Behavioural Processes 239 (2026) 105383

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

Developmental light colour influences adult phototaxis and locomotion in *Drosophila melanogaster*

Rui Han [a,*], Jun Zhang [b,*], Zi-Yu Guo [b], Xu-Hui Guo [b], Sheng-Yu Hou [b], Ke-Jing Feng [b], Yu-Xin He [b], Han-Ning Wu [b], Xin-Hui Chen [b]

[a] Tan Kah Kee College, Xiamen University, Zhangzhou, Fujian 363105, China
[b] School of Life Sciences, Xiamen University, Xiamen, Fujian 361102, China

ARTICLE INFO

Keywords:
Phototaxis
Developmental light colour
Locomotion
Behavioural plasticity
Drosophila melanogaster

ABSTRACT

Light colour is an important environmental factor influencing animal behaviour and its plasticity. Here, we investigated how developmental exposure to different light colours modulates adult phototaxis and locomotion in *Drosophila melanogaster*. Parental flies were allowed to mate and oviposit under one of five illumination conditions (white, blue, green, yellow, or red), and their offspring subsequently developed under the same light conditions from egg to eclosion. Using independent cohorts (20~25 adult males per light condition; $n = 231$ in total), phototaxis and locomotion were evaluated immediately after adult emergence and again following five days of recovery under white light. Flies developed under colour-filtered lights exhibited wavelength-congruent phototactic preferences, spending more time near the wavelength corresponding to their developmental light environment. In addition, individuals developed under long-wavelength lights (red) displayed significantly higher movement and active speeds than those developed under short-wavelength or white light. Notably, after five days of recovery under white light, both wavelength-specific phototactic preferences and enhanced locomotion were largely diminished, indicating that these effects are plastic and reversible. Together, our results demonstrate that the spectral environment during development can transiently modulate adult behavioural outputs, highlighting the importance of early-life light conditions in modulating adult insect behaviour.

1. Introduction

Light is one of the most fundamental ecological factors influencing animal behaviour across diverse environments (Bradshaw and Holzapfel, 2007; Warrant and Johnsen, 2013). Different wavelengths of light regulate circadian rhythms and strongly affect visually guided behaviours, including phototaxis, locomotion, and light avoidance (Blass and Gaffin, 2008; Ho et al., 2013; Hofstetter et al., 2005; Mascalzoni and Regolin, 2011; Paskin et al., 2014; Pauers et al., 2012). In insects, illumination serves not only as an environmental cue but also as a major factor modulating behavioural decisions and activity patterns (Dubowy and Sehgal, 2017; Helfrich-Förster, 2020; Lu et al., 2008). Phototaxis, an innate behavioural tendency widely observed among insects, provides a powerful behavioural framework for investigating how light environments shape behavioural rhythms and decision-making processes (Collett and Collett, 2000; Gorostiza et al., 2016).

Previous studies have extensively explored how light influences insect behaviour at the adult stage, particularly in relation to circadian regulation, learning and memory, and light avoidance (Helfrich-Förster, 2020; Lu et al., 2008, p. 200; Rieger et al., 2003). However, comparatively less attention has been paid to how light environments experienced during development shape behavioural outputs later in life. This question is particularly non-trivial in holometabolous insects, in which the larval and adult visual systems are anatomically and functionally distinct (Gilbert, 1994; Ready, 2002). The larval visual organ does not transform into the adult compound eye, which instead develops de novo during pupation, making it difficult to predict whether and how early-life light environments might influence adult visually guided behaviour and highlighting the need for behavioural-level investigations that directly test the persistence and plasticity of such effects.

Drosophila melanogaster is a well-established model organism for studying visually guided behaviour and behavioural regulation, as well as for dissecting the genetic and environmental bases of behaviour

* Corresponding authors.
E-mail addresses: hanrui@lolab-nthu.org (R. Han), jzhang@xmu.edu.cn (J. Zhang).

https://doi.org/10.1016/j.beproc.2026.105383
Received 25 January 2026; Received in revised form 31 March 2026; Accepted 25 April 2026
Available online 26 April 2026

(Bellen et al., 2010; Brembs, 2016; Gorostiza et al., 2016; Han et al., 2021, 2024a). Adult fruit flies exhibit robust phototactic responses and possess compound eyes containing multiple classes of photoreceptors that are most sensitive to short- and medium-wavelength light, whereas responses to longer wavelengths are relatively weaker (Hu and Stark, 1980; Salcedo et al., 1999; Yamaguchi et al., 2010). Behavioural responses to visual stimuli have also been shown to vary across genotypes and eye mutants, underscoring the genetic basis of visually guided behaviour in this species (Götz, 1964). Moreover, phototactic behaviour emerges early in development and has been reported to correlate with adult phototactic tendencies (Damulewicz et al., 2022; Humberg and Sprecher, 2017; Kane et al., 2013; Keene and Sprecher, 2012; Warrick et al., 1999), suggesting that early sensory environments may influence later behavioural expression. Nevertheless, whether spectral environments experienced during development exert persistent yet reversible effects on adult behaviour remains largely unexplored.

In behavioural studies of fruit flies, phototaxis is commonly assessed using light–dark choice paradigms or T-mazes by quantifying the proportion or duration of flies remaining near a light source within a fixed time window (Jacob et al., 1977; Shaw et al., 2020; Willmund, 1979). These methods provide reproducible and sensitive measures of visually guided choice behaviour. Locomotion represents another critical dimension of behavioural output that is tightly linked to motivational state and overall activity level. Previous studies have demonstrated that illumination can influence not only visual responsiveness but also locomotor activity indirectly, through modulation of arousal level and metabolic state (Donlea et al., 2011; Han et al., 2025a, 2026; Ho et al., 2013). Similar associations between illumination and activity have also been reported in other dipteran species, including cave-dwelling flies exhibiting attraction and activity rhythms under extreme or permanently dark environments (Stringer and Meyer-Rochow, 1997), highlighting the generality of light–activity relationships across *Diptera*.

Based on these considerations, the present study systematically examined how developmental exposure to different light colours (white, blue, green, yellow, and red) from egg to eclosion affects adult phototaxis and locomotion in *Drosophila melanogaster*. After eclosion, all groups were maintained under white light for five days to evaluate the reversibility of these effects. We hypothesized that developmental exposure to distinct wavelengths would induce colour-congruent phototactic preferences and modulate locomotor activity, but that these behavioural modifications would diminish following recovery under uniform white light. By integrating behavioural assays across both developmental and recovery phases, this study aims to elucidate how early-life spectral environments shape adult behavioural plasticity, thereby providing insight into how environmental illumination modulates insect behaviour.

2. Material and methods

2.1. Fly Strains

Wild-type fruit flies (*Drosophila melanogaster*, genotype *Canton-S*) were used throughout this study and obtained from the Bloomington Drosophila Stock Center (BDSC, RRID: BDSC_64349). Flies were maintained on standard cornmeal–yeast–agar medium in a temperature-controlled incubator at 25 °C and 60% relative humidity under a 12:12 h light–dark cycle (lights on at 08:00 and off at 20:00), unless otherwise specified. All experimental manipulations were performed under these controlled environmental conditions.

All behavioural experiments were conducted on male offspring (F1 generation) derived from controlled mattings. Virgin females were collected within 8 h after eclosion and used to establish mating pairs. Males were obtained from the same stock population. This procedure ensured that all matings involved virgin females and minimized variability associated with prior mating history. After eclosion, experimental male flies within the same treatment group were housed together in a single tube prior to behavioural testing to ensure uniform post-eclosion environmental exposure. Only adult males were used in all behavioural assays to avoid potential sex-related variation in phototaxis and locomotion.

2.2. Experimental design

To examine the effects of developmental light environment on adult phototaxis and locomotion, five experimental groups were established: white, blue, green, yellow, and red (Fig. 1A). After mating (Day 0), parental adults (F0) were maintained under the assigned light colour to allow oviposition. Here, Day 0 refers to the onset of oviposition and the beginning of F1 developmental time. All subsequent time points therefore correspond to the developmental timeline of the offspring (F1), rather than the parental generation. Offspring (F1) subsequently developed under the same light condition from egg to eclosion, after which newly eclosed males were collected for behavioural testing. Thus, each group experienced a continuous, colour-specific light environment spanning the post-mating period through development until adult emergence.

All illumination was provided by a Honeywell eye-care desk lamp (model HWX-02B Pro; rated power 45 W; nominal luminous flux 3200 lm), following the configuration described in Han et al. (Han et al., 2025b). Spectral composition for each treatment was generated by placing optical interference filters corresponding to the designated colours over the lamp. The emission spectra of all light conditions were measured using a spectroradiometer (380–780 nm, 5 nm resolution), confirming narrowband or quasi-monochromatic distributions for the coloured lights and a broadband profile for white light. The peak wavelength ranges were approximately 415–515 nm for blue, 470–570 nm for green, 540–640 nm for yellow, and 600–700 nm for red light (Fig. 1B).

To ensure that behavioural differences were not attributable to intensity differences, photon flux was calibrated using a quantum sensor and normalized across all colour conditions to approximately 4.24×10^{8} photons·m^{-2}·s^{-1} (≈0.42 μmol photons·m^{-2}·s^{-1}), thereby equating total photon input across spectral treatments. All experimental groups were maintained under a 12:12 h light–dark cycle throughout the entire experimental period, and no constant-light or constant-dark conditions were applied at any stage.

Mating was initiated on Day 0 by placing small groups of sexually mature *Canton-S* males and females together under their respective light conditions. Oviposition occurred continuously during Days 0–5, after which parental flies were removed to prevent overlapping generations. Eggs hatched within approximately 24 h under the same light conditions. Larval development lasted approximately 4–5 days, followed by a pupal stage of approximately 4–5 days, resulting in adult eclosion predominantly between Days 11–12 post-oviposition. Larvae were reared on standard cornmeal–yeast–agar medium in transparent culture vials and remained exposed to the same coloured light during the light phase of the 12:12 h cycle. Larval development lasted approximately 4–5 days at 25 °C.

Pupation occurred between Days 6–7 post-oviposition. Pupae remained in the same vials and continued to be exposed to the corresponding coloured light through the transparent vial walls during the light phase of the cycle; no shielding or covering was applied during the pupal stage. The pupal stage lasted approximately 4–5 days. Adult flies eclosed predominantly during the light phase between Days 11–12.

Newly eclosed males were collected within 8 h of eclosion and transferred to fresh food vials under the same illumination for the first round of behavioural assays (Day 12). For each behavioural assay, 20–25 adult males were tested per light condition. Specifically, phototaxis assays (measuring light preference) and locomotion assays (measuring movement activity and speed) were conducted using independent cohorts to avoid carry-over effects, yielding a total sample size of 231 flies (5 light conditions × 20–25 flies × 2 behavioural assays).

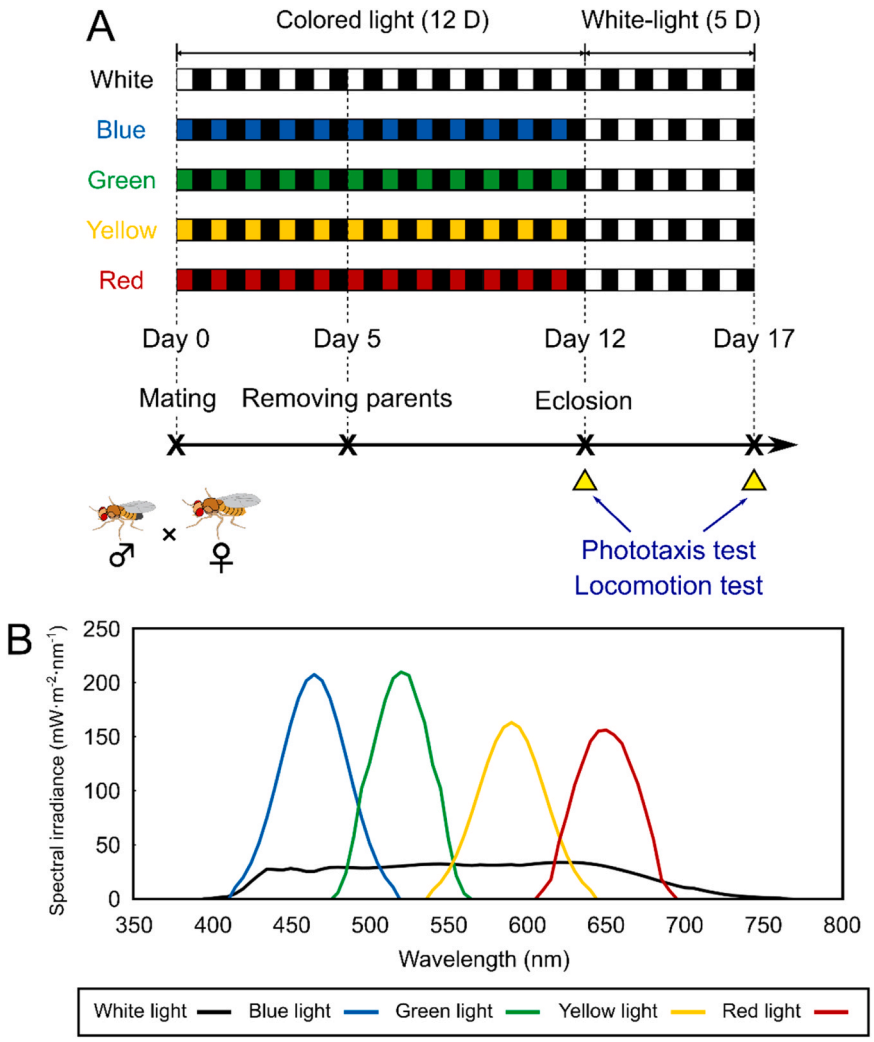

Fig. 1. Experimental design and spectral profiles of light stimuli. (A) Experimental timeline. On Day 0, *Canton-S* flies were allowed to mate and oviposit under five light conditions (white, blue, green, yellow, and red), all maintained under a 12 h light: 12 h dark cycle throughout development. Light was provided by a calibrated desk lamp fitted with colour-specific optical filters. Parental flies were removed on Day 5. Offspring developed under the same light conditions from egg to eclosion. Newly eclosed males were collected on Days 11–12 and tested for phototaxis and locomotion on Day 12. All groups were then transferred to white light for 5 days and retested on Day 17. (B) Spectral irradiance profiles of the five light conditions ($mW \cdot m^{-2} \cdot nm^{-1}$) as a function of wavelength (nm), measured at 5 nm intervals. All treatments were standardized to the same photon flux (~0.42 μmol photons·$m^{-2} \cdot s^{-1}$).

Following the initial behavioural tests, all experimental flies were transferred to identical white-light conditions for an additional 5 days (Day 12 to Day 17), constituting a recovery phase designed to assess the reversibility of developmental light-induced behavioural effects. On Day 17, the same sets of phototaxis and locomotion tests were repeated under standardized white-light conditions.

2.3. Tracking apparatus

For the phototaxis test, individual adult flies were transferred into transparent acrylic tubes (85 mm in length and 24 mm in diameter), which were evenly divided into four zones covered with blue, green, yellow, and red optical filters. Each fly was allowed to move freely within the tube for 120 s while being recorded from above. The photon flux of each colour stimulus during testing was kept consistent with that used in the rearing conditions to ensure comparable illumination intensity among colours.

All recordings were conducted in a darkened environment, with the only light source provided by the colour stimuli, thereby minimizing interference from external reflections. Behavioural recording and subsequent tracking analyses were performed blind to the rearing condition to minimize observer bias, and all tests were conducted under identical stimulus geometry and photon flux conditions.

For locomotion tests, individual flies were placed in empty tubes of the same size (85 mm × 24 mm) (Han et al., 2024b). Tubes were horizontally arranged on a white, matte background and separated by thin barriers to prevent visual interactions between individuals. Videos were captured using a high-resolution smartphone camera mounted on a fixed stand at a height of approximately 15–20 cm. Raw videos were standardized to 1920 × 1080 pixels at 15 frames per second (MP4 format, muted) for subsequent analysis. The movement of each fly was tracked frame by frame using a custom Python script that automatically extracted the x–y coordinates from each frame.

2.4. Data analysis

For the phototaxis test, video recordings were processed using a custom Python script to extract the x–y position of each fly frame by frame. The trajectory was divided into four predefined colour zones according to the optical filters covering the tube. For each individual, the total time spent in each zone during the 120 s test was calculated and expressed as a percentage of the total observation time, representing the

relative preference for each colour.

A phototaxis index (*PI*) was further computed to quantify colour-specific preference relative to the white-light control, defined as:

$$PI = Time\,spent\ (\%)_{rearing\ light\ color} - Time\,spent\ (\%)_{white\ light\ condition} \quad (1)$$

Positive *PI* values indicate stronger attraction to the test colour after rearing under the same colour, whereas negative values indicate reduced attraction relative to the white-light control. The *PI* was used for descriptive visualization only and was not subjected to inferential statistical testing.

For locomotion tests, the positional coordinates of each fly were extracted from videos using the same tracking algorithm. Instantaneous displacement between consecutive frames was computed to obtain movement trajectories over time. Three locomotion parameters were derived for each individual: activity level, defined as the proportion of frames in which the fly exhibited detectable movement; overall speed, defined as the total distance travelled divided by the total observation time; and active speed, defined as the total distance travelled divided by the duration of active movement only. All locomotor variables were calculated using frame-by-frame positional data smoothed with a short-term moving average to minimize background noise. Data were exported to CSV format and aggregated by individual before statistical analysis.

2.5. Statistical analysis

All statistical analyses were conducted using SPSS 22.0. Prior to analysis, data distributions were visually inspected for potential outliers. Although some extreme values were observed, these were retained because they were considered to reflect genuine biological variability rather than technical or measurement error; therefore, no data points were excluded from the final analyses. For phototaxis data obtained immediately after eclosion, a mixed-design general linear model (*GLM*) was applied, with test colour (blue, green, yellow, red) as a within-subject factor and rearing light condition (white, blue, green, yellow, red) as a between-subject factor. When a significant interaction was detected, planned pairwise comparisons were performed between each coloured-light–reared group and the white-light control under the corresponding test colour.

For phototaxis data after 5 days of white-light recovery, the same mixed-design *GLM* framework was applied. To assess recovery dynamics, a separate mixed-design *GLM* was used for phototactic preference toward the corresponding rearing colour, with Time (pre- vs post-recovery) as a within-subject factor and Rearing condition (blue, green, yellow, red) as a between-subject factor.

For locomotion tests, differences among rearing conditions (white, blue, green, yellow, red) were assessed using one-way ANOVA.

In all cases involving multiple post hoc comparisons, Bonferroni correction was applied. All tests were two-tailed, with $p < 0.05$ considered statistically significant. *P*-values between 0.05 and 0.10 were reported numerically in the figures as near-significant trends, consistent with conventions in behavioural studies. In addition, post hoc power analyses were conducted for the primary behavioural models based on the observed effect sizes ($\alpha = 0.05$) to evaluate sample size adequacy.

3. Results

3.1. Baseline phototactic responses under white-light rearing

To establish baseline phototactic responses under uniform illumination, we first examined flies reared exclusively under white light (Fig. 1). Newly mated flies were maintained under a 12 h white-light:12 h dark cycle from Day 0, and behavioural tests were conducted immediately after adult eclosion (Day 12) and again after five additional days of continued white-light exposure (Day 17). During each test, individual flies were allowed to freely explore a four-choice arena in which blue, green, yellow, and red zones were simultaneously present, and phototactic behaviour was quantified over a 120 s period.

The results showed that under white-light rearing, flies exhibited no significant differences in the proportion of time spent among the four coloured zones during the phototaxis test (Fig. 2A). A repeated-measures ANOVA revealed no significant main effect of colour ($F(3, 69) = 0.788$, $p = 0.504$, $\eta^2 = 0.033$; $n = 24$). The mean ± *SEM* proportions of time spent in each zone were 24.65 ± 1.19% (blue), 26.78 ± 1.13% (green), 24.05 ± 1.55% (yellow), and 26.19 ± 1.21% (red).After an additional five days of white-light rearing (Day 17), phototactic patterns remained statistically unchanged (Fig. 2B; $F(3, 69) = 1.283$, $p = 0.287$, $\eta^2 = 0.053$; $n = 24$), with comparable mean ± *SEM* proportions across colours (24.23 ± 1.15% for blue, 27.41 ± 1.39% for green, 23.02 ± 1.72% for yellow, and 25.35 ± 1.39% for red). Together, these findings indicate that under uniform white-light conditions, adult flies did not display any strong colour preference, providing a stable behavioural baseline for subsequent comparisons across different developmental light environments.

3.2. Effects of developmental light colour on phototaxis and locomotion

To examine whether developmental exposure to different light colours modulates adult phototactic behaviour, we compared flies reared under blue, green, yellow, or red light with those reared under white light, under identical test conditions. A mixed-design general linear model, with test colour as a within-subject factor and rearing light condition as a between-subject factor, revealed a significant interaction between test colour and rearing light condition ($F(12,330) = 7.811$, $p < 0.001$, partial $\eta^2 = 0.221$), while neither the main effect of test colour nor that of rearing light was significant.Post hoc comparisons with Bonferroni correction were then conducted to specifically test whether rearing under a given colour selectively enhanced phototaxis toward that same colour relative to white-light rearing. Under blue test light, flies reared in blue light spent significantly more time in the blue zone than those reared in white light ($p < 0.001$; Fig. 3A), demonstrating a clear enhancement of blue phototaxis induced by blue-light exposure during development. In contrast, under green and yellow test lights, flies reared under the corresponding light conditions exhibited a tendency toward stronger attraction compared to white-reared flies, although these differences did not reach statistical significance (green: $p = 0.084$; yellow: $p = 0.115$; Fig. 3B–C). In addition, a significant difference was detected under red test light, where red-reared flies showed a significantly altered phototactic response compared to white-reared flies ($p = 0.025$; Fig. 3D).

Additional comparisons revealed that the enhancement induced by developmental light exposure was selective rather than generalized: no consistent increases in phototaxis were observed for non-matching colour combinations, indicating that developmental illumination modulated wavelength-specific preference rather than overall phototactic sensitivity. This pattern was further illustrated by the phototaxis index (*PI*) heatmap (Fig. 3E), which showed maximal enhancement when the rearing colour matched the test colour.

We next examined whether developmental light colour also affected adult locomotor performance (Fig. 4). For activity level, a one-way ANOVA revealed no significant effect of rearing light ($F(4111) = 1.042$, $p = 0.389$, partial $\eta^2 = 0.036$), indicating that general movement frequency was not altered by developmental illumination (Fig. 4A). In contrast, rearing light exerted a significant effect on overall movement speed ($F(4111) = 6.605$, $p < 0.001$, partial $\eta^2 = 0.192$; Fig. 4B). Post hoc comparisons with Bonferroni correction showed that flies reared under red light tended to move faster than those reared under white light ($p = 0.001$), blue light ($p = 0.002$), green light ($p = 0.001$) and yellow light ($p < 0.001$). A similar pattern was examined for active speed; however, no significant effect of rearing light was detected ($F(4111) = 0.764$, $p = 0.551$, partial $\eta^2 = 0.027$; Fig. 4C).

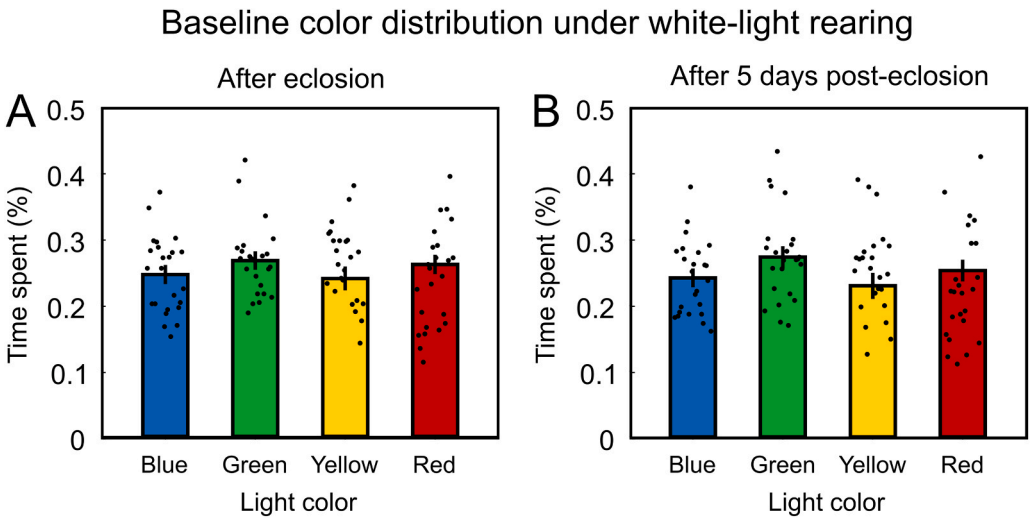

Fig. 2. Baseline colour distribution of fruit flies reared under white light.(A) Phototactic responses of flies reared under white light and tested immediately after eclosion (Day 12), and (B) after five additional days of continued white-light exposure (Day 17). The y-axis indicates the percentage of time spent in each coloured zone (blue, green, yellow, and red) during a 120 s four-choice phototaxis test. Each bar represents the mean ± *SEM* ($n = 24$). Data were analyzed using repeated-measures ANOVA, with colour as the within-subject factor.

Together, these results indicate that although developmental light colour does not influence general activity level or active speed, it significantly modulates overall movement speed, with red-light rearing associated with increased locomotor velocity.

3.3. *Effects of white-light recovery on phototaxis and locomotion*

To determine whether the phototactic modifications induced by developmental light exposure persist after removal of coloured illumination, we examined flies reared under blue, green, yellow, or red light and subsequently transferred to white light for five days following eclosion. Their wavelength-specific phototactic responses after recovery were compared with those of flies continuously reared under white light. A mixed-design general linear model, with test colour as a within-subject factor and rearing light condition as a between-subject factor, revealed no significant interaction between test colour and rearing condition after recovery ($F(12,330) = 0.473$, $p = 0.930$, partial $\eta^2 = 0.017$), indicating that the wavelength-dependent modulation observed immediately after eclosion was largely abolished following white-light exposure. Neither the main effect of test colour nor that of rearing light condition reached statistical significance, suggesting that phototactic behaviour converged across groups after recovery.

Comparisons with Bonferroni correction were then conducted to test whether developmental exposure to a given colour still produced a selective, colour-matched enhancement after recovery. Under blue, green, yellow, and red test lights, none of the corresponding colour-reared groups differed significantly from the white-reared control (all Bonferroni-corrected $p > 0.05$; Fig. 5A–D). No significant differences were detected for any non-matching rearing–test combinations either (all $p > 0.05$). These findings indicate that the colour-dependent phototactic biases observed immediately after eclosion were abolished after five days of white-light recovery. This attenuation was further illustrated by the phototaxis index (*PI*) heatmap (Fig. 5E), which showed markedly reduced contrast across rearing–test colour combinations compared with the strong colour-matched enhancement observed prior to recovery (Fig. 3E).

To further quantify recovery dynamics, we analyzed changes in phototactic preference toward the corresponding rearing colour before and after recovery using a mixed-design general linear model, with Time (Pre vs Post) as a within-subject factor and Rearing condition as a between-subject factor (Fig. 5F). The analysis revealed a significant main effect of Time ($F(1,87) = 23.390$, $p < 0.001$, partial $\eta^2 = 0.212$), indicating that, across all rearing conditions, phototactic preference toward the rearing-matched colour was significantly reduced after five days of white-light recovery. In contrast, neither the main effect of Rearing ($F(3,87) = 0.465$, $p = 0.707$, partial $\eta^2 = 0.016$) nor the Time × Rearing interaction ($F(3,87) = 0.558$, $p = 0.644$, partial $\eta^2 = 0.019$) reached statistical significance, suggesting that the magnitude of recovery did not differ significantly among the four rearing groups. Post hoc Bonferroni-corrected comparisons revealed that the reduction in preference was significant in the blue-reared group ($p = 0.002$), yellow-reared group ($p = 0.047$) and red-reared group ($p = 0.003$), but not significant in the green-reared groups ($p = 0.134$). The results demonstrate that developmental phototactic biases are largely reversible under neutral illumination.

We further examined whether white-light recovery also affected locomotor performance (Fig. 6). Relative to the locomotion levels measured immediately after eclosion (Fig. 4), all groups exhibited reduced locomotion after five days of recovery. However, one-way ANOVA revealed no significant effect of rearing light on activity level ($F(4111) = 0.410$, $p = 0.801$, partial $\eta^2 = 0.015$; Fig. 6A), overall movement speed ($F(4111) = 0.470$, $p = 0.758$, partial $\eta^2 = 0.017$; Fig. 6B), or active speed ($F(4111) = 0.601$, $p = 0.663$, partial $\eta^2 = 0.021$; Fig. 6C). These findings indicate that the locomotor differences observed immediately after development were no longer present following recovery.

4. Discussion

The present study demonstrates that the spectral composition of the light environment spanning the post-mating to eclosion period is associated with reversible changes in adult phototactic behaviour and locomotion in *Drosophila melanogaster*. Flies that developed under colour-filtered illumination exhibited transient, wavelength-specific biases in phototactic preference, whereas flies reared under red light showed a enhancement in overall movement speed. Importantly, after five days of recovery under neutral white light, both altered colour preferences and enhanced locomotion were largely diminished, indicating that these behavioural effects are plastic and reversible. Rather than reflecting permanent modifications of the visual system, our findings suggest that the developmental light environment biases adult behavioural output in a context-dependent and dynamic manner.

The compound eyes of adult fruit flies are most sensitive to short- to mid-wavelength light, with peak spectral sensitivity in the

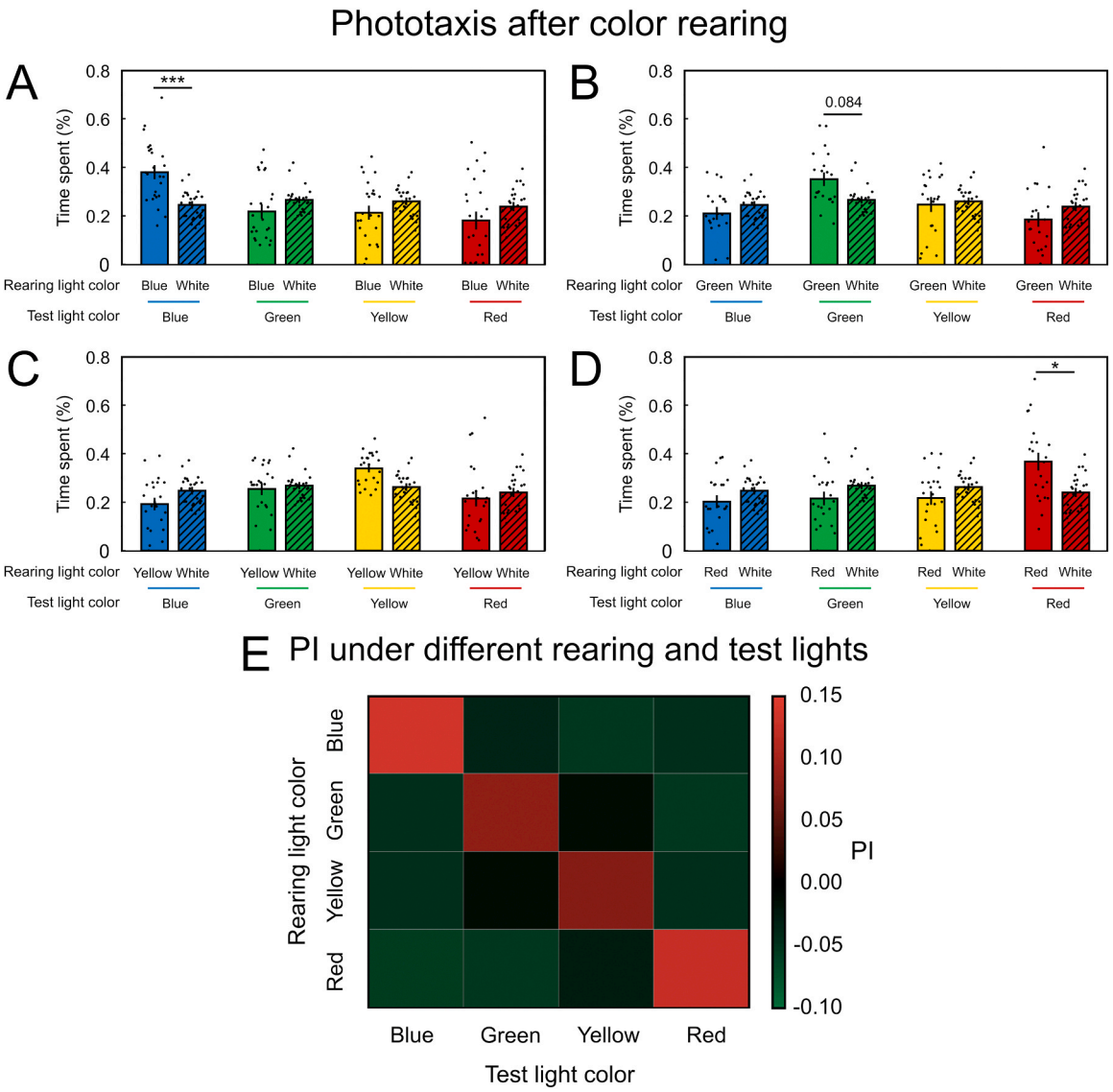

Fig. 3. Phototactic responses after colour rearing during development. (A–D) Phototactic responses of flies reared under blue (A), green (B), yellow (C), and red (D) light, each compared with those reared under white light during development. Bars represent the mean ± *SEM* proportion of time spent (%) in each colour zone during the 120 s test. Data were analyzed using a mixed-design general linear model with test colour as a within-subject factor and rearing light as a between-subject factor, followed by Bonferroni-corrected planned comparisons between each coloured-light-reared group and the white-light control under the corresponding test colour. $^{*}p < 0.05$, $^{***}p < 0.001$, p values are shown directly when $p < 0.1$. (E) Heatmap of the phototaxis index (*PI*) across different rearing and test light combinations, calculated as the difference between the time spent (%) under a given rearing light and that under white-light rearing for the same test colour.

ultraviolet–blue–green range, determined by the opsin composition of photoreceptors R1–R6 and R7/R8 (Hardie, 1979; Sharkey et al., 2020; Warrick et al., 1999). However, fruit flies are a holometabolous insect, and larval photoreception relies on simple light-sensitive organs that are anatomically and developmentally distinct from the adult compound eye, which forms de novo during the pupal stage (Gilbert, 1994; Ready, 2002). Therefore, the behavioural biases observed in this study are unlikely to represent a direct "transfer" of larval visual experience into adult photoreceptor tuning. A more parsimonious interpretation is that the light environment experienced across the post-mating to eclosion period may shape how adult behavioural responses are expressed, particularly during pupal development and early adult stages.

Consistent with this view, the wavelength-specific enhancement of phototaxis observed immediately after eclosion was largely eliminated following five days of exposure to white light. This convergence across groups does not necessarily imply a reversal of photoreceptor-level changes, but rather indicates that behavioural output is dynamically shaped by recent sensory environment. Even if molecular or cellular differences may exist as a result of development, prolonged exposure to a broadband spectrum may be associated with attenuation or modification of earlier behavioural biases, which may reflect the influence of recent sensory experience. This highlights that fruit fly phototaxis is not solely determined by developmental programming but remains subject to modulation by immediate environmental context.

Notably, our results showed that under long-wavelength illumination, specifically red light, developmental exposure selectively enhanced movement speed without increasing overall activity level, indicating a dissociation between locomotor frequency and kinematic output. Importantly, this dissociation was further supported by the absence of a significant increase in active speed, suggesting that flies did not move faster during active bouts, but instead exhibited greater overall displacement. A similar dissociation has been reported under visually deprived conditions, where overall activity does not necessarily increase (Damulewicz et al., 2022), suggesting that reduced visual input alone is insufficient to elevate general arousal. We therefore propose that the increased speed observed under red-light rearing may reflect an

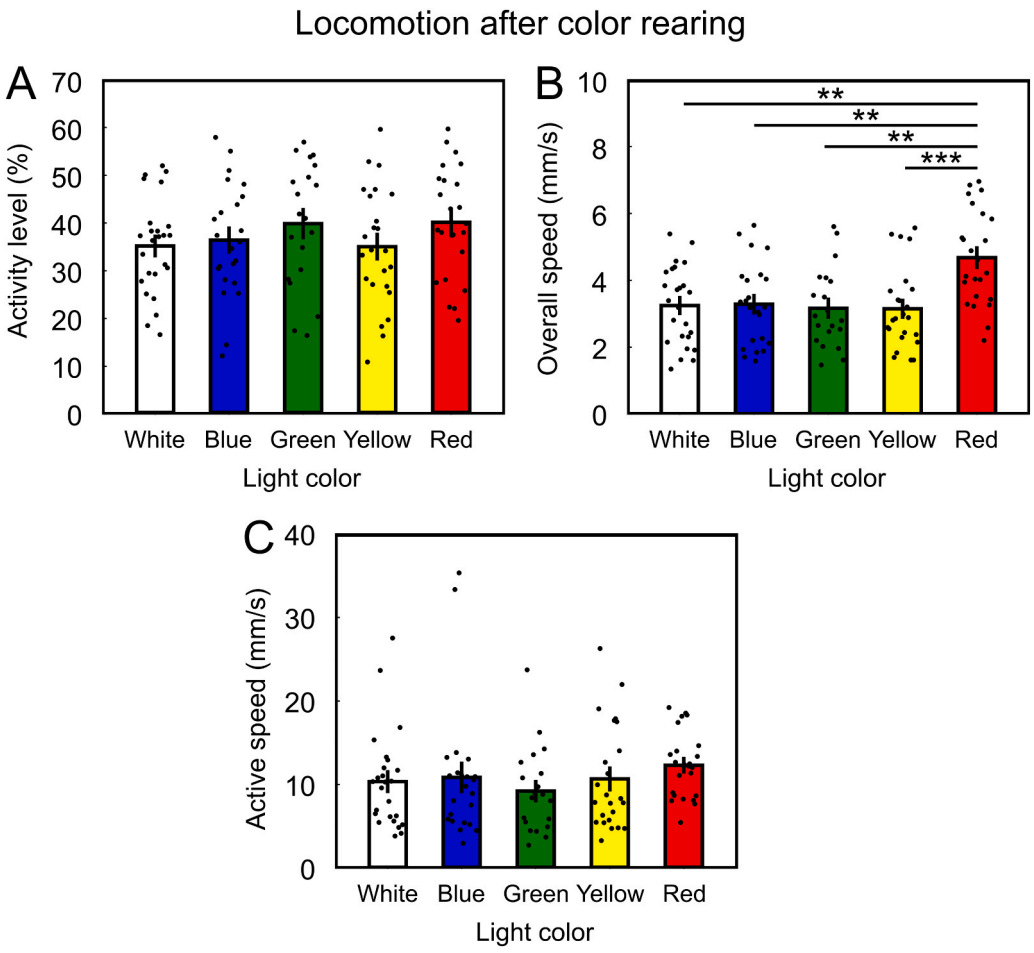

Fig. 4. Locomotion levels after colour rearing during development. (A–C) Activity level, overall movement speed, and active speed of flies reared under different light colours (white, blue, green, yellow, and red) during development. Each bar represents the mean ± *SEM* of the corresponding locomotion parameter. Data were analyzed using one-way ANOVA followed by Bonferroni-corrected post hoc comparisons. $^{**}p < 0.01$, $^{***}p < 0.001$.

adjustment in sensorimotor regulation under chronically low or less informative visual input, rather than a global activation of locomotor drive. Such compensatory modulation of motor output under limited sensory conditions has been described across animal systems, including enhanced reliance on nonvisual feedback for movement control (Kupers and Ptito, 2014; Mureli et al., 2017). Together, these observations suggest that prolonged exposure to low-visibility environments during development may bias sensorimotor integration toward mechanisms that selectively enhance locomotor velocity in adult flies. Thus, one possible interpretation is that prolonged exposure to red light during development may alter how locomotor output is expressed in adulthood, resulting in faster movement without a corresponding increase in general activity. This pattern suggests that developmental light environment can bias specific aspects of motor behaviour rather than inducing a global increase in arousal.

At the same time, we acknowledge that the increased locomotor output observed following transfer from red light to white light may partly reflect a startle-like response or a transient effect of environmental change. However, the absence of a corresponding increase in active speed suggests that this effect does not fully conform to a typical startle-induced increase in locomotor intensity. Future studies should directly quantify startle responses under controlled light-transition conditions (e.g., using high-temporal-resolution tracking or defined light-on paradigms) to disentangle transient arousal effects from longer-term developmental modulation of locomotor behaviour.

It is also important to recognize that in *Drosophila melanogaster*, light information is not transmitted solely through classical retinal photoreceptors; non-classical photic inputs, including extra-retinal photoreceptors and cryptochrome-dependent signaling, have been shown to contribute to light-driven behavioural modulation (Helfrich-Förster et al., 2001; Schlichting et al., 2019). In the present study, we did not directly assess neural activity or circuit-level changes, and therefore cannot provide direct evidence for modifications in visual system circuitry. Although the present study did not directly investigate neural pathways, such alternative sources of photic input may be consistent with the behavioural differences observed under red-light rearing conditions, rather than demonstrating specific neural mechanisms. Other indirect influences of red light, such as effects on circadian entrainment, physiological state, or hormonal signaling, may also contribute to this outcome. Future studies combining behavioural assays with neurophysiological or imaging approaches will be required to determine whether and how developmental light conditions influence underlying neural processing. Taken together, these considerations highlight that locomotor speed and activity frequency can be differentially shaped by developmental conditions and that multiple sensory pathways may be involved in translating environmental light history into adult behaviour.

Building on the possibility of non-classical photic pathways discussed above, an alternative explanation for the observed behavioural changes in phototaxis and locomotion is that they may reflect changes in light-dependent entrainment or physiological state mediated by extra-ocular photoreception, rather than modifications of visual processing per se. Previous studies have shown that prolonged exposure to altered light environments, including extended darkness, does not necessarily

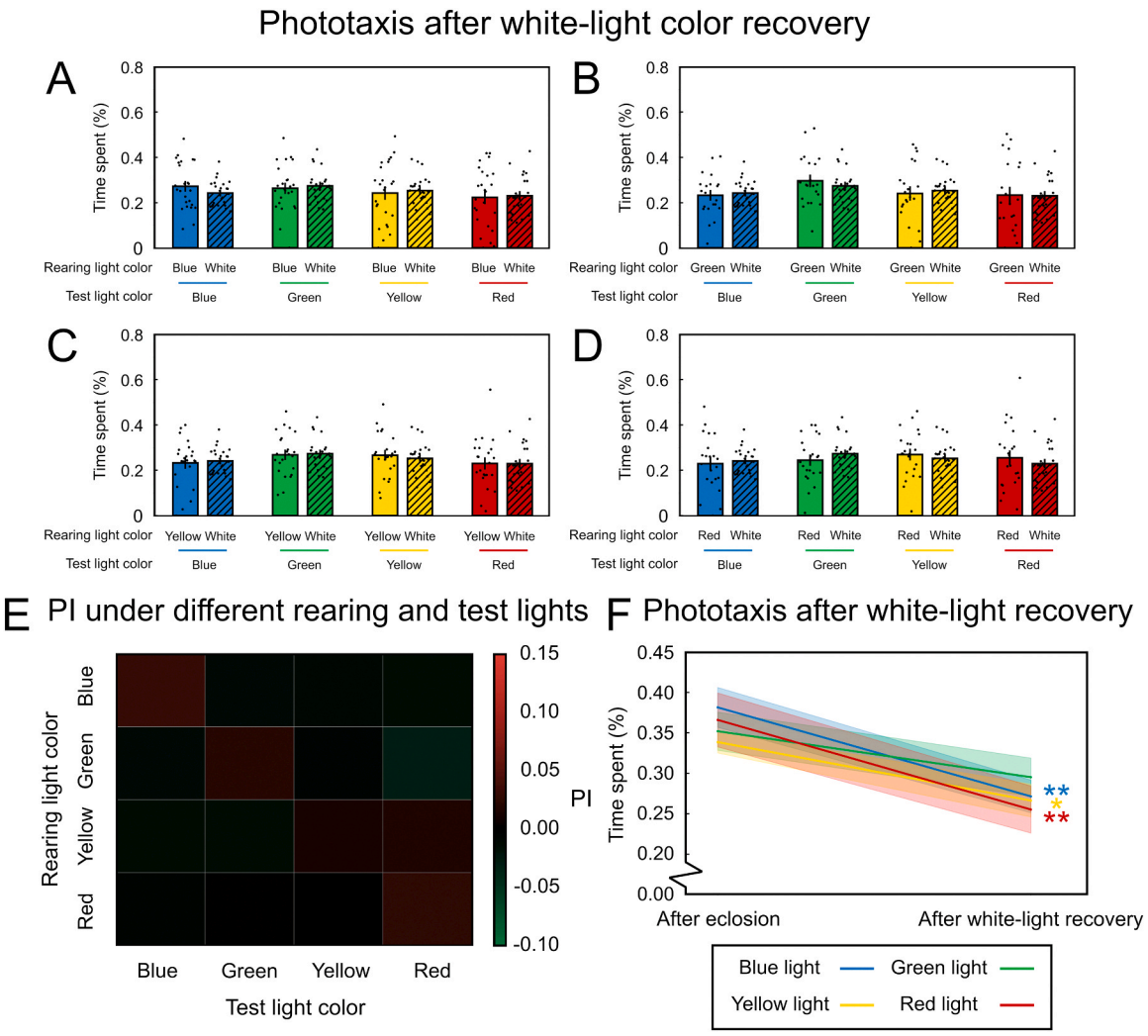

Fig. 5. Phototactic responses after 5 days of white-light recovery. (A–D) Phototactic responses of flies after 5 days of white-light recovery, shown in the same format as Fig. 3. Each bar represents the mean ± *SEM* proportion of time spent (%) in each colour zone during the 120 s test. Data were analyzed using a mixed-design general linear model followed by Bonferroni-corrected post hoc comparisons between each coloured-light–reared group and the white-light control under the corresponding test colour. (E) Heatmap of the phototaxis index (*PI*) across rearing and test light combinations after recovery. (F) Changes in phototactic preference toward the corresponding rearing colour before and after recovery. Each line represents flies reared under a specific colour during development. Data were analyzed using a mixed-design general linear model with Bonferroni correction. $^{*}p < 0.05$, $^{**}p < 0.01$.

alter spectral sensitivity of the visual system (Damulewicz et al., 2022), suggesting that behavioural differences may arise from non-retinal pathways or systemic regulation rather than changes in photoreceptor tuning. In this context, extra-ocular photoreceptors and cryptochrome-dependent pathways, which are known to contribute to circadian entrainment and light-dependent behavioural modulation (Helfrich-Förster et al., 2001; Schlichting et al., 2019), may play a role in shaping the observed responses. Importantly, the present study did not explicitly distinguish between visual and non-visual photic contributions, and therefore cannot determine whether the behavioural effects arise from changes in visual processing, extra-ocular light sensing, or their interaction. Future studies combining developmental and adult-stage light manipulations with genetic approaches (e.g., visual system mutants or cryptochrome-deficient lines) and temporally controlled light exposure paradigms will be necessary to disentangle these mechanisms.

Despite these findings, several limitations should be acknowledged. First, the present study was designed to examine the effects of continuous developmental light exposure across the post-mating to eclosion period, rather than to disentangle developmental and adult-stage contributions through separate exposure paradigms. Accordingly, adult-only exposure control groups were not included in the current experimental design, and thus we cannot fully exclude the possibility that part of the observed behavioural differences reflects contributions from adult light history in addition to developmental modulation. Second, we did not directly assess photoreceptor physiology or neural activity using electrophysiological recordings or calcium imaging. Consequently, our conclusions are restricted to the behavioural level and do not demonstrate changes in photoreceptor sensitivity or visual circuit function per se. Future studies combining developmental and adult-only light exposure paradigms with neural and molecular approaches will be essential to disentangle developmental versus immediate effects of light on sensory–motor behaviour.

In conclusion, this study provides evidence that the spectral composition of the developmental light environment can transiently bias visual preference and selectively enhance locomotor speed in *Drosophila melanogaster*, with red light exerting a uniquely strong effect on motor output. These changes are reversible upon re-exposure to neutral white light, underscoring the behavioural plasticity of flies in response to environmental illumination. Together, our findings offer a behavioural framework for future investigations into how specific wavelengths interact with sensory, motor, and circadian systems to

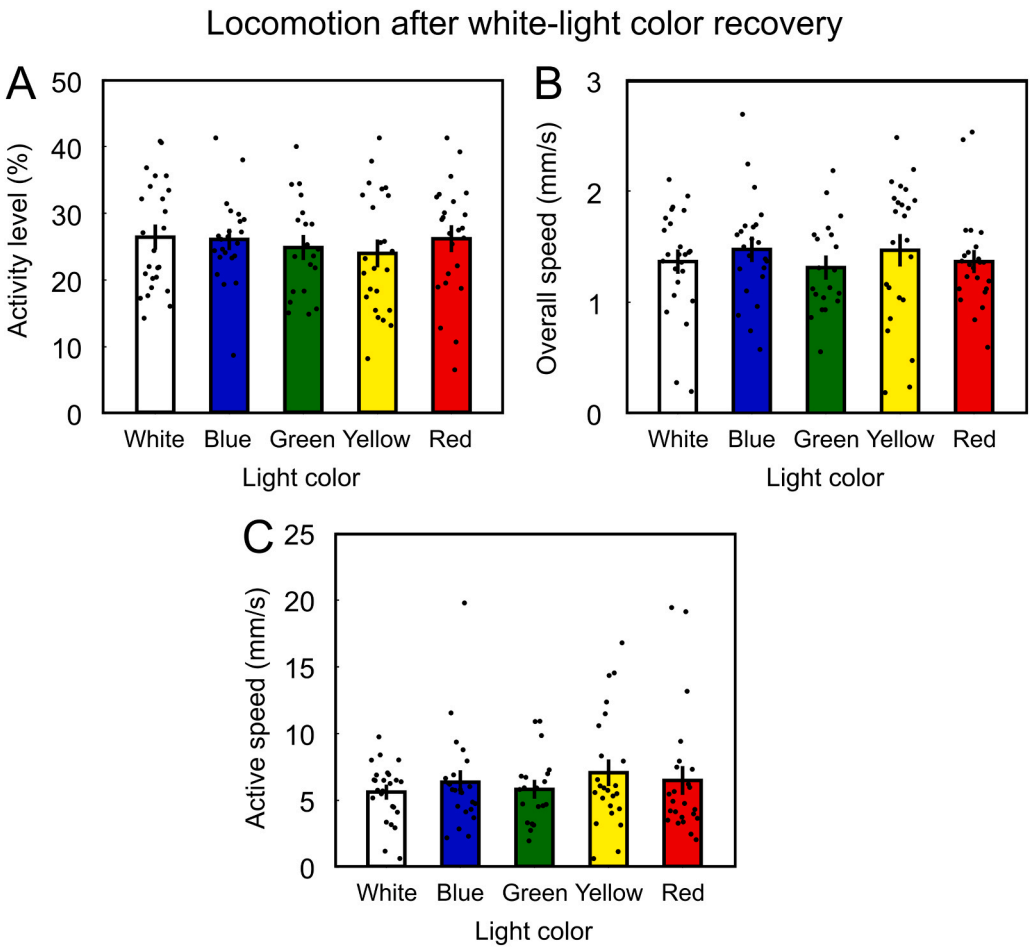

Fig. 6. Locomotion levels after 5 days of white-light recovery.(A–C) Activity level, overall movement speed, and active speed of flies after 5 days of white-light recovery. Each bar represents the mean ± *SEM* of the corresponding locomotion parameter. Data were analysed using one-way ANOVA followed by Bonferroni-corrected post hoc comparisons.

shape adaptive behaviour across developmental timescales.

CRediT authorship contribution statement

Xin-Hui Chen: Investigation. **Rui Han:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Zi-Yu Guo:** Resources, Methodology, Investigation, Data curation. **Jun Zhang:** Supervision, Project administration, Investigation, Conceptualization. **Sheng-Yu Hou:** Resources, Investigation. **Xu-Hui Guo:** Resources, Investigation. **Ke-Jing Feng:** Resources, Investigation. **Han-Ning Wu:** Resources, Investigation. **Yu-Xin He:** Resources, Investigation.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 32500921); the Fujian Provincial Natural Science Foundation of China (Grant No. 2024J08018); the Zhangzhou Natural Science Foundation Project (Grant No. ZZ2024J07); and the Young and Middle-aged Teachers' Education and Scientific Research Projects in Fujian Province (Science and Technology Category) (Grant No. JAT220508).

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

We are grateful to all the lab members for their suggestions and collaboration in this study. We also thank the National Demonstration Center for Experimental Life Sciences Education (Xiamen University) for providing the experimental environment and fruit fly.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.beproc.2026.105383.

Data availability

Data used for this study are provided as supplementary material (Data Table S1).

References

Bellen, H.J., Tong, C., Tsuda, H., 2010. 100 years of *Drosophila* research and its impact on vertebrate neuroscience: a history lesson for the future. Nat. Rev. Neurosci. 11, 514–522. https://doi.org/10.1038/nrn2839.

Blass, G.R.C., Gaffin, D.D., 2008. Light wavelength biases of scorpions. Anim. Behav. 76, 365–373. https://doi.org/10.1016/j.anbehav.2007.12.022.

Bradshaw, W.E., Holzapfel, C.M., 2007. Evolution of animal photoperiodism. Annu. Rev. Ecol. Evol. Syst. 38, 1–25. https://doi.org/10.1146/annurev.ecolsys.37.091305.110115.

Brembs, B., 2016. Genetic analysis of behavior in *Drosophila*. https://doi.org/10.7287/peerj.preprints.2491v1.

Collett, T.S., Collett, M., 2000. Path integration in insects. Curr. Opin. Neurobiol. 10, 757–762. https://doi.org/10.1016/S0959-4388(00)00150-1.
Damulewicz, M., Tyszka, A., Pyza, E., 2022. Light exposure during development affects physiology of adults in *Drosophila melanogaster*. Front. Physiol. 13. https://doi.org/10.3389/fphys.2022.1008154.
Donlea, J.M., Thimgan, M.S., Suzuki, Y., Gottschalk, L., Shaw, P.J., 2011. Inducing sleep by remote control facilitates memory consolidation in *Drosophila*. Science. https://doi.org/10.1126/science.1202249.
Dubowy, C., Sehgal, A., 2017. Circadian rhythms and sleep in *Drosophila melanogaster*. Genetics 205, 1373–1397. https://doi.org/10.1534/genetics.115.185157.
Gilbert, C., 1994. Form and function of stemmata in larvae of holometabolous insects, 39.
Gorostiza, E.A., Colomb, J., Brembs, B., 2016. A decision underlies phototaxis in an insect. Open Biol. 6, 160229. https://doi.org/10.1098/rsob.160229.
Götz, K.G., 1964. Optomotorische Untersuchung des visuellen systems einiger Augenmutanten der Fruchtfliege *Drosophila*. Kybernetik 2, 77–92. https://doi.org/10.1007/BF00288561.
Han, R., Huang, H.-P., Chuang, C.-L., Yen, H.-H., Kao, W.-T., Chang, H.-Y., Lo, C.-C., 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., Tan, Y.-H., Lo, C.-C., 2024a. Attractiveness Versus Stickiness: Behavioural Preferences of *Drosophila melanogaster* with Competing Visual Stimuli.
Han, R., Zhang, J., Chen, X.-H., Duo, J.-W., Li, Y.-Y., Chen, Y.-R., Chen, S.-T., Liu, L.-X., Lin, X.-H., 2026. Sex differences in locomotor recovery across mating contexts in *Drosophila melanogaster*. Behav. Process. 235, 105324. https://doi.org/10.1016/j.beproc.2025.105324.
Han, R., Zhang, J., Huang, G.-X., Yuan, R.-X., Lian, Y.-S., Zhao, M.-Y., Lu, Y.-Y., Huang, H., Wang, Y.-C., Chen, Y.-J., Lo, C.-C., Tan, Y.-H., 2025a. The effects of blue light on locomotion and cognition in early adult *Drosophila melanogaster*. J. Exp. Zool. A Ecol. Integr. Physiol. 343, 511–520. https://doi.org/10.1002/jez.2900.
Han, R., Zhang, J., Wang, R.-X., Xu, J.-X., Weng, K.-C., Wang, A.-X., Huang, H., Lu, Y.-Y., Chen, Y.-J., Wang, Y.-C., 2024b. The impact of different mating systems on locomotion in *Drosophila melanogaster*. J. Insect Behav. 38, 2. https://doi.org/10.1007/s10905-024-09866-7.
Han, R., Zhang, J., Xu, Y.-H., Xiao, Z.-X., Xie, M.-L., Xin, H.-R., 2025b. Differential spectral sensitivity influences mating, development, and reproduction in group-housed *Drosophila melanogaster*. J. Exp. Zool. Part A Ecol. Integr. Physiol. 343, 1231–1243. https://doi.org/10.1002/jez.70037.
Hardie, R.C., 1979. Electrophysiological analysis of fly retina. I: comparative properties of R1-6 and R 7 and 8. J. Comp. Physiol. 129, 19–33. https://doi.org/10.1007/BF00679908.
Helfrich-Förster, C., 2020. Light input pathways to the circadian clock of insects with an emphasis on the fruit fly *Drosophila melanogaster*. J. Comp. Physiol. A 206, 259–272. https://doi.org/10.1007/s00359-019-01379-5.
Helfrich-Förster, C., Winter, C., Hofbauer, A., Hall, J.C., Stanewsky, R., 2001. The circadian clock of fruit flies is blind after elimination of all known photoreceptors. Neuron 30, 249–261. https://doi.org/10.1016/S0896-6273(01)00277-X.
Ho, M., Mawji, N., Meng, S.W., Ng, D.T., Smyrnis, N.E., 2013. The effect of exposure to varying light wavelengths during development on locomotor speed of adult *Drosophila melanogaster*. Expedition 3.
Hofstetter, J.R., Hofstetter, A.R., Hughes, A.M., Mayeda, A.R., 2005. Intermittent long-wavelength red light increases the period of daily locomotor activity in mice. J. Circad Rhythms 3, 8. https://doi.org/10.1186/1740-3391-3-8.
Hu, K.G., Stark, W.S., 1980. The roles of *Drosophila* ocelli and compound eyes in phototaxis. J. Comp. Physiol. 135, 85–95. https://doi.org/10.1007/BF00660183.
Humberg, T.-H., Sprecher, S.G., 2017. Age- and wavelength-dependency of *Drosophila* Larval phototaxis and behavioral responses to natural lighting conditions. Front. Behav. Neurosci. 11. https://doi.org/10.3389/fnbeh.2017.00066.
Jacob, K.G., Willmund, R., Folkers, E., Fischbach, K.F., Spatz, H.Ch, 1977. T-maze phototaxis of *Drosophila melanogaster* and several mutants in the visual systems. J. Comp. Physiol. 116, 209–225. https://doi.org/10.1007/BF00605403.
Kane, E.A., Gershow, M., Afonso, B., Larderet, I., Klein, M., Carter, A.R., de Bivort, B.L., Sprecher, S.G., Samuel, A.D.T., 2013. Sensorimotor structure of *Drosophila* larva phototaxis. Proc. Natl. Acad. Sci. 110, E3868–E3877. https://doi.org/10.1073/pnas.1215295110.
Keene, A.C., Sprecher, S.G., 2012. Seeing the light: photobehavior in fruit fly larvae. Trends Neurosci. 35, 104–110. https://doi.org/10.1016/j.tins.2011.11.003.
Kupers, R., Ptito, M., 2014. Compensatory plasticity and cross-modal reorganization following early visual deprivation. Neuroscience & Biobehavioral Reviews. Multisens. Integr. Sens. Substit. Vis. Rehabil. 41, 36–52. https://doi.org/10.1016/j.neubiorev.2013.08.001.
Lu, B., Liu, W., Guo, F., Guo, A., 2008. Circadian modulation of light-induced locomotion responses in *Drosophila melanogaster*. Genes Brain Behav. 7, 730–739. https://doi.org/10.1111/j.1601-183X.2008.00411.x.
Mascalzoni, E., Regolin, L., 2011. Animal visual perception. Wiley Interdisciplinary Reviews Cognitive Science 2, 106–116. https://doi.org/10.1002/wcs.97.
Mureli, S., Thanigaivelan, I., Schaffer, M.L., Fox, J.L., 2017. Cross-modal influence of mechanosensory input on gaze responses to visual motion in *Drosophila*. J. Exp. Biol. 220 22182227. https://doi.org/10.1242/jeb.146282.
Paskin, T.R., Jellies, J., Bacher, J., Beane, W.S., 2014. Planarian phototactic assay reveals differential behavioral responses based on wavelength. PLOS ONE 9, e114708. https://doi.org/10.1371/journal.pone.0114708.
Pauers, M.J., Kuchenbecker, J.A., Neitz, M., Neitz, J., 2012. Changes in the colour of light cue circadian activity. Anim. Behav. 83, 1143–1151. https://doi.org/10.1016/j.anbehav.2012.01.035.
Ready, D.F., 2002. Drosophila compound eye morphogenesis: blind mechanical engineers? In: Moses, K. (Ed.), Drosophila Eye Development. Springer, Berlin, Heidelberg, pp. 191–204. https://doi.org/10.1007/978-3-540-45398-7_12.
Rieger, D., Stanewsky, R., Helfrich-Förster, C., 2003. Cryptochrome, compound eyes, hofbauer-buchner eyelets, and ocelli play different roles in the entrainment and masking pathway of the locomotor activity rhythm in the fruit fly *Drosophila melanogaster*. J. Biol. Rhythms 18, 377–391. https://doi.org/10.1177/0748730403256997.
Salcedo, E., Huber, A., Henrich, S., Chadwell, L.V., Chou, W.-H., Paulsen, R., Britt, S.G., 1999. Blue- and Green-Absorbing Visual Pigments of *Drosophila*: ectopic expression and physiological characterization of the R8 photoreceptor cell-specific Rh5 and Rh6 Rhodopsins. J. Neurosci. 19, 10716–10726. https://doi.org/10.1523/JNEUROSCI.19-24-10716.1999.
Schlichting, M., Weidner, P., Diaz, M., Menegazzi, P., Benetta, E.D., Helfrich-Förster, C., Rosbash, M., 2019. Light-mediated circuit switching in the *Drosophila* neuronal clock network. Curr. Biol. 29, 3266–3276.e3. https://doi.org/10.1016/j.cub.2019.08.033.
Sharkey, C.R., Blanco, J., Leibowitz, M.M., Pinto-Benito, D., Wardill, T.J., 2020. The spectral sensitivity of *Drosophila* photoreceptors. Sci. Rep. 10, 18242. https://doi.org/10.1038/s41598-020-74742-1.
Shaw, H.S., Larkin, J., Rao, Y., 2020. Phototactic T-maze behavioral assay for comparing the functionality of color-sensitive photoreceptor subtypes in the *Drosophila* Visual System. BioProtocol 10. https://doi.org/10.21769/BioProtoc.3558.
Stringer, I.A.N., Meyer-Rochow, V.B., 1997. Flight activity of Insects within a Jamaican Cave: in Search of the Zeitgeber. Invertebr. Biol. 116, 348–354. https://doi.org/10.2307/3226867.
Warrant, E.J., Johnsen, S., 2013. Vision and the light environment. Curr. Biol. 23, R990–R994. https://doi.org/10.1016/j.cub.2013.10.019.
Warrick, J.M., Vakil, M.F., Tompkins, L., 1999. Spectral sensitivity of wild-type and mutant *Drosophila melanogaster* Larvae. J. Neurogenet. https://doi.org/10.3109/01677069909083471.
Willmund, R., 1979. Light induced modification of phototactic behaviour of *Drosophila melanogaster*. J. Comp. Physiol. 129, 35–41. https://doi.org/10.1007/BF00679909.
Yamaguchi, S., Desplan, C., Heisenberg, M., 2010. Contribution of photoreceptor subtypes to spectral wavelength preference in *Drosophila*. Proc. Natl. Acad. Sci. 107, 5634–5639. https://doi.org/10.1073/pnas.0809398107.