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# IMPACT OF LIGHT PATTERNS ON PSYCHOPHYSICAL ACTIVITY: A FOCUS ON REPRODUCTIVE HORMONES IN MALE WISTAR RATS

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## Abstract:

**Light is a fundamental environmental factor that profoundly influences various physiological and behavioral processes in animals, including humans. This study aimed to investigate impact of light patterns on psychophysical behavior (such as depression-like behavior, anxiety-like behavior, social interaction), reproductive hormones (including testosterone, LH, FSH, and GnRH) in Wister rats. A total of twenty-eight male Wistar rats were grouped into four (4) groups: Group I: Control group which received normal light, Group II: Rats kept in total darkness (no light source), Group III: Rats expose to Tonic/constant light, Group IV: Rhythmic light (off and on flickering light). Data were analyzed using GraphPad Prism v9.0 version.Results demonstrated that exposure to rhythmic light, total darkness, and bright light induced depression-like behavior, with rhythmic light having the most pronounced effect. Anxiety-like behavior was heightened in rats exposed to rhythmic light, aligning with disrupted light-dark cycles inducing anxiety-like symptoms. Social interaction was negatively influenced by total darkness and bright light, while rhythmic light promoted positive social behavior. Reproductive hormone levels, including testosterone, LH, FSH, and GnRH, were significantly impacted by light patterns. Bright light exposure was associated with increased FSH levels, while rhythmic light suppressed FSH production. However, GnRH levels were elevated by bright light and reduced by other light patterns. Additionally, exposure to total darkness led to increased body weight in male rats, whereas rhythmic light was linked to reduced weight gain. The study underscores the intricate relationship between light patterns and physiological responses, contributing to a nuanced understanding of how light exposure influences behavior and hormonal regulation.**

## Keywords:

**light patterns, social depression, reproductive hormones.**

## Introduction

Light is a fundamental environmental factor that profoundly influences various physiological and behavioral processes in animals, including humans (Bellastella, 2014). Science has become much more interested recently in how changes in light patterns impact hormone regulation and psychophysical activity. Changes in people's natural light-dark cycles have been connected to a number of health issues, including sleep issues, mood swings, and cognitive deterioration (Cho *et al.*, 2017). The extensive use of artificial lighting and the rise in shift work have caused inconsistent exposure to light patterns, which might have an adverse effect on people's health and wellbeing (LeGates*et al.*, 2012). Understanding the complex interplay between hormone levels, behavior, and light exposure is crucial for basic study as well as prospective applications in areas like chronobiology, neurology, and human health (Moustafa, 2020). Light exposure, a significant environmental signal, controls the circadian rhythm, which in turn affects a number of physiological functions, including sleep-wake cycles, metabolism, and hormone synthesis (Steele et al., 2021; Boivin *et al.*, 2012). The hypothalamic suprachiasmatic nucleus (SCN), the main circadian pacemaker, regulates the timing of physiological and behavioral activities in response to cycles of light and dark (LeGates*et al.*, 2014). Unreliable shift work or constant exposure to artificial light can interfere with the circadian system's normal function, which can be harmful to one's health (Cho *et al.*, 2015; Pilorz*et al.*, 2016).

Reproductive health has an impact on the quality of life. An essential organ for the synthesis and metabolism of hormones is the testis. The gonadotropin-releasing hormone (GnRH), a key hypothalamic signal to the anterior pituitary, controls the process of spermatogenesis. This process is mediated by the hypothalamic-pituitary-testicular axis. In addition to being expressed in the brain, GnRH1 and its receptors are also found in the rodent testes, which regulate the generation of sperm of excellent quality (Ciaramella *et al.*, 2015). GnRH releases luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary, which control testicular activity and spermatogenesis (Ramaswamy and Weinbauer., 2015). Animals' testicular growth and regress are photoperiod-dependent, indicating that the production of melatonin on a circadian basis is an important part in these processes (Boyd, 1985). Gonadal hormones in male rats, particularly testosterone, have been revealed to have a significant role in behavior regulation. Through hereditary and non-genetic routes, testosterone affects brain regions involved in cognition, aggression, and sexual behavior (Arnold, 2009; Frye *et al.*, 2008). Additionally, testosterone levels fluctuate throughout the day, with mornings frequently seeing increases (Randler *et al.*, 2012). The natural circadian rhythm of testosterone secretion can be disturbed by disturbances in the light-dark cycle, which may have an effect on behavior and general wellbeing (Sellix, 2015).

Mammals have many physiological and behavioral processes regulated by endogenous biological rhythms; when these rhythms are out of sync, reproductive problems result. Melatonin, cortisol, thyroid-stimulating hormone (TSH), and to a lesser extent prolactin all exhibit endogenous circadian cycles (Souissi*et al.*,2022).

Studies on both people and animals have demonstrated that disturbed light patterns have a negative impact on mood, cognition, and general well-being (Evans *et al.*, 2017; Fonken *et al.*, 2010). For instance, studies on mice have shown that exposure to constant light, which disrupts the natural cycle of light and dark, can alter locomotion, increase anxiety-like behavior, degrade cognitive function, and interfere with hormone regulation (Bedrosian *et al.*, 2013; Fonken *et al.*, 2009). On the other side, studies utilizing light-dark cycles have demonstrated improvements in thinking ability, mood regulation, and general behavioral health (Ruby *et al.*, 2008; van der Zee *et al.*, 2008).

The present study could beimplacable for human health and wellbeing, particularly for nightclub employees, shift workers, prisoners, and other people who are exposed to low, flickering, or no light conditions. This is because the findings may help develop strategies for maximizing light exposure to support healthy behavior and hormone regulation.

**Materials and Methods**

The scope of this study is focused on male Wistar rats as the primary subjects. The selected psychophysical behaviors to be assessed include locomotor activity, anxiety-like behavior, and cognitive function. Reproductive hormone levels, particularly testosterone, was measured and correlated with behavior. The study examined the effects of three lighting conditions: light-dark cycles, constant light, and constant darkness.
Materials used in this study include: Capillary tube, Ethylenediamine tetra-acetic acid (EDTA) tube, Universal bottles, Plain Tube, Weighing scale, Dissecting Kit, Cold and Heat Centrifuge, Syringes and butterfly needle, Source of light, Saline solution, Beaker, Glass rod, Feeding and water troughs, formalin, Gloves, Permanent marker, Disinfectant, Cotton wool, Detergent, Cage, Hand gloves, ethanol, rags, maze, homogenizer, physiological saline, ketamine injection, distilled water, Eppendoff tube, PBF-Phosphate Buffer formalin, PBS- Phosphate Buffer Saline.

The research design that was selected for this study is a between-subjects design, with the independent variable being the different light pattern exposures: rhythmic light, constant/tonic light, and constant darkness. A total of twenty-eight (28) male rats was randomly assigned to one of the four experimental groups to ensure an equal distribution of potential confounding factors. This design allows for the comparison of the effects of different light patterns on psychophysical behavior and gonadal hormone levels. This study was carried out in the Department of Human Physiology, Faculty of Basic Medical Science, College of Health, Delta State University, Abraka, Delta state, Nigeria.

A total of 28 male Wistar rats was used in this study. The rats were obtained from the animal house of the faculty of Basic Medical Science, Delta State University, Abraka and which are approximately 8-10 weeks old at the beginning of the experiment. The animals were housed in clean and well-ventilated cages at the animal house. They were acclimatized for the period of 2 weeks prior to the experiment where they were fed daily with chow and clean tap water. The animals were maintained in accordance with the guidelines approved by the Animal Ethics Committee, Delta State University, Abraka, Nigeria. Rats that were infected with any form of disease or disorder were excluded from this study.

The twenty-eight (28) male rats were divided into four groups with each group containing seven (7) rats. The four groups were kept in four differently designed cages to suit the different treatment that was administered thus: Group I: Control group which received normal light; Group II: Rats kept in total darkness (no light source); Group III: Rats expose to Tonic/constant light and Group IV: Rhythmic light (off and on flickering light),

Housing and Light Exposure Conditions: The rats were housed individually in standard laboratory cages with bedding and ad libitum access to food and water. To ensure consistency, the temperature and humidity of the animal facility were maintained within a specified range. The rats were exposed to their respective light patterns in dedicated light-controlled chambers. The light conditions were carefully regulated using light-emitting diodes (LEDs) to provide the desired lighting conditions for each experimental group. Rhythmic Light: Rats assigned to this group were exposed to a short hour light-dark cycle. Constant Light: Rats assigned to the constant light group were exposed to continuous bright light, with an intensity of approximately 500 lux. The rats were continuously exposed to light without any periods of darkness. Constant Darkness: Rats assigned to the constant darkness group were housed in complete darkness throughout the experiment.

Behavioral psychophysical test includes open field test conducted according to the methods of Seibenhener and Wooten (2015).Depression levels in the rats were assessed using the forced swim test, a well-established paradigm for evaluating depressive-like behaviors in rodents (Yankelevitch-Yahav *et al.*,2015). Anxiety-like behaviors were assessed using the elevated plus maze and open field tests according to the method developed by Carola*et al.,* (2002). The behavior of rats in these tests were video-recorded and subsequently analyzed using behavioral analysis software. Measures such as time spent in open arms, closed arms, center zone, total distance traveled, and number of rearing behaviors were recorded to assess anxiety-like behaviors. Social interaction test was used. To initiate the test, rats are introduced to an arena containing a novel social stimulus, such as another rat and an object to the left and right of the test rat. Prior habituation to the testing environment and the use of the active phase of the rats' circadian cycle ensure consistent conditions. The test aims to measure behavior such as approach time, physical contact initiation, and general social exploration. These observations provide insights into the rats' responses to social stimuli and their interactions, potentially shedding light on psychosocial patterns. After recording the interactions through direct observation with the aid of a timer, the behaviors are analyzed to quantify the level of social interaction exhibited by the rats. Hormone Analysis Reproductive hormones were analyzed from serum using enzyme-linked immunosorbent assays (ELISA), to quantify hormone concentrations. Body weight of the experimental animals was determined at week 0 (before the experiment) and subsequent weeks and the last day of the experiment. Percentage weight gain was later calculated as follow:

Percentage weight gain (%)= $\frac{Final\ Weight - initial\ body\ weight\ (g)}{Initial\ body\ weight\ (g)}\ X\ 100$

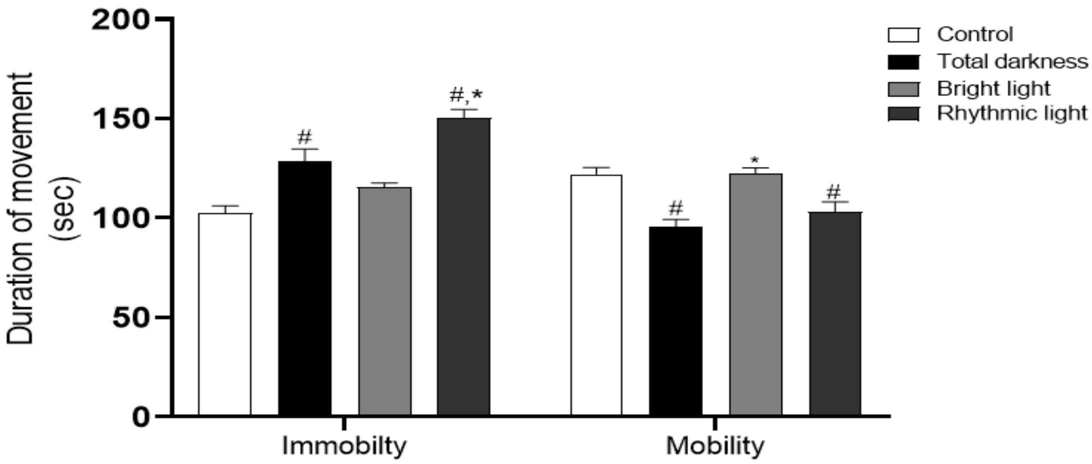
**Data Analysis**

All data were represented as Mean $\pm$ SEM using the Graph Pad Prism software, Inc., Lajolla, USA, version 8.4.3 with statistical difference at level $p< 0.05$ considered significant. Data analysis was done using one-way and two-way analysis of variance (ANOVA) followed by Turkey post hoc test for comparison between the experimental groups. $^{\#}p< 0.05$ is considered significant when all experimental groups (total darkness, bright light and rhythmic light) are compared to the control group. $^{*}p< 0.05$ is considered significant when bright light and rhythmic light is compared to the total darkness group.

**RESULTS AND DISCUSSIONS**

The results of the study suggest that exposure to rhythmic light, total darkness, and bright light can all induce depression-like behavior in male Wistar rats. All values are expressed as Mean±SEM. ANOVA followed by LSD’s multiple range tests. Values not sharing a common superscript differ significantly at P<0.05. The rats exposed to rhythmic light had the highest significantly (p<0.05)immobility time, followed by the total darkness and bright light. However, the rats exposed to the bright light did not show any significant difference with the control group immobility time.

**Figure.1 The impact of different light patterns (rhythmic light, tonic light and constant darkness) on depression-like behaviour using swim test in male *Wistar rats*.**




The results of this study are consistent with previous research that has shown that exposure to irregular or disrupted light-dark cycles can induce depression-like behavior in rodents according to Reppert and Weaver, (2002) and LeGates and Nelson, (2014). The rhythmic light used in this study is a type of irregular light-dark cycle that is characterized by periods of light and darkness that are of varying duration. This type of light exposure may disrupt the circadian rhythm of the rats, which can lead to depression-like symptoms. The results of this study also suggest that bright light may not always be beneficial for mood. While bright light therapy is often used to treat depression in humans, the results of this study suggest that it may actually induce depression-like behavior in rats. This may be because bright light can disrupt the circadian rhythm of the rats, which can lead to depression-like symptoms according to LeGates*et al.* (2012).

Also, current study evaluated another important psychophysical behavior, the anxiety-like behavior. The results of the study suggest that exposure to rhythmic light can induce anxiety-like behavior in male Wistar rats. Analysis of the time spent in each arm showed that there was no significant difference in the time spent in open arms for all the experimental groups. However, the rats exposed to rhythmic light showed significantly ($p<0.05$)reduced time of entry in the closed arm compared to the control group. This suggests that the rats exposed to rhythmic light were more hesitant to explore the closed arm, which is a common anxiety-related behavior in rodents.

**Figure.2 The effect of different light patterns on anxiety-like behavior using time of entry in male Wistar rats.**

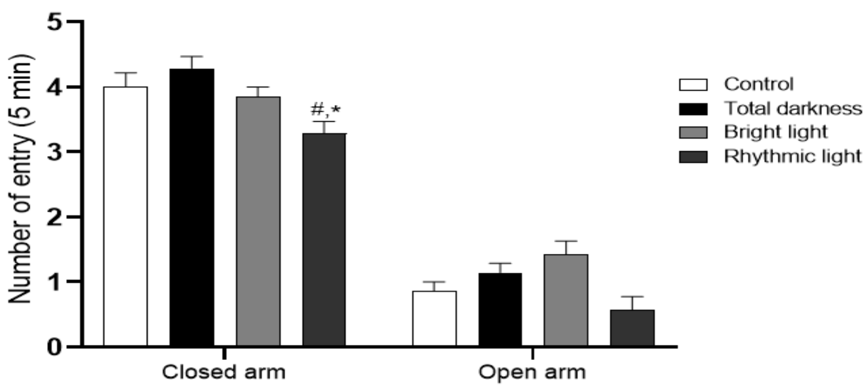
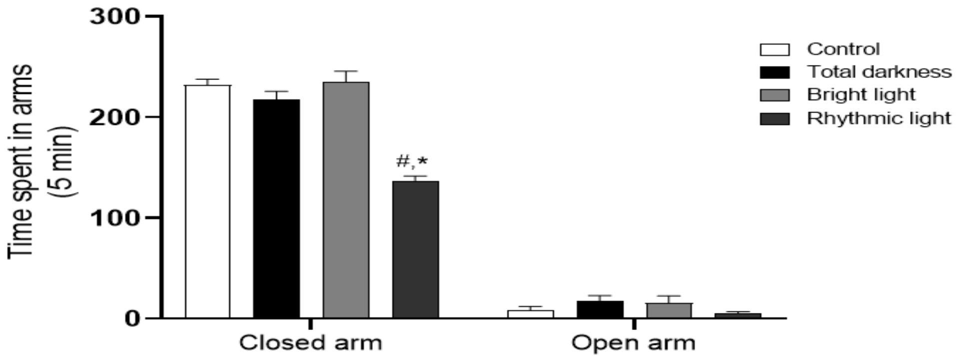


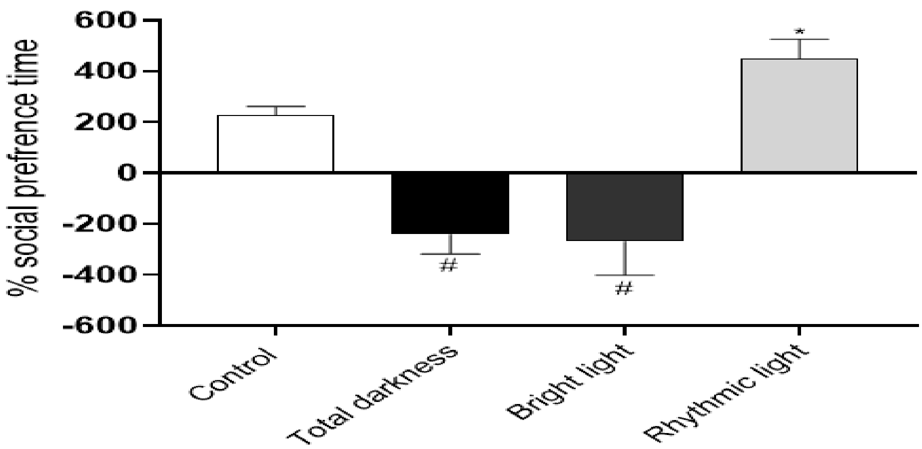
**Figure.2b: Different light patterns on anxiety-like behavior using time of entry**




These findings were consistent with previous research that has shown that exposure to irregular or disrupted light-dark cycles can induce anxiety-like behavior in rodents according to LeGates and Nelson, (2016). In another study by Reppert and Weaver, (2002) reported that rhythmic light may disrupt the circadian rhythm of the rats, which can lead to anxiety-like symptoms. The results of this study also suggest that bright light may not always be beneficial for anxiety. While bright light therapy is often used to treat anxiety in humans, the results of this study suggest that it may actually induce anxiety-like behavior in rats.

This study also evaluated the social interaction of the rats exposed to different light patterns. Rats exposed to total darkness or bright light showed negative social interaction, as measured by the NORT test (figure.3) while rats exposed to rhythmic light, however, showed significantly higher social interaction time compared to the control group. The results of these studies suggest that light can have a significant $p<0.05$ impact on social behavior in animal.

**Figure. 3 The effects of different light patterns on social interaction behavior with the aid of NORT test in male Wistar rats.**

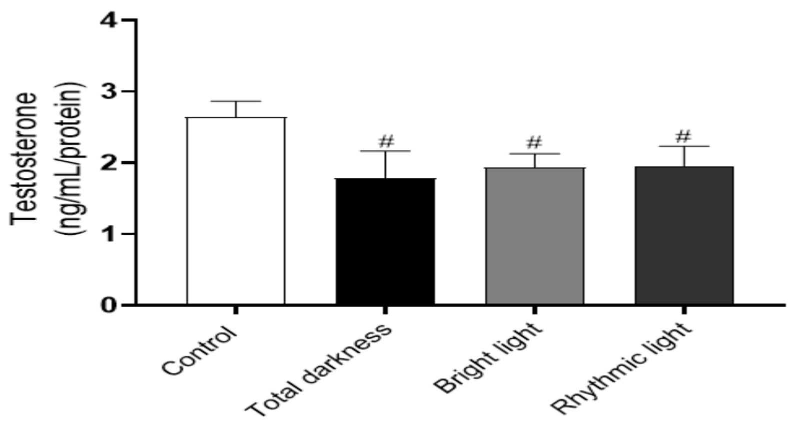


The findings of this study are consistent with a study by Abdulai-Saiku*et al.* (2017) who reported that rats exposed to bright light for 12 hours per day showed reduced social interaction compared to rats exposed to dim light for 12 hours per day. Another study by Provençal *et al.* (2012) found that mice exposed to constant light for 24 hours per day showed increased anxiety and decreased social interaction compared to mice exposed to a normal light-dark cycle.

## The effect of light patterns exposure on male reproductive hormones

This study further evaluated the effect of light patterns exposure on reproductive hormone levels specifically on serum testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and gonadotropin-releasing hormone (GnRH) levels in rats. The results showed that rats exposed to total darkness, bright light, or rhythmic light all had significantly reduced testosterone levels compared to the control group. This suggests that exposure to any type of light can suppress testosterone production in rats.

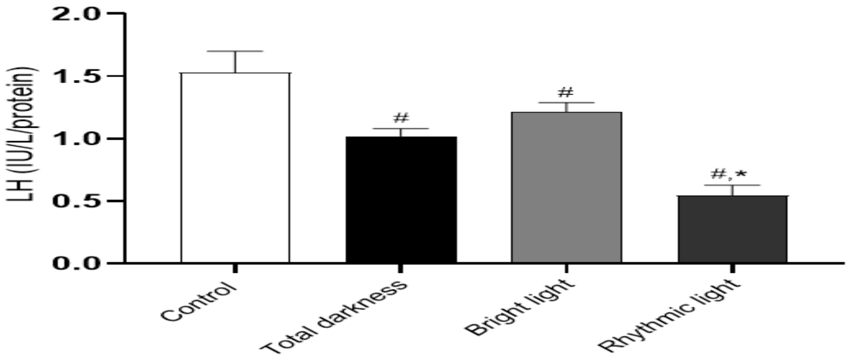
**Figure.4a: Effect of light patterns exposure onthe serum level of testosterone in male wistar rats**




This result agrees with the findings of Soreca *et al.* (2014) that investigated the effect of bright light at night on serum testosterone levels in healthy men. They reported that exposure to bright light at night suppressed testosterone production. Also, the study by Lewy *et al.* (1980) showed light pollution negative affects testosterone.

The results also showed that rats exposed to total darkness, bright light, or rhythmic light all significantly $p<0.05$ reduced LH levels compared to the control group. Rats exposed to rhythmic light had significantly lowered serum LH compared to those exposed to bright light, total darkness, and control. This suggests that exposure to any type of light can suppress LH production in rats.

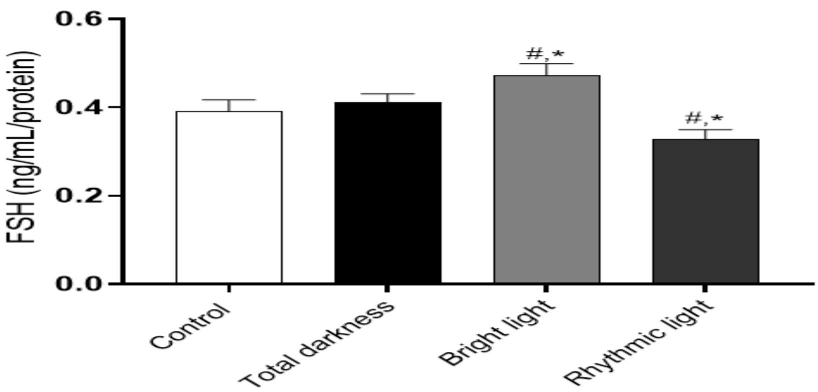
**Figure.4b: Effect of light patterns exposure on Wistar rats' serum Luteinizing Hormone (LH) levels**




The finding that exposure to any type of light can suppress LH levels in rats is consistent with the findings of other studies in humans and animals. Soreca *et al.* (2014) found that exposure to bright light at night suppressed LH levels in healthy men. Another study by Vandenbroucke *et al.* (2012) found that exposure to bright light during the day suppressed LH levels in healthy women. The suppression of LH levels by light exposure is thought to be due to the suppression of the production of gonadotropin-releasing hormone (GnRH) by the hypothalamus. GnRH is a hormone that stimulates the production of LH and follicle-stimulating hormone (FSH) by the pituitary gland. When GnRH levels are suppressed, LH levels will also be suppressed according to LeGates and Nelson, (2016). There are several possible explanations for why exposure to light can suppress LH levels. One possibility is that light exposure inhibits the production of melatonin by the pineal gland. Melatonin is a hormone that is produced in the dark and that helps to regulate the production of LH and other hormones. When light exposure inhibits the production of melatonin, it can lead to a decrease in LH levels (Evans *et al.*, 2017). Another possibility is that light exposure directly suppresses the production of GnRH by the hypothalamus. Light exposure has been shown to activate the sympathetic nervous system, which can in turn lead to the suppression of GnRH production according to Randler *et al.* (2012).

Also, we observed in this study that wistar rats exposed to bright light significantly $p<0.05$ increased FSH levels compared to the control group and rats exposed to total darkness or rhythmic light. However, rats exposed to rhythmic light had significantly reduced FSH levels compared to every other group. This suggests that bright light can stimulate FSH production in rats, while rhythmic light can suppress FSH production.

**Figure.4c: Effect of light patterns exposure on ratsserum Follicle-Stimilating Hormone (FSH) levels**



The finding that exposure to bright light can stimulate FSH production in rats is consistent with the findings of other studies in humans and animals. For example, a study by Cajochen*et al.* (2005) found that exposure to bright light during the day stimulated FSH production in healthy women. Another study by Lewy *et al.* (1980) found that exposure to bright light during the day stimulated FSH production in male rats. However, Wright *et al.* (2010) found that exposure to rhythmic light decreased FSH levels in healthy women. The stimulation of FSH production by light exposure is thought to be due to the stimulation of the production of gonadotropin-releasing hormone (GnRH) by the hypothalamus. GnRH is a hormone that stimulates the production of FSH and luteinizing hormone (LH) by the pituitary gland. When GnRH levels are increased, FSH levels will also be increased (Moustafa, 2020). The stimulation of FSH production by light exposure can have a number of consequences for reproductive health. For example, it can lead to an increase in fertility, an increase in sex drive, and an increase in breast development in women according to Evans *et al.* (2017).

**Figure. 4d: Effect of light patterns exposure on ratsserum Gonadotropin-releasing Hormone (GnRH) levels**

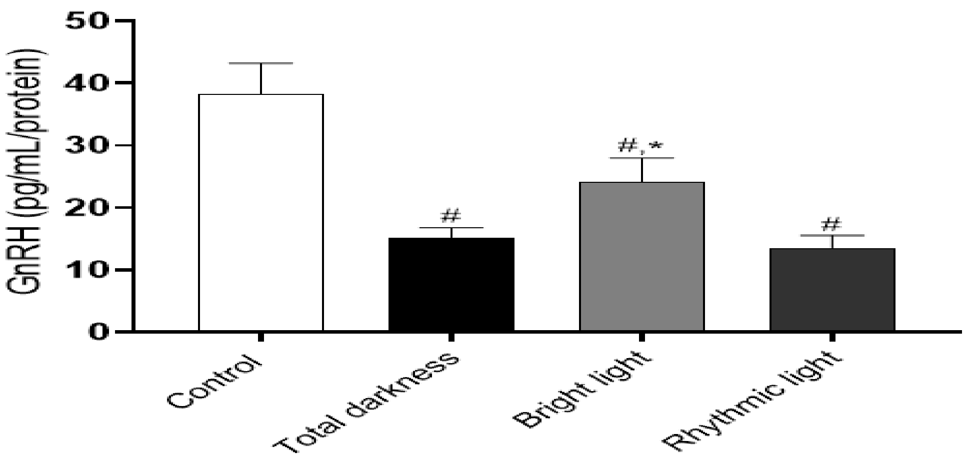



Figure.4d revealed the effect of different light pattern on serum Gonadotropin-releasing Hormone (GnRH). From the result, the rats exposed to total darkness, bright light and rhythmic light had significantly lowered serum GnRH levels. However, rats exposed to bright lights showed significantly higher levels of serum GnRH compared to rats exposed to total darkness and rhythmic light.

The finding suggesting that exposure to any type of light can suppress GnRH production in rats is consistent with the findings of other studies in humans and animals. However, rats exposed to bright light had significantly higher levels of GnRH compared to rats exposed to total darkness and rhythmic light. This suggests that bright light can stimulate GnRH production in rats, while total darkness and rhythmic light suppresses GnRH production. Lewy *et al.* (1980) investigated the effect of light exposure on the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus in male rats. They found that exposure to bright light during the day or night suppressed GnRH release, while exposure to darkness during the day stimulated GnRH release. The suppression of GnRH production by light exposure can have a number of consequences for reproductive health. For example, it can lead to a decrease in fertility, a decrease in sex drive, and a decrease in muscle mass (Chen *et al.*, 2016).

## Conclusion

This comprehensive study suggests that exposure to light can have a significant impact on physiological (reproductive hormone levels) and behavioral activities (in this case, depression, anxiety and social interactions) in response to cycles of light and dark. Exposure to light at night can suppress testosterone levels in men and FSH levels in women. This can lead to a decrease in fertility, sex drive, and muscle mass.

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