


RESEARCH ARTICLE

The Effects of Blue Light on Locomotion and Cognition in Early Adult *Drosophila melanogaster*

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ABSTRACT

Artificial light sources, particularly blue light, have raised concerns about their impact on biological health and behavior. In this study, we explored the effects of blue light on the locomotion and cognitive functions of early adult *Drosophila melanogaster*. Our experiments were conducted in a custom-designed behavioral arena to assess how blue light influences these parameters. We found that after ~2 weeks of blue light exposure, the locomotion of fruit flies, including movement speed and activity levels, significantly increased, but these effects disappeared by the third week. However, this exposure also led to a notable reduction in lifespan and had adverse effects on cognitive functions, including attention and short-term spatial memory. These findings may reveal the profound impacts of blue light on neural behavioral functions and lifespan, highlighting the importance of further research into the biological effects of blue light.

1 | Introduction

Light exposure is a key factor in regulating numerous physiological processes in organisms, from adjusting sleep-wake cycles (Boivin and James 2002; Stenvers et al. 2016), body temperature (Dijk, Cajochen, and Borbély 1991; Bunnell et al. 1992), and metabolism (Fonken and Nelson 2014; Melanson et al. 2018) to hormone secretion (Kostoglou-Athanassiou et al. 1998; Bedrosian and Nelson 2017), all of which are crucial for maintaining physiological homeostasis. The impact of natural and artificial light on biological systems has emerged as a significant area of scientific inquiry, with a focus on the proliferation of artificial light sources and their biological implications.

In recent years, with the widespread use of LED lights and digital devices, people have been increasingly exposed to blue light in their

work and personal lives, raising broad scientific concerns about the health impacts of artificial lighting, especially blue light. Blue light, which has high energy availability, affects physiological and behavioral processes in organisms (West et al. 2011; Wu et al. 2021). Prolonged exposure to blue light disrupts the circadian rhythms of organisms (Tähhämö, Partonen, and Pesonen 2019; Wahl et al. 2019) and even impairs retinal health (Tosini, Ferguson, and Tsubota 2016; Hatori et al. 2017).

The fruit fly (*Drosophila melanogaster*), a phototactic insect (Hu and Stark 1980; Kane et al. 2013) with a short lifespan, simple brain structure, and easily observable behavioral characteristics, has become an ideal model organism for studying the effects of light exposure. Previous studies have demonstrated that blue light significantly impacts the neural behavior of fruit flies, including increasing activity and changing attention and

Summary

- Blue light temporarily enhances locomotion in early adult fruit flies but significantly shortens their lifespan.
- Blue light reduces the attention of early adult fruit flies and impairs their short-term spatial memory.

memory, reflecting how light exposure directly affects the neural system and behavior of fruit flies (Nash et al. 2019; Song et al. 2022; Yang et al. 2022). Although previous studies have shown that blue light enhances brain activation in areas associated with locomotion and cognition, it is still unclear how blue light alters cognitive behavior in organisms.

This study aimed to explore how blue light affects the locomotion and cognitive behavior of early adult fruit flies. By exposing fruit flies to blue or white light during their rearing process, we compared the differences in lifespan, locomotion level, attention, and short-term spatial memory under these two lighting conditions. Through this research, we hope to better understand how blue light affects the behavior and physiological processes of fruit flies, providing scientific evidence for assessing the potential health risks of blue light.

2 | Materials and Methods

2.1 | Fly Strains

The female fruit flies were maintained in incubators at a steady temperature of 25°C and a relative humidity ranging from 40% to 60%. They were exposed to a 12-h light–dark cycle, where the light phase lasted from 8:00 a.m. to 8:00 p.m., followed by the dark phase from 8:00 p.m. to 8:00 a.m. For the purposes of the experiment, the control group was exposed to white light, while the experimental group was exposed to blue light. To set different light sources, we used white and blue LED stripes as the white and blue light sources, respectively. The LED stripes operate at a voltage of 12 V, with dimensions of 12 × 12 mm and an emitting surface of 12 mm, and with a power of 12 W/m. After adjustments, the average luminance of both white and blue light sources was set to ~400 lux. The blue light had a wavelength range of 400–500 nm, with a full width at half maximum (FWHM) of 425–475 nm. The white light covered the entire visible spectrum for fruit flies, with the blue light component (400–500 nm) contributing ~120 lux. The wild-type *Canton-S* strain, obtained from the Bloomington Drosophila Stock Center (BDSC), was used as a standard wild-type strain, along with the RRID BDSC_64349.

2.2 | The Tracking Apparatus

Our tracking apparatus was identical to the equipment described in our previous study (Chi et al. 2020; Han, Huang, et al. 2021) (Figure 1a). Briefly, the experimental setup consisted of a white circular platform surrounded by a 360° LED screen. The circular platform had a diameter of 85 mm and was surrounded by water to prevent the flies from escaping. The LED screen had a diameter of 200 mm and a height of 130 mm and was composed of 20 individual panels. Each panel

contained four 8 × 8 LED matrices made of yellow–green LEDs (1.2" KWM-30881CUGB). The LEDs on the screen could be individually controlled to switch on or off using an Arduino Shield MEGA2560. A CCD camera recorded the movement traces of the fruit flies. The movement traces captured in the video data were converted into analyzable *x* and *y* coordinates via Python 3.5.

2.3 | Experimental Design

Our experiment was derived from the classic Buridan's paradigm, in which wing-clipped fruit flies were sequentially placed within a 360° circular screen. When black visual stimuli appeared at both ends of the screen, due to negative phototaxis, the fruit fly moved back and forth between the two visual stimuli. In our previous studies, we observed that after the visual stimuli were presented for 60 s, the fruit flies continued moving back and forth between the positions where the stimuli had disappeared for ~90 s (Yen, Han, and Lo 2019; Han, Huang, et al. 2021). On the basis of these findings, we adopted the experimental setup used in previous studies, and divided the behavioral experiments into three stages: the prestimulus stage (90 s), the stimulus stage (60 s), and the poststimulus stage (90 s) (Figure 1b). No visual stimuli were presented during the prestimulus and poststimulus stages, whereas in the stimulus stage, two opposing 30° black visual stimuli were presented on the annular screen. The prestimulus stage assessed the locomotion levels of the fruit flies in the absence of visual stimuli, including their activity level, movement speed, and wobbling time ratio. In the stimulus stage, we evaluated the attention to the visual stimuli, measured by the fixation index (*FI*; see Section 2.4 for details). In the poststimulus stage, we tested the short-term spatial memory by examining their continued movement toward the location of the disappeared visual stimuli, which was also measured by the *FI*. Consistent with our prior experimental setup, in this study, we used 3–5-day-old fruit flies that were subjected to low-temperature anesthesia and the wings were shortened. Starting from the day after their wings were clipped, the flies were sequentially placed in an LED apparatus for experiments according to the purpose of the study. Figure 1c shows a representative trace of the fruit flies during their first behavioral test in this experiment (Figure 1c).

2.4 | Data Analysis

Our research objective was to analyze the impact of blue light on the behavior of early adult fruit flies. Based on previous studies (Chi et al. 2020; Han, Huang, et al. 2021; Han, Wei, et al. 2021; Han, Zhang, et al. 2024), to analyze the locomotion levels of fruit flies, we defined the activity level, movement speed, and wobbling time ratio. The activity level was calculated as the percentage of time points at which a fruit fly moved relative to all captured points, whereas the movement speed was calculated as the total distance moved divided by the duration of the prestimulus stage. The wobbling time ratio was defined as the percentage of the prestimulus stage in which the fruit fly moved < 0.3 mm, which is consistent with the definitions used in our previous studies.

Additionally, we analyzed the attractiveness of visual stimuli to fruit flies during the stimulus stage and poststimulus stage. We

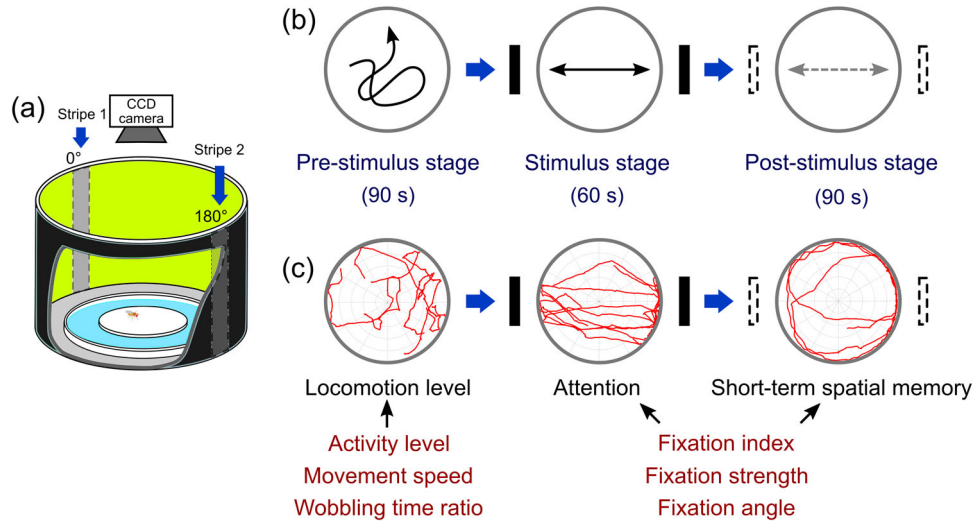


FIGURE 1 | Experimental apparatus and protocol for *Drosophila melanogaster*. (a) Following the test protocol and analysis methods mentioned in our previous study (Han, Huang, et al. 2021), we placed fruit flies on a white circular platform to analyze their movement traces. (b) The experiment consisted of three stages: the prestimulus stage (90 s), the stimulus stage (60 s), and the poststimulus stage (90 s). During each trial, an individual fruit fly was positioned at the center of the platform, where it was free to move throughout the trial. (c) Typical traces of the three stages. In the prestimulus stage without visual stimuli, the fruit fly locomotion level, such as activity level, movement speed, and wobbling time ratio, was tested. In the stimulus stage with two vertical visual stimuli, the fruit fly’s attention to visual stimuli, fixation index, fixation strength, and fixation angle were measured (see Section 2 for details). In the poststimulus stage after the disappearance of visual stimuli, the fruit fly’s short-term spatial memory, as well as the fixation index, fixation strength, and fixation angle, were measured (see Section 2 for details).

followed the definition of the *FI*, which quantifies the degree of fixation on the presented and disappearing positions of the visual stimuli (Han et al. 2021a; Han, Tan, and Lo 2024). Specifically, we represented the movement direction of fruit flies using vectors formed by the coordinates of two adjacent capture points, denoted by the angle θ on the screen’s speed vector projection. We divided the 360° range into 12 30° sectors and calculated the movement direction percentage for each, $p(\theta)$, every 5 s using a 15-s sliding window. To assess fixation behavior toward visual stimuli, we defined the *FI* as the difference between the orientation toward ($P(0^\circ; 180^\circ) = p(0^\circ) + p(180^\circ)$) and perpendicular to the stimuli ($P(90^\circ; 270^\circ) = p(90^\circ) + p(270^\circ)$). A positive *FI* indicates fixation toward the stimuli, and a larger absolute *FI* value suggests stronger fixation behavior.

$$FI = P(0^\circ; 180^\circ) - P(90^\circ; 270^\circ). \quad (1)$$

Next, consistent with our previous studies, we define σ_f from our past research as the “fixation angle” and *AS* as the “fixation strength” (Han, Wei, et al. 2021). In the radar plots, we draw a line representing the minimum second moment, and the direction of this line corresponds to σ_f , while the fixation strength is calculated as 1 minus the ratio of the minimum second moment to the maximum second moment.

According to our previous results (Han et al. 2021a), the average fixation strength of fruit flies in all prestimulus stages was ~ 0.05 . Therefore, in this study, on the radar plots, we used a fixation strength > 0.05 as the criterion for the presence of fixation behavior in fruit flies, which is indicated with bidirectional arrows.

Thus, the *FI* measures the fixation behavior in the direction of visual stimuli during the stimulus stage and poststimulus stage. The fixation angle and fixation strength are used to analyze the

angle of fixation orientation and the intensity of orientation in a specific direction during different stages.

2.5 | Statistical Analysis

In the analysis of all groups, data points that fell more than two standard deviations from the mean were considered outliers and excluded. Thus, the amount of data used in the first stage of the experiment (locomotion level testing) and the second and third stages (cognitive level testing) may not match the actual number of surviving fruit flies. Since the total duration of the behavioral experiments was 240 s, we observed that the fruit flies were not always moving. Upon analyzing the data, we found that outliers were primarily cases where the fruit flies remained completely static during a specific stage.

Therefore, in the locomotion level test, we excluded data that remained completely static during the prestimulus stage. For the cognitive level tests, if a fruit fly remained completely static during either the stimulus stage (60 s) or the poststimulus stage (90 s), its data were excluded from the analysis of attention and short-term memory.

Statistical analyses were conducted using Statistical Product and Service Solutions version 22.0 (SPSS 22.0). Independent samples *t* tests were used to analyze the differences in activity level, movement speed, wobbling time ratio, and *FI* between the white light-exposed group and the blue light-exposed group in each behavioral test. To analyze trends in the average fixation strength during the stimulus and poststimulus stages at different test time points between the two groups, polynomial regression models were employed. Linear regression, quadratic (squared), and cubic (cubed) polynomial regression models

Survival and locomotion level

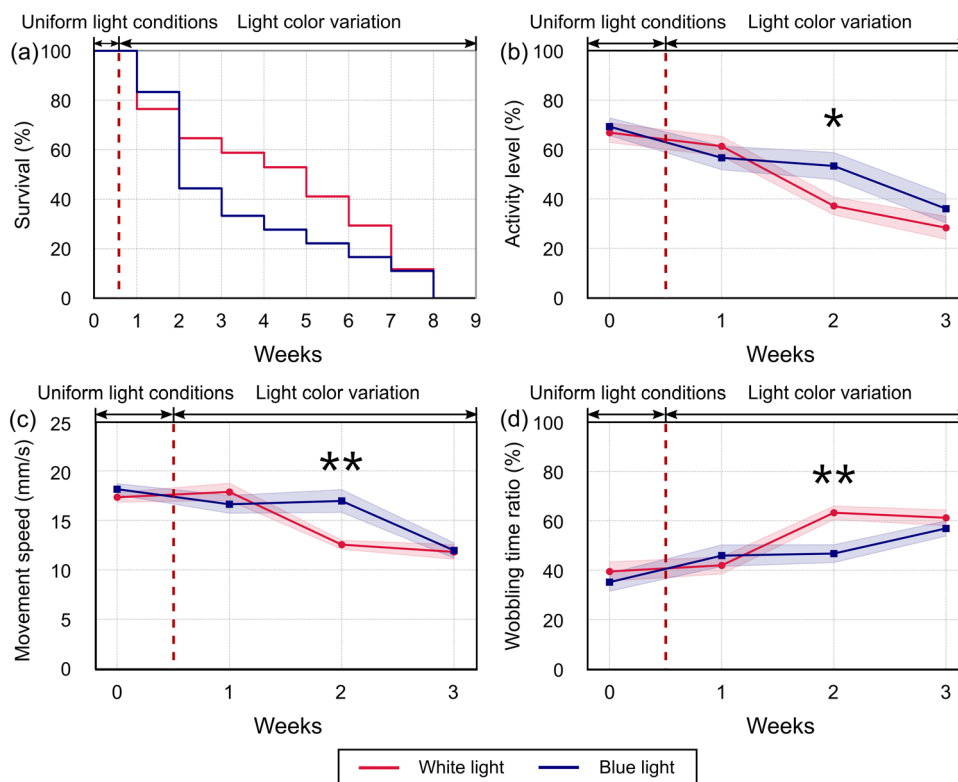


FIGURE 2 | Effects of blue light on the lifespan of fruit flies and the locomotion of early adult fruit flies. (a) We tracked the survival rates of two groups of fruit flies after exposure to 12 h of white light (red line) or 12 h of blue light (blue line). Initially, both groups were exposed to white light. After the first behavioral tests, we subjected the control group to a cycle of 12 h of white light followed by 12 h of darkness, while the experimental group was switched to 12 h of blue light followed by 12 h of darkness. We tracked the survival of fruit flies weekly. Over 9 weeks, the survival numbers for the white light group were 17, 13, 11, 10, 9, 7, 5, 2, and 0, whereas those for the blue light group were 18, 15, 8, 6, 5, 4, 3, 2, and 0. (b–d) We measured the activity level, movement speed, and wobbling time ratio of the fruit flies over the first 4 weeks. The numbers for the white light group during the 4 weeks of behavioral testing were 17, 12, 10, and 9, whereas those for the blue light group were 18, 15, 8, and 6. Two weeks after setting up the blue light, compared with the white light group, the blue light group presented increased activity levels, faster movement speeds, and a significant decrease in wobbling (* $p < 0.05$, ** $p < 0.01$, the shaded area indicates the standard error of the mean, SEM).

were used to analyze the behavioral patterns of fruit flies better. The regression models were as follows:

$$\text{Linear regression: } y = ax + b. \quad (2)$$

$$\text{Quadratic regression: } y = ax^2 + bx + c. \quad (3)$$

$$\text{Cubic regression: } y = ax^3 + bx^2 + cx + d. \quad (4)$$

Here, y represents the average fixation strength, x is the test time point, and a , b , c , and d are regression coefficients. The choice of regression model was based on the R^2 value, with higher-order regression models adopted if they significantly improved data fitting.

3 | Results

3.1 | Blue Light Enhances Locomotion in Early Adult Fruit Flies But Accelerates Their Aging

To investigate the effects of blue light on the lifespan and activity levels of fruit flies, we placed the flies in a custom-made

behavioral experimental apparatus to observe the behavior of different groups (Figure 1; see Section 2 for details). We clipped the wings of the flies 3–5 days after eclosion, divided them into two groups, and recorded the number of flies in each group (Figure 2a). During the first stage of the behavioral experiment, the flies were placed on a 360° green circular LED screen without black visual stimuli, and their activity level, movement speed, and wobbling time ratio were recorded (Figure 2b–d). Before the first behavioral experiment, both groups of flies lived under a 12-h white light and 12-h dark cycle; after the first behavioral experiment, we changed the light cycle for the experimental group to 12 h of blue light and 12 h of dark to compare the effects of blue light on the lifespan and locomotion levels of the flies. We conducted weekly survival tracking and tested the flies' activity level, movement speed, and wobbling time ratio once a week for 4 weeks to assess the impact of blue light on the locomotion of early adult fruit flies. Compared with those in the white light group, the survival rates of the flies in the blue light group significantly decreased by week 2 (Figure 2a). After the survival rate was measured in the first week, we conducted the first behavioral test. To determine whether the decrease in survival numbers for both groups of fruit flies in the second week was caused by the behavioral test, we tracked the survival numbers and rates of

Attention

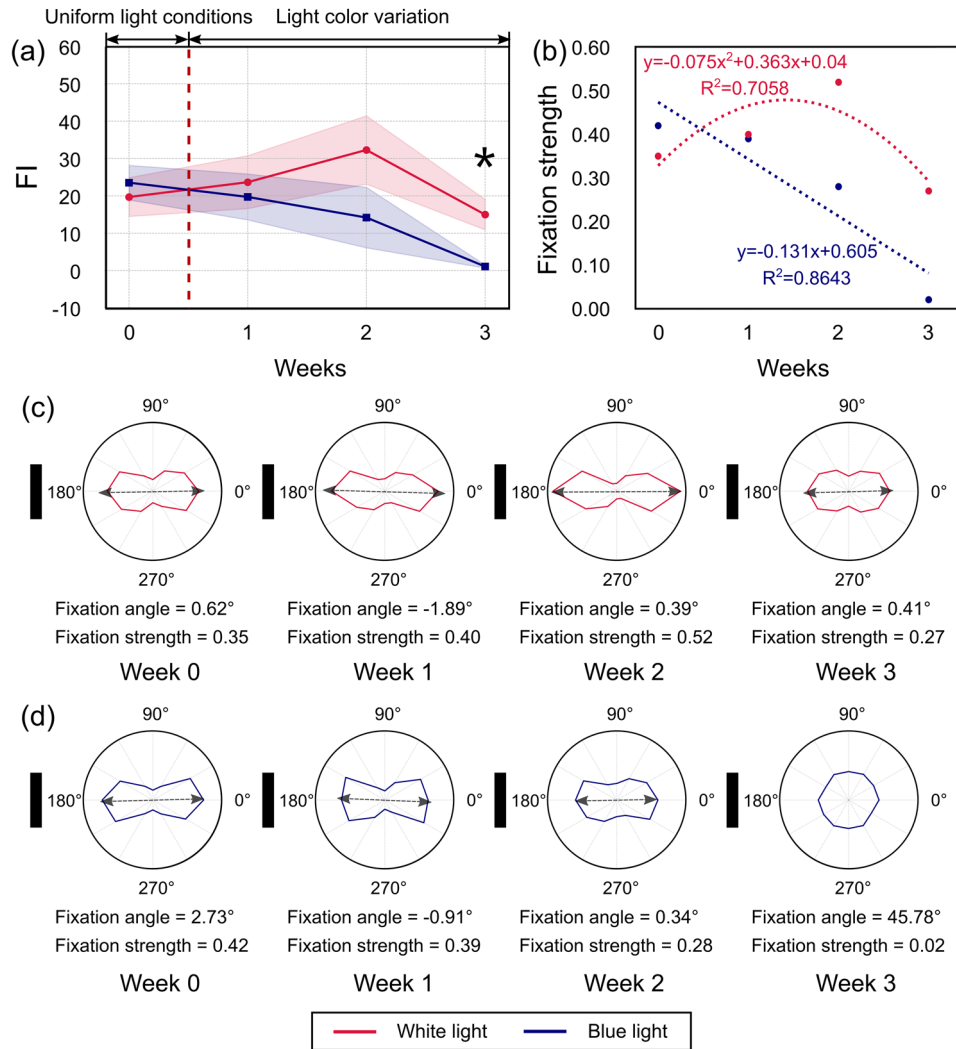


FIGURE 3 | Effects of blue light on the attention of early adult fruit flies. (a) During the attention test for fruit flies, the number of individuals in the white light group at different time points was 13, 12, 7, and 6, whereas the corresponding numbers in the blue light group were 15, 9, 4, and 4. Under uniform lighting conditions at week 0 and after blue light exposure to the experimental group from Weeks 1 to 3, the fixation index (*FI*, see Section 2 for details) for black visual stimuli was significantly lower in week 3 than in the white light group ($*p < 0.05$, the shaded area indicates the standard error of the mean, SEM). (b) In the stimulus stage, the average fixation strength of the fruit flies in both the blue light group and the white light group changed over time. The white light group exhibited a quadratic regression trend across the four tests ($y = -0.075x^2 + 0.363x + 0.04$, $R^2 = 0.7058$), whereas the blue light group exhibited a clear linear decline in fixation strength across the four tests ($y = -0.131x + 0.605$, $R^2 = 0.8643$). (c and d) Radar plots showing the effects of white and blue light exposure on the fixation angle and fixation strength of fruit flies when visual stimuli are presented. The results further revealed that as the duration of blue light exposure increased, the fixation behavior in response to visual stimuli decreased. Two weeks after blue light exposure, the fixation behavior on the visual stimuli disappeared (fixation strength = 0.02).

fruit flies without behavior tests (Figure S1, Table S1). During this process, the timing of blue light exposure and wing removal was consistent with that in Figure 2a, but no behavioral tests were performed. To track the impact of blue light on the survival rate of fruit flies more precisely, we refined the survival curve from weekly to daily intervals. When the survival curves shown in Figure 2a and Figure S1 were compared, we found that behavioral testing had no significant effect on the survival rate of the fruit flies. Surprisingly, compared with those in the white light group, the blue light group presented significantly greater activity levels and movement speeds in Week 2, whereas their wobbling time ratio was significantly lower (Figure 2b–d). These findings suggest that blue light may temporarily increase the

excitement and locomotion levels of fruit flies but ultimately affect their lifespan, thereby accelerating their aging.

3.2 | Blue Light Reduces the Attention of Early Adult Fruit Flies to Visual Stimuli

Next, we tested the effects of two vertical black visual stimuli presented in the fruit fly field of vision (the stimulus stage in Figure 1b) on fixation behavior in response to visual stimuli to understand the impact of blue light on the attention of early adult fruit flies (Figure 3). The results showed that for the control group exposed to white light, the fixation on visual stimuli was greatest in week 2

($FI = 32.30$, fixation strength = 0.52) (Figure 3a-c). We used polynomial regression models to fit the potential trends in the average fixation strength of fruit flies across different time points. The fitting results for the white light group via linear, quadratic, and cubic regression were as follows: linear regression: $y = 0.012x + 0.415$, $R^2 = 0.0219$; quadratic regression: $y = -0.075x^2 + 0.363x + 0.04$, $R^2 = 0.7058$; and cubic regression: $y = -0.0733x^3 + 0.475x^2 - 0.8617x + 0.81$, $R^2 = 1$. The low R^2 value for linear regression suggests that the data do not fit well with a linear model. Quadratic regression significantly improved the fit, whereas cubic regression achieved a nearly perfect fit, suggesting a risk of overfitting. Therefore, we concluded that quadratic regression is a reasonable choice for modeling the average fixation strength of the white light group. For the blue light group, the fitting results via linear, quadratic, and cubic regression were as follows: linear regression: $y = -0.131x + 0.605$, $R^2 = 0.8643$; quadratic regression: $y = -0.0575x^2 + 0.1565x + 0.3175$, $R^2 = 0.9975$; and cubic regression: $y = -0.0117x^3 + 0.03x^2 - 0.0383x + 0.44$, $R^2 = 1$. The results indicate that linear regression already provided a good fit for the blue light group. Therefore, we chose linear regression to model the average fixation strength of the blue light group.

Based on the polynomial fitting results, we observed that the attention of fruit flies in the white light group was the highest ~20 days after eclosion (Week 2 in Figure 3b). In contrast, early adult fruit flies exposed to blue light exhibited a persistent decrease in attention to visual stimuli; continuous exposure to blue light resulted in a sustained reduction in attention to visual stimuli ($R^2 = 0.8643$) (Figure 3b). Additionally, after each behavioral test, we created radar plots for the two groups, revealing differences in fixation strength between the blue light and white light groups (fixation strength = 0.52 in the white light group, fixation strength = 0.28 in the blue light group, week 2) (Figure 3c,d). By Week 3, the blue light group showed no fixation on the visual stimuli when presented, exhibiting behavior close to random movement (the right panel in Figure 3d).

3.3 | Blue Light Reduces the Short-Term Spatial Memory of Early Adult Fruit Flies

Furthermore, we tested the short-term spatial memory of early adult fruit flies in the poststimulus stage (Figure 4). The results showed that fruit flies exposed to white light presented the greatest decrease in short-term spatial memory performance at week 2 after eclosion, while those exposed to blue light consistently showed a decrease in short-term spatial memory (Figure 4a). Consistent with the attention analysis, we also used polynomial regression models to analyze changes in the average fixation strength of fruit flies at different time points. The fitting results for the white light group via linear, quadratic, and cubic regression were as follows: linear regression: $y = 0.012x + 0.14$, $R^2 = 0.0308$; quadratic regression: $y = -0.055x^2 + 0.287x - 0.135$, $R^2 = 0.5479$; and cubic regression: $y = -0.077x^3 + 0.52x^2 - 0.993x + 0.67$, $R^2 = 1$. We observed that the R^2 value for linear regression was low, whereas cubic regression carried a risk of overfitting. Therefore, we used quadratic regression to model the average fixation strength of the white light group in the poststimulus stage.

Similarly, the fitting results for the blue light group are as follows: linear regression: $y = -0.021x + 0.115$, $R^2 = 0.5158$;

quadratic regression: $y = -0.0175x^2 + 0.0665x + 0.0275$, $R^2 = 0.8023$; and cubic regression: $y = 0.0217x^3 - 0.18x^2 + 0.4283x - 0.2$, $R^2 = 1$. We found that linear regression already provided a good fit for the blue light group, so we used linear regression to model the average fixation strength of fruit flies in the blue light group. Similar to the changes in attention, the white light group exhibited stronger short-term memory ~20 days after eclosion (week 2 in Figure 4b), whereas the blue light group presented a strong decreasing trend ($R^2 = 0.5158$) (Figure 4b). Additionally, we used radar plots to visualize the fixation angle and fixation strength of the two groups of fruit flies toward the location of the visual stimuli after they had disappeared. We found that compared to when visual stimuli were present, the focus of the fruit flies toward the location of the stimuli was slightly lower after the stimuli had disappeared. After 3 weeks of blue light exposure, a weaker fixation strength was observed (Figure 4d, the two rightmost panels), indicating that the impact of blue light on fruit flies' short-term spatial memory was greater than the impact on fixation in the stimulus stage.

4 | Discussion

In the present study, we explored the effects of blue light on locomotion and cognition in early adult fruit flies. We found that during the second week of blue light exposure, the blue light group exhibited higher activity levels, faster movement speeds, and lower wobbling time ratios. However, the survival rate showed the opposite trend, with the survival rate of fruit flies in the blue light group decreasing more significantly than that in the white light group after 2 weeks of exposure. In terms of cognition, the cognition of fruit flies in the white light group peaked ~20 days after eclosion, whereas the cognition of fruit flies in the blue light group decreased with increasing exposure time. Cognitive differences between the two groups began to emerge in the second week of blue light exposure.

Our findings indicate that blue light exposure reduces the lifespan of fruit flies. In terms of locomotion, the fruit flies in the blue light group exhibited slightly lower locomotion levels than those in the white light group did in the early stages of blue light exposure (Week 1). However, their locomotion levels significantly exceeded those of the white light group at Week 2. Research has indicated that blue light can directly affect photoreceptors in the fruit fly brain, thereby influencing circadian rhythm cells (Helfrich-Förster 2020). In this study, although the 12-h light:12-h dark cycle remained constant, ensuring identical light exposure durations for both the blue light and white light groups, studies have shown that blue light exposure may affect the sleep duration of *Canton-S* genotype fruit flies during both the light and dark phases (Bond et al. 2024; Krittika and Yadav 2022). These changes in sleep patterns could further alter locomotion during wakeful periods. Additionally, another study demonstrated that blue light alters the expression of circadian rhythm genes in *w¹¹¹⁸* wild-type fruit flies, leading to a weakened circadian rhythm and slight reductions in behavioral activity during the early and middle stages of life (Huang et al. 2023). This finding may help explain the locomotion results observed in Week 1. On the other hand, considering the significant increase in locomotion levels observed in the blue

Short-term spatial memory

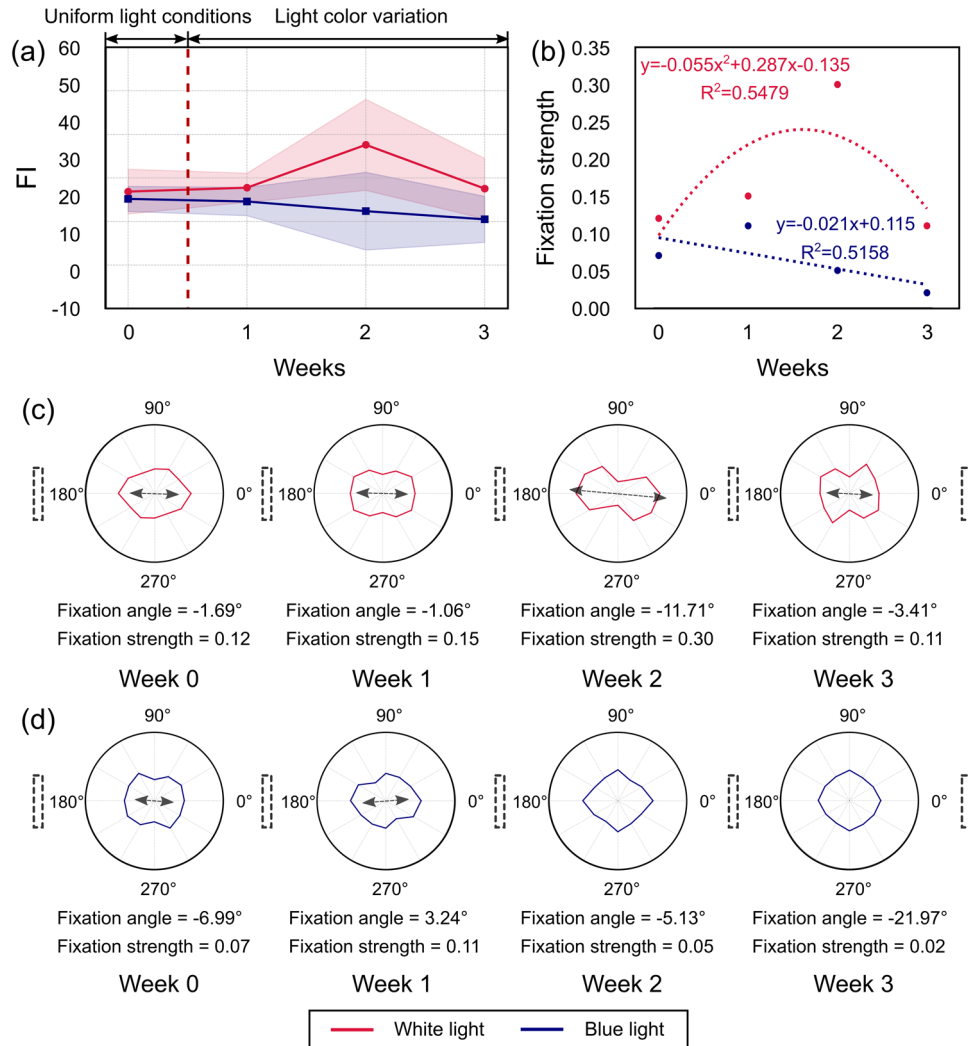


FIGURE 4 | Effects of blue light on the short-term spatial memory of early adult fruit flies. (a) The fixation index of the two groups of fruit flies toward the location where the visual stimuli disappeared matched the number of fruit flies used in the attention test at different time points ($*p < 0.05$, the shaded area indicates the standard error of the mean, SEM). (b) The average fixation strength of the fruit flies in the white light group exhibited a quadratic trend ($y = -0.055x^2 + 0.287x - 0.135$, $R^2 = 0.5479$), whereas the average fixation strength of the blue light group exhibited a strong linear declining trend ($y = -0.021x + 0.115$, $R^2 = 0.5158$). (c and d) Radar plots showing the impact of white and blue light on the fruit fly fixation angle and fixation strength when the visual stimuli disappeared, demonstrating that by Week 2 of blue light, the fruit flies were unable to maintain short-term spatial memory (fixation strength = 0.05 in Week 2, fixation strength = 0.02 in Week 3).

light group during Week 2, we propose that this result is related to a transient increase in locomotion in some blue light group fruit flies shortly before death. This conclusion is supported by previous research showing that some fruit flies exhibit green autofluorescence and irregular increases in locomotion levels shortly before death (Tower et al. 2019). Furthermore, fruit flies that experienced hypoxia-reoxygenation and died in early adulthood also presented relatively high activity levels shortly before death (Habib et al. 2021), potentially linking this phenomenon to metabolic changes. We suggest that there is a correlation between different locomotion levels in fruit flies. Thus, the observed increase in locomotion before death and the decline in survival rates in our study may be related to blue light affecting the metabolic levels of early adult fruit flies, potentially leading to premature aging and a shortened lifespan. Previous studies have suggested that blue light may increase

metabolic activity in organisms and that the high physiological costs of this activity could lead to premature depletion of energy reserves, thereby promoting early aging (Brainard et al. 2001; Chang et al. 2015). This process may be associated with metabolic pathways involving metabolites such as glutamate and riboflavin (Yang et al. 2022).

Notably, consistent with the results for activity level and movement speed, the wobbling time ratio was significantly lower in the blue light group than in the white light group. Research has indicated that wobbling involves changes in the stability of an organism due to alterations in the neural or muscular tissues of fruit flies, and the wobbling time ratio may be linked to behaviors associated with neurodegenerative diseases in fruit flies (Chi et al. 2020). We speculate that blue light accelerates energy depletion, thereby leading to more excitable

behavior, resulting in a lower wobbling ratio in adult fruit flies exposed to blue light than in those exposed to white light. However, eventually, it leads to premature aging in fruit flies and accelerates their death.

In this study, we discuss the impact of blue light on the behavior of fruit flies. We believe that blue light may influence behavioral performance by affecting neural functions. Research has demonstrated that blue light impacts the release of neurotransmitters and the activation of visual neural circuits. These neural signals may ultimately be transmitted to brain regions such as the mushroom body and ellipsoid body within the fruit fly's central complex (Helfrich-Förster 2020), both of which are directly associated with cognition in fruit flies (McGuire, Le, and Davis 2001; Neuser et al. 2008; Seelig and Jayaraman 2015; Han, Huang, et al. 2021). These findings may help explain why blue light exposure affects the attention and short-term memory of fruit flies. Additionally, chronic blue light exposure has been shown to accelerate aging in fruit flies (Yang et al. 2022), involving processes such as m6A methylation of aging-related genes, increased accumulation of reactive oxygen species (ROS), mitochondrial dysfunction, neuronal damage, and metabolic disturbances (Huang et al. 2023). Aging is typically accompanied by a decline in the cognition levels of fruit flies (Brandt and Vilcinskas 2013; Pacifico et al. 2018; König and Gerber 2022). Therefore, we speculate that the cognitive decline observed in this study may also be linked to blue light-induced accelerated aging. Furthermore, previous studies have indicated that blue light may influence behavior and cognitive levels by affecting neurotransmitters such as dopamine (Fasciani et al. 2020; Schilling et al. 2021; Carpena-Torres et al. 2023). Exposure to blue light may increase dopamine levels in organisms (Tian et al. 2021; Carpena-Torres et al. 2023; Lu and Tong 2024), and dopamine secretion is known to increase locomotion levels in organisms (Kelly et al. 1998; Ryczko et al. 2013; Ryczko and Dubuc 2017; Sotnikova, Efimova, and Gainetdinov 2020). These findings across different organisms may help explain why blue light exposure alters the locomotion levels of early adult fruit flies. However, long-term excessive activation of dopamine may lead to premature aging of neural cells (Ischiropoulos and Beckman 2003; Chakrabarti and Bisaglia 2023; Rademacher et al. 2024), which we speculate could also be associated with the cognitive impairments observed in fruit flies under blue light exposure in our study.

It is worth noting that the white light used in this study contained a portion of blue light, which might introduce some interference in the interpretation of the final experimental results. If the blue light component in the white light were completely filtered out, would the lifespan of fruit flies be extended, and would the damage to their locomotion and memory levels be mitigated compared with the current white light results? Future research should include additional experimental groups to analyze the behavioral performance of fruit flies under conditions where the blue light component is entirely excluded. Furthermore, to eliminate the influence of sex, this study used female fruit flies with relatively stable locomotion levels as the research subjects (Colomb et al. 2012; Yen, Han, and Lo 2019). However, studies have demonstrated differences in locomotion between male and female fruit flies (Burnet et al. 1988; Martin, Ernst, and Heisenberg 1999; Soyam and

Kannan 2024). Therefore, future research should further explore the differential effects of blue light on fruit flies of different sexes. Additionally, owing to the gradual decline in survival rates, the assessment of cognitive levels in the later stages of the experiment faced a shortage of fruit flies, which potentially affected the validity of the results. The effects of blue light on fruit flies in the middle-to-late stages of adulthood are also worth investigating. For example, does excessive blue light exposure in these stages lead to a higher wobbling time ratio compared with the white light group? Moreover, previous studies have shown that blue light exposure may have varying impacts on different genotypes of wild-type fruit flies, such as *Canton-S* and *w¹¹¹⁸* (Huang et al. 2023; Bond et al. 2024). Future research should focus on the effects of blue light on different genotypes of fruit flies over a longer lifespan to better understand these dynamics.

In summary, in the present study, we explored the effects of blue light on early-stage *D. melanogaster*. We found that although blue light temporarily increased the locomotion of fruit flies, it subsequently significantly shortened their lifespan. The premature depletion of energy reserves also temporarily decreased the wobbling time ratio. Additionally, the observed effects of blue light on the attention and short-term spatial memory of early adult fruit flies also suggest long-term degeneration of neural functions. As fruit flies age, this decline in cognition may become clearer. Future research should focus on the long-term effects of blue light on different types of fruit flies. This will not only help us understand the impact of light exposure on the physiological and cognitive functions of fruit flies but also provide important insights into the effects of light exposure on other organisms, including humans.

5 | Conclusion

This study revealed the effects of blue light on the behavior and physiological processes of early adult *D. melanogaster*, particularly in terms of locomotion and cognition. We found that compared with white light, exposure to blue light temporarily increased the activity levels and movement speeds of fruit flies. However, this exposure also significantly shortens the lifespan of fruit flies and adversely affects their cognitive functions, especially in areas such as attention and short-term spatial memory. Our research provides further insights into how blue light affects fruit fly behavior and physiology and lays an important foundation for future studies on the effects of blue light on other organisms.

Author Contributions

Rui Han planned and designed the study; acquired, analyzed, and interpreted the data; drafted and revised the manuscript; provided final approval for publication; and agreed to be accountable for all aspects of the work. Jun Zhang contributed to the study's conception and design. Guan-Xiong Huang, Ruo-Xi Yuan, Yun-Shan Lian, and Meng-Ying Zhao were involved in the study conception, design, and data collection. Yu-Yuan Lu, Hao Huang, Yu-Chen Wang, and Yi-Jie Chen assisted in data acquisition. Chung-Chuan Lo and Yi-Heng Tan helped set up the tracking apparatus and experimental protocol and assisted in analyzing and interpreting the data. All authors contributed significantly to the manuscript and approved the final version for publication.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

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