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Effects of light-dark cycle and light-emitting diode (LED) colors on the behavioral and physiological responses of *Phodopus roborovskii*

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Abstract. *Fuertes PJS, Aquino AM, Balones NA, Pajila JJL, Granadozin Jr. MP, Brillo SC. 2024. Effects of light-dark cycle and light-emitting diode (LED) colors on the behavioral and physiological responses of* Phodopus roborovskii. *Cell Biol Dev 8: 51-57.* The internal biological clocks regulate the 24-hour cycle of the circadian rhythm. Changes in the quantity or quality of light can disrupt the circadian rhythm as light influences the circadian clock synchronization, affecting the behavior and physiology of an organism. We determined the effects of different light-dark cycles and LED colors, specifically white, blue, and yellow, on the behavior and physiology of *P. roborovskii*. The test subjects were acclimated for one week. Five setups were utilized per LED color, particularly control (12L:12D) and treatment groups (24L:0D, 18L:6D, 6L:18D, 0L:24D). The amount of food intake (g), running wheel usage duration (min), sleep duration (min), and body weight (g) were recorded daily. Inferences regarding the melatonin and corticosterone levels were established from the behavioral assessment. The findings revealed a significant difference in sleep duration and running wheel usage. Results showed that white light at 18L:6D induced longer sleep duration, while blue light at 18L:6D resulted in shorter sleep duration. Moreover, blue light at 24L:0D promoted a longer duration of running wheel usage, whereas yellow light at 0L:24D impeded the use of the running wheel. Based on the behavioral data, it can be inferred that as melatonin levels increase throughout sleep onset, corticosterone levels decrease. Furthermore, there is no significant difference in the amount of food intake and body weight between the control and treatment groups. In conclusion, behavioral and physiological changes following light exposure were observed, disrupting the test subjects' circadian rhythm.

Keywords: Circadian rhythm, LED, light-dark cycle, *Phodopus roborovskii*

Abbreviations: LD cycle: Light-Dark cycle, LED: Light-Emitting Diode

INTRODUCTION

Biological clocks are internal mechanisms that regulate bodily processes, including circadian rhythms-24-hour cycles of mental, physical, and behavioral changes influenced by light and dark (National Institute of General Medical Sciences 2020; Rumanova et al. 2020). Specifically, the suprachiasmatic nucleus (SCN) houses the primary circadian pacemaker in mammals. The photoreceptors in their retina detect photic information. The information is then forwarded to the SCN through the retinohypothalamic tract. The SCN uses the data to synchronize the organism's circadian cycles with the environment (Alves-Simoes et al. 2015). Since light is the most crucial environmental aspect in synchronizing the circadian clock, some variations in exposure to and quality of light can disrupt circadian cycles, which could result in changes in the organism's body homeostasis, affecting everything from behavior to physiology. Bourgin and Hubbard (2016) mentioned that light can indirectly influence the behavior and physiology of mammals through body clock synchronization and the circadian cycle's phase adjustment.

Light can also directly affect organisms by stimulating sleep and alertness in nocturnal and diurnal species. The van der Merwe et al. (2019) study proved that the temporal pattern and light qualities affect rodents' daily locomotor activity. Furthermore, Roborovski dwarf hamsters have been used as experimental animals in biomedical research, especially in theriogenology, endocrinology, and circadian biology (Trimpert et al. 2020). Hamsters, like other rodents, can be valuable animal models in cancer research, infectious diseases, and behavioral studies. Additionally, they have fewer ethical and societal difficulties than when using larger mammals (Dutta and Sengupta 2019).

In the scientific review of Russart and Nelson (2018), it was mentioned that organisms' survival depends on their adaptation to their environment; hence, behavioral and physiological processes developed to correspond with light cycles to minimize the risks of survival and to optimize the accessible resources. Daily changes in the levels of light can be drastic and predictable, as are the behavioral and physiological functions in microorganisms, vertebrates, and invertebrates. As an exemplification in nocturnal rodents, studies show that the presence of light at night can disrupt the regular schedule of daily locomotor activities as well as alter the timing of food intake to daylight. However, exposure to dim light reduces anxiety-like behaviors such as rearing up often and spending more time in the open. Presented in the following are further discoveries regarding the biological and physiological effects of light on rodents.

Lighting conditions, such as intensity, duration, and wavelength, can disrupt circadian rhythms of metabolism (Wren-Dail et al. 2016). Dauchy et al. (2016) found that rats exposed to blue-enriched LED light consumed less food and water and showed slower growth rates compared to those under standard cool white fluorescent (CWF) light. In contrast, studies by Dauchy et al. (2019) and Voros et al. (2021) reported higher food intake and weight gain in mice exposed to LED lighting versus CWF lighting, indicating that LED light influences dietary habits and body weight. Similarly, Allen et al. (2022) noted that male rats, regardless of the lighting type, consumed more food than females, though no significant weight differences were found between LED and CWF groups.

We assert that there is a need to identify the effects of variations of light-dark cycles and LED colors on organisms, i.e., mammals, because exposure to such has an indirect and direct impact on their behavioral and biological responses. The behavior change affects how they interact with their own and other species within the environment, which may pose disturbances if left unchecked. Therefore, we proposed a study that aims to identify the effects of the light-dark cycle and varying light colors on the circadian control of behavior and physiology of the Roborovski hamster (*P. roborovskii*).

MATERIALS AND METHODS

Ethical statement

In compliance with the Animal Welfare Act of 1998 (RA 8485) and Administrative Order No. 40 Series of 1999 on Rules and Regulation on the Conduct of Scientific Procedures Using Animals, we secured authorization from the Bureau of Animal Industry (BAI) and Department of Agriculture (DOA) prior the conduct of the study. Researchers also underwent a 3-day animal training under the supervision of the Research Institute for Tropical Medicine (RITM). A specially designed housing facility fit for the experiment's set conditions was provided by the De La SalleAraneta University (DLSAU) Veterinary Hospital and utilized during the research. The test subjects, healthy male species of *P. roborovskii*, were purchased from a BAI-accredited facility, MOTS Animal House. In adherence to the RA 8485 and AO No. 40 recommendations, we used carbon dioxide to euthanize the animals at the end of the study.

Research design

An experimental research design was used to assess the effects of the light-dark cycle and different LED colors on the behavioral and physiological responses of *P. roborovskii* and to determine whether there is a significant difference in these responses between the control and treatment groups. Actual experimentation with test subjects exposed under control and experimental conditions was utilized to determine and establish the cause-and-effect relationship between the independent and dependent variables. Quantitative approaches and statistical techniques were employed to gather and analyze the data.

Test organisms

We used healthy individuals of *P. roborovskii* (n = 19; age, two months; weight, 19 to 25 g; all males), to observe its behavioral and physiological responses to varying light-dark cycles and colors of light. The same set was used to assess each LED color, given that a recovery phase was provided after every experiment.

Samples and sampling procedures

We purposely selected the subjects to form a homogenous population with the same characteristics. The test organisms were randomly grouped using a random wheel picker, with three subjects for the positive control group and four subjects for each treatment group. Specifically, the study population for this research is 19 male *P. roborovskii*, two months old, weighing 19 to 25 g. The sample size was made statistically valid by calculating the E value, which was equal to the total number of animals minus the total number of groups. To yield significant results, the value of E should be between 10 and 20 (Charan and Kantharia 2013). Computing for the E value provided E=19-5=14, within the acceptable limit.

Experimentation and data collection

The experiment was conducted at De La Salle Araneta University-Veterinary Hospital, Philippines which was suitable for the set conditions of the experiment. Five experimental setups were used to demonstrate the effects of varying light-dark cycles and different LED colors on the behavioral and physiological responses of *P. Roborovskii*. The experimental setups for each light color, specifically white, blue, and yellow, consisted of 24L:0D, 6L:18D, 12L:12D, 18L:6D, and 0L:24D light-dark cycle periods.

Acclimation

Hamsters were randomly divided into five groups, each with four males, except the 12L:12D standard cycle, which only had three. Each hamster was housed individually in a transparent polypropylene cage with a size of 11.38 × 6.5 inches and, by the minimum enclosure size for laboratory hamsters with a constant ambient temperature of 25.0 ± 1.0°C and relative humidity at 60% (Housing and Husbandry: Hamster | NC3Rs, 2021). Food (Vitakraft Premium Menu Vital food for hamsters) and water were always available ad libitum. All hamsters were allowed for a one-week acclimation period. They were subjected to a standard light-dark cycle (LD; 12 hours light/12 hours dark) for one (1) week before exposure to treatments of varying light-dark cycles. The light source was installed about 22 inches from the cages. Lighting was provided with a white light-emitting diode (LED), which is currently preferred due to its energy efficiency (Emmer et al. 2018; Dauchy et al. 2016).

Light treatment

To determine the effect of varying light-dark cycles and LED colors on the behavior and physiology of *P. roborovskii*, shelves with installed segments of LED strip lights were used as chambers to expose the hamsters under 400-450 lx of light intensity. Table 1 shows the light-dark cycle schedules introduced to *P. roborovskii* individuals. Following the acclimation period, each setup had four (4) hamsters subjected to 24L:0D, 6L:18D, 18L:6D, and 0L:24D cycles except 12L:12D cycle with three (3) hamsters. They were exposed to designated LED colors per week, specifically white, blue, and yellow, respectively, with an allotted 5-day recovery period after each exposure.

Positive control

Each of the four randomly assigned hamsters was subjected to 24L:0D, 6L:18D, 18L:6D, and 0L:24D cycle treatment, whereas the remaining three subjects were exposed to a 12L:12D standard cycle. The latter is needed to validate the experimental procedures.

Table 1. Light-dark cycles introduced to *P. roborovskii*

Code	Light/Dark (L/D) cycle	Light Period	Dark Period
A	24L:0D	7:00AM-7:00AM	-
B	6L:18D	7:00AM-1:00PM	1:00PM-7:00AM
C	12L:12D	7:00AM-7:00PM	7:00PM-7:00AM
D	18L:6D	7:00AM-1:00AM	1:00AM-7:00AM
E	0L:24D	-	7:00AM-7:00AM

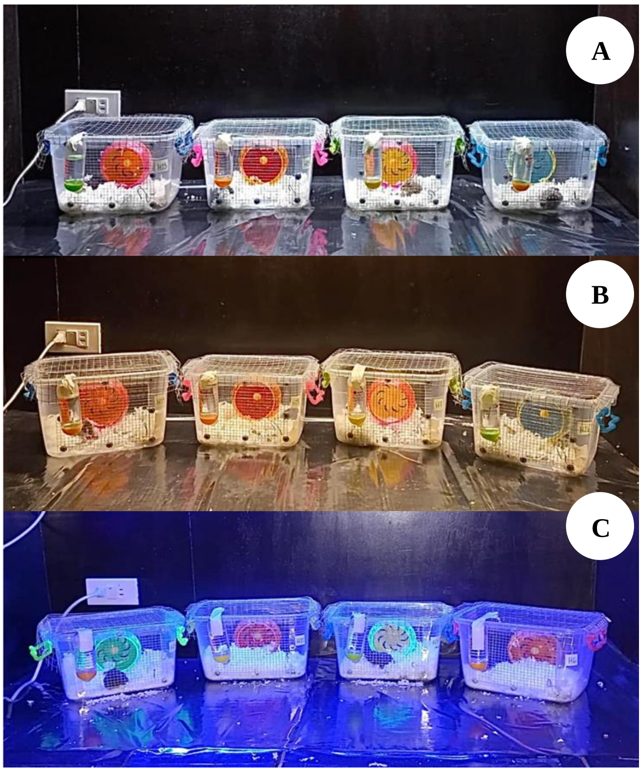

Figure 1. Experimental setups. A. White LED setup; B. Blue LED setup; C. Yellow LED setup

Behavioral testing

To accurately record the behavior of *P. roborovskii*, we used a closed-circuit television (CCTV) located 1.25 to 1.5 m away from the cages. This video trapping enabled us to observe locomotor activity, sleeping behavior, and feeding habits. Moreover, the behavioral responses were assessed daily. Regarding feeding behavior, the hamsters were given 8 g of food, with water provided ad libitum, following their daily food and water consumption based on the guidelines from McGill University Health Centre Research Institute - Laboratory Animal Biomethodology Workshop (2020). Food was provided at night (18:00) daily. To evaluate the amount of food intake, the difference between the allotted and leftover food mass (g) was calculated (Le Tallec et al. 2015).

To evaluate the effects of various lighting conditions on the locomotor activity of hamsters, the individual cages were equipped with running wheels. Based on the camera recordings, the time spent utilizing the running wheel was calculated and expressed in minutes (Bakeche et al. 2021). The usage of running wheels was defined as the time of occurrence of the first use of a running wheel (LA_{onset}) and the time of occurrence of the last use of the running wheel (LA_{offset}) (Le Tallec et al. 2015). The video recordings of sleeping behavior were analyzed to determine their sleeping duration. Additionally, time spent while sleeping was calculated and expressed in minutes (Bakeche et al. 2021).

Physiological testing

To evaluate the effects of varying light-dark cycles and LED colors on the physiology of *P. roborovskii*, we measured the body weight of hamsters to examine the effects on the feeding behavior. The body weight of each hamster was measured and recorded daily during the nocturnal phase.

In addition to the measurement of body weight, inferences regarding the levels of corticosterone and melatonin levels were established from the behavioral data. This is to determine the influence of light in association with variations in hormone levels. The investigation was followed by a recovery phase at the end of the week before conducting the same setups with a different light color.

Statistical analysis

Behavioral testing results: (i) amount of food intake, (ii) duration of running wheel usage, and (iii) sleep duration, and physiological testing results: (iv) body weight were analyzed with one-way ANOVA to determine the statistical difference among varying LD cycles at each of the LED color exposure. The data were represented as means ± SEM with statistical significance established at P-values of <0.05. A Post hoc test, particularly Dunnett's t-test, was used to determine which group was statistically significant or insignificant. The above statistical analyses were performed using Statistical Package for the Social Sciences (SPSS).

RESULTS AND DISCUSSION

Fonken et al. (2010) stated that light is the most potent entraining signal for the circadian clock. It is considered the most crucial environmental aspect in its synchronization; thereby, variations in the exposure and quality result in changes in the behavior and physiology of living things. Emmer et al. (2018) maintained that light influences the behavior and physiology of laboratory animals, emphasizing that light-dark cycle disruption disturbs biological rhythms resulting in widespread physiological consequences.

Behavioral responses

Food intake

As seen in Figure 2.A, the highest amount of food intake can be observed under white, yellow, and blue colors in 24L:0D, 6L:18D, 18L:6D, and 12L:12D and 0L:24D setups, respectively. On the other hand, the lowest amount of food intake can be correspondingly noted under blue and white colors in 24L:0D and 6L:18D, and 12L:12D, 18L:6 D, and 0L:24D setups. There is no significant difference in the average food intake of the subjects between the control and treatment setups (Table 2). This result is similar to Fonken et al. (2010), indicating no significant difference in the total 24-hour food consumption among the groups of mice. From our study, the mean food intake of the test organisms is approximately 2.29 to 3.08, which is within the average daily food consumption of hamsters (Kolynchuk 2015). This is supported by the research conducted by Rouibate et al. (2020), which showed that although there was an increase in food intake under experimental conditions compared to the control group, no statistically significant differences were observed between the groups. López-Espinoza et al. (2021) also produced the same results, depicting no significant differences in the total food consumption in any of the setups and thereby suggesting that the light-dark conditions did not influence the ability of the subjects to maintain the balanced intake.

Table 2. Behavioral responses under different LED colors via one-way ANOVA

LED Exposure	Food intake	Locomotor activity	Sleep duration	Body weight
White LED	0.068	3.949*	4.154*	0.851
Blue LED	0.456	4.988*	12.705*	0.528
Yellow LED	0.320	4.320*	13.572*	0.605

Note: $F^{P\text{-value}}$; significance level at P≤≤0.05 (95%)

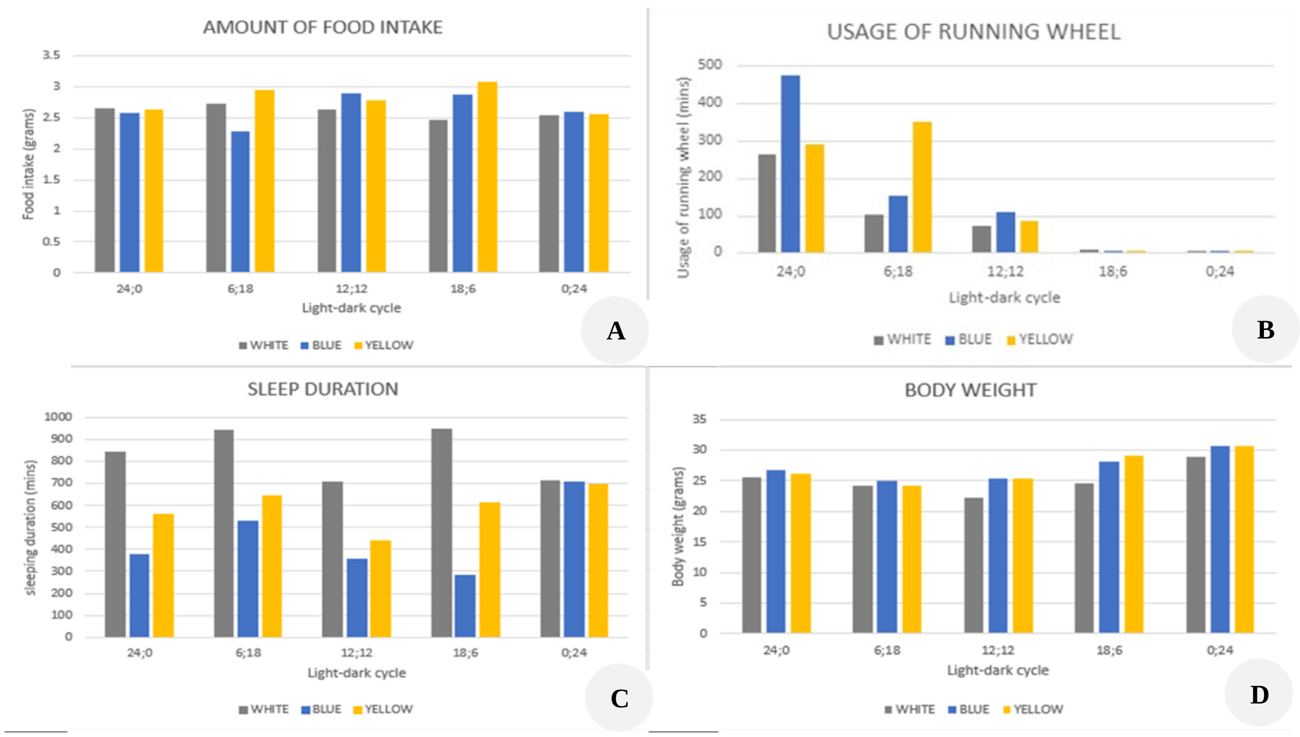

Figure 2. A-C. Mean amount of behavioral, and D. physiological responses measured in this study. A. Food intake; B. Usage of running wheel (locomotor activity); C. Sleep duration; D. Body weight in different light-dark cycles under white, blue, and yellow LED

Table 3. Dunnett's T-test post-hoc analysis results for locomotor activity under blue LED color

LED Exposure	Treatment	Pair	P-value	Interpretation
Blue LED	12L:12D	0L:24D	.830	Not significant
		18L:6D	.835	Not significant
		24L:0D	.045	Significant
		6L:18D	.989	Not significant

Table 4. Dunnett's T-test post-hoc analysis results for sleep duration under the different LED colors

LED Exposure	Treatment	Pair	P-value	Interpretation
White LED	12:12D	0L:24D	1.000	Not Significant
		18L:6D	.044	Significant
		24L:0D	.346	Not Significant
		6L:18D	.048	Significant
Blue LED	12L:12D	0L:24D	<.001	Significant
		18L:6D	.654	Not Significant
		24L:0D	.997	Not Significant
		6L:18D	.107	Not Significant
Yellow LED	12L:12D	0L:24D	<.001	Significant
		18L:6D	<.001	Significant
		24L:0D	.070	Not Significant
		6L:18D	.003	Not Significant

Locomotor activity

It can be observed from Figure 2.B that the highest duration of using the running wheel is depicted under blue, yellow, and white colors in 24L:0D, 12L:12D, and 0L:24D, 6L:18D, and 18L:6D setups, respectively. However, the lowest duration can be distinguished under white and yellow light exposure in 24L:0D, 6L:18D, 12L:12D, and 18L:6D and 0L:24D setups.

Table 2 shows that there is a significant difference in the locomotor activity of the test subjects exposed to white, blue, and yellow LED color, consecutively. There is no significant difference between the control and treatment groups in white and yellow LED colors. However, significant differences were observed among the treatment groups (0L:24D vs. 24L:0D; 18L:6D vs. 24L:0D; 24L:0D vs. 0L:24D; and 24L:0D vs 18L:6D). Table 3 shows that there is a significant difference between the 12L:12D and 24L:0D cycle setups in blue LED color. Overall, LED color and the light cycle duration both play a key role in affecting the locomotor activity of the test subjects, with certain cycle setups, especially continuous light or darkness, driving significant behavioral changes. In the study of Ferraro (2008), exposure of hamsters to constant light produced significantly and similarly longer free-running periods of the locomotor activity rhythm than the exposure of animals to constant dark. In a similar study, Van der Merwe et al. (2019) claimed that the degree of activity on nocturnal rodents is lowest under blue light during the daytime and is active at night. Furthermore, Alaasam et al. (2021) found that artificial light at night (ALAN) was sufficient to disturb nocturnal rest and increase nocturnal locomotor behavior.

Sleep duration

Figure 2.C depicts the average sleep duration for each light-dark cycle under white, blue, and yellow LED exposures. The highest sleep duration can be observed under white color in 18L:6D, 6L:18D, 24L:0D, 0L:24D, and 12L:12D cycles, respectively. On the contrary, the lowest sleeping duration can be seen under blue and yellow in 12L:12D, 18L:6D, 24L:0D, 6L:18D, and 0L:24D, respectively. Based on the findings in Table 4, the P-value resulted in 0.020, <0.001, and <0.001, showing a significant difference in the sleep duration of the subjects between the control and treatment groups under white, blue, and yellow LED color, consecutively.

Table 4 shows a significant difference between the 12L:12D cycle and 18L:6D and 6L:18D cycle setups in white LED color. Additionally, a significant difference in blue LED color can be seen between the 12L:12D and 0L:24D cycle setups. There are also significant differences between the three treatment groups, specifically the 0L:24D, 18L:6D, and 6L:18D cycles, and the positive control group of yellow LED color, which is the 12L:12D cycle setup. This suggests that the lighting cycles have an impact on the sleep duration across different LED colors. These results are supported by the study conducted by Fisk et al. (2018), delineating that the effects of prolonged exposure to white light on nocturnal rodents promote sleep while total darkness results in increased wakefulness. In a similar study conducted by Pilorz et al. (2016), the exposure of mice to blue color showed a significant difference between control and treatment setups. There was an observed delay in the sleep induction of the test subjects when they were exposed to blue light.

Physiological responses

Body weight

Figure 2.D exhibits each light-dark cycle's mean or average body weight subjected to white, blue, and yellow LED colors. The highest body weight was recorded under blue and yellow light colors in 24L:0D and 6L:18D, and 12L:12D and 18L:6D, correspondingly. The lowest body weight was perceived under white light color on all the LD cycles except 6L:18D but with only minimal difference. It can also be noted that in the 0L:24D cycle, blue and yellow light colors attained the same average body weight.

As presented in Table 4, there is no significant difference in the body weight of the Roborovski dwarf hamsters between the control and treatment groups under white, yellow, and blue LED color setup. This result was sustained by Dauchy et al. (2015), who conducted research on Sprague Dawley rats, who were consistently exposed to bright light for 6 to 10 weeks. The study's findings suggest no noticeable alteration in the body weight of the rats because of the light exposure. Unlike our study, Fonken et al. (2010) discovered that subjecting the mice to dim light and bright light increases the body weight and food consumption, in contrast to the standard LD cycle. As opposed, Rouibate et al. (2020) demonstrated that the disruption of the LD cycle affects the metabolism of the rodents characterized by the decrease in body weight of the control and treatment organisms, with a greater decrease in the latter, as food intake also decreases. In addition, the study of Zhang et al. (2015) concluded that short photoperiods in *P. roborovskii* led to decreased body mass compared to other long day photoperiods. As different factors may contribute to the body weight of animals, we should take into account other factors that led to varying body weights in our study, given a controlled setup to each animal aside from LED and light-dark cycles.

Melatonin and corticosterone levels

It has been stated that light is the most significant stimulus for the circadian clock. In particular, the light:dark (LD) cycle provides a cue that entrains individual clocks in the suprachiasmatic nucleus (SCN), the brain's central pacemaker, which regulates circadian rhythms and physiology, including humoral outputs. The most well-known circadian humoral outputs are melatonin and corticosterone. Thus, exposure to external lighting cues, depending on their intensity, duration, and wavelength, influences the release and suppression of these hormones. (Rumanova et al. 2020; Meléndez-Fernández et al. 2023).

According to Emmer et al. (2018), light-dark cycle disruption disturbs the biological rhythms of living organisms, inducing widespread physiological consequences. Physiological responses, such as melatonin and corticosterone secretion, are essentially coordinated by exposure to different light-dark conditions. Melatonin, sometimes called the sleep hormone, is an internal zeitgeber responsible for providing information concerning the length of the night (Rumanova et al. 2020). Moreover, melatonin is an important aspect of the circadian rhythm (Meléndez-Fernández et al. 2023).

Based on the findings from the behavioral data, it can be inferred that the melatonin levels increased as characterized by the longest sleep duration of the test subjects from the 18L:6D cycle setup under white LED, indicating that exposure to blue light delays melatonin secretion, increasing the alertness and wakefulness of the subjects. Meanwhile, the 0L:24D cycle setup exhibited longer sleep duration under blue and yellow LEDs. This confirms the results of Farhadi et al. (2016) that high plasma melatonin levels result from prolonged exposure to darkness. In contrast, it was established that continuous light exposure does not affect melatonin levels. These findings are evident in Table 4.

Several studies also implicated the relationship of corticosterone levels to the circadian rhythm of behavior. Pilorz et al. (2016) maintained that light exposure increases plasma corticosterone levels in laboratory rodents, influencing sleep duration and locomotor activity. Regarding the duration of sleep, Balbo et al. (2010) highlighted that sleep onset exerts an inhibitory effect on cortisol secretion while awakenings and sleep offset are accompanied by cortisol stimulation. Based on the findings of the study, as shown in Figure 2, an inversely proportional relationship between locomotor activity and sleeping behavior can be established. Concerning this, it can be inferred that as melatonin increases during sleep onset, the corticosterone level decreases. Results revealed that the 24L:0D setup exhibited the highest recorded locomotor activity under the blue LED. On the other hand, the 0L:24D setup demonstrated the lowest recorded locomotor activity under the yellow LED color. Contrastingly, the sleep duration of the setups under these light conditions was correspondingly lower and higher.

In conclusion, this study investigated the effects of varying light-dark cycles and LED light colors on the circadian control of behavior and physiology in *Phodopus roborovskii*. Statistically significant differences were observed in sleep duration and locomotor activity across different light-dark setups and LED colors, indicating that light exposure disrupts circadian rhythms. Specifically, blue LED light delayed melatonin secretion, reducing sleep duration and increasing locomotor activity, while prolonged darkness elevated melatonin levels, promoting longer sleep durations. Conversely, no significant differences were found in food intake and body weight between control and treatment groups, suggesting that these variables were less sensitive to changes in light conditions. The findings highlight the critical role of LED color and light cycle duration in regulating circadian behaviors, with continuous light or darkness causing notable disruptions. Behavioral data further inferred changes in melatonin and corticosterone levels, emphasizing the physiological implications of altered light exposure. While the 7-day experimental period yielded valuable insights, future studies should extend the exposure duration, explore additional biological parameters, and investigate the interplay between behavior and hormonal changes to better understand the circadian regulation in *P. roborovskii* under varying photoperiods and light wavelengths.

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