under ambient light conditions while the group subjected to darkness had 0 hour of ambient light. Other two groups were exposed to 12 hours of blue and red lights at night (12 AL) and 12 hours of ambient light during the day (12L) summarised as 12L: 12AL. All treatments lasted for ninety days, rats were fed with normal rat pellets and clean water *ad libitum* and average daily ambient temperature ranges between 27 °C and 29 °C was recorded in the facility. Ethical guidelines of the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) was followed during this study. Though authors acknowledge the weak vision of Albino rats, their availability for research in the facility and their adaptability to biomedical research was sufficient reasons to make them the model of choice.

Neurobehavioural changes

Two rats were randomly selected from each replicate light treatment totalling six rats per treatment. Morris Water Maze (MWM) and Open Field Test (OFT) were used as measure of cognition/memory and anxiety. MWM was done three days prior to sacrifice (day 58 and 88) while the OFT was done at the early hours of the day of sacrifice (day 60 and day 90).

Morris Water Maze (MWM): This was done according to the method described by [15]. In brief, the rats were placed in a pool of water where they must swim to a circular hidden escape platform which served as the learning face (latency to find the platform). This was done four times for two minutes each using the four cardinal directions (East, West, North and south). As the animals became more familiar with the task, they were able to find the platform more quickly. After the learning phase was the annulus count (memory). The platform was removed and the rats introduced to the pool to locate the area where the platform was previously placed and this indicated the memory capacity of the rat.

Open Field Test (OFT): Open field is used for measuring anxiety and exploration as well as locomotion. The open field apparatus was constructed of plywood and measured 72 cm x 72 cm with 72 cm walls. Blue visible lines were drawn on the floor, with a marker; the lines divided the floor into sixteen 18 cm x 18 cm^2 squares. A central square (18 cm x 18 cm) was drawn in the middle of the open field [16]. Each rat was then given a score for total locomotor activity that was calculated as the sum of line crosses and number of rears according to [17].

Animal sacrifice and Sample Collection for Neurotransmitters

Three rats were randomly selected from each treatment aside from the one used for OFT. The rats were sacrificed by quick decapitation and brain tissues were harvested immediately and placed in iced normal saline, perfused with the same solution to remove blood, blotted on filter paper and frozen at - 80 °C.

Preparation of Brain Tissue Homogenate for Neurotransmitters Estimation: The frozen tissues were cut into small pieces and homogenized in phosphate buffer (pH 7.4), then centrifuged at 4000 rpm for 15 minutes at 4 °C and the supernatant removed.

Preparation of Sample by Hydrolysis: One gram of homogenous sample was weighed into a stoppered 250 ml conical flask, 100 ml of 6 M HCl was added to the sample, stoppered and heated in an oven for 16 hours to hydrolyse the sample. The mixture obtained was filtered through a double layered Whatman No 42 Filter paper into another 250 ml Conical Flask and stoppered. The hydrolysate obtained was stored at -4 °C prior till analysis.

Determination of Dopamine: Two millilitre of the above hydrolysate was pipetted into a 30 ml test tube; 10 ml of buffered ninhydrin reagent was added, heated in boiling water for 15 minutes, cooled to room temperature, after cooling, 3 ml of 50 % Ethanol was added to the sample.

Zero to five microgram per millilitre (0-5 µg/ml) working standard Dopamine was prepared from each stock dopamine solution to get the gradient factor. The working standards were heated with the buffered ninhydrin reagent as done with the sample hydrolysate above. The absorbances or transmittance of sample buffered heated hydrolysate and working standards were measured at the wavelength of 360 nm on a Cecil Spectrophotometer.

Dopamine was calculated in umol/mg using the formula:

$$\text{Dopamine } \left(\frac{\mu\text{mol}}{\text{mg}}\right) = \text{Absorbance of sample extract} \times \text{Gradient factor} \times \text{Dilution factor}$$

Determination of Serotonin: Two millilitre of hydrolysate sample above was pipetted into 100 ml conical flask; 50 ml of solvent mixture Methanol, Methylene chloride and acetonitrile (ratio 4:2:1) were added to dissolve and extract all the serotonin. The mixture was thoroughly shaken in the 250 ml separating funnel and the lower organic layer was discharged into a 50 ml volumetric flask. One millilitre of TCA (5 %) and 2 ml 10 M NaOH were added and made up to mark with the Solvent mixture and stoppered. This extract was stored at -4 ^{0}C till analysis was done.. Serotonin working standard stock mixture (0-20 umol/mg) were prepared from 100umol/mg stock serotonin and treated similarly like sample with 5 % TCA plus 2 ml NaOH. Absorbances of sample as well as working standard solutions were read on the Cecil Spectrophotometer at a wavelength of 415 nm.

Serotonin was calculated in umol/mg using the formula:

$$\text{Serotonin } \left(\frac{\mu\text{mol}}{\text{mg}}\right) = \text{Absorbance of sample extract} \times \text{Gradient factor} \times \text{Dilution factor}$$

Data Analysis

Data obtained were expressed as means ± SEM. The means were analyzed by one-way Analysis of Variance (ANOVA). Significant means were separated using Student-Newman Keuls (SNK), P values < 0.05 were considered statistically significant. T-test was used to analyse the difference between the effect of light treatment on memory and learning ability, neurotransmitter (serotonin and dopamine) at days 60 and 90.

Results

Neurobehavioural Changes

The effects of different light treatments on learning (latency to find the platform) and memory (annulus) ability in the rats, on days 58 and 88, are shown in Table 1. There was no significant difference ($p > 0.05$) in the latency to find the platform (learning ability) between the days of exposure. Exposure of the rats to blue light elicited an increase in learning and memory with exposure, with annulus count being significantly increased ($p = 0.01$) as against the control. A non-significant decrease in latency to find the platform and annulus count with exposure, was however observed in rats exposed to red light and darkness. The neurobehavioral responses in the Open field maze in rats exposed to different light spectra on days 60 and 90 are in Table 2. The grooming frequency in the rats with exposure was not significant ($p > 0.05$). However, rearing activity was significant under blue light ($p = 0.01$) and darkness ($p = 0.04$). The frequency of line crossing was also significant under blue light ($p = 0.00$).The frequency of freezing activity in the rats was highly significant ($p = 0.01$) under red and blue light. But the time spent in the centre square, the stretch-attend posture, urination and defecation counts were not significantly different ($p > 0.05$) with days of exposure.

Light Treatment	Latency to find platform (sec)			Annulus count		
	Day 58	Day 87	T-test	Day 58	Day 88	T-test
Ambient Light	25.50±5.56[a]	24.25±8.57[a]	0.63	2.60±0.93[a]	6.80±1.32[a]	0.25
Blue (470 nm)	19.43±4.95[a]	27.23±4.77[a]	0.45	2.40±0.40[a]	6.40±0.75[a]	0.01*
Red (665 nm)	25.50±3.72[a]	23.33±4.66[a]	0.65	1.80±0.66[a]	1.60±0.25[b]	0.49
Darkness	25.22±5.52[a]	16.95±3.68[a]	0.85	4.80±0.97[a]	3.20±0.58[b]	0.89

Means with the same superscript in a column are not significantly different ($p > 0.05$)

Table 1: Effect of different light treatment on learning (latency to find the platform) and memory (annulus count) ability in Wistar albino rat at day 58 and 88

		Light Treatment							
Parameters	Days	Ambient Light	T-test	Blue	T-test	Red	T-test	Darkness	T-test
Grooming	60	40.56±15.80[b]	0.63	43.38±19.08[b]	0.99	89.19±15.01[ab]	0.64	119.10±30.59[a]	0.49
	90	24.74±0.75[b]		24.30±2.23[b]		74.18±6.44[ab]		88.56±9.32[a]	
Rearing	60	15.40±1.12[b]	0.88	23.40±1.56[a]	0.01*	11.20±0.58[c]	0.28	5.20±0.48[c]	0.04*
	90	12.80±1.46[ab]		8.00±0.31[b]		19.00±0.71[a]		14.80±1.24[ab]	
Line crossing	60	47.00±7.40[a]	0.29	52.00±2.60[a]	0.00*	12.00±1.10[b]	0.11	8.00±0.63[b]	0.06
	90	22.00±1.60[a]		19.00±2.10[a]		30.00±4.80[a]		14.00±1.80[a]	
CSD	60	5.20±0.57[a]	0.33	3.20±0.31[b]	0.15	1.00±0.01[c]	0.20	2.30±0.37[b]	0.91
	90	6.70±0.40[a]		0.84±0.12[c]		2.30±0.37[b]		4.50±0.72[b]	
SAP	60	1.00±0.45[a]	0.08	1.40±0.51[a]	0.37	0.40±0.40[a]	0.67	1.40±0.51[a]	1.00
	90	0.80±0.37[a]		1.00±0.32[a]		0.20±0.20[a]		1.00±0.55[a]	
Freezing	60	154.21±20.28[a]	0.40	31.93±9.50[b]	0.01*	102.70±8.68[a]	0.01*	150.50±13.37[a]	0.09
	90	165.30±36.92[a]		169.50±5.21[a]		35.03±5.30[b]		3.00±0.63[b]	
Urination	60	1.60±0.93[b]	0.36	5.40±1.20[a]	0.63	3.00±1.30[b]	1.00	0.80±0.49[b]	0.24
	90	6.60±1.20[a]		4.80±1.10[a]		5.20±0.97[a]		3.00±0.63[b]	
Defecation	60	2.40±0.40[b]	0.42	3.60±0.68[b]	0.20	5.80±0.74[a]	0.84	4.00±0.63[b]	0.82
	90	3.20±0.50[a]		2.20±0.20[a]		4.20±0.37[a]		0.60±0.40[b]	

* Mean significant between day 60 and day 90 at $p < 0.05$ (T-test)

Table 2: Neurobehavioural responses in Open field maze in rats exposed to different spectra of light at day 60 and 90

Neurotransmitter Changes

There was no significant difference ($p > 0.05$) in the Dopaminergic activity at day 60, but the highest activity was observed in rats exposed to darkness (0.58 ±0.04 µmol/mg), followed by blue light treatment (0.45 ±0.07 µmol/mg) and least in rats exposed to red light treatment (0.37 ±0.05 µmol/mg) when compared to ambient light control (0.38 ±0.03 µmol/mg; Table 3). Significant increases ($p < 0.05$) were observed in the Dopamine concentrations of the rats exposed to darkness (0.63±0.04 µmol/mg), and red light (0.55±0.02 µmol/mg) compared to the control (0.42±0.03 µmol/mg). Dopamine activities between the days of exposure were significantly different ($p = 0.02$) in the rats exposed to red light.

Serotonin activities in the rats under all the light treatments at days 60 and 90 were not significantly different ($p < 0.05$). On day 60,

serotonin activity (1.53±0.02 µmol/mg) was highest in the rats exposed to darkness while on day 90 its activity (1.64±0.03 µmol/mg) was highest in those exposed to red light compared to the control groups (1.47±0.09 µmol/mg). Serotonin activities between the days of exposure were significantly different (p = 0.015) in the rats exposed to blue light (Table 3).

The activity trend of dopamine (A) and serotonin (B) in rats exposed to the different lights at night is shown in Figure 1. Dopamine and serotonin increased significantly ($p < 0.05$) with time in the rats exposed to red light. Serotonin increased significantly ($p < 0.05$) with time in the rats exposed to blue light while dopamine reduced with time in the rats exposed to blue light.

Light treatment	Dopamine (µmol/mg)				Serotonin (µmol/mg)			
	Day 60	Day 90	T-test	% increase	Day 60	Day 90	T-test	% increase
Ambient Light	0.38 ±0.03[a]	0.42±0.03[b]	0.450	10.53	1.35±0.02[a]	1.47±0.09[a]	0.259	8.89
Blue (470 nm)	0.45 ±0.07[a]	0.43±0.06[b]	0.789	-4.44	1.41±0.01[a]	1.53±0.03[a]	0.015*	8.51
Red (665 nm)	0.37 ±0.05[a]	0.55±0.02[ab]	0.024*	48.65	1.39±0.11[a]	1.64±0.03[a]	0.098	17.99
Darkness	0.58 ±0.04[a]	0.63±0.04[a]	0.410	8.62	1.53±0.02[a]	1.62±0.06[a]	0.194	5.88

Means with the same superscript in a column are not significantly different ($P > 0.05$).

*Mean significant between day 60 and day 90 at $p < 0.05$ (T-test)

Table 3: The activities of neurotransmitters in rats on exposure to light for 60 and 90 days

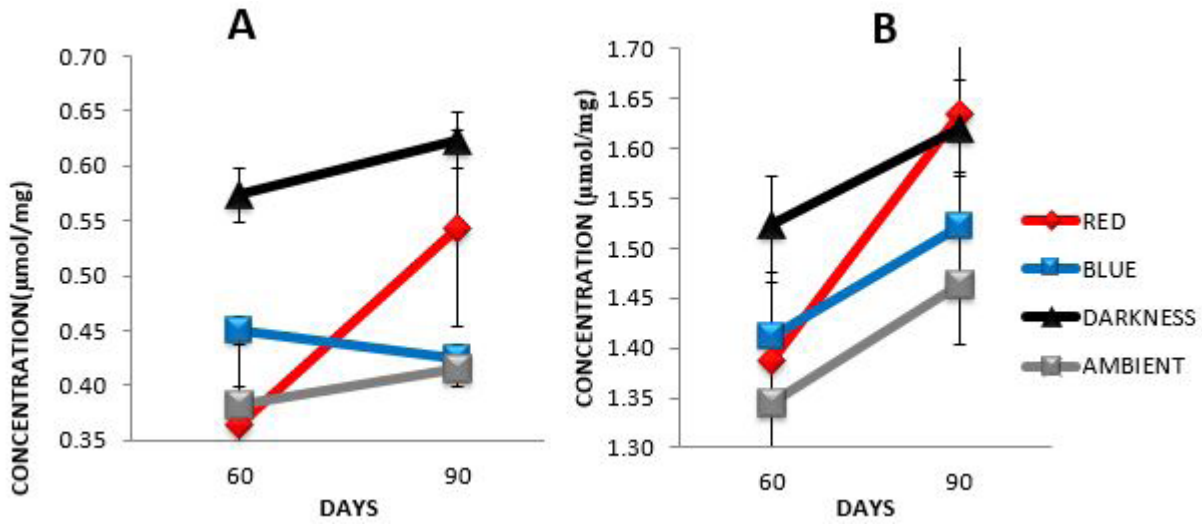

Figure 1: Trend in the activities of dopamine **(A)** and serotonin **(B)** in rats exposed to monochromatic lights at night

Discussion

Open-field test provides simultaneous measures of locomotion, exploration and anxiety. Rats exposed to blue light treatments after day 60 displayed higher mobility and exploratory activity and lower anxiety, this is evidenced by the increased number of the lines crossed, the number of rears and decreased immobilization (freezing). Walsh and Cummins [18] described a high frequency of line crossing and rearing as indicators of increased locomotion and exploration and/or a lower level of anxiety. The present study shows that blue light seems to enhance such behavioural activities. The study of Chellappa *et al*. [19] also confirmed that exposure to blue-enriched light increased psychomotor activity. Rats exposed to darkness and red light at day 60 showed reduced mobility and exploration. Borniger *et al*. [20] had earlier reported that mice exposed to dim light at night during the first 3 weeks of life had increased anxiety as adults.

Grooming-test is a tool for evaluating anxiety-like behaviours in rats [21]. The increase in the rate of grooming, defecation and freezing in the rats exposed to red light and darkness in this study, may be indicative of increased anxiety. Voiculescu *et al.* [22] stated that a significantly higher number of defecation suggests increased anxiety. The reduced mobility and exploration rates observed among rats exposed to red light at day 60 in this study therefore may probably be adduced to increased anxiety. After sixty days, reduced anxiety was observed in the rats exposed to red light. The increased number of rears, line crossing and decrease in immobilization among rats exposed to red light at day 90 confirmed the reduced anxiety in the rats. This observation coincides with the elevated activities of serotonin and dopamine at day 90 under red light. Sinha and Ray [23] submitted that decreased immobilization (freezing behaviour) indicates a low level of anxiety. In contrast, the rats exposed to blue light at day 90 showed less exploration which is indicative of anxiety.. This may be suggestive of a time dependent decrease of dopamine with prolonged exposure.

The MWM is a test of spatial learning which is assessed across repeated trials and reference memory which is determined by the preference for the platform area when the platform is absent (probe trial) [15]. Wistar rats exposed to different light treatments over a period were capable of learning the location of the escape platform, as revealed by the decline noticed in the latency to find the platform with subsequent trials at day 60 and progressive reduction at day 90. However, learning was evident in rats exposed to blue light. This agrees with the report of [24] that the blue light spectrum enhances memory. Long term exposure to blue light seemed to improve memory in the rats while memory deficit was evident in rats exposed to darkness and red light treatment.

Dopamine and serotonin are neurotransmitter s that play important roles in the vertebrate nervous system [11]. Dopamine is important in neuronal circuitry that controls reward and regulates movement [12]. Dopamine is also associated with a state of euphoria while serotonin regulates mood and pleasure [13,25]. In our study, serotonin and dopamine concentrations were higher in rats exposed to darkness but significantly higher with time in the rats exposed to red light. Brain serotonin depletion has been shown to lead to anxiety, decrease in exploration and impairs short term memory in rats [22]. Therefore, an increase in locomotion and exploration in rats exposed to blue light at day 60, and in red light at day 90 might be linked to the increase in serotonin activity at these respective periods. Hence, the observed behavioural changes in this study might be linked to the changes in the activity of serotonin. This could also be responsible for the progressive learning ability observed [22].

Previous studies have shown that reduction in the activity of dopamine and serotonin decreased with age [9], and was responsible for some behavioural changes in old age such as depression [7]. Hensler [8] submitted that elevation in serotonergic neurotransmission enhanced attention and recognition of positive emotional material. Administration of red lights to aged people may serve to ameliorate this effect.

Conclusion

Light treatments affected locomotion, exploration and anxiety in Wistar rats, but this varied with days of exposure and spectra of light. Blue light spectrum enhanced memory and learning while darkness and red light led to memory deficit. In addition, this study has provided information that red light increased the activities of dopamine and serotonin. Blue light reduced the activity of dopamine and increased the activity of serotonin with time of exposure. Further research should be conducted on the underlying basis of the ability of blue and red light at night to improve locomotory activities, enhance memory and reduce depression.

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