Analysis of Zeitgeber-Timed Blue Light Exposure & Circadian Rhythm Fragmentation in Aged Rat Models

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Abstract

Analyzing Silva et al.'s (2023) original research on the investigation of zeitgeber-timed blue light therapy effects on circadian rhythm fragmentation in aged rat models confirmed significant activity suppression during blue light exposure with the most pronounced effects occurring during the inactive phase differences between blue and post-blue light treatment conditions. Additionally, this research was extended to examine changes over 42 days, encompassing baseline, treatment, and post-treatment phases, which revealed phase-dependent temporal responses. Early zeitgeber blocks showed rebound effects post-treatment, while later blocks demonstrated sustained activity suppression. This extension serves as a waypoint to analyze whether treatment periods throughout a time span have varying degrees of effects on circadian rhythm, as measured by function stability. Aging has been shown to have an impact on circadian rhythm function. As seniors age, the rates of neurodegenerative diseases increase, indicating that the biological and mechanistic processes of the brain are affected due to slower cell turnover, which leads to changes in hormonal production and ultimately affects the suprachiasmatic nucleus. Applying blue light treatment in rat models has been shown to increase stability between different time periods in a circadian rhythm cycle, suggesting that this therapy can provide more predictable and robust results. This research could be expanded to analyze treatment doses, light intensity, and wavelength specificity to fully utilize these mechanisms in care settings.

Introduction

Aging is a natural, time-dependent process that involves changes at the molecular, cellular, and organismal levels over time. While aging itself is not considered a disease, senescence refers to the gradual process accompanying aging and the declines associated with it, such as the degeneration of cells in the body that makes it more susceptible to diseases. A wide range of factors can influence the aging process, including lifestyle choices, environmental factors, and genetic predispositions. Arguably, the most profound influence of aging is on circadian rhythms. Circadian rhythms are biological processes that follow an approximately 24-hour cycle, regulating key physiological functions such as sleep-wake cycles, metabolism, hormone secretion, and body temperature. (Reddy, Reddy, & Sharma, 2023). As aging progresses, circadian rhythm and synchronization further deteriorate in response to external cues.

One of the critical structures responsible for regulating these rhythms is the suprachiasmatic nucleus (SCN), a small cluster of neurons located in the hypothalamus just above the optic chiasm. (Ma& Morrison, 2023) This synchronization is vital for regulating physiological processes that exhibit a 24-hour periodicity, such as the sleep-wake cycle, hormone secretion, and body temperature (Colwell, 2011). Henceforth, as one ages, the SCN changes, including but not limited to the fragmentation of rest-activity rhythms, decreased rhythm amplitude, a short circadian period, and difficulty resynchronizing rhythms to external light-dark cycles. These changes refer respectively to disruptions of sleep and activity patterns, a weakening of the body's internal signal, a faster internal clock, and challenges in adapting to environmental time cues. The SCN structure loses neurons, weakening the condition and its ability to produce a synchronized circadian rhythm. Neurotransmitters such as GABA, glutamate, and vasoactive intestinal peptides weaken the correlation between the SCN neurons, making their signals less precise. (Duffy, Zitting, & Chinoy, 2015) This effect is evident in aging individuals, as a common feature of

circadian rhythm alterations is the tendency toward early morning awakening, often occurring involuntarily.

The most important feature is its role in synchronizing the body with environmental cues, notably light. The SCN receives direct input from the retina via the retinohypothalamic tract, which sends light signals to the retina and the suprachiasmatic nucleus (SCN). This pathway helps integrate with the body's internal clock and external dark cycles by modulating hormone secretion, including melatonin, which controls sleep cycles (Brainard et al., 2001). It allows the body to detect light levels and adjust circadian rhythms accordingly. This light information helps synchronize the internal biological clocks found throughout the body with the external day-night cycle (Hastings et al., 2018). By coordinating these processes, the SCN plays a crucial role in maintaining homeostasis and supporting adaptive behaviors aligned with the external environment (De Assis & Oster, 2021)

Light therapy utilizes specific wavelengths to regulate physiological and psychological processes, primarily thoughts and their influences on the circadian system. Different wavelengths have different effects, with blue light, which is approximately 450-950nm, being partially effective. This is due to its interaction with intrinsically photosensitive retinal ganglion cells (ipRGCs), which express melanopsin, a key photopigment in circadian entrainment. (Berson et al., 2002). Light therapy impacts the brain through multiple pathways. One approach is through the suppression of melatonin, in which blue light exposure increases wakefulness and alertness, making it a valuable intervention for sleep disorders and circadian rhythm misalignments (Gooley et al., 2010). Excessive exposure to blue light can disrupt circadian rhythms and reduce melatonin production, which may indirectly affect the balance of neurotransmitters such as dopamine and serotonin, crucial for mood regulation, attention, and overall cognitive function. FMRI studies have also shown that blue light activates the prefrontal cortex and hippocampus, enhancing memory and executive function (Vandewalle et al., 2009). These effects are attributed to the activation of

intrinsically photosensitive retinal ganglion cells (ipRGCs); the increased activation in these regions suggests potential application in treating cognitive declines associated with aging and neurodegenerative diseases.

Given the known effects of aging on circadian rhythms and activity patterns, it is important to explore potential interventions that could help mitigate these disruptions. Aging leads to significant disruptions in circadian rhythms, largely due to degeneration of the SCN and decreased neurotransmitter signaling between SCN neurons (Duffy et al., 2015). This deterioration weakens synchronization with external cues, causing fragmented sleep-wake cycles and cognitive decline. In Alzheimer's disease and other mental disorders, circadian desynchronization worsens the side effects that come with such diseases, such as agitation, confusion, and sundowning (Figueiro et al., 2014). One such intervention is blue light therapy, which has been shown to influence circadian regulation and activity levels. To assess its effects, this study examines whether blue light exposure improves the consistency of daily activity patterns in aged rats. Based on previous findings, we hypothesize that blue light therapy will enhance the stability of daily activity patterns in aged rats, reducing variability in activity levels and improving overall activity regulation. Precisely, we predict that post-treatment activity will show increased consistency compared to pre-treatment levels, reflecting a stronger alignment with circadian rhythms.

This extension project not only builds on the work of Silva et al. (2023) but also seeks to deepen our understanding of how targeted light therapy could actively counteract the biological degradation of circadian regulation in aging. By introducing blue light at a specific circadian time (ZT 0-6), we aim to determine whether this controlled exposure can reinforce the SCN’s diminished ability to synchronize internal rhythms. What makes this study particularly significant is the inclusion of both young and aged rats, allowing us to isolate age-dependent effects and explore whether the same treatment yields differential neurobehavioral outcomes. This comparative approach could inform age-specific intervention,

suggesting that blue light may be more than just a general enhancer of activity, and could serve as a neuroprotective agent in older organisms. The original project and this replication may offer a noninvasive pathway to improve quality of life in the elderly population by optimizing circadian alignment through tailored light exposure.

Replication

Methods

Subjects:

In the original paper, 33 sixteen-month-old male Wistar rats weighing 650-690g were individually housed in polypropylene cages of 40 x 32 x 17 cm, in a soundproof room. Twelve or more Wistar rats were incorporated as subjects to understand their response to certain conditions. However, they were also younger (3 months old) and were used to compare age-based variance in the effects on circadian rhythm following blue light exposure. Food in the form of rodent chow, Labina, and Purina; 3.5 kcal/g—and water was freely available. Twelve young rats, 3 months old, were used to evaluate the ontogenetic effect on the circadian rhythmicity pattern and the effect of blue light exposure on the youthful circadian rhythm. There was no disturbance to the rats except for refilling bedding, food, and water.

All rats were individually housed in Polypropylene cages (40 x 32 x 17 cm). The temperature was controlled (24 ± 1°C) with a humidity of about 50%, and the impact of sounds would be negligible due to soundproof features.

Light Conditions

Lights were turned on at 6:00am Zeitgeber Time and turned off at 18:00 ZT. Subjects were placed on a 12h:12h light:dark cycle, controlled by white lamps of 350 lux during photophase, and 2 lux during scotophase, regulated through 350 lux with white lamps for the light cycle and 2 lux for the dark cycle.

Blue light exposure occurred during the first half of the light phase (ZT 0-6) at 150 lux, with a peak wavelength ranging from 470 to 495 nm. The blue light was applied to simulate the peak effect of sunlight on the circadian rhythm, possibly altering activity patterns and providing stability. Activity sensors attached to the cages tracked the rats' movements. This gave data for their rhythm robustness, phase shifting, stabilization, and rest fragmentation.

Procedures

The aged rats were first given time to adjust to the experimental room to ensure that stress did not interfere with the results. For 14 days, they remained on a set light-dark cycle (350 lux light, 2 lux dark) with a peak wavelength of 450-470 nm before starting blue light therapy. Once the researchers recorded their baseline activity, they were then exposed to an additional blue light (150 lux, 470-495 nm) during the first half of the light phase (ZT 0-6) for another 14 days. To determine whether the effects of blue light persisted over time, the animals were returned to their original light-dark cycle for an additional 14 days before being euthanized. Additionally, their movements were tracked continuously throughout the experiment. The young control group followed the same process at first, remaining on the 14-day light-dark cycle to establish their normal activity patterns. After that, they were also exposed to blue light (150 lux, 470-495 nm) from ZT 0 to 6 for another 14 days. In total, both groups spent 28 days in the experiment to examine how blue light affected their circadian rest-activity rhythms.

Data Analysis Plan

To evaluate the results and methods of Silva et al. (2023), we conducted a replication study using data sets containing locomotor records of aged rats from osf.io, which specifically consists of quantifiable data

indicating the activity movements of the rats as monitored through the sensors. Using RStudio and R, as well as readxl, dplyr, tidyr, ggplot2, lubridate, effsize, lme4, emmeans, and car libraries, we replicated the data visualizations by generating two violin plots with box plot overlay: one comparing Blue light treatment versus White light, and another for the Post-White Blue treatment for the aged rats. To replicate the original study's statistical analysis, we used Cohen's d test, a Two-Sample t-test, and a Wilcoxon test to compare the mean activity levels and standard deviations between the Baseline group and the Post-Blue group. Violin plots were chosen to effectively illustrate the variability within each phase and ZT block since they highlight the distribution of activity levels. These plots help to reveal the consistently higher activity in the Baseline phase, as shown in the graphs. This replication aimed to confirm the accuracy and validity of the results reported in the original paper. Since we compare the Baseline and Post-Blue groups, Cohen's d test helps us understand whether there is a standardized difference in activity levels and how large this difference is. This is crucial for determining whether blue light therapy has a significant impact on activity levels. The Two-Sample t-test helps us test the hypothesis of the mean activity levels of the two groups being statistically different from each other, supporting the alternative hypothesis stating that the true difference in means between group Baseline and group PostBlue is not equal to 0 given a low p-value. Likewise, in case if the data is skewed or contains outliers that might contradict assumptions of the t-test, the Wilcoxon test provides a non-parametric test for comparing the two groups' activity levels.

Analysis

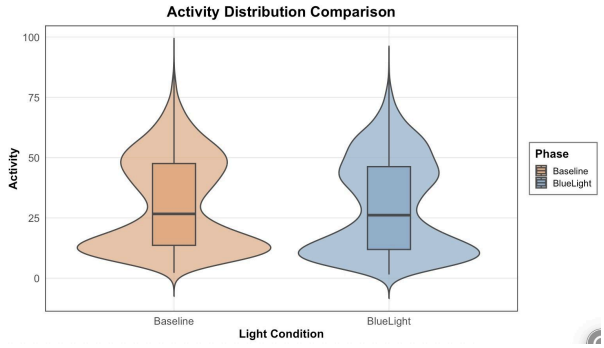


Figure 1: Blue Treatment vs. White

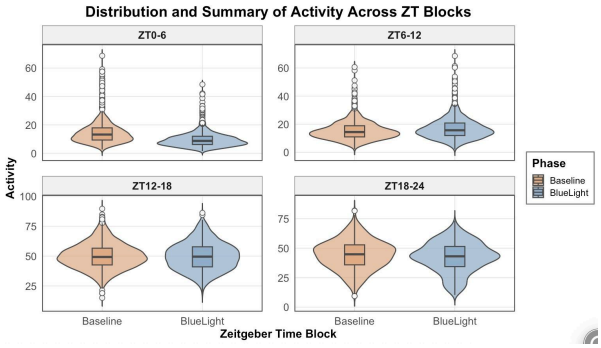


Figure 2: Post White vs. Blue Light

Analysis of Activity Level Shifts:

Using the illustration of violin plots and the data above, the first figure shows the changes in median activity as well as the variability throughout the 24-hour rest-activity distribution in aged rats under baseline white-light versus timed blue-light conditions. To directly evaluate the effects of blue light exposure on the distribution and dispersion of locomotor activity across each circadian phase, the second figure divides the data into four Zeitgeber time blocks (ZT0-6, ZT6-12, ZT12-18, and ZT18-24).

Overall mean activity with blue light decreased from 31.0 to 29.8 units throughout a 24-hour cycle, with a linear mixed-effects model corroborating this mild suppression (estimate = –1.390, SE = 0.596, t = –2.332, $p < 0.05$). This data shows consistency when looking at the phases individually.

Phase Types

1. **Inactive Phase (ZT0-6)**: The most pronounced effect occurred during the primary inactive phase, with mean activity decreasing from 15.0 ± 8.80 (n=1008) under baseline conditions to 9.80 ± 5.61 (n=1008) following blue light exposure. This robust difference ($t(2014) = 15.864$, $p <$

2.2×10^(-16)) yielded a medium-to-large effect size, with the 95% confidence interval for the mean difference ranging from 4.57 to 5.86 units.

2. **Transition Phase (ZT6-12)**: This period showed a modest but significant increase in mean activity from 15.5 ± 6.85 to 17.2 ± 8.16 (t(2014) = -4.973, p = 7.15×10^(-7), F(1,2014) = 24.7).
3. **Active Phase (ZT12-18)**: Activity during the peak activity period remained virtually unchanged (50.1 ± 11.1 vs. 49.9 ± 11.7; t(2014) = 0.383, p = 0.702, F(1,2014) = 0.146, d = 0.02), indicating preservation of normal active behaviors.
4. **Early Evening (ZT18-24)**: A small but statistically significant decrease in activity was observed from 44.3 ± 12.7 (n=936) to 42.4 ± 12.8 (n=1008) (t(1942) = 3.298, p = 9.91×10^(-4), F(1,1942) = 10.88).

The interquartile range decreased significantly under blue light, although the median activity remained approximately 25 units in both phases. This suggests that blue-light therapy successfully decreased extremes of rest and activity, thereby strengthening the stability of the circadian rhythms in aged rats.

Blue-light exposure had the greatest stabilizing effect across 6-hour Zeitgeber blocks during the animals' inactive phase (ZT0–6), when mean activity decreased from 15.0 (SD = 8.80, n = 1008) under baseline to 9.80 (SD = 5.61, n = 1008) post-treatment. This was a highly significant difference (t(2014) = 15.864, p < 2.2 × 10^(-16)), with a medium–large effect size and a 95% confidence interval for the mean difference of [4.57, 5.86] units. While peak activity (ZT12–18) stayed constant (mean = 50.1 ± SD = 11.1 vs. mean = 49.9 ± SD = 11.7; t(2014) = 0.383, p = 0.702, d = 0.02), mean activity in ZT6–12 increased slightly from 15.5 (SD = 6.85) to 17.2 (SD = 8.16; t(2014) = –4.973, p = 7.15 × 10^(-7)). Activity decreased marginally from 44.3 (SD = 12.7, n = 936) to 42.4 (SD = 12.8, n = 1008) during the early evening block (ZT18–24) - a tiny but significant effect (t(1942) = 3.298, p = 9.91 × 10^(-4)).

Phase & ZT-Block Table

Phase	ZT-Block	Mean	Standard Deviations (SD)	Number of sample rows in group (n)
Baseline	ZT 0-6	15.0	8.80	1008
Baseline	ZT 6-12	15.5	6.85	1008
Baseline	ZT 12-18	50.1	11.1	1008
Baseline	ZT 18-24	44.3	12.7	936
Post-Blue	ZT 0-6	12.2	7.04	1008
Post-Blue	ZT 6-12	13.8	6.58	1007
Post-Blue	ZT 12-18	43.0	13.1	1008
Post-Blue	ZT 18-24	41.1	11.2	1008

Blue treatment vs. White:

The blue and white treatment plots indicate medians at relatively similar levels, suggesting that the effects of those treatments are not significant. Throughout the ZT blocks, this pattern was also consistently visualized, but white light treatment showed higher median lines and overall elevated activity levels compared to blue light treatments. Quantitative analysis revealed that the median activity levels ranged from ~48-46 at the lowest. Following blue light treatment, median activity levels spanned from ~43-42 at the lowest. This connects to ZT blocks 12-18 and 18-14. ZT 0-6 and ZT 6-12 showed activity levels for white light in lower intervals, initially at about 13-15. Blue light also showed increasing activity levels, ranging from 11.5-12.5, which is still lower than the results for white light. Overall, white light treatment elicited greater activity levels, with minimal differences in range; however, these differences were relative to the activity levels of blue light treatment.

Post-White Blue:

The second figure compares the activity patterns across different periods of the Baseline and Post-Blue light phases. The x-axis represents Zeitgeber Time (ZT) blocks, which are divided into four segments: ZT 0-6, ZT 6-12, ZT 12-18, and ZT 18-24. These ZT blocks correspond to different phases of the light-dark cycle. During ZT 0-6 and ZT 6-12, which correspond to the light phase, the post-blue light violin plots have clustered data points ranging from activity levels 0 to 60, with a median of lower activity, around 15-20. With the most dense points between 5 and 25 levels, the distributions are narrower, which may indicate less variability. The aged rats are less active during this time than during the other ZT phases. The baseline activity levels have wider activity distributions, with activity levels ranging from 0 to 75 and a median activity roughly around 25-30, meaning there is more variability or sporadic activities before blue light exposure. This suggests that blue light exposure helped the aged rats better regulate their circadian rhythms, leading to more consistent and predictable activity patterns with less overall variability after exposure.

Discussion

The findings suggest that blue light exposure alters activity patterns in aged rats, reducing nighttime movement and producing effects beyond the treatment periods. (Ryabinina et al. (2024)) This suggests that blue light exposure significantly reduced rat's nocturnal activity. This suppression of nighttime movement was particularly notable because, under normal conditions, rats tend to be more active during the night, reflecting their natural circadian rhythms. This has potential implications for understanding how light-based treatments might regulate circadian rhythms in aging populations, as the aging population tends to experience more sleep disruption during the night on average (Miner 7 Kryger,

2017). The suppression of activity in response to blue light may provide insights into how artificial lighting environments impact individuals with disrupted sleep cycles, such as those with neurodegenerative diseases or sleep disorders, as well as the older population. For example, senior care facilities may benefit from incorporating specific lighting strategies to improve the sleep-wake cycles of their residents. Similarly, individuals with sleep disorders that experience disturbed circadian rhythms could potentially benefit from exploring optimal lighting conditions, such as blue light or white light, as a non-invasive intervention to improve their quality of life. Further research is needed to examine the reversibility of blue light's effects and whether similar patterns occur across different age groups or species, as well as to evaluate the long-term effects of these types of interventions on sleep and cognitive function (Ryabinina et al., 2024).

Blue light exposure helped the aged rats regulate their circadian rhythms, with post-blue light exposure coinciding with a decrease in overall activity variety. The results suggest that the effects of blue light exposure persist beyond the treatment period, indicating a potential long-term impact on circadian regulation. Further studies should investigate whether these persistent effects are consistent over extended periods. If the results were persistent, this could imply significant benefits in improving exposure to certain types of light, depending on the time of day and the age group exposed.

Research related to sleep disorders that impact circadian rhythms has found similar results, with timed light exposure proving beneficial in regulating the body's natural clock (Gooley, 2008). The human sleep-wake cycle is highly sensitive to light; exposure to bright light at specific times of day can shift the circadian clock, allowing for the potential delay of sleep onset. Due to this sensitivity, further studies may benefit from more detailed investigations of light therapy in humans, potentially focusing on timed lighting and light intensity.

Extension

Materials & Methods

Subjects

This study aims to understand the varying impact of blue and white light on specifically aged Wistar rats. The same 33 male rats will be utilized, and they will all be around 16 months old. The Wistar rats will weigh around 650-690g and will be housed in polypropylene cages with dimensions of 40 x 32 x 17 cm. The environment will be maintained at a room temperature of 24 ± 1°C with a humidity level of 50%. To ensure that sensory feedback does not create unwanted variability in results, the housing method will incorporate soundproofing features. As for nutrition, rodent food will be made available in reasonable increments and quantities that are proportional to the rat's characteristics, but this should be relatively constant between all subjects.

Lighting Conditions

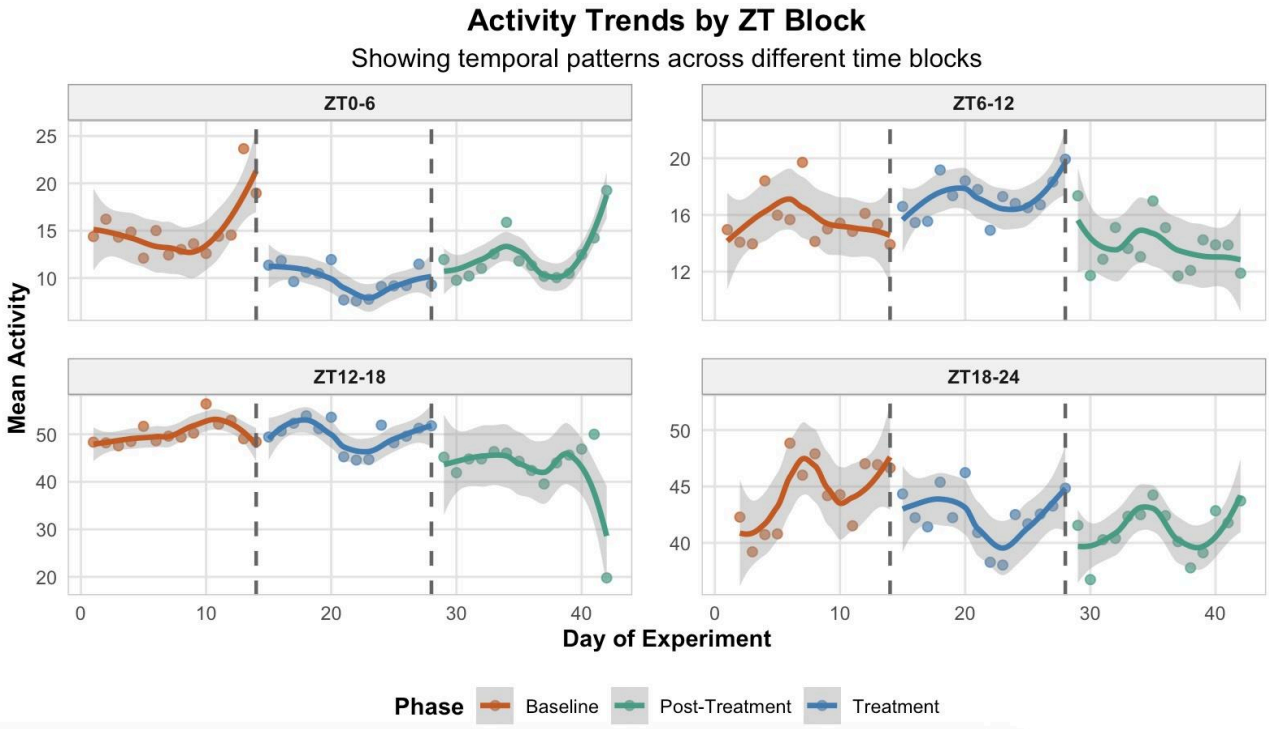
The lighting, being critical for this study, will be provided in clear and calculated intervals throughout the experiment. This will utilize the Zeitgeber Time (ZT) system, which is known to imitate the light-dark cycles a rat would naturally encounter. Specifically, the rats will undergo a light cycle with lights turning on at 6:00 AM on Zeitgeber Time (ZT) and being turned off at 6:00 PM ZT. There is a 24-hour light-dark cycle, with 12 hours of light and 12 hours of dark. This will be stimulated by a 350 lux set of white lamps for the light cycle and 2 lux for the dark cycle. For blue light exposure, this occurs during the first half of the light phase, which is 0-6 ZT, at 150 lux, with a peak wavelength of 470-495 nm. This blue light will be used to simulate the effect of sunlight on the rat's circadian rhythm. This can cause alterations to activity levels, but may also improve or worsen stability.

Procedures

The overall procedure component involves initially conditioning the rats to the experimental room so that they do not feel stressed in a way that would cause an impact on the results. Over 2 weeks, there will be a consistent light-dark cycle mentioned in the 'Lighting Conditions' section. However, this will be without the blue light therapy in which the peak wavelengths would be 450-470 nm and associated with the white light at lux 350 during the light phase and lux 2 at dark phase. To clearly observe the study's outcomes, the rats will be placed on a light-dark cycle without blue light again.

In this extension study, we developed the experiment by including baseline, treatment, and post-treatment periods, as described above. By using Linear Mixed Effects Models, linear regression for Slopes, and LOESS Smoothing, we will be able to extend our analytical understanding of the relationship between blue-light exposure and activity levels. The LOESS Smoothing will help us create clearer trends of how the data is coming together to present a relationship between the mentioned characteristics of the experiment. Additionally, the linear regression for Slopes will help us measure the progression and development of changes in activity levels across different levels of exposure to light and types of light (blue or white), specifically during each exposure phase. Lastly, the Linear Mixed Effects model can help us see the changes in activity levels while accounting for redundancy in data and repeated outcomes across the ZT blocks. We can see through this how the ZT blocks impact the outcomes of our light types and phases.

Analysis



Comparative Analysis

The presented data demonstrates distinct trends of activity levels among each ZT block, in which activity levels decrease post-treatment after implementation of the blue-light therapy, but also during treatment. The decrease is greatly noticeable in the initial, ZT0-6 application of the blue light treatment, in which there is a difference of at least 10 in activity levels. Comparatively, however, the activity levels greatly contrast with the ZT0-6 phase in the next, ZT6-12, time block in which the activity is highest with the treatment state compared to the post-treatment and baseline phase. This same trend is seen in the ZT12-18 phase as well, in which the post-treatment timeline is trending downward while the treatment phase is relatively level, along with the baseline phase. Additionally, the activity levels across all four time blocks appear to vary significantly in their trends over time, not just between different phases.

Phase Shift

As time passes, we observe a general downward trend in post-treatment activity, which suggests that the blue-light treatment tends to have an overall activity-suppressing effect. This is especially seen in the post-treatment phases in the night-time section which can align with the overall goal of aiming to mediate activity levels between specific time blocks associated with preferred activity levels. For example, the early ZT blocks associated with the day-time represent a sharp upward shift in activity post-treatment. Over time, the activity gradually decreases and eventually stabilizes at a lower level during the post-treatment nighttime ZT18-24 block. The transition from the baseline phase to the treatment and post-treatment phases represents a general association between the blue light treatment and the mediation of the circadian rest rhythm, which is tuned to specific times of the day.

Data Extraction

The following trends are observed

1. **ZT Block 0-6:** Baseline activity mean is ~13-16, treatment reduces activity to ~8, post-treatment activity reaches a steep ~20. Overall, a large rebound is seen in the post-treatment phase.
2. **ZT Block 6-12:** Baseline activity mean ranges from ~14-16.5, treatment effect increases sharply to ~20 and post-treatment activity steeply decreases to ~12. A generally high treatment spike is seen with suppression following post-treatment.
3. **ZT Block 12-18:** The baseline mean ranges from a high ~50-55, but this time, no change is seen in the treatment phase, and a highly sharp drop is seen post-treatment, with levels ~40. The treatment is greatly effective within 10 days.
4. **ZT Block 18-24:** Baseline mean ranges from ~42-48, treatment activity levels fall between ~43 on average with post-treatment remaining consistent with treatment activity levels at ~43.

There is generally a trend in which the activity levels remain sustained after treatment relative to baseline, indicating the SCN promoting stability in the circadian rhythm cycles of the aged rats.

Discussion

Our findings aimed to understand the trends and rates of change in aged rat activity over time, categorized into baseline, treatment, and post-treatment periods, using methods that included rate of change analysis, phase-specific slopes, and a mixed-effects model. Overall, the trend reveals that the activity of aged rats increased during the baseline phase, especially during nighttime (ZT18-24). This decrease is slight during treatment and continues to decline post-treatment, re-aligning the rats' Circadian rhythm with their standard 12–12 h light–dark cycle (Ryabinina et al., 2024). This reinforces the original hypothesis that exposing aged rats to blue light can increase the robustness of their circadian rhythm and reduce circadian rhythm dysfunction. However, our extension further shows that the activity of aged rats varies across the different time zone blocks, suggesting that the effectiveness of blue light treatment differs over time. As such, our analysis concludes that blue light treatment reduces locomotor activity in aged rats. While its effects are lasting, they also vary depending on the time of day.

Several limitations were faced in the process of conducting our extension. First, limitations from the original study’s data carry over to our analysis. The study used albino rats, whose age-related loss of photoreceptors exceeds that of pigmented rats due to their vulnerability to light-induced damage, caused by the reduced pigmentation of Retinal Pigment Epithelium (RPE) cells and decreased melanin production in the retinas of albino rats (De Vera Mudry et al., 2013). Therefore, the effects of blue light exposure on pigmented rats were not taken into consideration. Additionally, our findings open the door to further investigation into the yet unexplained effects of blue light treatment on aged rats. This includes the

sharp increase in locomotor activity during the nighttime, during the active phase of aged rats, and the surprising finding that blue light exposure does not accelerate the decline in total activity count of aged rats during the 10 hours they are most active (M10). Finally, further studies are needed to understand the neural basis of blue light exposure effects on Circadian rhythms, particularly its impact on the Neuropeptidergic content of the suprachiasmatic nucleus.

Application of Blue Light Therapy in Nursing Homes

Whereas this paper examined the short-term effect of blue light on albino rats after 14 days of exposure, these results do not explore the effects of longer periods of exposure on aged rats, nor on human subjects. This is important because neurobiological differences between rats and humans alter the relationship between sleep and aging—for instance, while human studies suggest that aging reduces deep, restorative sleep, the sleep duration of laboratory rats has been noted to increase with aging (McKillop & Vyazovskiy, 2020). This highlights the limitations of using rats as model organisms in developing medical treatments for humans.

In future studies, extended observations of the effects of blue light exposure, combined with human trials, would provide insight into the longevity of these effects and help us better understand how light therapy can benefit long-term care and senior residents in their daily lives. For instance, a 2011 study (Royer et al., 2012) proposed that exposing long-term care elderly residents to 4 weeks of blue light exposure improved cognition and reduced anxiety. Further studies should also be conducted with participants in more controlled environments and with more rigorous monitoring processes. While practical environments, such as the nursing home, offer contextual data on the behaviour of target subjects (A. Aziz, H., 2017), controlled environments are repeatable and allow researchers to filter out “random

events" that occur in a natural setting (Torday, J. S., & Baluška, F., 2019). Ultimately, Blue light therapy can be incorporated into nursing homes through a variety of interventions, including light boxes, ceiling-mounted light-emitting diode systems, and "dawn-dusk simulation therapy," which adjusts light levels during sleep (Zhang, M., et al., 2022). In doing so, Blue light therapy could be a promising intervention that helps decrease cognitive symptoms in elderly residents.

Blue light therapy could be further extended to treatments for Neurodegenerative diseases. Neurodegenerative diseases are primarily treated with pharmaceuticals, such as Rivastigmine, which is used to treat Alzheimer's disease by increasing acetylcholine levels, thereby allowing nerve cells to communicate (Birks, J.S. et al., 2015). However, since this often poses the risk of negative reactions such as nausea, physical therapies such as light therapy are attractive due to their affordability, safety, and easy implementation (Liu et al., 2020). Past studies have depicted the success of blue light therapy in treating neurodegenerative diseases; for instance, a 2023 study (Chen et al., 2023) revealed that exposing institutionalized adults with dementia to blue-enriched light therapy over 10 weeks led to increased sleep efficiency and latency, the effects of which continued for up to 6 months (Royer, N. Ballentine, P. Eslinger, 2012). This research encourages further investigation into the potential use of blue light therapy in treating neurodegenerative diseases.

Conclusion

Our replication provides evidence that the research conducted by (Silva et. al 2023) represents a promising non-invasive and non-pharmacological treatment for regulating circadian rhythm dysfunctions. Blue light therapy is effective in stabilizing circadian rhythms by ensuring the predictability of movement during the different Zeitgeber-Times. Blue light mimics natural light, and when exposed to subjects during certain time periods, are able to modulate the circadian rhythm with the reduction in circadian fragmentation and improved temporal organization of behavior patterns. Limitations to this study must also be addressed, as albino rats exhibit a heightened sensitivity to light exposure due to the absence of melanin, resulting in a decreased threshold for photoreceptor activation. This finding highlights the need for cross-species applications in human subjects. Future research should address these limitations and include studies on wavelength specificity, brightness intensity, and long-term safety considerations in human models. This treatment shows promise in treating circadian rhythm dysfunction. It may open new avenues for non-pharmacological research that can be easily applied to clinical and vulnerable populations, effectively improving the quality of life for patients suffering from neurological disorders.

Open Data Set

All raw and processed data & code used in this study are available in our Github repository: https://github.com/JeremyManwaring/Blue-Light-Circidian-Rythym-Extension.git

Sources Cited

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