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Effects of Coffee on *Drosophila melanogaster*: Concentration-Dependent Enhancement and Sleep Deprivation Recovery

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ABSTRACT

The effects of sleep deprivation on organisms have become an important research topic. Prolonged sleep deprivation not only affects locomotion and cognition in humans but also has a significant negative effect on the lifespan of organisms. This study investigated the effects of weekly continuous light exposure (16, 20, or 24 h) as a method of sleep deprivation on the behavior of *Drosophila melanogaster* and evaluated the potential recovery effects of caffeine and other components in coffee on fruit flies. The results revealed that weekly sleep deprivation reduced the activity level and short-term spatial memory of fruit flies. In contrast, moderate coffee intake (0.1 g/L caffeine content) not only had positive effects on nonsleep-deprived fruit flies but also reduced the negative effects caused by sleep deprivation. Our study assessed the short-term and long-term effects of coffee during the sleep deprivation process and examined the relationships between these effects and the aging of fruit flies. Through this research, we aim to gain a deeper understanding of the mechanisms by which coffee affects sleep deprivation and provide new insights and references for future studies.

1 | Introduction

The effects of sleep deprivation on organisms have become an important research topic (Pilcher and Huffcutt 1996; Elliott et al. 2014; García et al. 2021). Prolonged sleep deprivation not only affects the health and locomotion of organisms (Du et al. 2022) but also has significant negative impacts on cognitive functions (Killgore 2010). Previous studies have shown that sleep deprivation leads to a series of behavioral and physiological problems, including memory impairment (Kim et al. 2021), reduced motor coordination (Umemura et al. 2022), and decreased immune function (Moldofsky et al. 1989). As a result, how to effectively reduce the negative reactions caused by sleep deprivation remains an issue worth exploring.

Coffee, a common central nervous system stimulant, is widely used to relieve fatigue (Herden and Weissert 2020) and increase alertness (Akosua et al. 2023). Caffeine in coffee works

by blocking adenosine receptors, reducing the sedative effects of adenosine and thereby increasing neural activity levels (Fiani et al. 2021). Additionally, coffee contains polyphenols and antioxidants, which are believed to have neuroprotective and lifespan-extending potential (Socała et al. 2020). Although studies have shown that caffeine has positive effects on locomotion and cognition (Xie et al. 2021; Olopade et al. 2021; Du et al. 2022), its effects on the behavioral and physiological changes caused by sleep deprivation at different caffeine concentrations remain unclear.

Drosophila melanogaster (fruit fly) is an ideal model organism widely used in behavioral, genetic, and neuroscience research (McGurk et al. 2015; Hales et al. 2015). Owing to its short life cycle, simple genome, and ease of manipulation, the fruit fly is convenient for studying the effects of sleep deprivation and coffee. Furthermore, the physiological and behavioral changes in flies at different life stages have been extensively studied, making them ideal subjects for research on long-term effects (Chi et al. 2020; Overman et al. 2022). The lifespan of flies varies depending on the nutritional ratio of the culture medium, temperature, genotype, etc. (Kawaguchi et al. 2016; Landis et al. 2020; Mołoń et al. 2020). Typically, the lifespan of flies can be divided into three main stages: young (average of 10–20 days), middle-aged (35–45 days), and old (60–70 days; Bushey et al. 2010). This age division allows us to assess the effects of different treatments accurately at various life stages. Additionally, the survival rate, locomotion, and cognition are key indicators of the physiological function of fruit flies (Neuser et al. 2008; Jans et al. 2021; Cai et al. 2022), and evaluating these factors helps us explore the impact of sleep deprivation and coffee.

In summary, this study used activity level, average movement speed, and the wobbling time ratio as indicators of locomotion and short-term spatial memory performance as indicators of cognition. We investigated the behavioral and physiological consequences of acute and severe sleep deprivation by exposing fruit flies to prolonged light durations (16, 20, or 24 h) once a week beginning in early middle age. We hypothesized that more severe sleep deprivation would lead to progressively greater impairments in locomotion, cognition, and survival. In a separate experiment, we examined the long-term effects of different caffeine concentrations (0.1, 0.5, and 1.0 g/L) on flies that were not sleep deprived. We hypothesized that low doses of caffeine might have beneficial effects on lifespan and behavior, whereas high doses might have detrimental effects. Finally, to evaluate whether caffeine could mitigate the adverse consequences of sleep deprivation, we tested whether adding 0.1 g/L caffeinated coffee to the diet of sleep-deprived flies would improve their behavioral performance and survival. We hypothesized that caffeine supplementation after sleep deprivation would partially restore locomotor and cognitive functions. By repeatedly testing flies throughout their lifespan, we aimed to assess the time course of these effects and to explore the interactions between sleep deprivation, caffeine intake, and aging. These findings may help clarify the mechanisms by which caffeine modulates the negative consequences of sleep loss and provide insight into its potential neuroprotective properties.

2 | Materials and Methods

2.1 | Fly Strain

The fruit flies were cultured in an incubator at a stable temperature of 25°C and a humidity level of approximately 50%. Under normal conditions, the flies were kept under a 12-h light/12-h dark cycle, with light from 8:00 AM to 8:00 PM and darkness from 8:00 PM to 8:00 AM. These light and dark conditions were regulated by a timer controlling white fluorescent lamps. The wild-type fruit flies (genotype *Canton-S*) were obtained from the Bloomington *Drosophila* Stock Center (BDSC), RRID: BDSC_64349.

2.2 | The Sleep Rebound Test

To study the effects of different degrees of sleep deprivation on the behavior of fruit flies, we first tested the effectiveness of sleep deprivation in our experimental setup. We maintained the fruit flies under a normal 12-h light/12-h dark cycle in the incubator until day 11 posteclosion. On day 11, the flies were divided into different groups according to the degree of sleep deprivation (SD) (12-h:12-h, 16-h:8-h, 20-h:4-h, 24-h:0-h light/dark cycles): Ctrl (12 hL:12 hD), SD (16 hL:8 hD), SD (20 hL:4 hD), and SD (24 hL:0 hD). A timer was set to control the light/dark cycle, and daylight lamps were used to simulate natural light, with paper covering the lamps to reduce the light intensity. After 1 day of sleep deprivation, the flies were returned to the 12-h light/12-h dark cycle on day 12, and their sleep duration during the light period on day 12 was tested as a measure of sleep rebound.

During the 36 h from day 11 to day 12, we recorded the movement of the flies via a Mrobo D3 night vision HD motion camera, with video output in AVI format, a resolution of 1920 × 1080P, and a frame rate of 24 fps. The video setup included eight evenly distributed test tubes, each containing one fly, separated by dividers to prevent interference. For effective tracking, the video was edited into eight individual clips, each containing one test tube, converted to 1920i resolution in MP4 format, and muted. Movement tracking was then performed via a Python 3.5 script that used optical flow technology to track and record the traces of the flies. This was achieved by analyzing the visual displacement of objects between consecutive frames, capturing position changes, and accurately plotting their movement paths as x and y coordinates in pixels.

Following the sleep duration calculation methods mentioned in previous studies (Ho and Sehgal 2005; Li et al. 2009), we analyzed the activity levels of the flies at 30-s intervals, recording any continuous 5-min period of static sleep. Finally, we compared the total sleep duration under light conditions on day 12 across different groups to determine the impact of varying degrees of sleep deprivation on the behavior of fruit flies.

2.3 | The Arena and Tracking System

Our behavioral experimental setup is consistent with those used in our previous studies (Chi et al. 2020; Han, Wei,

et al. 2021; Han, Huang, et al. 2021; Han, Tan, and Lo 2024). The area in which the fruit flies move consists of a white circular platform with a diameter of 85 mm. The platform is surrounded by a 20 mm wide water channel to prevent the wing-clipped flies from escaping. The platform is encircled by a 360° LED screen composed of 20 independent panels, each consisting of four 8 × 8 LED matrices. We used yellow–green light, which is highly sensitive to fly vision, as the color of the LEDs (1.2″ KWM-30881CUGB, with a peak wavelength of 573 nm). The entire LED screen has a diameter of 200 mm and a height of 130 mm. The LED system is connected to a personal computer via an Arduino board (Arduino Shield MEGA2560) and allows individual control of each LED to present different visual stimuli at specific times. Additionally, a CCD camera positioned directly above the platform captures the movement traces of the flies on the platform and generates high-resolution videos. These motion data are extracted via a Python 3.5 script.

2.4 | The Behavior Test

To study the locomotion and short-term spatial memory of fruit flies under different conditions, we first froze 3-day-old fruit flies and removed one-third to one-half of their wings, allowing them to rest for 2 days. From day 5 to day 47, we placed the fruit flies in the LED setup for a weekly behavior test. Our experiment was modified on the basis of the classic Buridan's paradigm (Yen et al. 2019; Han, Wei, et al. 2021). In Buridan's paradigm, wing-clipped fruit flies are placed between two black visual stimuli on a circular screen. Owing to their scototaxis, the flies moved back and forth between the two visual stimuli (Götz 1980; Strauss and Pichler 1998; Neuser et al. 2008; Colomb et al. 2012). Our previous research revealed that when visual stimuli are presented for 60 s or longer, fruit flies continue to shuttle between the positions of the stimuli for a period even after the stimuli disappear, guided by their short-term spatial memory.

The setup of this study is consistent with that used in our previous studies (Yen et al. 2019; Han, Huang, et al. 2021). Briefly, our experiment is divided into three stages: the pretraining stage, training stage, and posttraining stage. The pretraining stage lasts for 90 s, during which all LED lights are on, allowing the flies to move in a fully illuminated environment. We recorded the random movement traces of the flies and calculated their locomotion levels, including activity level, average movement speed, and wobbling time ratio (see Data Analysis section for details).

In the training stage, two vertical black stripes, each 30° wide, appeared at 0° and 180° on the LED screen. This stage lasted for 60 s. In the posttraining stage, the black stripes disappeared. During this stage, we calculate the score of the flies moving between the positions of the missing visual stimuli, which is defined as the performance index (see Data Analysis section for details). This stage lasted for 90 s. We used a CCD camera to capture images at 15 frames per second and used Python 3.5 to track the position of the flies in the images, storing this information in TSV-formatted files.

2.5 | The Sleep Deprivation Test

To study the effects of different degrees of sleep deprivation on fruit flies, we conducted sleep deprivation tests. The first behavioral test (see The Behavior Test section for details) was performed on day 5 posteclosion, without sleep deprivation, for any group. The first sleep deprivation session was conducted on day 11 posteclosion, with four sleep deprivation groups set up as in the sleep rebound test (Ctrl [12 hL:12 hD], SD [16 hL:8 hD], SD [20 hL:4 hD], and SD [24 hL:0 hD]). One day of sleep deprivation was subsequently performed every 7 days, followed by a behavioral test the next day to evaluate the impact of different degrees of sleep deprivation on locomotion and short-term spatial memory (see Data Analysis section for details). To observe the long-term effects of different experimental conditions, all behavioral measurements in the sleep deprivation, caffeine concentration, and recovery tests were repeatedly measured every 7 days on the same cohort of fruit flies and were consistently performed between 1:00 PM and 5:00 PM (zeitgeber times 5–9) to ensure experimental consistency. After each behavioral test, the fruit flies were returned to the incubator and maintained on a 12-hL/12-hD cycle.

2.6 | The Caffeine Concentration Test

To study the effects of different caffeine concentrations on the behavior of fruit flies, we based our approach on previous research. We purchased Nescafé coffee (3% caffeine, lot number 22990012; Shin et al. 2010) and prepared media with different caffeine concentrations. The caffeine concentrations were modified from a previous study (Nall et al. 2016): 0.1, 0.5, and 1.0 g/L. To eliminate the influence of noncaffeine components in coffee, we included a decaffeinated coffee group (DC COF). We purchased Nescafé decaffeinated coffee (< 0.3% caffeine, lot number 22621902; Shin et al. 2010) and prepared media with the same weight as the 0.1 g/L caffeine group, ensuring that the concentration of noncaffeine components in the decaffeinated coffee matches that in the 0.1 g/L caffeine group. The Nescafé coffee and the Nescafé decaffeinated coffee were sourced from the official Nestlé website.

Consistent with the sleep deprivation test, the first behavioral test was conducted on day 5 posteclosion. At this time, all groups used media without coffee. On the second day after the first behavioral test, different amounts of coffee were added to the media according to the experimental design (DC COF, CAF (0.1 g/L), CAF (0.5 g/L), or CAF (1 g/L)). Behavior tests were then conducted weekly.

2.7 | The Recovery Test

To study the recovery effects of coffee on the behavior of fruit flies after sleep deprivation, we selected groups with significant behavioral impairments following sleep deprivation, specifically those with a 20-hL/4-hD cycle and a 24-hL/0-hD cycle (see Results section for details). We added media with a caffeine concentration known to improve behavior (0.1 g/L; RE [20 hL:4 hD + 0.1 g/L] and RE [24 hL: 0 hD + 0.1 g/L]).

Consistent with the sleep deprivation test, the first behavioral test was conducted on day 5 posteclosion, without any additional conditions for the different groups. On the second day after the first behavioral test, the fruit flies were transferred to media containing 0.1 g/L caffeine. Starting on day 11, sleep deprivation was performed every 7 days, followed by behavioral tests the next day.

We compared the behavioral data of the recovery groups (RE [20 hL:4 hD + 0.1 g/L] and RE [24 hL:0 hD + 0.1 g/L]) with those of the control group from the sleep deprivation test (Ctrl [12 hL:12 hD + 0 g/L]), the sleep deprivation groups (SD [20 hL:4 hD] and SD [24 hL:0 hD]), and the caffeine concentration group from the caffeine concentration test (CAF (0.1 g/L)). This comparison aimed to explain the effects of coffee (or caffeine) in reducing the impact of sleep deprivation.

2.8 | Data Analysis

Our study aimed to analyze the changes in locomotion levels and short-term spatial memory at different time points across three sets of tests (sleep deprivation test, caffeine concentration test, and recovery test). All behavioral data were recorded via the LED setup (see The Behavior Test section for details). We used a Python 3.5 script to record the position of each fly frame by frame and tracked their movement trajectories as x and y coordinates.

To analyze the locomotion levels of the fruit flies, we defined the activity level, average movement speed, and wobbling time ratio. The activity level was calculated as the percentage of time points when the flies were moving relative to all captured points within a unit of time. The average movement speed was calculated as the total movement distance divided by the total time. The wobbling time ratio was defined as the percentage of time units where the flies moved less than 0.3 mm, which is consistent with the definition used in our previous study (Chi et al. 2020; Han, Zhang, et al. 2024).

Additionally, on the basis of our previous research, we quantified the short-term spatial memory index of the flies during the posttraining stage, continuing to use the previously established performance index (PI) (Han, Huang, et al. 2021) but modifying the calculation method. Briefly, we recorded the angle of the vectors formed by every two adjacent positions of the flies, representing the angle θ toward the LED screen. We recorded the distribution of all vectors over the 90 s of the posttraining stage and divided the 360° range into twelve 30° sectors to calculate the movement direction percentage for each, $p(\theta)$. We defined the initial performance index as the sum of the percentages directed 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 initial PI indicates approach behavior toward visual stimuli, whereas a negative initial PI indicates avoidance behavior.

$$\text{initial PI} = P(0^\circ;180^\circ) - P(90^\circ;270^\circ) \quad (1)$$

Finally, to compare the PI between different groups and track the long-term effects under different conditions, we normalized the initial PI mean of the first behavioral test for each group to 1. We then divided the initial PI of each subsequent test by the initial PI mean of the first test for that group, resulting in the standardized PI used for comparisons between different groups.

2.9 | Statistical Analysis

For each group, the initial behavioral test included ≥ 20 fruit flies; therefore, > 300 fruit flies were used across all the conditions. During the analysis of all conditions, any data point that deviated from the mean by more than two standard deviations or showed no movement at all was considered an outlier and excluded. Additionally, since the total duration of the behavioral test was 240 s, we observed that fruit flies were not always in motion throughout the experiment. Therefore, the amount of data used in the analysis of locomotion and short-term memory may not exactly match the actual number of surviving fruit flies. We used SPSS 22.0 for the statistical analysis. Descriptive statistics were used to interpret the sleep duration of fruit flies under light conditions from day 11 to day 12 in the sleep rebound test, and one-way ANOVA was used to analyze the sleep rebound duration among different groups on day 12.

Behavioral data—including activity level, average movement speed, wobbling time ratio, and performance index (PI)—were analyzed via generalized linear mixed models (GLMMs) in SPSS 22.0. In these models, treatment and time were set as fixed effects, and individual fly ID was treated as a random effect to account for repeated measurements. When a significant interaction between treatment and time was detected, pairwise comparisons were conducted via the least significant difference (LSD) method.

For survival data, we adopted two complementary analytical approaches. First, the mean lifespan was compared across groups via one-way ANOVA. Second, to provide a more comprehensive evaluation of survival patterns, we performed Kaplan–Meier survival analysis. We first conducted an overall log-rank test to compare survival differences among all groups, followed by pairwise log-rank tests to assess specific group-level differences. To control for false positives due to multiple comparisons, p values were adjusted via the Bonferroni correction.

3 | Results

3.1 | The Sleep Rebounds After Sleep Deprivation

To evaluate the impact of sleep deprivation on sleep patterns, we compared sleep duration across groups exposed to varying durations of light (Figure 1A). Flies subjected to extended light exposure on day 11 (16, 20, or 24 h) presented disrupted sleep patterns during the subsequent 12-h period (Figure 1B). On day 12, all the sleep-deprived groups exhibited a rebound in sleep duration, with the extent of rebound positively correlated with the duration of prior light exposure. In particular, compared with control flies, flies in the 20-h and 24-h light

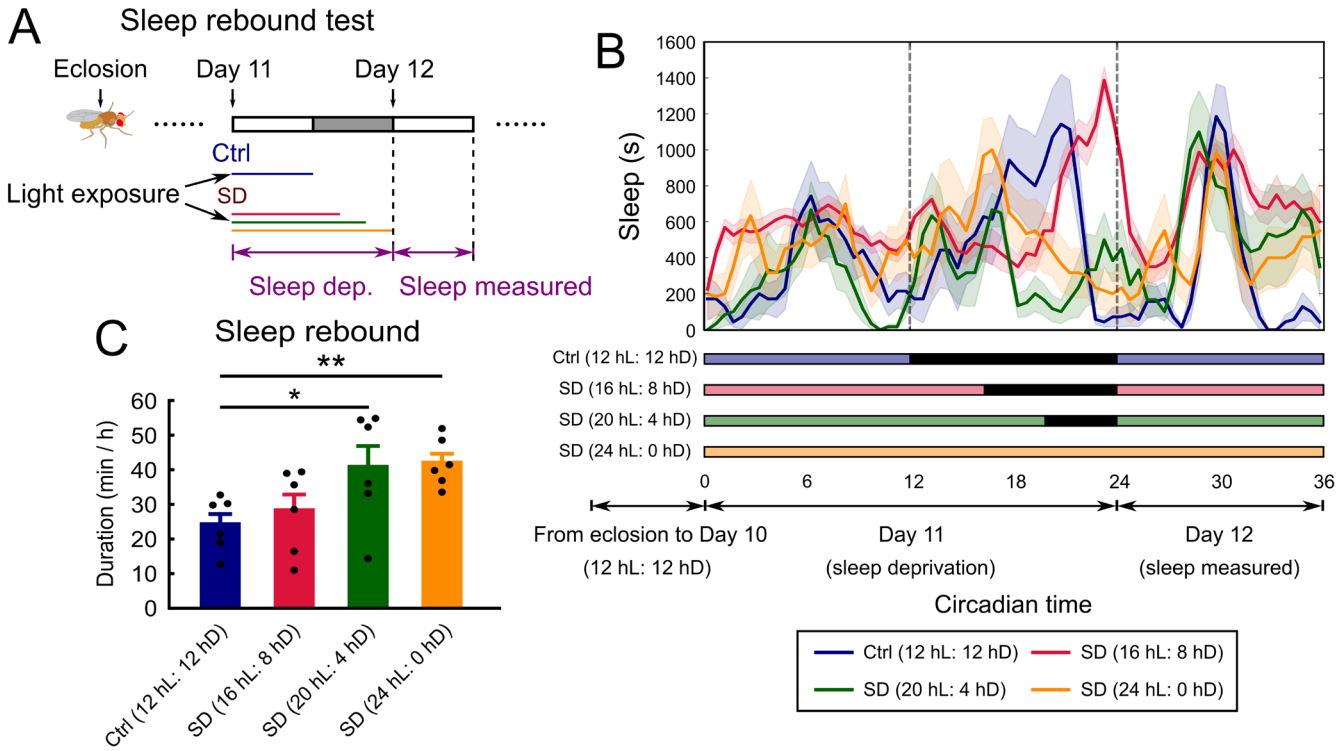

FIGURE 1 | Sleep rebound and sleep rhythms in *Drosophila melanogaster* following sleep deprivation. (A) Experimental timeline. Flies were maintained under a 12:12 h light:dark cycle until day 10 posteclosion. On day 11, the groups were exposed to different light conditions: 12 h light:12 h dark (blue line), 16 h light:8 h dark (red line), 20 h light:4 h dark (green line), or 24 h constant light (orange line). Sleep was recorded following 1 day of treatment. (B) Sleep profiles over a 36 h period spanning the sleep deprivation phase and recovery. The y-axis represents sleep duration (s), and the x-axis indicates elapsed time. The shaded areas represent the standard error of the mean. (C) Average sleep duration during the 12 h light period on day 12. The bars represent the mean hourly sleep duration for each group; individual data points are shown as black dots. Asterisks indicate statistically significant differences between groups. The error bars represent the standard error of the mean (**$p < 0.01$, *$p < 0.05$; one-way ANOVA).

groups presented significantly increased sleep (Figure 1C). These results confirm the effectiveness of our sleep deprivation protocol and support its use in subsequent behavioral and recovery experiments.

3.2 | Effect of Sleep Deprivation

To assess the effects of sleep deprivation on survival and behavior, flies were tested weekly from day 5 posteclosion (Figure 2A,B). The sleep-deprived groups showed a slight reduction in survival from week 3 (day 26) onward (Figure 2C). In the analysis of the mean lifespan, no statistically significant differences were observed among the different sleep deprivation conditions (Figure 2D). This finding was further supported by Kaplan–Meier survival analysis and log-rank tests. The overall log-rank test revealed no significant differences in survival curves across groups ($\chi^2 = 1.34$, $p = 0.72$). Nevertheless, a subtle trend was noted whereby more severe sleep deprivation conditions were associated with slightly reduced survival (Figure 2C,D), suggesting a mild dose-dependent negative impact of prolonged light exposure on lifespan.

Additionally, we evaluated the impact of different levels of sleep deprivation on the locomotion of fruit flies (Figures 3A–C, S1 and Table S1). The results indicated that significant differences in activity levels were observed only under 20-h and 24-h continuous light conditions and only transiently on day 26 (Figures 3A and S1A). Moreover, sleep deprivation had minimal effects on average movement speed (Figures 3B, S1B and Table S1), and the wobbling time ratio was not significantly affected by sleep deprivation (Figures 3C, S1C and Table S1).

On the other hand, to evaluate the impact of sleep deprivation on the cognition of fruit flies, we tested their short-term spatial memory after visual stimuli disappeared. We found that sleep deprivation significantly affects the short-term spatial memory of fruit flies, with greater degrees of deprivation leading to earlier impairment (Figures 3D, S1D and Table S1). The results revealed that in the group subjected to 24 h of continuous light before the behavioral test, short-term spatial memory began to decline significantly beginning on day 12. The other groups also showed a significant decline starting on day 26 posteclosion. By day 33, short-term spatial memory was almost completely lost in the sleep-deprived groups. In contrast, fruit flies maintained under a 12-h light/12-h dark cycle presented a marked decline in short-term spatial memory only by day 40.

3.3 | Effects of Different Caffeine Concentrations

After introducing different caffeine concentrations to the media following the initial behavioral test, we examined their

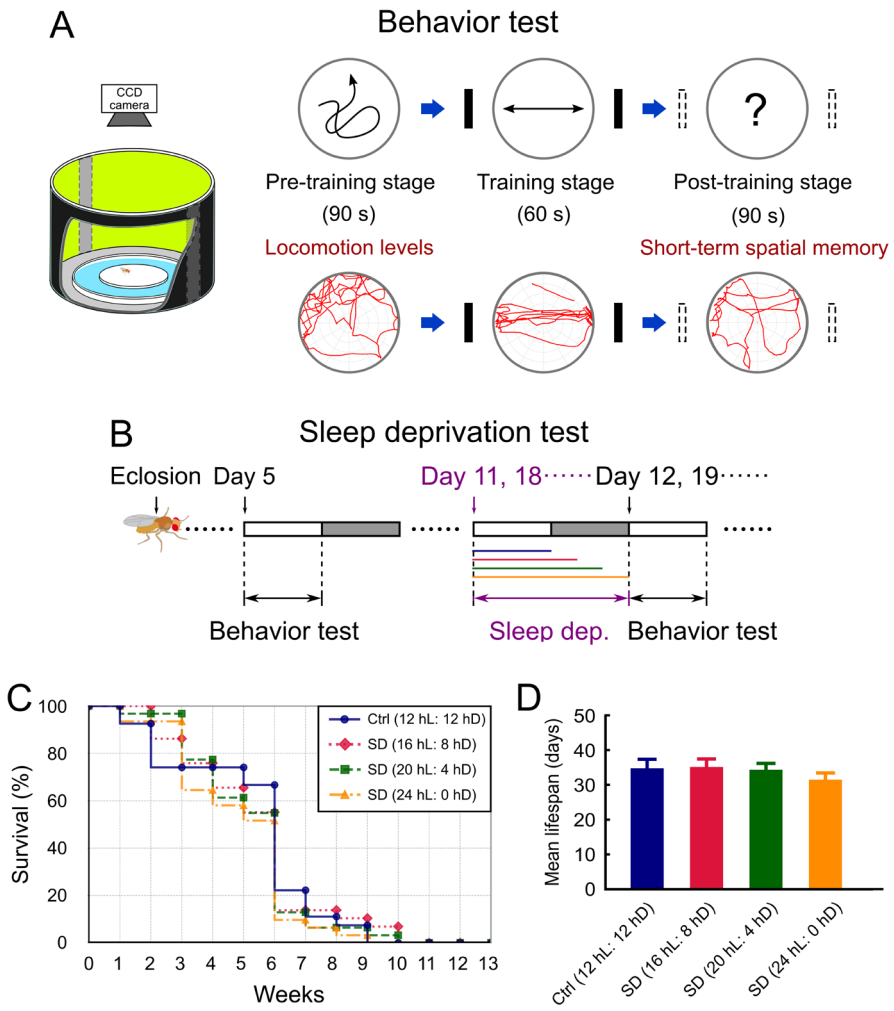

FIGURE 2 | Behavioral assay and survival under sleep deprivation. (A) Schematic of the behavioral apparatus and experimental stages. The behavioral assay comprised pretraining, training, and posttraining. Locomotion levels were assessed during pretraining. Short-term spatial memory was evaluated during posttraining. Typical movement trajectories across stages are shown. (B) Experimental timeline for sleep deprivation. The flies were divided into four groups: 12:12 h light:dark (blue line), 16:8 h (red), 20:4 h (green), and 24:0 h (orange). Sleep deprivation began on day 11 posteclosion and recurred weekly, with behavioral testing the following day. (C) Weekly survival rates during behavioral assays, expressed relative to day 5 posteclosion(week 0, 100%). Survival was further tracked after the final test. (D) The mean lifespan of the four groups of fruit flies. The error bars represent the standard error of the mean.

long-term impact on survival (Figure 4A). Flies exposed to 1 g/L caffeine significantly reduced survival soon after exposure (Figure 4B,C), and Kaplan–Meier survival analysis confirmed this effect. Log-rank tests revealed that the 1 g/L caffeine group had a significantly shorter median lifespan than the other groups did (Ctrl vs. CAF (1 g/L): $\chi^2 = 19.64$, Bonferroni-corrected $p < 0.001$; DC COF vs. CAF (1 g/L): $\chi^2 = 32.28$, Bonferroni-corrected $p < 0.001$; CAF (0.1 g/L) vs. CAF (1 g/L): $\chi^2 = 18.90$, Bonferroni-corrected $p < 0.001$; CAF (0.5 g/L) vs. CAF (1 g/L): $\chi^2 = 24.61$, Bonferroni-corrected $p < 0.001$). In contrast, no significant differences in survival were observed among the Ctrl, DC COF, and CAF (0.1 g/L) and CAF (0.5 g/L) groups. Interestingly, flies fed with decaffeinated coffee also presented an increased mean lifespan compared with those in the control group, suggesting that noncaffeine components of coffee may contribute positively to lifespan, although the difference in median survival time between the DC COF and Ctrl groups was not statistically significant ($\chi^2 = 4.51$; Bonferroni-corrected $p > 0.05$).

We also tested the effects of different caffeine concentrations on the locomotion of fruit flies (Figures 5A–C, S2 and Table S2). We found that coffee with high caffeine concentrations significantly reduced the activity level of fruit flies. Starting from day 26, caffeine concentrations of ≥0.5 g/L significantly reduced the activity level of fruit flies (Figures 5A, S2A and Table S2). Notably, although there was no significant difference in activity between the decaffeinated coffee group and the control group, the activity of the decaffeinated group also did not differ significantly from that of the groups treated with various caffeine concentrations at most time points (Table S2). Therefore, it remains unclear whether the observed reduction in activity was caused by caffeine itself or by noncaffeine components present in coffee. Similar to the activity level results, the reduction in movement speed due to coffee occurred earlier (starting from day 12; Figures 5B, S2B and Table S2). With respect to the wobbling rate, adding coffee significantly increased the wobbling time ratio of fruit flies. This effect appeared as soon as the coffee was added (day 12). However, the decaffeinated coffee group also presented

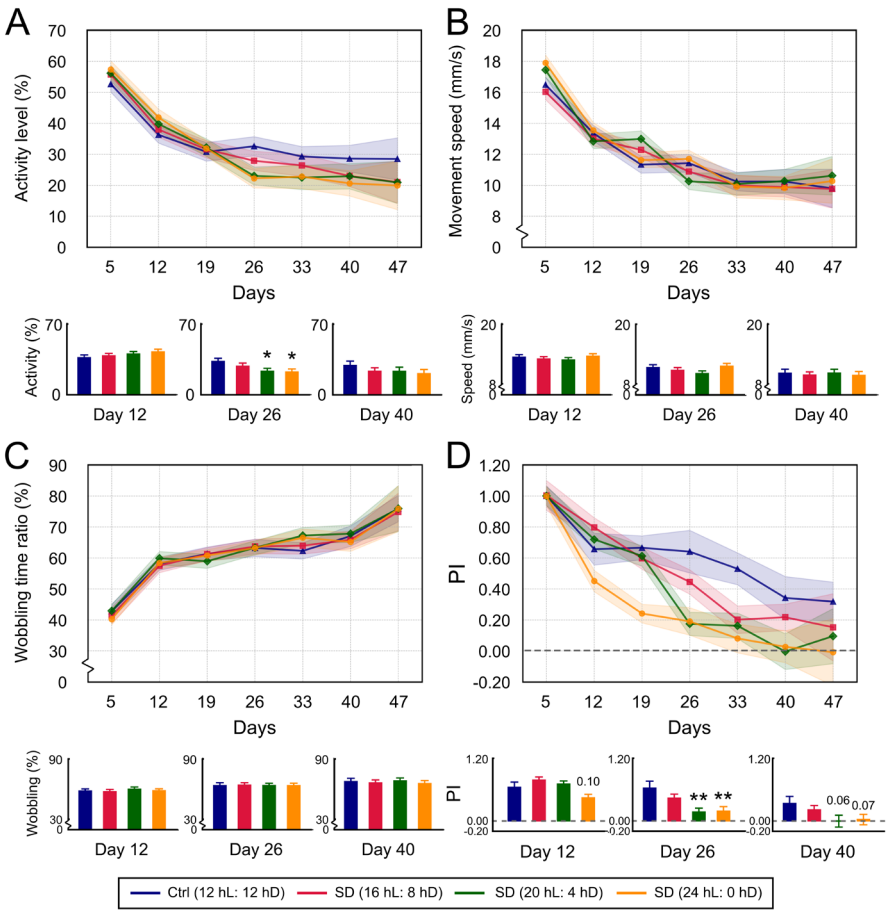

FIGURE 3 | Locomotion levels and short-term spatial memory in the sleep deprivation test. (A–D) Activity level, movement speed, wobbling time ratio, and time course of short-term spatial memory (represented by the normalized PI) under different degrees of sleep deprivation. The y-axis represents the different observation levels, and the x-axis indicates days posteclosion. The shaded areas indicate the standard error of the mean for each condition; bar graphs compare groups at days 12, 26, and 40. Significance is indicated above the bars and reflects pairwise comparisons between each sleep deprivation group and the control group (**$p < 0.01$, *$p < 0.05$, $p < 0.1$ values are shown directly via GLMM).

an increased wobbling time ratio, indicating that the effects of noncaffeine components on the wobbling time ratio cannot be excluded (Figures 5C, S2C and Table S2).

Additionally, we evaluated the effects of different caffeine concentrations on the cognitive abilities of fruit flies (Figures 5D, S2D and Table S2). We found that decaffeinated coffee did not significantly enhance cognition. Furthermore, beginning on day 12, coffee with different caffeine concentrations significantly improved the short-term spatial memory of fruit flies. However, beginning at early middle age (day 26), relatively high caffeine concentrations (1 g/L) had a negative effect on short-term spatial memory. We speculate that this may be due to the higher caffeine concentration reducing the locomotion of fruit flies, thus affecting their cognition, or it may be due to the negative effects of high caffeine concentrations on their cognitive functions.

3.4 | Effect of Caffeine on Recovery From Sleep Deprivation

We found that sleep deprivation negatively affected the locomotion and short-term spatial memory of fruit flies, whereas different concentrations of caffeine had positive effects on their lifespan and short-term spatial memory. Therefore, we further explored whether caffeine could mitigate the effects of sleep deprivation on fruit flies. Our previous results revealed that continuous 20-h or 24-h light exposure significantly deprives fruit flies of sleep, whereas a moderate caffeine concentration (0.1 g/L) has positive effects on their short-term spatial memory. In contrast, higher caffeine concentrations (≥ 0.5 g/L) negatively affect locomotion, and a high concentration of caffeine (1 g/L) also impairs cognitive performance. Therefore, we established two recovery groups, one with 20 h of light and 4 h of darkness supplemented with 0.1 g/L caffeine and the other with 24 h of light and 0 h of darkness supplemented with 0.1 g/L caffeine, to evaluate the recovery effects of caffeine on sleep-deprived fruit flies.

Consistent with the previous sleep deprivation and caffeine concentration tests, no additional conditions were applied to the different groups during the first behavioral test. After the first behavioral test, coffee with different caffeine concentrations was added to the media, and sleep deprivation was applied every day before the second behavioral test (Figure 6A). The results revealed that the difference in survival curves between the 20-h light sleep deprivation group and the recovery group receiving 0.1 g/L caffeine did not reach statistical significance

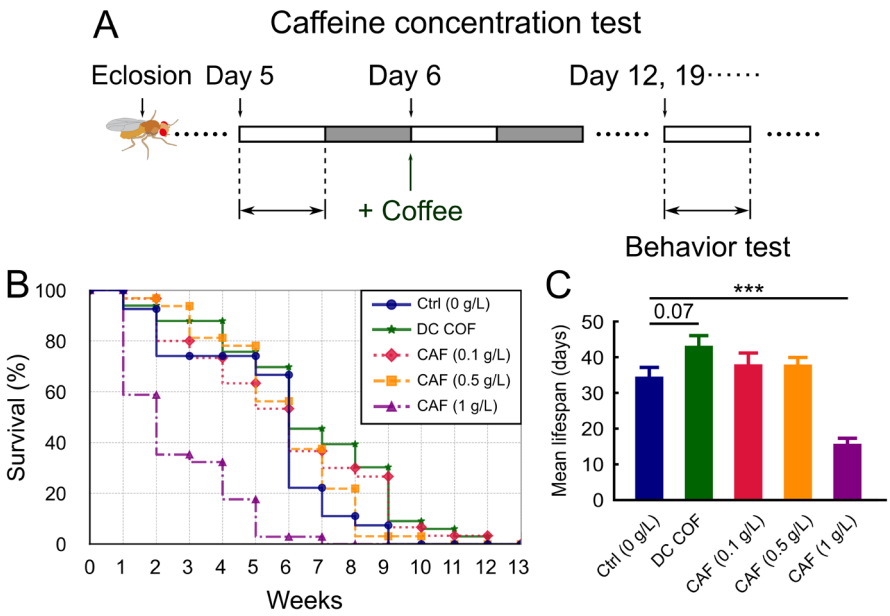

FIGURE 4 | Schematic diagram and survival data from the caffeine concentration test. (A) Experimental timeline. The behavioral tests began on day 5 posteclosion and were repeated weekly. Coffee was added to the medium on day 6, after the first test, to assess the long-term effects of caffeine. (B, C) Weekly survival rates and mean lifespan during behavioral assays. Groups were defined by caffeine concentration: 0 g/L (decaffeinated coffee; green line), 0.1 g/L (red), 0.5 g/L (yellow), and 1 g/L (purple). The shaded areas indicate the standard error of the mean for each condition. The Ctrl (0 g/L) group corresponds to the control group in the sleep deprivation test. These panels show the same measurements as in Figure 2 (***$p < 0.001$, $p < 0.1$ values are shown directly; one-way ANOVA).

(purple line vs. green line; $\chi^2 = 2.50$, Bonferroni-corrected $p > 0.05$; Figure 6B). However, the mean lifespan analysis indicated a slight positive effect of caffeine intake following sleep deprivation, yet this result did not reach statistical significance ($p = 0.11$; Figure 6C).

When locomotion was assessed, we found that from day 26, sleep deprivation affected the activity levels of fruit flies (Figure 3A). However, adding caffeine after sleep deprivation did not significantly increase their activity levels (Figures 7A, S3A and Table S3). The movement speed followed a similar pattern to that of the activity levels (Figures 7B, S3B and Table S3). Our earlier results revealed that caffeine intake increased the wobbling time ratio, but this effect was not substantially altered by sleep deprivation (Figures 7C, S3C and Table S3).

Finally, we tested the recovery effect of coffee on the short-term spatial memory of sleep-deprived fruit flies (Figures 7D, S3D, Table S3). Our results confirmed that adding 0.1 g/L caffeine to coffee significantly reduced the negative impact of SD on short-term spatial memory. This positive effect appeared from day 12 and continued until day 47, which was the middle-aged to aged adulthood stage of the fruit flies.

4 | Discussion

In this study, we investigated the effects of different degrees of weekly sleep deprivation on the behavior of *Drosophila melanogaster* and evaluated the potential restorative effects of coffee. Our results showed that weekly exposure to 20 or 24 h of light had negative effects on the survival rate, locomotion level, and short-term spatial memory of the flies. However, long-term moderate coffee consumption (0.1 or 0.5 g/L caffeine) improved short-term spatial memory. Additionally, moderate coffee intake (0.1 g/L caffeine) could alleviate the negative effects of short-term sleep deprivation.

We noted that in our study, the peak of sleep rebound occurred in the second half of the day following sleep deprivation. This pattern was especially evident under 16-h and 20-h continuous light conditions. This seems to contradict the previous literature, which reports that the peak of rebound sleep typically occurs immediately after the end of deprivation. We believe that this discrepancy may be related to the method and timing of sleep deprivation used in our study. Previous studies have generally employed two approaches for sleep deprivation in fruit flies: mechanical shaking/disturbance without altering the light–dark cycle and sleep deprivation by prolonging light exposure. In studies in which mechanical disturbance was used to induce sleep deprivation (Huber et al. n.d.), flies showed immediate sleep rebound following disturbance. We believe that this is because mechanical interference drastically disrupts the sleep pattern of flies, triggering rapid physiological and behavioral responses. After the mechanical disturbance ends, recovery mechanisms are activated immediately, similar to a "high-consumption followed by urgent energy recovery" process. In contrast, for deprivation via prolonged light exposure, the timing of rebound depends on the light schedule. Some studies have implemented intermittent light pulses during the dark phase to achieve sleep deprivation (Liu and Zhao 2014). We believe that such manipulations cause substantial circadian rhythm disruption, prompting flies to fall asleep immediately to restore physiological balance once deprivation ends. In our study, under 16-h and 20-h light conditions, the light-on time was the same as that of the control group. Therefore, the circadian rhythm

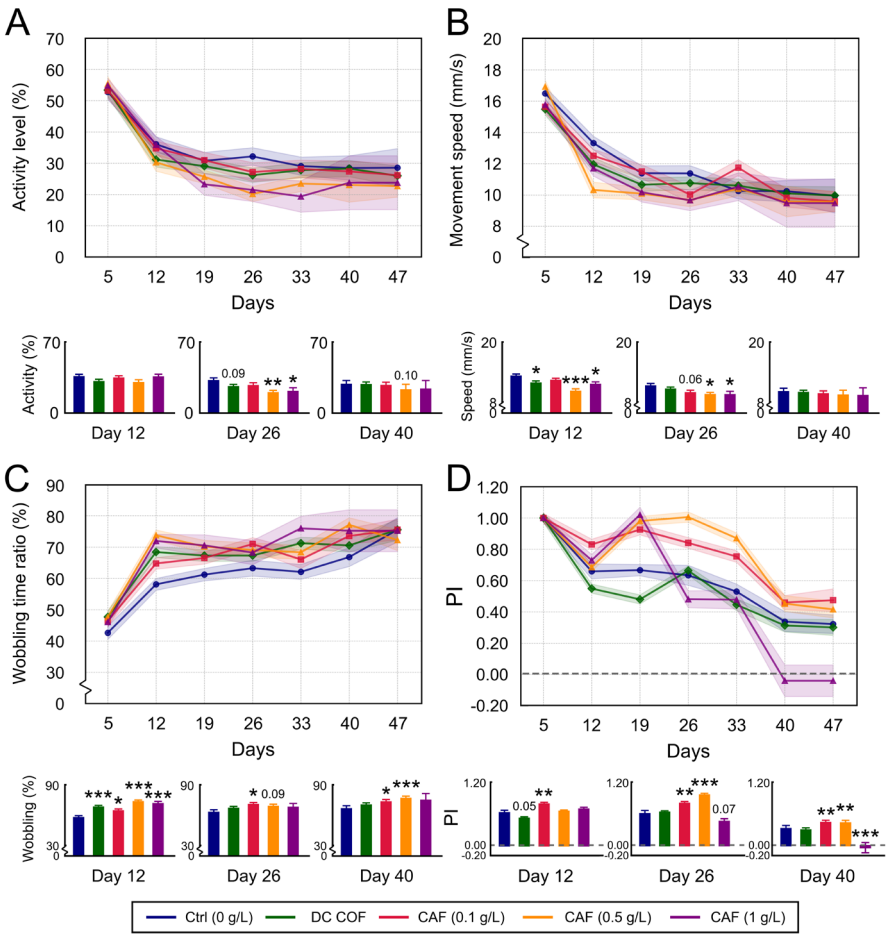

FIGURE 5 | Locomotion levels and short-term spatial memory in the caffeine concentration test. (A–D) Activity level, movement speed, wobbling time ratio, and time course of short-term spatial memory (represented by the normalized PI) under different caffeine concentrations. These panels are based on the same measurements as those in Figure 3 (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, $p < 0.1$ values are shown directly via GLMM).

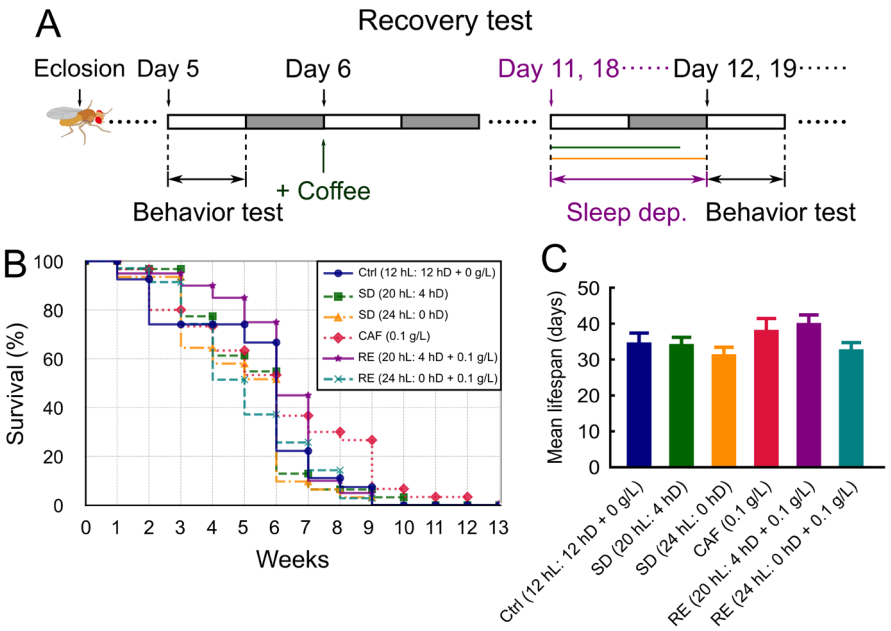

FIGURE 6 | Schematic diagram and survival in the recovery test. (A) Flies were assigned to six groups: Control (12:12 h light:dark + 0 g/L caffeine; blue), sleep deprivation (20:4 h; green), sleep deprivation (24:0 h; yellow), caffeine 0.1 g/L (red), recovery (20:4 h + 0.1 g/L caffeine; purple), and recovery (24:0 h + 0.1 g/L caffeine; teal). The control and sleep deprivation data correspond to Figure 2; caffeine-only data are from Figure 4. (B, C) Weekly survival rates and mean lifespan during behavioral assays. These panels correspond to the same metrics as those in Figure 2.

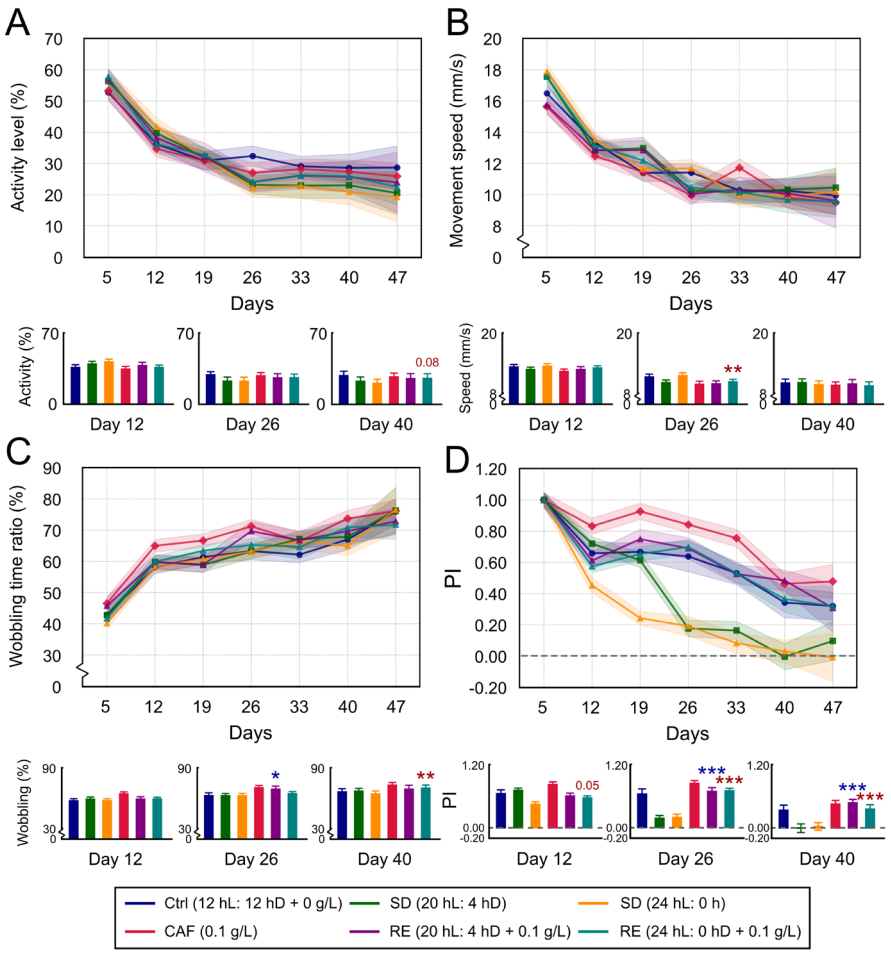

FIGURE 7 | Locomotion levels and short-term spatial memory in the recovery test. (A–D) Activity level, movement speed, wobbling time ratio, and the time course of short-term spatial memory (represented by the normalized PI) after sleep deprivation and caffeine recovery. The control and sleep deprivation data correspond to Figure 3; caffeine-only data are from Figure 5. These panels are based on the same measurements as those in Figure 3. The blue asterisks and numbers indicate differences among the SDs (20:4 h) and REs (20:4 h + 0.1 g/L); the red asterisks and numbers indicate differences among the SDs (24:0 h) and REs (24:0 h + 0.1 g/L). Significance is indicated above the bars (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, $p < 0.1$ values are shown directly via GLMM).

was not greatly disrupted; only the nighttime sleep duration was reduced—this is similar to the situation of "staying up late but needing to wake up at a fixed time." Previous studies have demonstrated that flies are generally alert during light–dark transitions (Ho and Sehgal 2005). Moreover, under the 16-h and 20-h light conditions in our study, the flies had already obtained partial sleep during the night, so no immediate rebound occurred when the light resumed. In contrast, under the 24-h continuous light conditions shown in Figure 1B, the circadian rhythm was severely disrupted, and no clear sleep rebound appeared in the second half of the day—this result is consistent with those of previous studies (Kirszenblat et al. 2018). For this reason, and based on the observations in Figure 1B, we set all behavioral testing time points to the second half of the light period on the day following sleep deprivation for the locomotion and cognition assays.

Interestingly, for the wild-type *Canton-S* strain of *Drosophila melanogaster*, day 26 posteclosion appeared to be a critical time point, from which significant differences in various behavioral parameters began to emerge across the sleep deprivation test, caffeine concentration test, and recovery test. Although the aging trajectory of wild-type flies varies across genotypes—for example, the Harwich strain results in accelerated reproductive capacity, developmental rate, body weight, and lifespan compared with those of *Canton-S* flies—day 26 is not necessarily a universally applicable benchmark (Zakharenko et al. 2024); however, day 26 for the Canton-S strain corresponds to approximately middle age (Bushey et al. 2010). Therefore, the conclusions drawn in this study may help elucidate the impact of coffee on middle-aged flies. The behavioral changes observed around this time point are speculated to be associated with physiological changes occurring at this stage. First, as age increases, the resistance of the fly to external stressors such as sleep deprivation decreases (Vienne et al. 2016). Second, oxidative stress and inflammatory responses increase during aging, further exacerbating the negative effects of external stress on the body (Le Bourg 2001; Belyi et al. 2020). These physiological changes increase the likelihood that middle-aged flies are affected by sleep deprivation, leading to significant declines in behavior and cognitive function. Additionally, owing to our experimental setup, the effects of sleep deprivation and coffee (or caffeine) on the flies may accumulate over their lifespan. Future research should further clarify the impact of these effects on flies and

assess the relationship between coffee (or caffeine) and aging via additional physiological indicators.

Additionally, our study suggests that caffeine intake after sleep deprivation may have a positive effect on the aging trajectory of fruit flies. Since our study only assessed locomotor and memory performance, future research could incorporate additional behavioral indicators to further validate the beneficial effects of coffee (or caffeine) intake after sleep deprivation. These indicators may include attention tasks to reflect cognitive capacity (Han et al. 2025), foraging behavior (Promislow et al. 2022), mating behavior (Economos et al. 1979), and social behavior (Brenman-Suttner et al. 2020), which may serve as indirect markers of aging.

In this study, we employed a protocol of acute and severe sleep deprivation once per week, with the aim of specifically observing the effects of short-term but intense circadian disruption on individual organisms. This design simulates real-life scenarios such as shift work or staying up late during exams, where individuals experience acute sleep loss followed by a recovery period. Previous studies have shown that acute and severe sleep deprivation has pronounced negative effects on diet (Lombardo et al. 2020), arousal, and cognitive performance (Tassi et al. 2012), and emotional state (Pires et al. 2016). These findings help explain the phenomena observed in our fly experiments. Furthermore, by repeated intense sleep deprivation weekly, we found that the resulting damage to the flies accumulated over time and that a single day with a normal sleep rhythm was insufficient for recovery.

Although our research focused on the effects of acute and severe sleep deprivation, the impact of chronic mild sleep deprivation also requires further investigation. Previous studies have shown that even slight sleep interruption can affect an organism's circadian rhythm, leading to long-term health consequences (Medic et al. 2017). Continuous mild sleep deprivation may affect cognition, emotion, and health (Liew and Aung 2021; Khoo et al. 2024). Moreover, prolonged sleep deficiency may impact brain plasticity, weakening learning and memory (Frank 2019; Ochab et al. 2021). We believe that chronic mild sleep deprivation may have cumulative effects on health and behavior that are different from those of acute severe sleep deprivation, potentially leading to slight but progressive impairments in locomotion and cognition. Future research should further explore the effects of chronic mild sleep deprivation to fully understand its impact on organisms.

We observed that the locomotion and survival of fruit flies were directly related to caffeine concentration. Previous studies have reported that high concentrations of caffeine (2.5 and 1.25 g/L) significantly reduce the lifespan of fruit flies (Nikitin et al. 2008), which is consistent with our findings. This may be because high caffeine concentrations suppress the activity of transposable elements, thereby causing genomic instability and ultimately exerting severe effects on lifespan (Nikitin et al. 2008). In addition, our results showed that coffee containing 0.5 and 1.0 g/L caffeine reduced the activity levels and movement speed of the flies. These phenomena may be attributed to the cumulative effects of long-term caffeine intake on locomotor behavior. We speculate that high concentrations of caffeine may lead to behavioral inhibition and reduced locomotion in fruit flies. Additionally, prolonged caffeine intake may cause the flies to overexert energy, leading to subsequent decreases in activity levels and movement speed. However, our results revealed that the decaffeinated coffee group did not differ significantly from the control group or from the caffeinated coffee group. This finding suggests that another possible explanation is that noncaffeine components in coffee may also have negative effects on the locomotion of fruit flies. In groups with higher caffeine concentrations, the concentration of noncaffeine compounds was greater. Unfortunately, our current study could not exclude the potential interference of these noncaffeine components. Future studies should be designed more precisely to explore the specific effects of noncaffeine substances on fly behavior.

Previous studies have indicated that the wobbling time ratio in flies is related to aging (Chi et al. 2020). Our results revealed that different caffeine concentrations in coffee increased the wobbling time ratio, but sleep deprivation did not significantly change the wobbling time ratio of the flies. Additionally, the decaffeinated coffee group presented an increased wobbling time ratio. Therefore, we speculate that noncaffeine components in coffee may increase the wobbling time ratio in flies. Regrettably, our study cannot exclude the impact of caffeine in coffee on the wobbling time ratio. Research has suggested that coffee affects the central nervous system, potentially causing tension or tremors (Winston et al. 2005), which could explain the increased wobbling. As a central nervous system stimulant, caffeine increases neural activity by increasing dopamine and norepinephrine release, which may lead to muscle coordination issues and increased wobbling (Fiani et al. 2021). The lack of a significant impact of sleep deprivation on the wobbling time ratio indicates that wobbling is more sensitive to coffee (or caffeine) than to sleep deprivation stress. This might be because the physiological stress from weekly acute sleep deprivation is insufficient to significantly alter neural activity, whereas the effect of coffee (or caffeine) is more pronounced.

Regarding the effects of coffee (and caffeine) on sleep, previous studies have shown that caffeine suppresses sleep, but this suppressive effect depends on the sucrose concentration in the diet. Under extremely low sucrose conditions, caffeine has little effect on sleep, yet caffeine reduces food intake in fruit flies, thereby indirectly reducing their sleep (Keebaugh et al. 2017). This finding may partly explain our observation that coffee helped restore cognitive function after sleep deprivation. Coffee consumption may have indirectly reduced the sleep requirements of the flies, thereby enhancing their daytime cognitive performance and arousal level. However, many questions remain unanswered. To what extent do the noncaffeine components of coffee contribute to the restoration of cognitive function after sleep deprivation? Is the restorative effect of caffeine due to increased arousal during behavioral testing, or does caffeine reduce the overall need for sleep in flies? Future studies should more precisely isolate coffee components and conduct daily sleep monitoring in fruit flies to clarify these issues.

Notably, the taste of decaffeinated coffee differs from that of regular caffeinated coffee, as decaffeinated coffee is subjected to chemical solvents or physical processing to remove caffeine. During this process, some volatile compounds and aromatic

substances are inevitably lost, leading to noticeable differences in taste, such as reduced sweetness, bitterness, and acidity (Choo et al. 2017; Shofinita et al. 2024). However, despite their stronger bitterness, people tend to prefer caffeinated coffee. This may be because of its stimulating effects rather than its taste alone. Previous studies have shown that genetic variants associated with the physiological effects of caffeine (such as arousal) have a greater influence on coffee consumption than do variants associated with bitter taste receptors (TAS2R genes; Cornelis and van Dam 2021). These findings may also help explain the effects of coffee observed in fruit flies in our study: the impact of coffee on flies may result more from its stimulatory properties than from its taste.

In addition, this study considered other differences between decaffeinated and caffeinated coffee in addition to taste. Owing to its significantly lower caffeine content, decaffeinated coffee has a weaker stimulating effect on the central nervous system. However, since some other components of coffee are still retained in decaffeinated coffee, it can still have positive effects on the body—albeit to a lesser extent than caffeinated coffee—in terms of its antioxidant and anti-inflammatory properties (Vicente et al. 2014). Our study revealed that decaffeinated coffee positively affected the survival of fruit flies, suggesting that components other than caffeine in coffee may also contribute to increased survival rates, which is consistent with previous findings (Cano-Marquina et al. 2013; Nieber 2017). Studies have shown that polyphenols and antioxidants in coffee have neuroprotective and lifespan-extending properties. Moreover, the chlorogenic acid and flavonoids present in coffee have antioxidant effects, neutralizing free radicals and reducing cellular damage (Socała et al. 2020). Additionally, other bioactive components in decaffeinated coffee, such as polysaccharides and phenolic compounds, may promote health by modulating the immune system and anti-inflammatory responses. The benefits of decaffeinated coffee observed in our study are consistent with these findings, suggesting that these bioactive compounds may play an important role in increasing the survival rates of fruit flies.

Our study used the *Canton-S* strain of *Drosophila melanogaster*, but different genotypes may exhibit varying sensitivities to sleep deprivation (Hendricks and Sehgal 2004; Wu et al. 2018). In addition, our study revealed that coffee intake enhances short-term memory in fruit flies. Previous research has demonstrated that caffeine promotes arousal in fruit flies through dopaminergic signaling, with these dopaminergic neurons projecting to the central complex and mushroom bodies of the fly brain (Nall et al. 2016). These brain regions are well established as being directly involved in memory processes (McGuire et al. 2001; Seelig and Jayaraman 2015; Turner-Evans and Jayaraman 2016; Barnstedt et al. 2016). Therefore, we speculate that the memory-enhancing effect of coffee may result from caffeine's direct action on neural circuits that regulate short-term memory. Moreover, our findings indicate that coffee intake has a notable restorative effect on cognitive function after sleep deprivation. This may be attributed to caffeine indirectly reducing sleep demand and thereby increasing arousal in fruit flies (Segu and Kannan 2023). Previous studies have highlighted the role of specific ring neurons in the ellipsoid body (EB) of the fly brain in regulating sleep and wakefulness. The activation or inhibition of these neurons significantly alters the sleep patterns of flies (Andreani et al. 2022; Yan et al. 2023; Singh et al. 2023). The ellipsoid body is also considered a key region within the central complex responsible for short-term memory (Su et al. 2017; Han, Huang, et al. 2021). Future neural functional research should explore the role of specific genetic variations and neural circuits in regulating the effects of sleep deprivation and coffee on fruit flies.

In summary, our study demonstrated that severe weekly sleep deprivation negatively affects the behavior and survival of fruit flies, whereas moderate coffee consumption has protective and recovery effects. The critical period around day 26 highlights that the impacts of these treatments may be related to the life stages of the flies. Our study also underscores the importance of further research on chronic mild sleep deprivation, the stimulatory effects of coffee on wobbling, and the beneficial components of decaffeinated coffee. Understanding the genetic and neural basis of these effects will further elucidate the mechanisms involved, providing valuable insights into the interactions among sleep, coffee, and behavior.

Author Contributions

Rui Han: conceptualization, methodology, data curation, investigation, formal analysis, supervision, funding acquisition, visualization, writing – original draft, writing – review and editing, resources, project administration. **Jun Zhang:** investigation. **Hao Huang:** investigation, resources, data curation. **Yi-Jie Chen:** data curation, investigation, resources. **Yu-Yuan Lu:** data curation, investigation, resources. **Yu-Chen Wang:** data curation, investigation, resources. **Chung-Chuan Lo:** methodology. **Yi-Heng Tan:** methodology. **Jia-Yi Li:** data curation. **Bao-Wei Pan:** data curation. **Ya-Lin Zhang:** conceptualization, supervision, project administration.

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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 raw data, code, and associated metadata for this paper are available at https://figshare.com/articles/dataset/Ethology/28666520.

References

Akosua, A., N. Adwoa, and Y. A. Asantewaa. 2023. "The Effect of Caffeine Consumption on Alertness: A Review of Literature." *Journal of Food Science and Human Nutrition* 2: 28–36.

Andreani, T., C. Rosensweig, S. Sisobhan, E. Ogunlana, W. Kath, and R. Allada. 2022. "Circadian Programming of the Ellipsoid Body Sleep Homeostat in Drosophila." *eLife* 11: e74327. https://doi.org/10.7554/eLife.74327.

Barnstedt, O., D. Owald, J. Felsenberg, et al. 2016. "Memory-Relevant Mushroom Body Output Synapses are Cholinergic." *Neuron* 89: 1237–1247. https://doi.org/10.1016/j.neuron.2016.02.015.

Belyi, A. A., A. A. Alekseev, A. Y. Fedintsev, et al. 2020. "The Resistance of *Drosophila melanogaster* to Oxidative, Genotoxic, Proteotoxic, Osmotic Stress, Infection, and Starvation Depends on Age According to the Stress Factor." *Antioxidants* 9: 1239. https://doi.org/10.3390/antiox9121239.

Brenman-Suttner, D. B., R. T. Yost, A. K. Frame, J. W. Robinson, A. J. Moehring, and A. F. Simon. 2020. "Social Behavior and Aging: A Fly Model." *Genes, Brain and Behavior* 19: e12598. https://doi.org/10.1111/gbb.12598.

Bushey, D., K. A. Hughes, G. Tononi, and C. Cirelli. 2010. "Sleep, Aging, and Lifespan in Drosophila." *BMC Neuroscience* 11: 56. https://doi.org/10.1186/1471-2202-11-56.

Cai, Y. D., S. I. Hidalgo Sotelo, K. C. Jackson, and J. C. Chiu. 2022. "Assaying Circadian Locomotor Activity Rhythm in Drosophila." In *Circadian Clocks*, edited by T. Hirota, M. Hatori, and S. Panda, 63–83. Springer US.

Cano-Marquina, A., J. J. Tarín, and A. Cano. 2013. "The Impact of Coffee on Health." *Maturitas* 75: 7–21. https://doi.org/10.1016/j.maturitas.2013.02.002.

Chi, H., L. Sun, R.-H. Shiu, et al. 2020. "Cleavage of Human Tau at Asp421 Inhibits Hyperphosphorylated Tau Induced Pathology in a Drosophila Model." *Scientific Reports* 10: 13482. https://doi.org/10.1038/s41598-020-70423-1.

Choo, E., B. Picket, and R. Dando. 2017. "Caffeine May Reduce Perceived Sweet Taste in Humans, Supporting Evidence That Adenosine Receptors Modulate Taste." *Journal of Food Science* 82: 2177–2182. https://doi.org/10.1111/1750-3841.13836.

Colomb, J., L. Reiter, J. Blaszkiewicz, J. Wessnitzer, and B. Brembs. 2012. "Open Source Tracking and Analysis of Adult Drosophila Locomotion in Buridan's Paradigm With and Without Visual Targets." *PLoS One* 7: e42247. https://doi.org/10.1371/journal.pone.0042247.

Cornelis, M. C., and R. M. van Dam. 2021. "Genetic Determinants of Liking and Intake of Coffee and Other Bitter Foods and Beverages." *Scientific Reports* 11: 23845. https://doi.org/10.1038/s41598-021-03153-7.

Du, P., C. Du, R. Wang, et al. 2022. "Caffeine Combined With Taurine Improves Cognitive Function and Locomotor Performance in Sleep-Deprived Mice." *Journal of Functional Foods* 99: 105298. https://doi.org/10.1016/j.jff.2022.105298.

Economos, A. C., J. Miquel, R. Binnard, and S. Kessler. 1979. "Quantitative Analysis of Mating Behavior in Aging Male *Drosophila melanogaster*." *Mechanisms of Ageing and Development* 10: 233–240. https://doi.org/10.1016/0047-6374(79)90037-X.

Elliott, A. S., J. D. Huber, J. P. O'Callaghan, et al. 2014. "A Review of Sleep Deprivation Studies Evaluating the Brain Transcriptome." *Springerplus* 3: 728. https://doi.org/10.1186/2193-1801-3-728.

Fiani, B., L. Zhu, B. L. Musch, et al. 2021. "The Neurophysiology of Caffeine as a Central Nervous System Stimulant and the Resultant Effects on Cognitive Function." *Cureus* 13: e15032. https://doi.org/10.7759/cureus.15032.

Frank, M. G. 2019. "Sleep and Brain Plasticity." In *Sleep, Memory and Synaptic Plasticity*, edited by S. K. Jha and V. M. Jha, 107–124. Springer.

García, A., J. D. Angel, J. Borrani, C. Ramirez, and P. Valdez. 2021. "Sleep Deprivation Effects on Basic Cognitive Processes: Which Components of Attention, Working Memory, and Executive Functions Are More Susceptible to the Lack of Sleep?" *Sleep Science* 14: 107–118. https://doi.org/10.5935/1984-0063.20200049.

Götz, K. G. 1980. "Visual Guidance in Drosophila." In *Development and Neurobiology of Drosophila*, edited by O. Siddiqi, P. Babu, L. M. Hall, and J. C. Hall, 391–407. Springer US.

Hales, K. G., C. A. Korey, A. M. Larracuente, and D. M. Roberts. 2015. "Genetics on the Fly: A Primer on the Drosophila Model System." *Genetics* 201: 815–842. https://doi.org/10.1534/genetics.115.183392.

Han, R., H.-P. Huang, C.-L. Chuang, et al. 2021. "Coordination Through Inhibition: Control of Stabilizing and Updating Circuits in Spatial Orientation Working Memory." *eNeuro* 8. https://doi.org/10.1523/ENEURO.0537-20.2021.

Han, R., Y.-H. Tan, and C.-C. Lo. 2024. "Attractiveness Versus Stickiness: Behavioural Preferences of *Drosophila melanogaster* With Competing Visual Stimuli." *Journal of Insect Physiology* 159: 104716. https://doi.org/10.1016/j.jinsphys.2024.104716.

Han, R., T.-M. Wei, S.-C. Tseng, and C.-C. Lo. 2021. "Characterizing Approach Behavior of *Drosophila melanogaster* in Buridan's Paradigm." *PLoS One* 16: e0245990. https://doi.org/10.1371/journal.pone.0245990.

Han, R., J. Zhang, G.-X. Huang, et al. 2025. "The Effects of Blue Light on Locomotion and Cognition in Early Adult *Drosophila melanogaster*." *Journal of Experimental Zoology. Part A, Ecological and Integrative Physiology* 343: 511–520. https://doi.org/10.1002/jez.2900.

Han, R., J. Zhang, R.-X. Wang, et al. 2024. "The Impact of Different Mating Systems on Locomotion in *Drosophila melanogaster*." *Journal of Insect Behavior* 38: 2. https://doi.org/10.1007/s10905-024-09866-7.

Hendricks, J. C., and A. Sehgal. 2004. "Why a Fly? Using Drosophila to Understand the Genetics of Circadian Rhythms and Sleep." *Sleep* 27, no. 2: 334–342. https://doi.org/10.1093/sleep/27.2.334.

Herden, L., and R. Weissert. 2020. "The Effect of Coffee and Caffeine Consumption on Patients With Multiple Sclerosis-Related Fatigue." *Nutrients* 12: 2262. https://doi.org/10.3390/nu12082262.

Ho, K. S., and A. Sehgal. 2005. "*Drosophila melanogaster*: An Insect Model for Fundamental Studies of Sleep." *Methods in Enzymology* 393: 772–793. https://doi.org/10.1016/S0076-6879(05)93041-3.

Huber, R., S. L. Hill, C. Holladay, et al. n.d. "Sleep Homeostasis in *Drosophila melanogaster*."

Jans, K., K. Lüersen, and G. Rimbach. 2021. "*Drosophila melanogaster* As a Model Organism to Study Lithium and Boron Bioactivity." *International Journal of Molecular Sciences* 22: 11710. https://doi.org/10.3390/ijms222111710.

Kawaguchi, T., Y. Umezaki, C. Ito, and K. Tomioka. 2016. "Interaction Between Sexes and Between Different Circadian Phenotypes Affects Lifespan in the Fruit Fly *Drosophila melanogaster*." *Physiological Entomology* 41, no. 1: 48–58. https://doi.org/10.1111/phen.12124.

Keebaugh, E. S., J. H. Park, C. Su, R. Yamada, and W. W. Ja. 2017. "Nutrition Influences Caffeine-Mediated Sleep Loss in Drosophila." *Sleep* 40: zsx146. https://doi.org/10.1093/sleep/zsx146.

Khoo, S. Y., W. H. Lai, S. H. On, et al. 2024. "Resting-State Electroencephalography (EEG) Microstates of Healthy Individuals Following Mild Sleep Deprivation." *Scientific Reports* 14: 16820. https://doi.org/10.1038/s41598-024-67902-0.

Killgore, W. D. S. 2010. "Effects of Sleep Deprivation on Cognition." In *Progress in Brain Research*, edited by G. A. Kerkhof and H. P. A. van Dongen, 105–129. Elsevier.

Kim, S.-M., S. Zhang, J. Park, et al. 2021. "REM Sleep Deprivation Impairs Learning and Memory by Decreasing Brain O-GlcNAc Cycling in Mouse." *Neurotherapeutics* 18: 2504–2517. https://doi.org/10.1007/s13311-021-01094-7.

Kirszenblat, L., D. Ertekin, J. Goodsell, Y. Zhou, P. J. Shaw, and B. van Swinderen. 2018. "Sleep Regulates Visual Selective Attention in Drosophila." *Journal of Experimental Biology* 221: jeb191429. https://doi.org/10.1242/jeb.191429.

Landis, G. N., D. Doherty, and J. Tower. 2020. "Analysis of *Drosophila Melanogaster* Lifespan." In *Aging: Methods and Protocols*, edited by S. P. Curran, 47–56. Springer US.

Le Bourg, É. 2001. "Oxidative Stress, Aging and Longevity in *Drosophila melanogaster*." *FEBS Letters* 498: 183–186. https://doi.org/10.1016/S0014-5793(01)02457-7.

Li, X., F. Yu, and A. Guo. 2009. "Sleep Deprivation Specifically Impairs Short-Term Olfactory Memory in Drosophila." *Sleep* 32: 1417–1424. https://doi.org/10.1093/sleep/32.11.1417.

Liew, S. C., and T. Aung. 2021. "Sleep Deprivation and Its Association With Diseases- a Review." *Sleep Medicine* 77: 192–204. https://doi.org/10.1016/j.sleep.2020.07.048.

Liu, Z., and Z. Zhao. 2014. "Effects of Light Interruption on Sleep and Viability of *Drosophila melanogaster*." *PLoS One* 9: e105678. https://doi.org/10.1371/journal.pone.0105678.

Lombardo, C., A. Ballesio, G. Gasparrini, and S. Cerolini. 2020. "Effects of Acute and Chronic Sleep Deprivation on Eating Behaviour." *Clinical Psychologist* 24: 64–72. https://doi.org/10.1111/cp.12189.

McGuire, S. E., P. T. Le, and R. L. Davis. 2001. "The Role of Drosophila Mushroom Body Signaling in Olfactory Memory." *Science* 293: 1330–1333. https://doi.org/10.1126/science.1062622.

McGurk, L., A. Berson, and N. M. Bonini. 2015. "Drosophila as an In Vivo Model for Human Neurodegenerative Disease." *Genetics* 201: 377–402. https://doi.org/10.1534/genetics.115.179457.

Medic, G., M. Wille, and M. E. Hemels. 2017. "Short- and Long-Term Health Consequences of Sleep Disruption." *Nature and Science of Sleep* 9: 151–161.

Moldofsky, H., F. A. Lue, J. R. Davidson, and R. Gorczynski. 1989. "Effects of Sleep Deprivation on Human Immune Functions." *FASEB Journal* 3: 1972–1977. https://doi.org/10.1096/fasebj.3.8.2785942.

Mołoń, M., J. Dampc, M. Kula-Maximenko, et al. 2020. "Effects of Temperature on Lifespan of *Drosophila melanogaster* From Different Genetic Backgrounds: Links Between Metabolic Rate and Longevity." *Insects* 11: 470. https://doi.org/10.3390/insects11080470.

Nall, A. H., I. Shakhmantsir, K. Cichewicz, S. Birman, J. Hirsh, and A. Sehgal. 2016. "Caffeine Promotes Wakefulness via Dopamine Signaling in Drosophila." *Scientific Reports* 6: 20938. https://doi.org/10.1038/srep20938.

Neuser, K., T. Triphan, M. Mronz, B. Poeck, and R. Strauss. 2008. "Analysis of a Spatial Orientation Memory in Drosophila." *Nature* 453: 1244–1247.

Nieber, K. 2017. "The Impact of Coffee on Health." *Planta Medica* 83: 1256–1263. https://doi.org/10.1055/s-0043-115007.

Nikitin, A. G., S. Navitskas, and L.-A. N. Gordon. 2008. "Effect of Varying Doses of Caffeine on Life Span of *Drosophila melanogaster*." *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 63, no. 2: 149–150. https://doi.org/10.1093/gerona/63.2.149.

Ochab, J. K., J. Szwed, K. Oleś, et al. 2021. "Observing Changes in Human Functioning During Induced Sleep Deficiency and Recovery Periods." *PLoS One* 16: e0255771. https://doi.org/10.1371/journal.pone.0255771.

Olopade, F. E., O. M. Femi-Akinlosotu, A. J. Adekanmbi, O. O. Ighogboja, and M. T. Shokunbi. 2021. "Chronic Caffeine Ingestion Improves Motor Function and Increases Dendritic Length and Arborization in the Motor Cortex, Striatum, and Cerebellum." *Journal of Caffeine and Adenosine Research* 11: 3–14. https://doi.org/10.1089/caff.2020.0017.

Overman, K. E., D. M. Choi, K. Leung, J. W. Shaevitz, and G. J. Berman. 2022. "Measuring the Repertoire of Age-Related Behavioral Changes in *Drosophila melanogaster*." *PLoS Computational Biology* 18: e1009867. https://doi.org/10.1371/journal.pcbi.1009867.

Pilcher, J. J., and A. I. Huffcutt. 1996. "Effects of Sleep Deprivation on Performance: A Meta-Analysis." *Sleep* 19: 318–326. https://doi.org/10.1093/sleep/19.4.318.

Pires, G. N., A. G. Bezerra, S. Tufik, and M. L. Andersen. 2016. "Effects of Acute Sleep Deprivation on State Anxiety Levels: A Systematic Review and Meta-Analysis." *Sleep Medicine* 24: 109–118. https://doi.org/10.1016/j.sleep.2016.07.019.

Promislow, D. E. L., T. Flatt, and R. Bonduriansky. 2022. "The Biology of Aging in Insects: From Drosophila to Other Insects and Back." *Annual Review of Entomology* 67: 83–103. https://doi.org/10.1146/annurev-ento-061621-064341.

Seelig, J. D., and V. Jayaraman. 2015. "Neural Dynamics for Landmark Orientation and Angular Path Integration." *Nature* 521: 186–191.

Segu, A., and N. N. Kannan. 2023. "The Duration of Caffeine Treatment Plays an Essential Role in Its Effect on Sleep and Circadian Rhythm." *SLEEP Advances* 4: zpad014. https://doi.org/10.1093/sleepadvances/zpad014.

Shin, J.-W., J.-H. Wang, J.-K. Kang, and C.-G. Son. 2010. "Experimental Evidence for the Protective Effects of Coffee Against Liver Fibrosis in SD Rats." *Journal of the Science of Food and Agriculture* 90: 450–455. https://doi.org/10.1002/jsfa.3838.

Shofinita, D., D. Lestari, R. Purwadi, et al. 2024. "Effects of Different Decaffeination Methods on Caffeine Contents, Physicochemical, and Sensory Properties of Coffee." *International Journal of Food Engineering* 20: 561–581. https://doi.org/10.1515/ijfe-2024-0013.

Singh, P., A. Aleman, J. J. Omoto, et al. 2023. "Examining Sleep Modulation by Drosophila Ellipsoid Body Neurons." *eNeuro* 10. https://doi.org/10.1523/ENEURO.0281-23.2023.

Socała, K., A. Szopa, A. Serefko, E. Poleszak, and P. Wlaź. 2020. "Neuroprotective Effects of Coffee Bioactive Compounds: A Review." *International Journal of Molecular Sciences* 22: 107. https://doi.org/10.3390/ijms22010107.

Strauss, R., and J. Pichler. 1998. "Persistence of Orientation Toward a Temporarily Invisible Landmark in *Drosophila melanogaster*." *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 182: 411–423.

Su, T.-S., W.-J. Lee, Y.-C. Huang, C. T. Wang, and C. C. Lo. 2017. "Coupled Symmetric and Asymmetric Circuits Underlying Spatial Orientation in Fruit Flies." *Nature Communications* 8: 139.

Tassi, P., S. Schimchowitsch, O. Rohmer, et al. 2012. "Effects of Acute and Chronic Sleep Deprivation on Daytime Alertness and Cognitive Performance of Healthy Snorers and Non-Snorers." *Sleep Medicine* 13: 29–35. https://doi.org/10.1016/j.sleep.2011.06.017.

Turner-Evans, D. B., and V. Jayaraman. 2016. "The Insect Central Complex." *Current Biology* 26: R453–R457.

Umemura, G. S., F. Furtado, F. C. dos Santos, F. C. Dos Santos, B. D. S. B. Gonçalves, and A. Forner-Cordero. 2022. "Is Balance Control Affected by Sleep Deprivation? A Systematic Review of the Impact of Sleep on the Control of Balance." *Frontiers in Neuroscience* 16: 779086. https://doi.org/10.3389/fnins.2022.779086.

Vicente, S. J. V., Y. S. Queiroz, S. L. D. Gotlieb, and E. A. F. d. S. Torres. 2014. "Stability of Phenolic Compounds and Antioxidant Capacity of Regular and Decaffeinated Coffees." *Brazilian Archives of Biology and Technology* 57: 110–118. https://doi.org/10.1590/S1516-89132014000100016.

Vienne, J., R. Spann, F. Guo, and M. Rosbash. 2016. "Age-Related Reduction of Recovery Sleep and Arousal Threshold in Drosophila." *Sleep* 39: 1613–1624. https://doi.org/10.5665/sleep.6032.

Winston, A. P., E. Hardwick, and N. Jaberi. 2005. "Neuropsychiatric Effects of Caffeine." *Advances in Psychiatric Treatment* 11: 432–439. https://doi.org/10.1192/apt.11.6.432.

Wu, K. J., S. Kumar, Y. L. Serrano Negron, and S. T. Harbison. 2018. "Genotype Influences Day-To-Day Variability in Sleep in *Drosophila melanogaster*." *Sleep* 41: zsx205. https://doi.org/10.1093/sleep/zsx205.

Xie, G., X. Huang, H. Li, P. Wang, and P. Huang. 2021. "Caffeine-Related Effects on Cognitive Performance: Roles of Apoptosis in Rat Hippocampus Following Sleep Deprivation." *Biochemical and Biophysical Research Communications* 534: 632–638. https://doi.org/10.1016/j.bbrc.2020.11.029.

Yan, W., H. Lin, J. Yu, et al. 2023. "Subtype-Specific Roles of Ellipsoid Body Ring Neurons in Sleep Regulation in Drosophila." *Journal of Neuroscience* 43: 764–786. https://doi.org/10.1523/JNEUROSCI.1350-22.2022.

Yen, H.-H., R. Han, and C.-C. Lo. 2019. "Quantification of Visual Fixation Behavior and Spatial Orientation Memory in *Drosophila melanogaster*." *Frontiers in Behavioral Neuroscience* 13: 215. https://doi.org/10.3389/fnbeh.2019.00215.

Zakharenko, L. P., M. A. Bobrovskikh, N. E. Gruntenko, D. V. Petrovskii, E. G. Verevkin, and A. A. Putilov. 2024. "Two Old Wild-Type Strains of *Drosophila melanogaster* Can Serve as an Animal Model of Faster and Slower Aging Processes." *Insects* 15: 329. https://doi.org/10.3390/insects15050329.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.