Home-Based Mice Colony Explores the Effect of Light Therapy on the Cognitive Functions of Circadian Rhythm Disruption in Mice

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***Abstract*—The fast and non-invasive modulation of brain activity has shown promising therapeutic potential for treating neurological disorders. Gamma-frequency light therapy has been proven effective in treating neurodegenerative diseases. However, its broader therapeutic potential remains unclear. In this study, light therapy was used to treat cognitive dysfunction induced by circadian rhythm disruption in mouse models. First, a light therapy treatment device was built to produce flickering LED light at a 30 Hz gamma frequency. Second, a home-based mouse colony and an artificial circadian rhythm induction chamber were built to induce circadian rhythm disruption in mice during different patterns of light-dark cycles. Circadian rhythm induction was confirmed by video tracking and analysis. Mouse behavior tasks, including the Morris water maze test, forced swim test, and open field test, tested the spatial learning and memory, depression levels, and stress levels of the mice, respectively. The results showed that circadian rhythm disruption affected the spatial learning and memory of the mice but had negligible effects on depression and stress levels. Light therapy was able to improve the spatial learning and memory dysfunction in a specific group of circadian-disrupted mice. This study demonstrates the feasibility of light therapy as a treatment for circadian rhythm disruption. More efforts are needed to understand the mechanisms underlying the effects of light therapy and feasibility for human use.**

***Keywords*—light therapy, circadian rhythm, circadian rhythm disruption, spatial learning and memory, anxiety, depression**

I. Introduction

Circadian Rhythm Disruption (CRD) is a neurological disorder that heavily impacts daily life and has been linked to various health issues, including obesity, diabetes, cardiovascular diseases, and cognitive function decline [1]. The boundary between day and night is becoming increasingly blurred due to frequent exposure to digital devices, electric light, and the modernization of society, leading to CRD [2]. CRD affects millions of people worldwide, and it has been shown to play a role in increasing the risks of many neurodegenerative diseases [3].

Light therapy is a non-invasive, practical, and promising treatment for CRD and other neurological disorders [4]. Light therapy involves exposing subjects to light as a form of therapy and has been found to reduce glial cell activation and neuroinflammation. A specific method of light therapy involves exposing subjects to 30–40 Hz flicker light to induce gamma oscillations of neuronal activity in the brain [5]. However, the direct link between light therapy and its therapeutic effect is not fully understood, and there is significant unexplored therapeutic potential. Therefore, this study aims to investigate whether light therapy can alleviate the cognitive decline associated with CRD in mouse models.

To achieve this goal, a mouse colony, a circadian rhythm induction chamber, and a light therapy device were built for this research. The cognitive functions of spatial learning and memory, depression, and anxiety were tested using the Morris water maze test, forced swim test, and open field test [6]. Then, light therapy was applied to the mice, and the cognitive functions were re-tested. This study seeks to determine whether light therapy affects cognitive functions (spatial learning and memory, anxiety, and depression). By testing the effects of light therapy on CRD-induced cognitive decline, this research aims to provide valuable insight into extending light therapy treatment to neurological disorders and exploring further therapeutic techniques.

II. Literature Review

Light therapy is a rapid, non-invasive treatment that utilizes exposure to light as a form of therapy. A specific method of light therapy involves exposing subjects to 30-40 Hz of flickering light to induce gamma oscillation of neuronal activity in the brain [5]. It has been found to reduce glial cell activation and neuroinflammation. Light therapy is quick (lasting only about one hour) [7], non-invasive, and does not require much professional equipment, making it highly practical and applicable for home use. This specific treatment has demonstrated

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therapeutic potential in mouse models of Alzheimer's disease [8], Seasonal Affective Disorder [4], and other neurodegenerative diseases [4, 9]. However, the direct link between light therapy and its therapeutic effects has not been fully revealed. A lack of thorough understanding of light therapy also means that it has plenty of unexplored therapeutic potential.

Circadian Rhythm Disruption (CRD) is a neurological disorder that heavily impacts our daily lives. The circadian rhythm acts as the internal clock of the body by regulating the sleep-wake cycle and various hormone functions [10]. However, the recurring stimulus of digital devices, electric light [11], and the modernization of society push workers to sleep late, participate in night shifts, or suffer from jet lag [2]. The boundary between day and night is becoming ambiguous [12], leading to CRD.

The disruption of the internal clock has resulted in health issues such as obesity, diabetes, and cardiovascular diseases [13]. Moreover, CRD also affects cognitive function [6]. For example, a study has shown that CRD is linked to an increased risk of major depression, anxiety disorders, bipolar disorder, and schizophrenia. Since CRD is an urgent issue, a thorough understanding of circadian rhythm is needed before exploring further therapeutic techniques.

Natural circadian rhythm is primarily induced by solar light-dark cycles [14]. In the human eye, in addition to the image-forming photoreceptors, there is another group of retina ganglion cells that can also act as photodetectors and participate in the process of circadian rhythm generation [15]. Those retina ganglion cells are called intrinsically photosensitive Retinal Ganglion Cells (ipRGCs). ipRGCs project into the brain and send photo-sensory information to a nucleus called the Suprachiasmatic Nucleus (SCN) (Fig. 1). The SCN sends broad projections to many brain areas and serves as a major circadian rhythm pacemaker. The presence of protein expression regulatory networks in the SCN neurons leads to the circadian oscillation of neural functions. Once the circadian rhythm is generated, the network maintains the same oscillation rhythm without further input [16].

In natural environments, the activities of mice are subject to solar light and develop a circadian rhythm period of around 24 hours [17], similar to human beings. Since the ipRGC-SCN pathway in both humans and mice is evolutionarily conserved, it is reasonable to use mice to model the human circadian rhythm period. Different lighting patterns in the mouse colony can induce different patterns of circadian rhythms, and LED lights have been employed to artificially recreate light-dark cycles and affect the photosensitive information received in the brains of mouse models [18].

When exposed to light, the SCN sends information to corresponding brain areas responsible for cognitive functions [10]. For example, the SCN is related to the hippocampus, as it exhibits oscillatory capacity where clock timing impacts synaptic plasticity [10]. The SCN could also impact intracellular signaling pathways, affecting learning and memory formation. As circadian rhythms are crucial to our bodies, so is CRD. CRD has also been shown to play a role in increasing the risks of many neurodegenerative diseases [15].

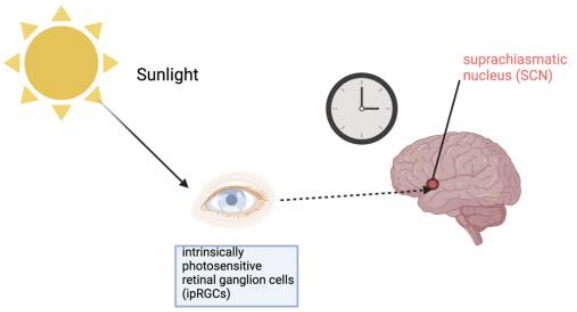


Fig. 1. A schematic diagram of the mechanism behind circadian rhythm regulation. Photo-sensory information from sunlight is received by intrinsically photosensitive Retinal Ganglion Cells (ipRGCs) and then sent to the Suprachiasmatic Nucleus (SCN).

III. Methods

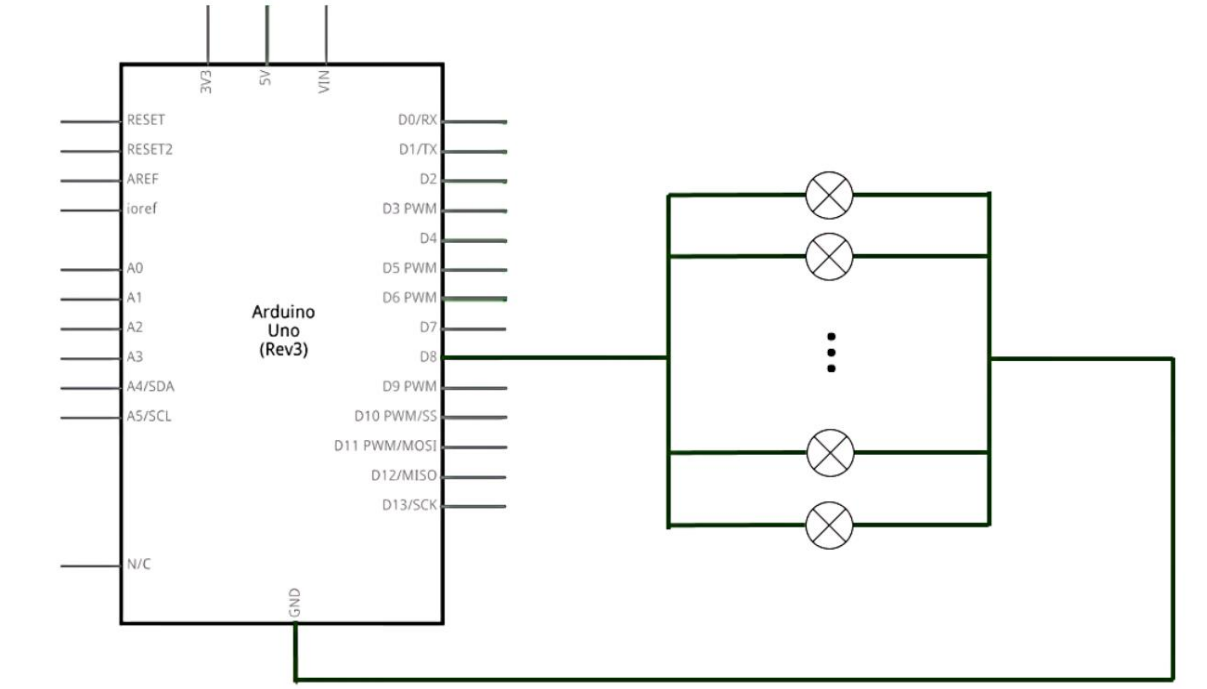
A. Constructing the Light Therapy Device

To investigate the effect of light therapy on the cognitive function of CRD mice, two important experimental apparatuses were constructed: a light therapy device and a circadian rhythm induction chamber. The light therapy device was designed to induce gamma frequency neuro-oscillation in the mouse brain, and the circadian rhythm induction chamber was used to induce CRD in the mice.

1) Hardware for light therapy device

To achieve the therapeutic effect of light therapy, a light therapy device was assembled using basic materials such as white Light-Emitting Diodes (LEDs), due to the lack of available devices on the market. Previous research has used white LED lights as a source of emission for light therapy. This study utilized a 30 Hz gamma frequency, which has been shown to have a beneficial therapeutic effect [5]. The brightness used in this study was 3,000 lux, which has proven effective in inducing neuro-oscillation in the brain [19].

The LED light used in this study was 0.5 meters long, with 4 bands of LED light, each containing 10 light bulbs (Figs. 2a and 2b). Each light bulb used 20 mA of current and 5 V of voltage. The LED lights were connected to an Arduino UNO R3 board (Figs. 2b and 2c), which was controlled by a computer. The 30 Hz flickering was generated through code written in the Arduino Uno software. The code controlled the electrical level of the ground output to vary from "High" to "Low," which caused the LED light band to flicker by switching between high and low electrical levels to produce the alternating light effect. The 30 Hz frequency was achieved by coding two 16 ms delays for every 1000 ms [1000 ms/(16 ms×2) $\approx$ 30], approximating 30 Hz.



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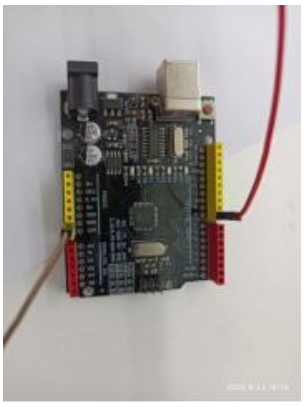

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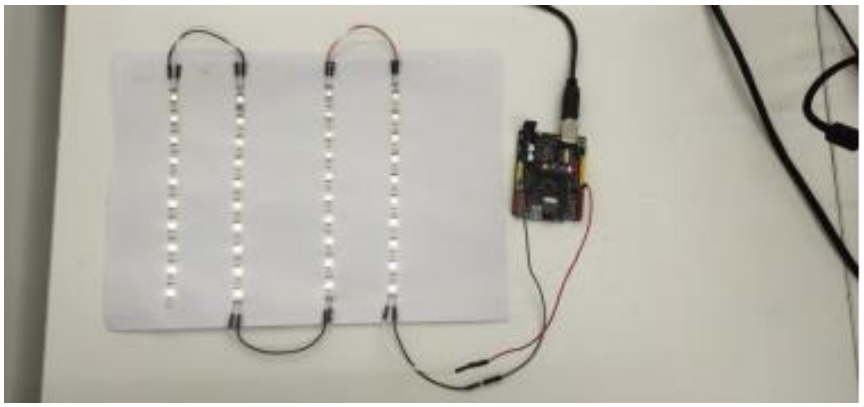

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Fig. 2. The hardware of the light therapy device. Fig. 2a shows a schematic diagram of the electrical circuit of the Arduino UNO R3 board that controls the light flickering. Fig. 2b is a photo of the actual Arduino Board. Fig. 2c shows the Arduino board connected to the LED band, which was used to apply light therapy.

B. Building the Circadian Rhythm Inducing Chamber

To investigate the impact of CRD on cognitive functions using mouse models, a home-based mouse colony was established according to the Guide for the Care and Use of Laboratory Animals, 8th edition. During the experiment, the mice were kept in standard transparent plastic cages (41.2 cm × 30 cm × 26.5 cm) with access to normal food and water.

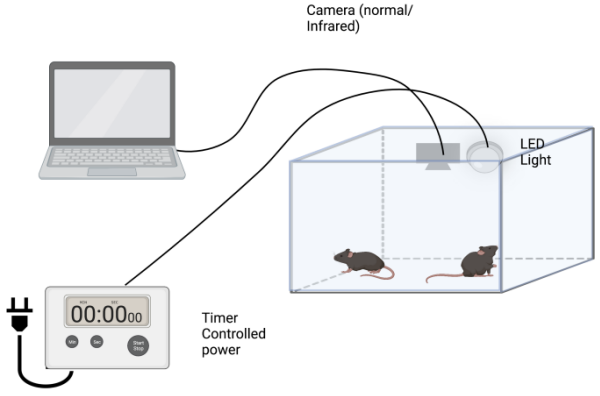

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Fig. 3. Circadian rhythm induction chamber. a: Schematic diagram of the mouse colony and circadian rhythm induction chamber setup. b: Photo of the custom-built circadian rhythm induction chamber housing 4–5 mice in a light-proof cage at room temperature. c: Light-proof cloth completely covering the cage.

To induce CRD in mice, different light-dark patterns were generated using LED lights, which have been shown to entrain the circadian rhythm of mice [14]. The LED lights and a camera were placed on top of the cages, with the LED lights connected to a power supply with a programmable timer to control the duration of light exposure for each group. The disruption of the circadian rhythm was monitored through the activity of the mice under light and dark conditions, which was recorded using a digital infrared camera connected to a computer (Fig. 3b). The circadian rhythm induction chamber was fully separated and shielded from external light using a light-proof cloth (Fig. 3c). The entire colony was maintained at a temperature of 25 ± 3°C with 50 ± 10% humidity, following the animal care guidelines mentioned previously.

IV. Circadian Rhythm Disruption and Cognitive Tests

After completing construction of the apparatuses, CRD was induced in the mouse colony. The CRD was verified using motion tracking and analysis. Once the circadian rhythm of the mice was fully disrupted, cognitive tests were performed to determine whether CRD affected the cognitive function of the mice.

A. Animals and Home-Made Environment

All animal work was conducted under the supervision of Enable Biotechnology (Shanghai) Co., Ltd., which approved the use of animals in this study and ensured adherence to ethical guidelines. Adult wild-type C57BL/6 male mice aged 9–12 weeks were used in this study to avoid metabolic changes due to growth and to prevent age-related effects on cognitive functions [3]. The mice were housed in the customized circadian rhythm induction chambers described earlier, with the walls of the cages polished and re-designed to prevent escape.

The mice were kept in different light-dark cycles for 2 weeks, followed by different behavioral tests. All experiments were conducted at 25 ± 3°C and other factors were kept constant. Mice were housed in groups of four in separate light-dark cycle environments. The control group was the normal circadian rhythm 12 h light – 12 h dark cycle (12L-12D, also called group 1). The unbalanced cycle of the 18 h light – 6 h dark cycle (18L-6D, also called group 2) was designed to simulate an extended circadian rhythm of sleep deprivation due to light exposure from digital devices. The reversed 12 h dark – 12 h light cycle (12L-12D-R, also called group 3) group was set to mimic jet lag or a night shift.

B. Tracking of the Circadian Rhythm

1) Procedure for tracking circadian rhythm

Mice are nocturnal animals [20] and typically exhibit more activity during the dark phase and less during the light phase [21]. Motion activities of mice indicate circadian rhythm activities [22]. To track the circadian rhythm of the mice, real-time video recordings were made, followed by post-hoc analysis of the videos to track the motion activities of the mice over 24-hour circadian cycles, a commonly used method in laboratories [23]. During the light phase, videos were captured with white LED light (Fig. 4a). During the dark phase, the white LED light was kept off, and the videos were captured with infrared light and an infrared camera (Fig. 4b). It has been shown that infrared light (with a wavelength of 820–880 nm) is not detected by either photoreceptor or ipRGC in the mouse retina [24]. Thus, the addition of infrared light did not affect the circadian rhythm of mice.

To track the motion activities of mice from the video recordings, the machine learning software TopScan was used to automatically extract motion activity data from the videos (Fig. 4c). Other software, such as DeepLabCut, can also be used for motion tracking from video recordings. Although DeepLabCut is the most advanced available technology [25] for tracking mouse activity and behavior, it requires substantial computational resources and can be challenging to implement. Therefore, TopScan was used as it is a more accessible method with high efficiency and accuracy.

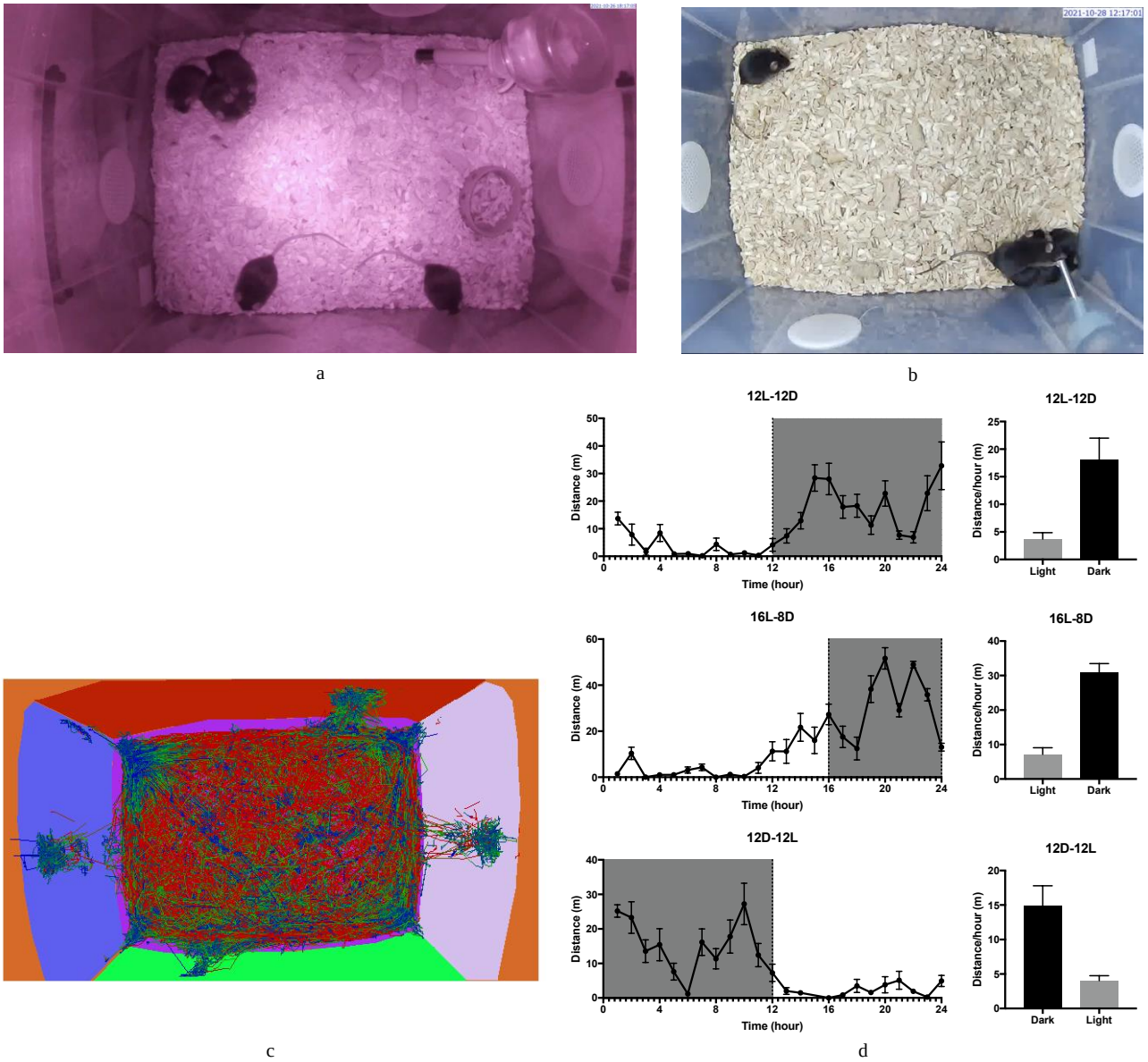

Fig. 4. Motion tracking analysis from the video recordings of mice reveals the induction of different circadian rhythms. a and b: Sample image of the IR video recording of the mice in the dark phase with infrared light and light phase with LED light. c: Motion tracking traces of the 4 mice from video analysis. d: Distance traveled per hour by mice at different circadian times.

2) Results of circadian rhythm tracking

The locations of the mice in the cage were extracted every hour during the 24-hour period, and the moving distance of each mouse was calculated for each hour. The motion activities over 24 hours are shown in Figs. 3b and 3c. The motion activities during the dark phase were significantly greater than during the light phase. For the 18L-6D group, the higher activities of the mice were restricted to the 8-hour dark phase, indicating the successful induction of the circadian rhythm disruption. For the 12L-12D-R group, the reversed circadian rhythm was also successfully induced (Fig. 4d).

C. Behaviors and Cognitive Tests Overview

CRD is known to affect spatial learning and memory as well as depression and anxiety, which are also influenced by sleep. Therefore, three widely accepted behavioral tests were chosen: the Morris water maze test for spatial learning and memory, the forced swim test for depression, and the open field test for anxiety. Fig. 5 provides a timeline overview of the different cognitive tests performed.

D. Morris Water Maze

1) Morris water maze test procedure

The Morris water maze was used to test the spatial learning and memory ability of mice [26]. During the training phase, the mice learned how to navigate when swimming in the water and find a hidden platform in the water, as shown in the visual representation of Fig. 6a. A circular tub was filled with a mixture of water and white paint, and a platform (10 cm in diameter) was submerged in one quadrant as a hidden platform. The start locations were randomized to one of four equidistant locations. Each

mouse was trained for four days, with four trials conducted each day. Each trial had a cut-off time of 60 seconds. After each trial, the mice were placed on the platform for 15 seconds. A probe trial was conducted after the last training session of each day, with the platform removed, and the mice allowed to swim freely for 60 seconds. If a mouse found the platform within 60 seconds, the trial was considered successful. The success rate (number of successful trials/total number of trials) of each day was used to evaluate the learning and memory ability of each group. The latency time for successful trials was also used to evaluate the learning and memory ability of the mice, with shorter latency times indicating better learning and memory ability. This cognitive test was performed based on the behavioral test described by Vorhees and Williams. The top-view camera was used to track the location of the mice during all sessions (Fig. 6b), and the videos were analyzed offline to extract the trajectory of the mice in each trial.

2) Morris water maze test results

Over the 4 days of training, mice in the 12L-12D group showed an increased success rate (around 50% on day 1 to 80% on day 4) and decreased latency time (from 37.03 ± 5.8 s to 14.15 ± 2.842 s), indicating they learned and retained memories. However, mice in group 2 showed a transient increase in success rate during day 3 but returned to baseline by day 4 (as shown in Fig. 6). Latency time decreased from 36.67 ± 4.963 s to 20.78 ± 4.656 s. However, statistical analysis found no increase in learning and memory. For mice in the 12L-12D-R group, success rate also showed a transient increase on day 3 but returned to baseline by day 4 (Fig. 6). Latency time ranged from 27.80 ± 6.473 s to 24.56 ± 5.406 s with no significant difference over 4 days of training. Hence, spatial learning and memory did not increase in these mice. These results suggest that CRD patterns decreased spatial learning and memory ability in mice from both the 18L-6D and 12L-12D-R groups.

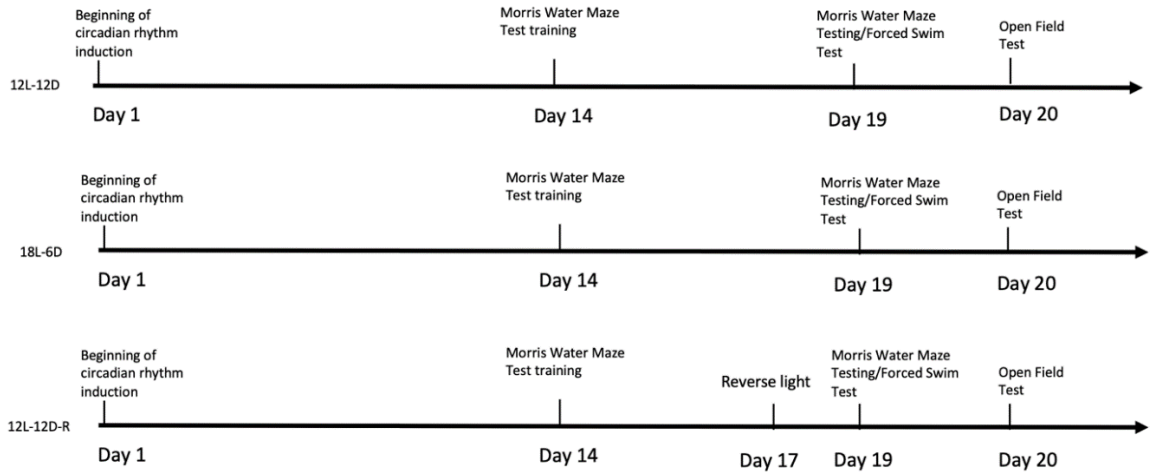

Fig. 5. Experimental timeline. On Day 1, circadian rhythm disruption began for the different light-dark cycle groups. Morris Water Maze training started on Day 14, followed by Morris Water Maze and forced swim testing on Day 19, and open field testing on Day 20. The reverse lighting schedule for the 12L-12D-R group began on Day 17, two days before behavioral testing.

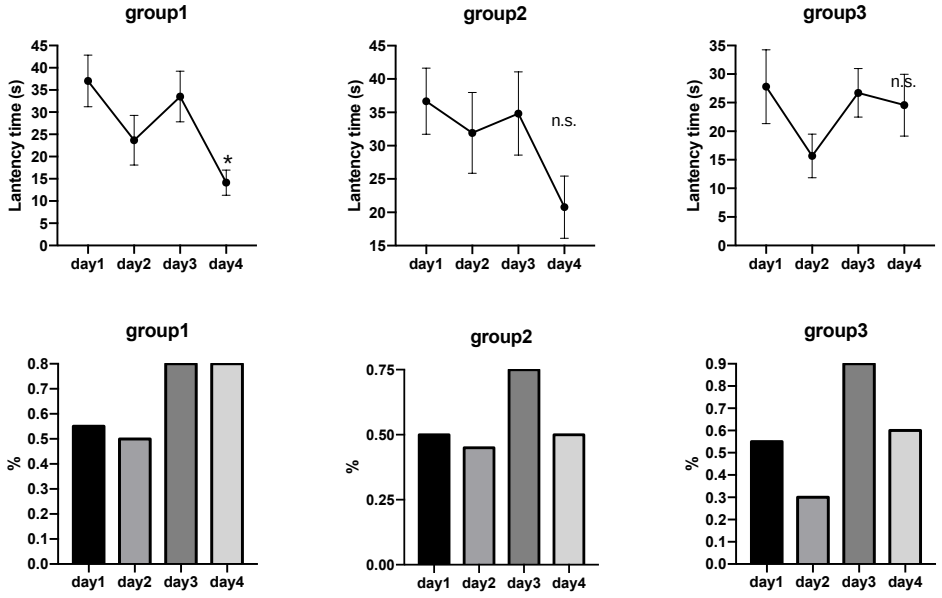

Fig. 6. Morris water maze test reveals the dysfunction in spatial learning of mice after circadian rhythm disruption. The graphs show latency time (left) and success rate (right) for mice finding the platform across 4 training days.

E. Forced Swim Test

1) Forced swim test procedure

The forced swim test evaluated the depression levels of the mice. Though mice can swim, they generally dislike water. When placed in water, mice initially swim out of fear but eventually give up when escape proves impossible. The time mice continue swimming before giving up indicates depression severity, with longer swim times signifying less depression. The test followed procedures described by [27]. For each mouse, a pre-test and test trial were conducted. For the pre-test, mice were placed in a vertical cylinder with 25 cm water (23–25°C) for 15 minutes. Afterward, mice were removed, dried in a heated enclosure, and returned to their cages. The test was conducted 24 hours post pre-test. For the test trial, each mouse was placed in the cylinder for 5 minutes. The entire swim test was video-recorded and subsequently assessed for the following behaviors: immobility (floating passively in the water without struggling) and swimming (active movements to keep the head of the mice above the water).

2) Forced swim test results

The mean test result for Group 1 (12L-12D, the control group) was 369.4 ± 144.3 s. Group 2 (18L-6D) averaged 465.2 ± 166.5 s while Group 3 (12L-12D-R) averaged 120.4 ± 30.79 s. Though mice in Group 3 appeared to give up swimming sooner, one-way ANOVA found no significant difference between groups in the forced swim task, suggesting circadian rhythm patterns did not influence depression levels in mice (Fig. 7).

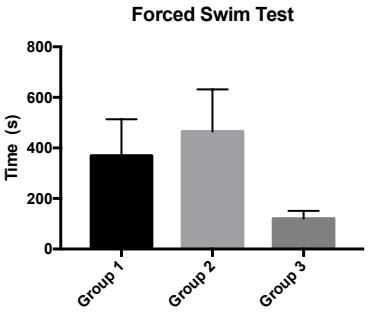

Fig. 7. The forced swim test reveals that mice with different circadian rhythms showed no significantly different levels of depressive behavior.

F. Open Field Test

1) Open field test procedure

The open field test evaluated anxiety levels in mice. Naturally, mice fear open fields due to danger from predators [28]. However, mice also explore novel areas out of curiosity. The balance between avoidance and exploration depends on anxiety levels [28]. Time spent in the central open field after placement indicates their anxiety level. The longer the mice spend in the open field, the less anxious they are.

Mice from different groups were placed in an open field area for 900 seconds. The total travel distance, travel distance in the surrounding area and central area, entry frequency into the surrounding and central area, and the time mice spent in the surrounding area and the central area were quantified. The open field test was conducted following the instructions described by Seibenhener and Wooten. The mice were placed in a soundproof and moderately illuminated cubic chamber. At the beginning of each test, the animals were gently placed in the center of the arena and allowed to explore. The exploratory activity in the open field was recorded on video using a camera and then analyzed using video tracking software.

2) Open field task results

To evaluate the anxiety level of mice, the metrics employed were the total distance, the surrounding/total ratio, central to total ratio, entry-time to surrounding distance, entry time to central, time to surrounding, and time to central. Mice traveled greater distances and spent more time in the surrounding area than the central area. For example, the ratio of distance traveled in the surrounding area exceeded 90% across all groups. This is consistent with mice generally preferring not to remain in open field areas.When comparing different groups, the results showed that the mice showed no difference in the total travel distance and the travel distance, entry frequency, or time spent in the surrounding and central area (Fig. 8). The test measured traveling distance, traveling ratio in surrounding and center, entry time to surrounding and center, and time spent in surrounding and center of the mice. The results showed no significant difference among mice from different groups and our circadian rhythm patterns did not influence the anxiety levels of the mice.

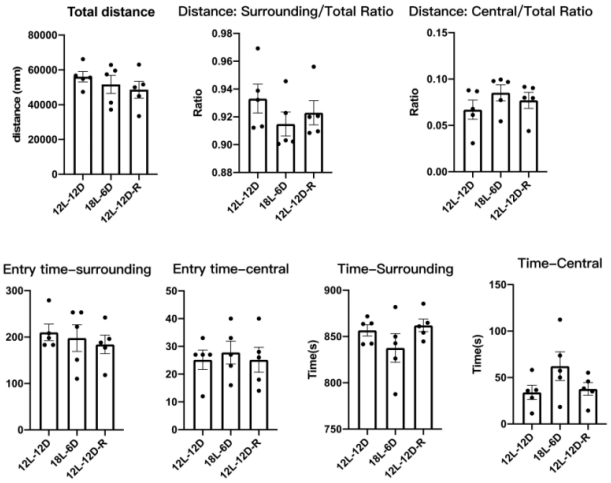

Fig. 8. The open field test reveals that mice with different circadian rhythms did not exhibit significantly different levels of stress behavior.

G. The Procedure of Light Therapy

Neurons in our nervous system use electrical signals to carry information. At a meta-level, our brain develops different oscillatory electrical rhythms of different frequencies [29]. These rhythms are associated with different brain states and cognitive functions. Various factors, such as light, can stimulate brain oscillatory rhythms. In the retina, visible light activates photoreceptors (rods and cones), and the information is transmitted to retinal ganglion cells and higher-order visual processing pathways [30] like the visual cortex. The oscillatory electrical rhythms of the visual cortex could propagate to other brain areas. For example, the light flickering could induce a brain-wide oscillation through the visual pathway. Some brain rhythms are found to exert

therapeutic effects on cognitive deficiencies. Specifically, the gamma frequency (30–40 Hz) of brain rhythm induced by LED light flickering through the visual pathway is shown to be able to improve the cognitive functions of patients with Alzheimer's disease [5]. The capacity of improving cognitive functions of this non-invasive method could be extended to other contexts, for example, CRD-induced cognitive function decline. To explore this possibility, LED light flickering of 30-40 Hz was used to cause CRD and test the cognitive functions. For mice from 16L-8D and 12L-12D-R groups, the mice were exposed to 30-40 Hz LED light flickering for 30 minutes for 5 days before the behavior tasks.

H. Results of Light Therapy

Due to CRD-induced deficiency only existing in spatial learning and memory manifested in the Morris water maze test, the Morris water maze task was the only one tested with light therapy. As expected, after 4 days of training, mice from the 12L-12D group (group 1) showed both an increase in success rate (from 5% to 40%) and decreased latency time (from 43.45 ± 0.000 s to 24.78 ± 8.856 s)for finding the platform in the Morris water Maze task. Our previous data have shown that mice with CRD were not able to show significantly better performance after being trained, indicating a deficiency in spatial learning and memory. As depicted in Fig. 9, after light therapy, the mice from the 18L-6D group (group 2) showed both increased success rate (from 0% to 45%) and decreased latency time (60.00 ± 0.000 s to15.72 ± 4.550 s) in the Morris water maze task, indicating that light therapy was able to correct extended light exposure CRD induced spatial learning and memory deficits. However, for mice from reversed 12L-12D-R cycle group (group 3), although the success rate and latency time on day 4 were higher than on day 1 (success rate going from 0% to 10%, latency time going from 60.00 ± 0.000 s to 20.83 ± 15.53 s), the success rate and latency time over 4 days of training fluctuated a lot (especially on Day 3, where the success rate and latency time were the same as on Day 1) and the difference was also not statistically significant. Thus, it is hard to determine whether after light therapy treatment the mice from this group have improved spatial learning and memory capacity. In conclusion, these results have shown that light therapy exerted therapeutic effects on CRD-induced spatial learning and memory deficits, in a pattern-specific manner.

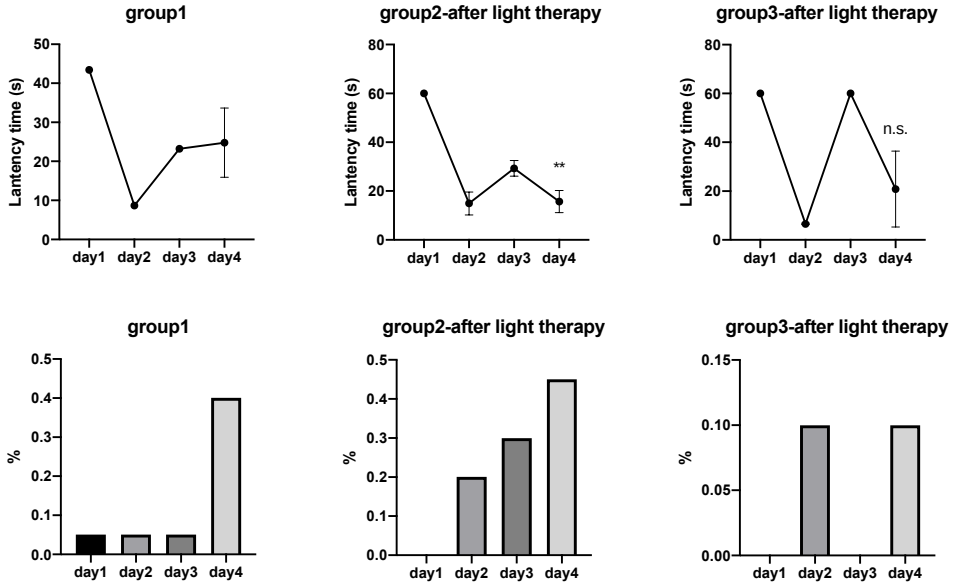

Fig. 9. Light therapy improves spatial learning dysfunction in mice with circadian rhythm disruption. The figure shows the latency time and success rate (%) of mice during the Morris water maze test after light therapy.

V. Discussion

A. Pathogenesis and Causality

It is promising to use light therapy to improve CRD-induced cognitive dysfunction. The question is how light therapy can influence the impact on cognitive functions like learning and memory, and whether there are any mechanisms specific to circadian rhythm. Research showed that 30 Hz flickering light inhibited glial cell activation in the brain [5]. Thus, one hypothesis is that light therapy reduces neuroinflammation induced by various stresses, including CRD [31]. CRD would thus induce metabolic changes in the brain that lead to neuroinflammation.

At the Neuro-molecular level, CRD may affect the formation of synaptic plasticity, which is the foundation of learning and memory [32]. As CRD leads to a deteriorating learning abilities in mice, another possible explanation would be that CRD would disrupt our sleep-wake cycles [33]. Therefore, circadian rhythm could also directly influence the method of organizing and processing information during sleep [34].

The long-term CRD illustrated by troubled sleep or reversed sleeping cycles is associated with anxiety and depression in mice or humans [35]. However, the CRD induced in this study did not have any impact on depression or anxiety levels. One potential reason would be that the time of CRD was not long enough to induce

significant changes in anxiety levels and depression. More research is needed to test the effect of light therapy upon prolonged CRD in mice needs to determine whether CRD affects anxiety and depression levels in mammals.

B. Mechanism of Light Therapy

The mechanism behind the functioning of light therapy is still unknown, but several hypotheses can be suggested. One possibility is that light therapy affects neuroinflammation and gene expression. The brain secretes excess proteins to maintain homeostasis [1]. Similar to light therapy for Alzheimer's disease, gamma oscillation helps clear faulty proteins and other waste material, allowing better expression of the gene that controls synaptic plasticity and enabling microglial cells to deal with inflammation. Gamma oscillations could also inhibit the expression of genes that promote neuroinflammation or promote a gene that counters neuroinflammation [1]. This would reduce inflammation and decrease the expression of the gene that promotes it. The next step is to determine whether this hypothesis is true. One potential way is to test whether light therapy affects mice that have been targeted with hippocampal neuroinflammation or other inflammation-related areas of the brain that control spatial learning and memory. Further investigation is needed on the effect of light therapy on the Rev-erbα (reverse erythroblastosis virus alpha) gene and other clock genes, as research has shown that these genes are mediators between inflammation and circadian rhythmicity [36].

Another hypothesis for how light therapy might affect our cognitive abilities is that it is similar to Low-Level Laser Therapy (LLLT), a concept in photobiology that has been shown to induce specific gene expression, wound healing, or even nerve regeneration through the expression of cytokines and growth factors [37]. Therefore, a comparative study on the effects of light therapy and LLLT should be tested to determine whether photobiology is the key to uncovering the mechanisms behind light therapy.

C. Light Therapy vs Melatonin Ingestion

The usage of light therapy has proven to be quick, simple, non-invasive, and successful against cognitive dysfunction of spatial learning and memory. Compared to the most common method against CRD, which is the ingestion of melatonin tablets [38], light therapy is superior because melatonin ingestion can cause headaches, nausea, and drowsiness [39]. Additionally, melatonin can interact with other drugs, such as anticoagulants and anti-platelet drugs, anticonvulsants, and contraceptive drugs [39], whereas light therapy does not have any of these side effects. Therefore, light therapy is a safer and more convenient option for treating CRD-induced cognitive dysfunction.

D. Improvements to Be Made for Human Use

Although this study used mice to test the effects of light therapy on CRD-induced cognitive dysfunction, adapting light therapy to more animal models and conducting experiments on humans would be necessary before clinical and commercial trials. In terms of clinical use, the maintenance and setup costs of light therapy would be relatively low, as any empty room with light that has a frequency of 30 Hz could be adapted for this purpose. For commercial use, the construction of small, portable gadgets with 30 Hz light in the form of an eye mask or goggles would be a low-cost and potentially high-reward product. However, further research is needed to confirm the safety and efficacy of light therapy in humans before it can be widely used clinically or commercially.

E. Limitations

Although revealing, this study includes a few limitations. The first limitation is the biological differences between mice and humans, which means that our conclusions from the mouse model may not apply to humans. Mice are nocturnal animals, and their natural circadian rhythm may differ from that of humans. While mice and human brains share similar fundamental functions, the human brain is much more complex and may respond differently to CRD [40]. For example, the prefrontal cortex of the human brain (which controls cognitive functions) is much more developed than the mice's brain [41] which could lead to either more negative impacts. Moreover, the genes controlling the circadian rhythm in humans and mice are inherently different, which is why they might possess different properties and mechanisms of control that have not been taken into account.

Ideally, the correlation between circadian rhythm patterns and human performance in cognitive behavior tasks would be observed. However, considering that the daily lives of humans vary a lot, it is difficult to control the independent variables and tease out the effects of CRD. Hence, the C57BL/6 mouse model was chosen to explore the issue. It is the most commonly used mammal animal model in biomedical studies [42]. It has a relatively stable and uniform genetic background and is relatively easy to handle, with standard behavior tasks available to test different cognitive behaviors of mice. However, caution should be taken when extrapolating the results to humans, and further research is needed to confirm the safety and efficacy of light therapy in humans.

The second limitation of this study arises from the use of traditional methods of cognitive testing (Morris water maze test, forced swim test, open field test). A potential drawback is that the cognitive function testing may be limited, and there is room for improvement in the range of cognitive functions targeted [43]. The three cognitive functions targeted in this study were chosen because they are each different and their behavior task is easy to build or access. However, further exploration with more experimental equipment is needed. For example, neurophysiological screening can be used to test sensory and motor function, autonomic reflexes, and the continuous performance task or Multiple-Choice Serial-Reaction Test to assess the vigilance of mice. Furthermore, electrophysiological methods could be used to explore more complex social behaviors, such as information processing, decision-making, emotional regulation, and pain sensation.

Thirdly, the behavior task chosen (Morris water maze, forced swim test, open field test) might be limited in reflecting the cognitive function targeted (spatial learning, depression levels, and anxiety). The Morris water maze test might by itself is not sufficient to assess whether spatial learning and memory is the sole factor improved in CRD mice, as other cognitive factors come into play, such as motor skills that may also affect the results. More experiments targeting motor skills and motivation should be done to truly discern the full extent of light therapy.

Finally, the number of mice and data used in this study was limited. Although TopScan is a useful tool, the computational power required to track 5 mice simultaneously (as shown in Fig. 4) for long periods of time was too much of a burden. Hence, the motion of only 4 mice was tracked. In future explorations, more computational power is needed in order to track larger amounts of mice and get better results.

F. Potential Harms

Although light therapy has a lot of potential, there might be some risks to human usage. Research has shown that flicker light with more than 100 Hz of frequency could induce seizures in humans [44, 45]. While 30 Hz flickering light is safe for most people, it may be harmful to patients diagnosed with epilepsy, as it could trigger seizures and make them unsuitable for this type of treatment.

Another potential issue is that exposure to 30 Hz flickering light for 30 minutes to an hour can be unpleasant and may cause discomfort to the eyes with long-term exposure. To address this issue, exploring light with different colors and frequencies could help identify a light that is more effective, less unpleasant, and has fewer harmful effects on vision. It is important to carefully evaluate the safety and efficacy of any new light therapy techniques before they are widely used in clinical or commercial settings.

G. Future Opportunities

The next step is to experiment with light therapy on other animal models, such as monkeys, which have gene expression and circadian rhythms closer to humans. Further research on the effects of light therapy on monkeys could provide a better understanding of whether light therapy could work in humans. Light therapy should also be applied with different frequencies, colors, and wavelengths or compared to other types of photobiological technologies to explore its potential for treating other neurodegenerative diseases and understand its full capacity, as there may be an optimum setting for each cognitive function or neurological disorder that it targets.

In the future, it may be possible to compensate for the side effects of CRD in humans. With further research, the side effects of our daily CRD could be minimized, leading to increased productivity and a faster pace of life. When light therapy becomes a more mature technology, it could potentially provide a cure for all diseases related to CRD, completely eradicating it from people's lives.

The ultimate clinical and commercial goal of this technology is to create a practical, portable, and wearable device that uses different wavelengths of flickering light, which could be widely used and easily accessible. This would allow for more people to benefit from the therapy and potentially improve their cognitive function and overall well-being.

VI. Conclusion

In this study, the efficacy of light therapy in treating cognitive dysfunction related to circadian rhythm disruption has been explored and proven. Specific apparatus, such as the circadian rhythm induction chamber and the light therapy device, were built and designed for this study. A reliable laboratory and investigational environment were established, and the mice's circadian rhythms were intentionally disrupted for the experiment. Light therapy was then administered to alleviate their cognitive dysfunctions. The mice's cognitive abilities were assessed both with and without light therapy treatment.

The results demonstrated the effectiveness of light therapy in improving spatial learning and memory deficits induced by extended light exposure circadian rhythm disruption. However, light therapy did not significantly affect the depression and anxiety levels of the mice, as measured by the Forced Swim test and Open Field test.

Further research is needed to thoroughly explore the full potential of light therapy, including its effectiveness on other animal models and for different cognitive functions, as well as any potential side effects and risks. While initial results are promising, more evidence is required before concluding that light therapy could provide a cure for all diseases related to CRD. However, if properly developed and validated, light therapy treatments have the potential to lead to improved cognitive function and wellbeing for many people suffering from CRD-related conditions. An ultimate goal of this research could be the development of a practical, portable, and wearable device that uses specific wavelengths of flickering light, making this technology widely accessible and potentially life-changing for many people.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Robin Dao conceived of the presented idea, performed the calculations, analyzed the data, worked on the technical details, and wrote the paper. All authors discussed the results together and Pingchuan Ma proofread the paper. All authors had approved the final version.

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