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BEHAVIOURAL IMPLICATIONS OF EXPOSURE TO ULTRAVIOLET AND INFRARED LIGHTS ON WISTAR ALBINO RAT

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ABSTRACT

The crucial role played by different light wavelengths can evoke different behavioural responses in animals, especially when introduced at night. This study investigated the effects of exposure to near Ultraviolet (UV) and near Infrared (IR) lights on exploratory, memory and antidepressant behaviour of male Wistar albino rat (*Rattus norvegicus*). Thirty weaned male rats (30.02±5.82 g) were exposed to daylight (6 AM to 6 PM) and 6 hours of artificial lights of varying wavelengths (UVA-365 nm, UVA-396 nm, IRA-850 nm and IRA-940 nm) at night for 90 days. The control groups were exposed to darkness (DRK) and ambient light (AML), respectively. Light treatments and control were replicated five times. Behavioural outcomes were measured using the Open Field Test (OFT), Forced Swim Test (FST) and Novel Object Recognition Test (NOR). The highest immobility time in FST was highest in DRK (110.00±6.33 s) while UVA 365 nm had the lowest immobility time (41.60±23.72 s). In the OFT, rats exposed to ultraviolet A (365 nm) light showed significantly ($p < 0.05$) higher exploratory and non-depressive behaviour: centre square duration (44.40±10.46 s), grooming duration (110.80±28.05 s), rearing duration (103.40±38.56 s). Rats exposed to UVA (396 nm) had the highest discrimination index for the novel object (0.03) in NOR test. In conclusion, exposure of male Wistar rats to ultraviolet and infrared lights of varying wavelengths had significant impact on the depressive, memory and exploratory behaviour.

Keywords: Behaviour, Ultraviolet light, Infrared light, Wistar albino rat

INTRODUCTION

Natural light, sunlight, is a crucial aspect of the environment that has considerable impact on the physiology of animals. It serves as a major source of information and energy, affecting the life-sustaining behaviour, development, and wellbeing of animals (Longcore and Rich, 2004). Exposure to sunlight plays significant roles in facilitating sight, regulating circadian rhythms which acts internal biological clocks that regulate various physiological processes such as hormone secretion, sleep-wake cycle and general metabolism (Rea *et al.*, 2010). The daily pattern of sunlight exposure has been greatly altered for years through the introduction of artificial light from diverse sources, especially at night (Gaston *et al.*, 2015). Artificial light disrupts the natural light exposure pattern to animals and is recognized as a global threat especially at night (Longcore and Rich, 2004;

Gaston *et al*., 2015). The direct and indirect effects of light on animal physiology is dependent majorly on the nonvisual pathway of light as it enters through the eyes and converted to electrical and nerve signals by special photoreceptors called Intrinsically Photosensitive Retinal Ganglion Cells (ipRGCs) – Pickard and Sollars, 2012. The ipRGCs project to several direct and indirect sites in the brain including the hypothalamus, midbrain, thalamus, amygdala, cerebral cortex, hippocampus, and striatum (Pickard and Sollars, 2012).

Studies have shown that exposure to light significantly enhances brain function, alertness and performance of activity (Cajochen *et al*., 2000; Phipps-Nelson *et al*., 2003; Perrin *et al*., 2004; Vandewalle *et al*., 2006) and also significantly affect the behaviour or emotions of animals by inducing depressive -like behaviours in rodents (Ashkenazy *et al*., 2009; Fonken *et al*., 2009). Conversely, these reported effects depended on the wavelength of the light, duration of exposure and time of exposure (Ashkenazy *et al*., 2009; Fonken *et al*., 2009).

Ultraviolet light (UV) has been linked to mood enhancement and relaxation and anxiety (Sivamani *et al*., 2009). Multiple studies using insects (Schultheiss *et al*., 2016; Negelspach *et al*., 2018), fishes (Shcherbakov *et al*., 2012; Novales Flamarique, 2013), reptiles (Bajer *et al*., 2011), birds (Smith *et al*., 2002; Secondi *et al*., 2012; James *et al*., 2018) and mammals (Tyler *et al*., 2014) have shown that UV and IR wavelengths are important behavioural determinants in foraging, orientation/disorientation and attraction/repulsion, reproduction, circadian rhythms and mood such as anxiety and depression.

Several studies on the effects of light on the physiology of Wistar rat have been conducted in Nigeria (Dedeke *et al*., 2017; Kehinde *et al*., 2023). However, a large portion of the works reported the effects of conventional light colours as red, blue, yellow and green on these organisms. Thus, leaving a gap of knowledge on the effects of other light wavelengths such as the ultraviolet and infrared light that are also exposed to animals and humans. A recent study carried out in Nigeria reports that the invisible light spectrum can have profound impact on the behaviour and neurotransmitter levels in Albino Rats (Dedeke *et al*., 2021). There is the need to document the findings on the effects of invisible wavelengths (ultraviolet and infrared) on the behaviour of rat.

This study was therefore designed to determine the pathological changes observed in selected brain sites of Wistar Albino rat exposed to ultraviolet and infrared lights, as well as, identify the behavioural changes associated with such exposure.

MATERIALS AND METHODS

Study Area

The study was conducted in the Experiment Unit of the Animal House at the Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria (Latitude: 7.2292, Longitude: 3.4364).

Study Animals

Thirty weaned male rats (30.02±5.82 g) were bought from Crystal Animal Farms, Abeokuta, Ogun State. The rats were acclimatized in well-ventilated plastic cages of 15 cm × 7 cm × 7 cm for 7 days under normal room temperature and fed with pelletized feed (10% body weight daily) and water (*ad libitum*).

Study Design

A completely randomized study design was employed for the study (Table 1). After acclimatization, the rats were divided into six (6) different light treatments of one (1) rat per treatment and replicated five times. The light treatments were: Ultraviolet light A (UVA - 365 nm), Ultraviolet light A (UVA – 396 nm), Infrared light A (IRA – 850 nm), Infrared light A (IRA – 940 nm), Ambient light (AML - positive control) and Darkness (DRK - negative control).

Table 1: Study design

Light Treatments	Wavelength (nm)	Number of Rats in each Replicate Cage	Replicates	Number of Rats Per Treatment
Ultraviolet A (UVA)	365	1	5	5
Ultraviolet A (UVA)	396	1	5	5
Infrared A (IRA)	850	1	5	5
Infrared A (IRA)	940	1	5	5
Ambient Light (AML)	Control (positive)	1	5	5
Darkness (DRK)	Control (negative)	1	5	5

Light Exposure

Study animals were exposed to the different light treatments (UVA 365 nm, UVA 396 nm, IRA 850 nm, IRA 940 nm, AML and DRK) for 6 hours daily at night (8 pm – 1 am) for 90 days (chronic exposure) (USEPA, 2023). The lights were generated from 3 watts LED lights powered by an inverter.

Behavioural analysis

At the end of 90 days of exposure, one rat from each light treatment and its replicate were subjected to the following behavioural tests prior to sacrifice: Forced Swim Test (FST), Novel Object Recognition Test (NOR) and Open Field Test (OFT). The OFT and NOR tests were conducted first, following 24 hours rest period before the FST.

Forced Swim Test (FST)

The FST was conducted in a 20 cm diameter by 60 cm height cylinder filled with water at 23 ± 1 °C as described by Porsolt *et al.* (2001) and Yankelevitch-Yahav *et al.* (2015) - Plate 1a. Each rat was placed in the FST cylinder for 5 minutes and video recorded. The water was changed after each test session and videos were scored by an observer for the following behaviours: immobility (time spent immobile) and mobility (time spent mobile).

Novel Object Recognition test (NOR)

The NOR test was conducted in an open field of 60 cm × 60 cm open field by 50 cm high walled wooden chamber in standard lighting conditions with two different kinds of objects (Plate 1b). Both objects were consistent in height and volume but different in

shape, colour and appearance. The NOR test was divided into three phases with 24-hour interval in-between (Lueptow, 2017). First, in the habituation phase, each rat was placed in the NOR test field without any object for 10 minutes. Second, in the familiarization phase, the rat was exposed to the NOR test field with two identical objects (A1 and A2) for 10 minutes. Third, in the test phase, the rat was introduced into the NOR test field with one familiar object from the previous phase and a novel/ different object (A1 and B1) for 10 minutes. All behaviour during each phase was video recorded and scored by an observer for the following: Time spent with familiar object and time spent with novel object. The Discrimination Index (DI) was calculated as the discrimination measure (DM) divided by the total time spent exploring both novel object and familiar object where DM is time spent exploring novel object - time spent exploring familiar object.

Open Field Test (OFT)

The OFT was carried out in a 60 cm × 60 cm open field surrounded by 50 cm high walled wooden chamber in standard lighting conditions as shown in Plate 1c (Vollert *et al.*, 2011). The floor of the field was divided into a 4 × 4 grid with a central zone (4 squares in the middle) and a periphery zone (squares close to the field walls). Each rat was placed in the OFT test field for 5 min and video recorded. The OFT test field was clean with 70% ethanol after each test session. Videos were scored by an observer for the following behaviours: Centre Square Entries, Centre Square Duration, Peripheral Square Entries, Peripheral Square Duration, Groom Frequency, Grooming Duration, Rearing Frequency, Rearing Duration; Freezing Frequency, Freezing Duration, and Time Spent Moving (Crawley, 1985).

Statistical Analysis

Data collected were analyzed using One-way Analysis of Variance (ANOVA). Mean (±Standard deviation) were compared and further separated using the Student Newman -Kuel's multiple range test with least significant difference at $p < 0.05$.

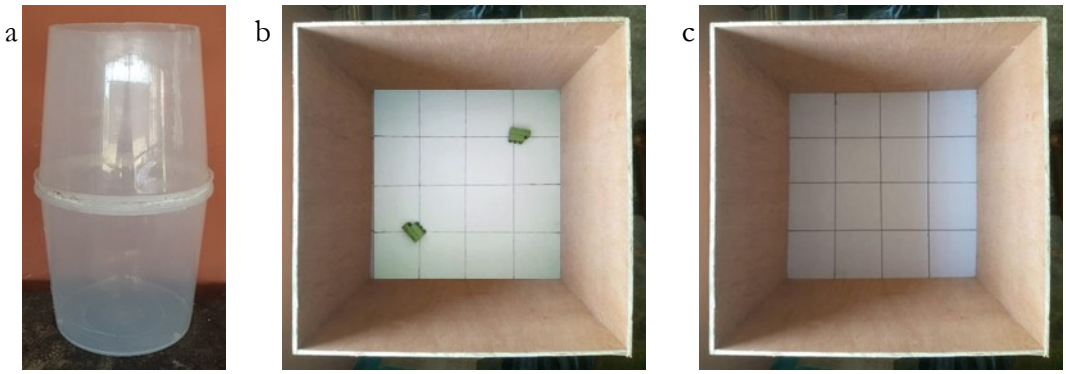

Plate 1: Forced Swim test container (a), Novel Object Recgonition Test Maze (b) and Open Field Test Maze (c)

RESULTS

Forced Swim Test (FST)

The results of the FST (Table 2) showed that immobility was significantly ($P < 0.05$) higher in rats exposed to DRK (110.00±6.33 s) and UVA 396 nm (78.20±4.97 s) and lowest in UVA 365 nm (41.60±23.72 s). In ascending order, the immobility time followed this pattern: UVA 365 nm < AML < IRA 940 nm < IRA 850 nm < UVA 396 nm < DRK. Mobility was significantly ($P < 0.05$) lower in rats exposed darkness (250.00±6.33 s), UVA 396 nm (281.80±4.97 s), IRA 850 nm (294.20±4.39 s), IRA 940 nm (297.60±14.95 s) compared to the positive control (315.40±4.04 s) while UVA 365 nm (318.40±23.72 s) had the highest mobility. In ascending order, the immobility time followed this pattern: DRK < UVA 396 nm < IRA 850 nm < IRA 940 nm < AML < UVA 365 nm.

Table 2: Mobility and Immobility duration during FST (Mean±SD)

Light Treatment	Immobility (s)	Mobility (s)
	(*n* = 5)	
Darkness	110.00±6.33[c]	250.00±6.33[a]
Ultraviolet A 365nm	41.60±23.72[a]	318.40±23.72[c]
Ultraviolet A 396nm	78.20±4.97[b]	281.80±4.97[b]
Ambient light	44.60±4.04[a]	315.40±4.04[c]
Infrared A 850nm	65.80±4.39[b]	294.20±4.39[b]
Infrared A 940nm	62.40±14.95[b]	297.60±14.95[b]

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

Novel Object Recognition test (NOR)

The results of the NOR test (Table 3) showed that the exploration duration during the training phase was significant highest in rats exposed to ambient light (75.00±28.75 s) and lowest in rats exposed to IRB (21.40±6.81 s). The other light treatments were not significantly different but showed different exploration duration following this pattern: DRK > IRA > UVA > UVB (Figure 2). During the test phase, there were no statistically significant differences in exploration duration between the familiar object and novel object. Conversely, rats exposed to UVA 396 nm had a positive discrimination index (0.02) compared to the negative and positive control (DRK: -0.22 and AML: -0.14) respectively with high negative discrimination index. Other light treatment groups were also negative.

Table 3: Novel and Familiar Object Exploration duration during NOR (Mean±SD)

Light treatments	Training phase	Testing phase	
	Object A + B (s)	Object A (s)	Novel Object (s)
		(*n=5*)	
Darkness	43.80±13.96[a]	38.80±1.79[a]	24.60±5.95[a]
Ultraviolet A 365nm	32.60±11.68[a]	26.80±6.15[a]	25.40±5.51[a]
Ultraviolet B 396nm	26.20±6.27[a]	23.80±10.76[a]	25.20±2.69[a]
Ambient light	75.00±28.75[b]	33.60±17.17[a]	25.60±3.58[a]
Infrared A 850nm	38.40±13.82[a]	19.40±5.51[a]	17.80±2.17[a]
Infrared B 940nm	21.40±6.81[a]	34.40±14.66[a]	25.4±4.93[a]

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

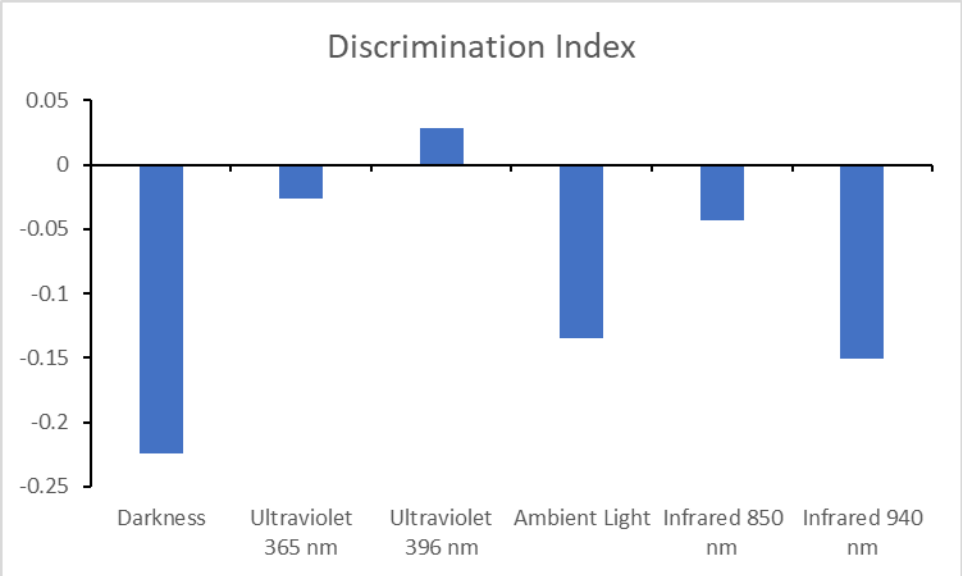

Figure 2: Discrimination Index for novel object during NOR Test Phase

Open Field Test (OFT)

Centre square entries (CSE) was significantly higher in rats exposed to UVA 365 nm (12.60±3.51) compared with the positive and negative controls (AML: 6.00±1.59, DRK: 4.20±1.31) respectively while the least significant CSEs was recorded in those exposed to UVA 396 nm (3.00±1.42) – Table 4. There was no significant difference in centre square duration when compared with the positive control but was significantly higher in UVA 365 nm (44.40±10.46 s) compared with UVA 396 nm (10.00±3.81 s). Grooming frequency was significantly higher in rats exposed to IRA 850 nm (34.80±22.95) compared with the positive

and negative controls (AML: 11.80±3.77, DRK 10.20±5.17) respectively while other light treatments were not significantly different from the positive and negative controls. There were no significant differences observed in grooming duration, rearing frequency and duration, and freezing frequency and duration of rats exposed to all light treatments. Defecation was significantly higher in rats exposed to IRA 940 nm (4.40±2.7) compared with the positive control (0.00±0.00) while other light treatments were not significantly different from the positive and negative controls. There was no significant difference observed in total time spent moving in rats exposed to all light treatments.

DISCUSSION

The results from this study shows that ultraviolet and infrared light have significant but varying effects on the behaviour of male Wistar albino rats. FST is a behavioural study of depressive-like behaviour in rodents (Yankelevitch-Yahav *et al*., 2015) because it can induce physiological stress via forced swimming (Porsolt *et al*., 2001). The time spent immobile during FST is an indication of depressive-like symptoms in rodents. The highest and significant immobility recorded in this study was in the rats under total darkness and was consistent with the study of Gonzalez and Aston-Jones (2008) who reported that the % mean duration of immobility was higher in rats under darkness than those under ambient condition (+151%, P = 0.08). Studies had shown that several neurotransmitters and hormones that regulate mood and depression are down-regulated at night and up-regulated during the day (Saladin *et al*., 2014). Exposure to total darkness may reduce levels of monoamines such as serotonin (5-HT), dopamine, and norepinephrine, neurotransmitters which are implicated in mood regulation and depression (Cryan *et al*., 2002) as reflected in increased immobility for rats exposed to darkness in this study. Studies indicate that light exposure enhances serotonergic activity, while darkness can diminish it (Cryan *et al*., 2002; Dedeke *et al*., 2021). The highest mobility recorded in the rats exposed to ultraviolet A in this study supports the claim by Sivamani *et al.* (2009), which links ultraviolet to mood enhancement and relaxation and anxiety.

The Novel Object Recognition test (NOR) is used to evaluate cognition, especially recognition memory (Lueptow, 2017). The most commonly used measure is the discrimination index which is not influenced by differences in exploration time and falls between -1 and +1 (Lueptow, 2017). LeGates *et al.* (2012) demonstrated that ultradian lighting—light exposure following cycles shorter than the typical 24-hour circadian rhythm—could increase the discrimination index in rodents as shown in rats exposed to UVA 365 nm and UVA 396 nm in this study. The discrimination index, a measure of the ability to differentiate between familiar and novel objects, reflects improvements in recognition memory and cognitive function. This enhancement is linked to the activity of melanopsin-expressing neurons, which are sensitive to light intensity and wavelength. Melanopsin-based signalling pathways influence mood, cognition, and alertness, independent of the traditional rod and cone photoreceptor systems in the retina (Hattar *et al*., 2002; LeGates *et al*., 2012). Conversely, Faborode *et al.* (2022) revealed that exposure to prolonged unpredictable light significantly impairs spatial learning and memory in rats. This impairment is attributed to oxidative stress and increased levels of pro-inflammatory cytokines, particularly tumour

necrosis factor-alpha (TNF-α), within the hippocampus with no significant effects of aberrant lighting on object recognition memory or locomotor activity.

Table 4: OFT parameters (Mean±SD)

Parameters	LIGHT TREATMENT					
	Darkness	**Ultraviolet 365nm**	**Ultraviolet 396nm**	**Ambient light**	**Infrared 850nm**	**Infrared 940nm**
CSE	4.20±1.31^{a}	12.60±3.51^{c}	3.00±1.42^{a}	6.00±1.59^{ab}	11.00±7.69 ^{b}	7.00±1.59^{ab}
CSD(s)	19.60±12.90^{a}	44.40±10.46^{b}	10.00±3.81^{a}	21.80±12.80^{ab}	43.60±21.84^{b}	22.80±9.34^{ab}
PSE	4.20±1.31^{a}	12.60±3.51^{a}	3.00±1.42^{a}	5.60±1.15^{a}	10.60±7.20^{a}	7.00±1.59^{a}
PSD (s)	580.40±12.9^{c}	555.80±10.43^{a}	590.20±3.97^{c}	578.40±12.74^{ab}	556.40±21.84^{a}	577.20±9.34^{ab}
GF	10.20±5.17^{a}	12.60±0.90^{a}	13.20±4.77^{a}	11.80±3.77^{a}	34.80±22.95^{b}	11.60±8.21^{a}
GD (s)	46.80±14.54^{a}	106.00±36.77^{a}	110.80±28.05^{a}	83.20±21.61^{a}	97.40±29.33^{a}	73.80±62.41^{a}
RF	40.20±7.09^{a}	46.60±17.23^{a}	40.40±19.20^{a}	32.40±12.43^{a}	38.20±18.34^{a}	40.40±10.67^{a}
RD (S)	95.60±17.51^{a}	103.40±38.56^{a}	75.80±47.89^{a}	80.40±35.51^{a}	80.20±52.86^{a}	92.80±21.76^{a}
FF	6.20±3.04^{a}	2.60±0.55^{a}	4.80±1.93^{a}	7.40±4.45^{a}	4.00±2.24^{a}	4.00±3.17^{a}
FD (s)	12.80±5.50^{a}	5.40±1.95^{a}	11.60±6.88^{a}	26.80±26.61^{a}	17.00±9.36^{a}	12.20±9.45^{a}
Defecation	3.40±1.15^{ab}	3.20±2.17^{ab}	1.60±2.31^{ab}	0.00±0.00^{a}	3.20±2.17^{ab}	4.40±2.7^{b}
TSM (s)	505.20±24.48^{a}	445.80±14.28^{a}	462.20±37.36^{a}	470.00±51.16^{a}	465.80±34.24^{a}	481.60±55.83^{a}

Means with the same superscript in a row are not statistically significantly different ($p > 0.05$). CSE: Centre Square Entries; CSD: Centre Square Duration; PSE: Peripheral Square Entries; PSD: Peripheral Square Duration; GF: Groom Frequency; GD: Grooming Duration; RF: Rearing Frequency; RD: Rearing Duration; FF: Freezing Frequency; FD: Freezing Duration; TSM: Time Spent Moving

The open field test (OFT) provides concurrent measure of exploratory, locomotory and general activity and anxiety behaviour in rodents (Gould *et al*., 2009). Rats exposed to ultraviolet (365 nm) recorded higher centre square entries and duration, rearing frequency and duration and decreased freezing frequency and behaviour which are indicators of mobility, exploratory activity and lower anxiety (Walsh and Cummins, 1976). The significant increase in grooming and defecation observed in rats exposed to infrared light (850 nm and 940 nm) and darkness are indication of anxiety-like behaviours (Estanislau, 2012) and suggests increased anxiety (Voiculescu *et al*., 2016).

CONCLUSION

Exposure of male Wistar albino rats to ultraviolet and infrared lights of different wavelengths has significantly varying effects on depressive, memory and exploratory behaviour. The findings from this study infer that ultraviolet light can be used to improve memory and encourage exploratory activities while infrared light and darkness can induce depressive behaviour. Further research on other behaviours is recommended to understand the far-reaching impact of ultraviolet and infrared light. alpha (TNF-α), within the hippocampus with no significant effects of aberrant lighting on object recognition memory or locomotor activity.

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