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Light-induced disruptions elevate norepinephrine and impair cognition in honey bees

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With 4 figures

Abstract: Light exposure is a primary cue that shapes circadian rhythms in many organisms, yet the harmful effects of artificial lighting are becoming increasingly apparent. We examined the impact of low-intensity illumination (2.3 µmol/s/m²) on young honey bees (*Apis mellifera*) and found that continuous light significantly increased mortality and impaired olfactory learning and memory. Although norepinephrine (NE) levels peaked at midnight under all conditions – normal light/dark, continuous darkness, and continuous light – bees exposed to constant light had consistently higher NE concentrations. Elevating NE levels in bees maintained under a normal light cycle produced similar deficits in survival and cognition, reinforcing the link between NE and light-induced stress. These results provide the first evidence that NE, like octopamine, contributes to stress responses and cognitive impairment in bees and underscore the importance of understanding how altered lighting environments influence insect physiology and behavior.

Keywords: Circadian rhythms; artificial illumination; olfactory learning; foraging efficiency; biogenic amines; *Apis mellifera*

1 Introduction

Honey bees served as some of the earliest insect models in chronobiology. Researchers in the early twentieth century highlighted the biological importance of clock-regulated behavior (Beer & Helfrich-Förster 2020a; Kleber 1935; Galizia et al. 2011), including how circadian rhythms relate to social environment, behavioral plasticity, and task-related activities (Abreu et al. 2018). This study addresses the gap in knowledge on how light exposure influences biogenic amine metabolism, survival, and foraging ability in bees. Previous work suggests that worker bees develop circadian rhythms after embryonic stages (Eban Rothschild & Bloch 2012; Moore 2001). This indicates that *Apis mellifera* does not display circadian rhythmicity at emergence and that its neural clock continues maturing after emergence (Beer & Helfrich-Förster 2020b). Young bees care for newly emerged individuals and often move both day and night without circadian patterns (Bloch 2010; Eban Rothschild & Bloch 2012; Moore 2001). Stable circadian locomotor activity arises during the first two weeks of adulthood, though this varies widely among individuals and social groups (Bloch & Meshi 2007; Bloch et al. 2002; Toma et al. 2000).

Honey bee larvae as young as three days old may exhibit sleep behavior and possess three adult sleep stages (Eban Rothschild & Bloch 2008). Evidence suggests that the social environment influences circadian rhythms in bees. Younger bees that lived with older foragers developed approximately 24-hour locomotor activity rhythms at earlier ages and showed stronger circadian patterns than those reared exclusively with age-matched peers (Meshi & Bloch 2007). Moreover, in the absence of older foragers, younger bees began foraging sooner (Dong et al. 2023b). Light cycles also affect honey bee sleep. The daily foraging activities of *Apis dorsata* are coordinated by lunar and seasonal cues (Young et al. 2021). In *Apis cerana*, worker bees exhibit diurnal rhythms under both constant light and constant dark conditions, with free-running periods exceeding 24 hours in constant light and falling below 24 hours in constant darkness. These observations underscore that manipulating light cycles powerfully alters diurnal activity patterns (Shimizu et al. 2001). Recently, Kim et al. (2024) reported that *A. mel-*

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lifera foragers exposed to constant light for 79 hours slept less and may have experienced more stress, as evidenced by more frequent sleep disruptions caused by nestmates and a marked preference for darker resting areas compared to bees kept in constant darkness.

Disrupted light exposure substantially impacts reproduction, physiology, and circadian rhythms in *A. mellifera* and other organisms. Continuous light severely reduces reproduction in *A. mellifera*, whereas a 12:12-hour light cycle preserves queen fecundity at levels similar to long-term darkness, with egg-laying peaking during illuminated periods (Shpigler et al. 2022). Reduced light exposure heightens lipid and protein stores in honey bees, approximating a winter physiology (Fluri & Bogdanov 1987). Light exposure combined with thiamethoxam synergistically damages circadian locomotor rhythms in bees (Tackenberg 2019). Similar patterns are found in other taxa. Varying light intensities during feeding disturbs life cycles and lowers survival rates in male *Spodoptera frugiperda* and *S. litura* larvae (Xu et al. 2024). Artificial nighttime illumination also shortens moth feeding durations, contributing to population declines and impaired pollination services (van Langevelde et al. 2017).

Norepinephrine and its invertebrate equivalent, octopamine, are stress-related neurotransmitters found across Arthropoda, Mollusca, and Chordata (Adamo 2008). In humans, noradrenaline is essential for cognition, including attention, memory, and executive function (Holland et al. 2021). In mice, hindering the norepinephrine transporter disrupts fear conditioning (Pantoni et al. 2020). Although the role of noradrenaline in insect learning or stress is presently undocumented, its invertebrate counterpart, octopamine, is well studied, and, relevantly, is involved in insect stress responses (Mezheritskiy et al. 2024), and can influence memory retention in *A. mellifera* exposed to toxins (Khooshe-Bast et al. 2024).

We hypothesized that shifts in the early-life light environment affect honey bee metabolism, development, survival, learning and memory. This study aims to address the knowledge gap on how alterations in light cycles, capable of modifying circadian rhythms, impact biogenic amine metabolism, and the survival and foraging ability of bees. We evaluated the effects of bright, continuous artificial light relative to a 14:10-hour light/dark cycle (LD) on olfactory learning and memory after seven days of exposure. We further measured biogenic amine levels, focusing on brain NE, and tested whether increasing NE levels under normal LD conditions would compromise olfactory learning.

2 Materials and methods

2.1 Honey bee colonies and light treatments

We used three colonies of *Apis mellifera* that were kept in the experimental apiary of the Kunming Southwest Biodiversity Laboratory in Langstroth wooden boxes. All colonies were healthy, as determined by standard inspection practices (Imdorf et al. 1987). Each colony had approximately 12,000 bees (determined by Lieberfelder's estimate) and six frames, of which two were filled with honey and pollen at approximately the same levels (Dainat et al. 2020). In all experiments, we used matched treatments so that the control and experimental treatments used bees from the same colonies and, therefore, with similar genetic backgrounds.

To obtain marked bees of known age, the queen was confined to an empty brood comb for one day, during which she laid eggs in the empty cells. After her release, the eggs developed under the care of nurse bees. One day before the emergence of adult bees, we placed this comb in an incubator (34 °C and 70% relative humidity, RH). We gently and individually marked all bees, each cohort with a different color, upon their emergence with paint (Edding 750) on their thoraces. These marked bees were then reintroduced into the colonies.

The division of labor between bees is closely related to age, and bees transition from tasks as nurse bees to middle-aged bees that can begin working outside the nest at 12 days of age (Robinson et al. 1992; Johnson 2008; Rueppell et al. 2007). Therefore, this work transition takes the bees from the dark nest to the bright outside environment. We used bees when they were 8, 9 and 14 days old to encompass this transition. Although younger bees (< 12 days of age) tend to have poorer olfactory learning than older bees, we compared the effects of our light treatment with bees of the same age at each age point.

When the bees reached 7 days of age, we transferred 900 marked bees (300 per colony) into nine transparent plastic boxes of a size (14 cm × 12 cm × 5 cm) that provided sufficient space to prevent overcrowding. These boxes have 50 holes drilled on three sides, each with a diameter of 2 mm (150 holes in total), to provide adequate ventilation. (Fig. S1). We provided a 30% sucrose solution (w/v) through three feeders inserted into the bee box (two on the sides and one on the lid, Fig. S1). Each feeder consisted of a 2.5 ml microcentrifuge tube drilled with two 1 mm diameter holes through which 2 ml of the sugar solution was dispersed.

We kept the bees in two separate incubators: one with a normal 14:10 light/dark (LD) cycle at the time of year at which we conducted this study and one with continuous artificial lighting (constant light) at 34 °C and 70% relative humidity (RH) (Shi et al. 2020). LD bees received a light cycle of 14 h light (2.3 µmol/s/m^2) followed by 10 h of dark which followed the sunrise and sunset times at that time. The constant light treatment group was continuously illuminated with fluorescent lights at a level chosen to match the mean level of outdoor light at the colony entrances (2.3 µmol/s/m^2).

This light level is fairly low compared to natural average light intensities throughout the day. However, *A. mellifera* is a cavity nester and workers are therefore normally not exposed to high daylight levels. Bees inside the nest could

be exposed to light penetrating the entrance, which would be reduced compared to full sunlight. However, there are two contexts in which bee workers are exposed to higher light levels. The first context is swarming, a natural phase of colony reproduction in which the swarm is temporarily outside while it seeks a new nest site (Winston 1991). Swarms choose different locations but can often be observed in trees or bushes where they are shaded and protected by leaves and thus experience lower light levels. Secondly, when colony temperatures increase, bees can cluster outside the nest, forming a "bee beard" to reduce unwanted heat production in the nest (Stabentheiner et al. 2021). In the tree cavities that are the preferred natural nest sites of *A. mellifera* (Winston 1991), workers in bee beards would likely also be shaded by leaves and branches. We, therefore, used a fairly low light level corresponding to what bees would experience just at their hive entrances.

We measured light levels with the same photometer placed inside the bee box (Fig. S1). In the illuminated incubator (Intelligent incubator, PRX-250B, Ningbo Safe Experimental Instrument Co., Ltd.), the mirrored sides evenly distributed the light (color temperature is 6500 K, Fig. S1). Bees experienced these two different treatments for 1, 2, or 7 days.

2.2 Exp. 1: Testing learning and memory

To test whether constant light would harm bee cognitive abilities, we measured bee olfactory learning and memory with classical olfactory conditioning via the Proboscis Extension Reflex (PER) assay. The bees were harvested in the morning when they were 8, 9, and 14 days (corresponding to their exposure to light treatments in the incubator for 1, 2, or 7 days). We used 30 bees per colony and performed three replicates per treatment type in each of the four exposure durations (1, 2, or 7 days of exposure).

We harnessed the bees using chill anesthesia (−20 °C for 5 min) to reduce their movements and then placed each bee in a 0.6 ml centrifuge tube with its tip cut off so that only the head and proboscis were exposed and moved freely. Each bee was then fed 10 µL of pure 30% sucrose solution (w/v). We then waited for 2 h and tapped the bees on the antennae with a wood toothpick dipped in this sucrose solution to test their response (day 1 $_{response}$ = 74.4%, day 2 $_{response}$ = 73.3% and day 7 $_{response}$ = 74.4%).

All learning tests began at 8 am on each test day. We placed each bee in the airflow of the apparatus, which was clean and humidified at 90% HR, for 10 s for the bee to acclimate and then added hexane as reward odor (purity: 98%, CAS: 66-25-1, Aladdin Reagent Database Inc. Shanghai, China) dissolved in n-Hexane (GC = 98.0%, CAS# 110-54-3, Tianjin Fengchuan Chemical Reagent Co., Ltd) at (1:10 v/v) for 6 s. In the first 3 s, we recorded if the bee extended its proboscis. In the next 3 s, the bee received the 30% sucrose solution reward by touching the toothpick to its antennae and then immediately moving the toothpick to its proboscis while it continued to experience the odor. We conducted six learning trials with an intertrial interval of 10 min (Brandes et al. 1990). We then tested bee memory 2 h, 6 h, and 12 h after the last learning trial. In these memory tests, we recorded whether the bee extended its proboscis within 3 s of being exposed to the rewarded odor and if the bees were not rewarded with sucrose solution.

2.3 Exp. 2: Measurement of head biogenic amino acid levels

We next investigated the impact of light cycle disturbances on the levels of three biogenic amines in honey bee brains: octopamine, dopamine, and norepinephrine (*NE*). We conducted preliminary measurements with octopamine and dopamine (see Results), but because only found significant effects of light treatment on *NE*, we only measured *NE* for the remainder of our study.

The treatments consisted of a normal 14:10 h light/dark cycle (LD), continuous darkness, and continuous light (CL). To address the largely unknown effects of prolonged light exposure on circadian rhythms and noradrenaline levels, we exposed bees to continuous light for 7 days and then sampled individuals at ages 8, 9, and 14 days (corresponding to 1, 2, and 7 days following the week-long treatment) to assess temporal changes in noradrenaline content. At each time point, we sampled 30 bees from each of three different colonies, pooled them into nine groups of 10 individuals, and repeated the sampling three times for each colony. Sampling was conducted at three distinct intervals throughout the day – 10:00 am to 11:00 am (A), 7:00 pm to 8:00 pm (B), and 11:00 pm to 12:00 am (C) – to capture daily fluctuations in NE levels.

All samples were stored at −40 °C until thawed and processed. For each sample, we pooled two bee brains from the same colony in a 1.5 mL microcentrifuge tube containing 200 µL of protein precipitation solution (0.4 mol/L perchloric acid diluent, 2.6 mM Na2S2O5, and 2.7 mM EDTA). We ground the sample with an electric grinder (Tiangen) at 8000 rpm, then added another 200 µL of the protein precipitation solution, vortexed the mixture, and centrifuged it at 4 °C and 13000 rpm for 30 min. We filtered the resulting supernatant through a 0.22 µm needle filter to remove impurities before transferring it into an HPLC sample vial. The chromatographic setup included a Waters 1525 binary HPLC pump (Singapore), an autosampler, a reverse-phase C18 column (4.6 mm × 250 mm, 5 µm particle size) maintained at 39 °C, and a Waters 2489 UV detector (Singapore) set at 264 nm, with signals recorded and processed using Breeze v2.0 software. The mobile phase consisted of 100 mL acetonitrile, 1.7 mM sodium 1-octane sulfonate, 64 mM anhydrous sodium dihydrogen phosphate, and 50 µM EDTA, adjusted to pH 3.0 with citric acid. We filtered this mobile phase through a 0.22 µm membrane and degassed it in an ultrasonicator (SB-800D,

Scientz, 40 kHz) for 30 min. During detection, the mobile phase flow rate was held at 1.0 mL/min. We identified and quantified NE (purity 97%, CAS: 55-27-6 from OKA) using external standards. Before each analytical run, we injected five known concentrations of each amine (0.25, 0.74, 2.22, and 6.67, and 20 μg/mL) and quantified the amine content by comparing sample peak areas to those of the corresponding standards (Dong et al. 2023a).

2.4 Exp. 3: Effects of consuming NE on bee learning and memory

In a separate PER experiment, we examined the effects of elevating NE levels in the bee brain on learning and memory. We used norepinephrine hydrochloride (purity < 97%, CAS: 55-27-6, from OKA, Yunnan Shanming Technology Co., Ltd.). We placed 30 bees in a queen cage (10 cm × 5 cm × 5 cm) with sugar candy and maintained them in an incubator at 23–27 °C and 70% relative humidity, under the same LD conditions described above. The control bees were fed with a 30% sucrose solution, while the treatment group received a 30% sucrose solution supplemented with 100 μg NE/mL. All bees had *ad libitum* access to their respective solutions. After 7 days, we immobilized the bees with cold anesthesia (−20 °C for 5 min) and harnessed them for the PER assay, as described previously. Once harnessed and awake, the experimental bees were given two doses of 20 μL sucrose solution (30% w/v) containing 100 μg NE/mL, 30 minutes apart. Preliminary trials showed that bees maintained under our conditions did not reach satiety after these volumes. Control bees received two identical doses of pure sucrose solution without NE. We allowed 90 minutes after the final feeding before initiating the PER assay.

We also monitored mortality under different treatments. In the light cycle experiments, LD and CL groups were housed in separate climate chambers, but received identical conditions of temperature and humidity, differing only in their light cycles. Each feeding box initially contained 100 bees, and we conducted four replicates. In the NE experiment, bees from the LD sucrose-only group and the LD sucrose-plus-NE group were kept in the same climate chamber and exposed to the same temperature, humidity, and 14:10 h light cycle. The NE treatment group received a sucrose solution with 100 μg NE/mL, while the control received sucrose only. We began with 50 bees per feeding box and conducted nine replicates. We recorded bee mortality in all treatment groups daily, at the same time each morning, for seven consecutive days.

2.5 Statistics

We used the JMP Pro V16.1.0 software for our analyzes. To analyze biogenic amine levels, we ran mixed models (REML algorithm); the light treatment and the number of treatment days (days) and their interaction as fixed effects. In these analyses, colony was a random effect. The NE content at three time points of light exposure was analyzed with one-way ANOVA (JMP Pro V17.0.0). To analyze the effects of light on bee learning, we focused on the sixth and final learning trial and ran a mixed model (REML algorithm) with treatment (LD or constant illumination), duration of treatment (days) and their interaction as fixed effects. Colony was a random effect in this model. To analyze the effects of NE feeding on bee learning, we focused on the sixth and final learning trial and ran a mixed model (REML algorithm) with treatment (NE or control) as a fixed effect. Colony was a random effect in these models. We removed nonsignificant interactions in our final models. To make all corrected pairwise comparisons, we used Tukey Honestly Significant Difference (HSD) tests. We used GraphPad Prism v7 to analyze survival data. Upon publication, all data in this study will be freely available at zenodo.org (DOI 10.5281/zenodo.14373394).

3 Results

3.1 Exp. 1: constant exposure to light reduced olfactory learning

In their sixth and final learning trial, constant light bees had significantly lower learning (64% lower on average for all pooled treatment durations) than LD bees ($F_{1,534}$ = 6.40, P < 0.0001). There was no significant effect of the duration of treatment ($F_{2,534}$ = 1.21, P = 0.30) and no significant interaction of treatment × duration of treatment ($F_{2,532}$ = 1.27, P = 0.28, Fig. 1A). Colony accounted for < 1% of the model variance.

3.2 Constant exposure to light reduced bee memory

Bees exposed to constant light had significantly lower memory (measured in terms of correct PER responses) than LD bees (63% lower memory at the 2 h memory test point for all treatment durations pooled, treatment: $F_{1,256}$ = 136.79, P < 0.0001) (Fig. 1B). Bee memory declined over time when measured at 2 h, 6 h and 12 h (memory time point: $F_{2,1292}$ = 20.27, P < 0.0001, Tukey HSD test, P < 0.05). The duration of treatment was not significant ($F_{2,1524}$ = 2.48, P = 0.08, Fig. 1B). No interactions were significant: duration of treatment × treatment ($F_{2,1599}$ = 0.77, P = 0.46), duration of treatment × memory time point ($F_{2,1281}$ = 0.04, P = 0.97), duration of treatment × memory time point ($F_{4,1281}$ = 0.12, P = 0.98) and duration of treatment × treatment × memory time point ($F_{4,1281}$ = 0.27, P = 0.90). Colony accounted for < 1% of the model variance.

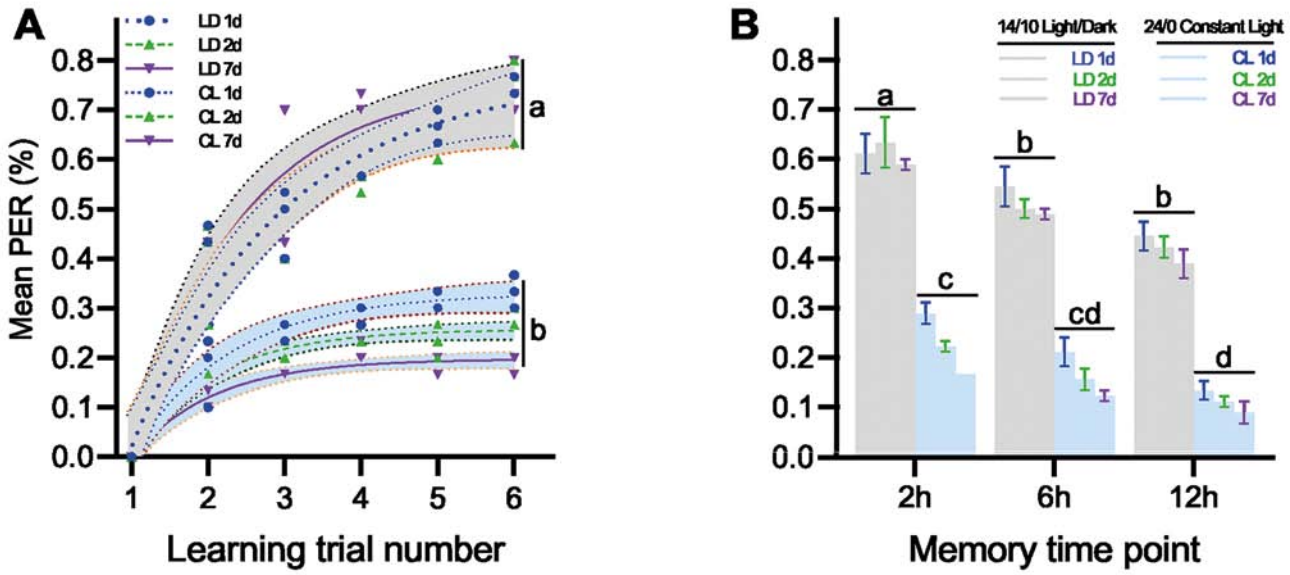

Fig. 1. Constant light harmed the learning and memory of honey bees. There were no significant effects on the duration of treatment. (A) Bees that received 14/10 light/dark (LD) treatment had, as a group, significantly higher learning in their sixth learning trial than constant light (CL) bees. The shaded regions show 95% confidence intervals. (B) Constant light also reduced bee memory (means and 95% confidence intervals are shown). For both graphs, different letters show significant differences (Tukey HSD tests, $P < 0.05$, 1 day $n = 91$ bees, 2 days $n = 91$ bees and 7 days $n = 90$ bees, LD treatment $n = 270$ bees and constant light treatment $n = 270$ bees).

3.3 Exp. 2: no effect of light exposure cycles on octopamine or dopamine levels in honey bee brains

We conducted preliminary analyses of multiple biogenic amines, but found no effect of light treatment (LD or CL) on brain octopamine levels: no significant effects of treatment ($F_{1,30} = 0.25$, $P = 0.62$), duration ($F_{2,30} = 0.78$, $P = 0.47$), or the interaction × duration ($F_{1,30} = 0.75$, $P = 0.39$). Colony accounted for 2% of model variance (sample size: 1 day $n = 4$ bees, 2 days $n = 13$ bees and 7 days $n = 18$ bees, LD treatment $n = 13$ bees and constant light treatment $n = 22$ bees). For dopamine, we focused on just the longest exposure duration (7 days), and also found no significant effect of treatment ($F_{1,14} = 0.57$, $P = 0.46$). Colony accounted for 7% of model variance (sample size: LD treatment $n = 9$ bees and constant light treatment $n = 9$ bees). Given strong preliminary data on showing an effect of treatment on NE (see below), we focused on NE for the remainder of our experiment.

3.4 Effect of light exposure cycles on NE levels in honey bee brains

Disrupting the light cycle increases the relative content of NE in honey bee brains. On the first day, bees exposed to continuous darkness (Tukey HSD test, $P < 0.0107$) and continuous light (Tukey HSD test, $P < 0.0011$) had significantly higher NE levels than bees under a normal light/dark cycle. On the second day, bees exposed to continuous darkness had significantly higher NE levels (Tukey HSD test, $P < 0.0021$) compared to those under normal light conditions. By the seventh day, NE levels in bees under both continuous darkness (Tukey HSD test, $P < 0.0001$) and continuous light (Tukey HSD test, $P < 0.0001$) were significantly higher than those in bees under normal light conditions. Additionally, NE levels in bees exposed to continuous light were higher than those in bees exposed to continuous darkness (Tukey HSD test, $P < 0.0272$).

The NE content of bees sampled in the afternoon did not show significant numerical differences due to their different treatment states. However, continuous light significantly increased the NE levels of bees sampled at midnight. On the second day, bees with continuous illumination had the highest NE content, significantly higher than bees with continuous darkness (Tukey HSD test, $P < 0.0108$). On day 7, continuous light bees showed significantly higher levels of NE than normal bees (Tukey HSD test, $P < 0.0035$), but there was no significant difference compared to continuous darkness bees (Tukey HSD test, $P < 0.3150$).

On the first day, there were no significant differences between bees with continuous darkness and continuous light at different sampling times. For normal bees, NE levels in the afternoon (Tukey HSD test, $P < 0.001$) and at midnight (Tukey HSD test, $P = 0.0002$) were higher than in the morning, but there was no significant difference between the two. There is a significant difference in the NE content between bees in different light treatments (Tukey HSD test, $P = 0.0007$), with normal bees having the lowest NE content. There were significant differences between the sampling time points (Tukey HSD test, $P < 0.0001$). Although there

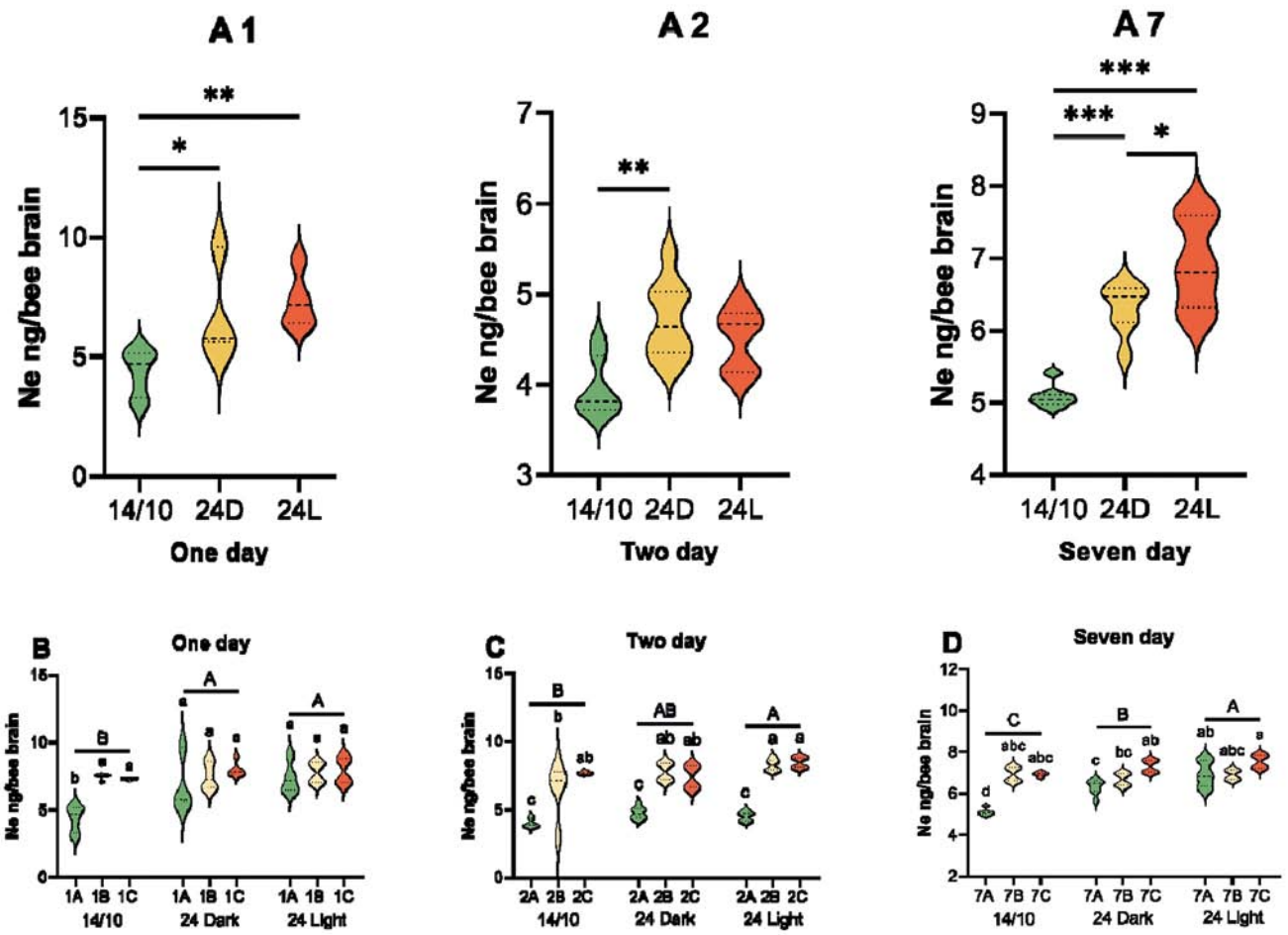

Fig. 2. Persistent light exposure increased brain NE levels. For A1, A2, and A7, the level of NE in the brain of normal bees is shown for a 14/10 light/dark cycle 14/10 ($N_{A1\ 14/10} = 14$, $N_{A2\ 14/10} = 14$, $N_{A7\ 14/10} = 14$), constant dark (24 Dark) ($N_{A1\ 24D} = 14$, $N_{A2\ 24D} = 18$, $N_{A7\ 24D} = 14$) and constant light (24 Light) ($N_{A1\ 24L} = 18$, $N_{A2\ 24L} = 10$, $N_{A7\ 24L = 18)\ on}$ one day, two days, three days, and seven days. Figures B–D show the NE levels in bee brains for one day at three sampling times: 10:00(A), 20:00(B)($N_{1B\ 14/10} = 12$, $N_{1B\ 24D} = 12$, $N_{1B\ 24L} = 12$, $N_{2B\ 14/10} = 10$, $N_{2B\ 24D} = 12$, $N_{2B\ 24L} = 12$, $N_{7B\ 14/10} = 12$, $N_{7B\ 24D} = 12$, $N_{7B\ 24L} = 12$) and 0:00(C) ($N_{1C\ 14/10} = 12$, $N_{1C\ 24D} = 12$, $N_{1C\ 24L} = 12$, $N_{2C\ 14/10} = 12$, $N_{2C\ 24L = 12,}$ $N_{7C\ 14/10 = 12,}$ $N_{7C\ 24L = 12,\ N\ 7C\ 24L = 12}$).

is no significant difference between afternoon and midnight, the average value of midnight is higher. Furthermore, NE at both time points was significantly higher than in the morning (Tukey HSD test, $P = 0.0002$; $P < 0.0001$).

On the second day, there was a significant difference in NE content between bees with different light treatments (Tukey HSD test, $P = 0.0011$), with bees under continuous light significantly higher than under normal conditions. However, there was no significant difference between bees in continuous darkness and bees in continuous light and normal conditions. There were significant differences in NE content at different sampling times (Tukey HSD test, $P < 0.0001$). Normal bees have significantly higher NE levels in the afternoon (Tukey HSD test, $P < 0.0001$) and at midnight (Tukey HSD test, $P < 0.0001$) than in the morning, while continuous darkness bees have significantly higher NE levels in the afternoon (Tukey HSD test, $P < 0.0001$) and at midnight (Tukey HSD test, $P < 0.0001$) than in the morning. Bees with continuous light also have significantly higher levels of NE in the afternoon (Tukey HSD test, $P < 0.0001$) and at midnight (Tukey HSD test, $P < 0.0001$) than in the morning. The trend of variation in the NE content in three different light treatments of bees is consistent.

By day 7, there were significant differences between bees treated with different light conditions (Tukey HSD test, $P < 0.0001$) and continuous light bees had the highest levels of norepinephrine. Furthermore, there were significant differences in NE content between different sampling times (Tukey HSD test, $P < 0.0001$). For normal bees, NE levels in the afternoon (Tukey HSD test, $P < 0.001$) and at midnight (Tukey HSD test, $P < 0.001$) are significantly higher than those in the morning. The NE content of bees in continuous darkness is significantly higher at midnight than at dawn (Tukey HSD test, $P = 0.0002$), but there is no significant difference between the NE content in the afternoon and the two time points. For bees under continuous illumination, there

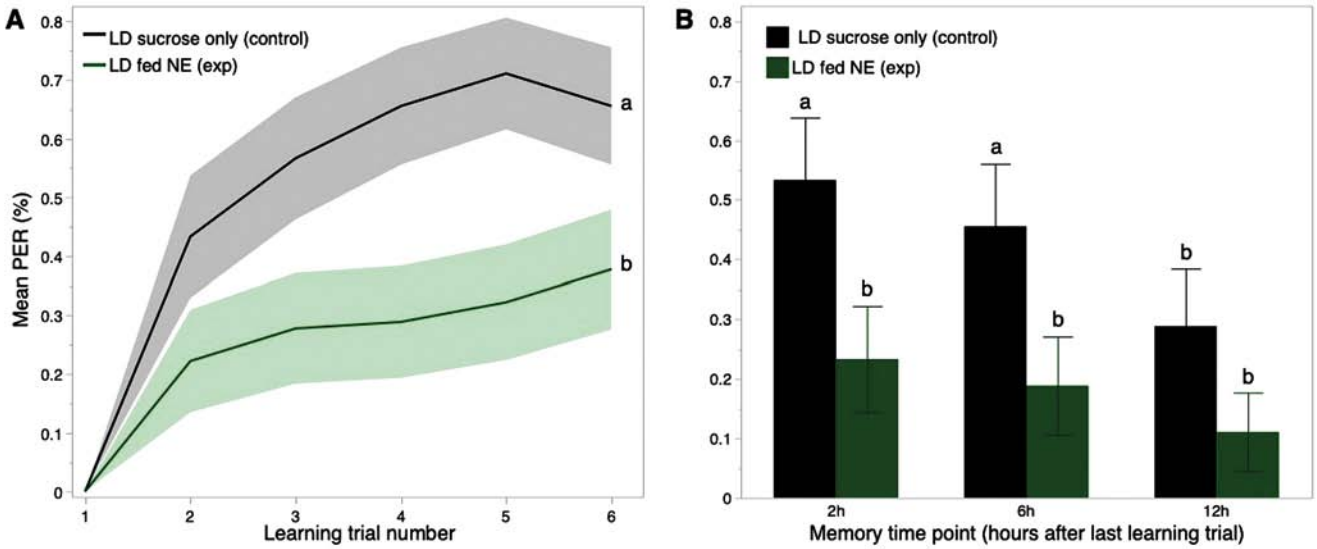

Fig. 3. NE feeding reduced bee learning and memory. The bees that were 14 years old were fed sucrose (NE, exp) only without norepinephrine (control) under the 14/10 light dark (LD) treatment condition. NE feeding reduced (A) bee learning (shaded regions show 95% confidence intervals) and (B) bee memory (means and 95% confidence intervals shown with error bars). We tested n = 180 bees, 90 $_{\text{LD sucrose alone,}}$ and 90 $_{\text{LD fed }NE}$. Different letters indicate significant differences (Tukey HSD test, $P < 0.05$).

were no significant differences in NE content between the three time points, but the average NE value at midnight was the highest. The NE content of bees in the three light treatments is highest at midnight than in the afternoon (Tukey HSD test, $P = 0.0064$) and in the morning (Tukey HSD test, $P < 0.0001$), and the effects of treatment at different lighting times are consistent.

3.5 Exp. 3: feeding LD bees NE reduced learning

Similarly to exposure of bees to constant light, feeding LD bees NE reduced their learning, as measured in the sixth and final learning trial (42% lower PER responses on average for NE-fed bees). There was a significant effect of treatment ($F_{1,176} = 14.77$, $P = 0.0002$), and the colony accounted for < 1% of the model variance.

Bee memory declined over time when measured at 2 h, 6 h and 12 h (memory time point: $F_{2,358} = 11.81$, $P < 0.0001$, Tukey HSD test, $P < 0.05$, Fig. 3B), and there was a significant effect of treatment ($F_{1,176} = 27.59$, $P < 0.0001$) because the bees fed NE had a lower memory PER than the bees not fed NE (56% lower average memory when tested at the 2 h time point). The time point of memory of the treatment × memory time was not significant ($F_{2,356} = 1.36$, $P = 0.26$). The colony represented 2% of the model variance.

3.6 Exp. 4: constant light increased bee mortality

Constant artificial illumination for 7 d resulted in significantly higher mortality than normal treatment (log rank Mantel-Cox test: $P_{\text{Colony1,2,3,4}} < 0.0001$, Fig. 4). Feeding bees NE also reduced bee survival compared to controls not fed NE (log rank Mantel-Cox test $P = 0.0001$).

4 Discussion

Light is a primary *zeitgeber* for circadian rhythms, and its disruption is known to impair sleep and increase physiological stress in bees (Helfrich-Förster 2018). Sleep deprivation alone impairs memory retention (Hussaini et al. 2009) and disrupts complex, memory-dependent behaviors such as waggle dancing (Klein et al. 2010). Our findings support these observations. We showed that constant, low-intensity light (2.3 µmol/s/m²) impairs olfactory learning and memory in honey bees, increases mortality, and elevates brain norepinephrine (NE) levels. Compromised olfactory learning and memory in *Apis mellifera* workers undermines their ability to remember rewarding foraging sites and communicate these resources to nestmates. Successful navigation, foraging efficiency, and waggle dancing rely on well-developed learning and memory (Sherman & Visscher 2002; Grüter & Farina 2009; Farina et al. 2012). These impairments intensified with longer light exposure, and extended training did not mitigate the cognitive deficits. This outcome reinforces previous reports that sleep deprivation impairs memory in bees (Hussaini et al. 2009) and that artificial light reduces learning and memory in mice (Song et al. 2021).

Prolonged light exposure also significantly increased bee mortality. Similarly, feeding bees sucrose solutions containing NE under normal light/dark cycles reduced survival, linking NE regulation and light-induced stress. This relationship parallels findings in *Drosophila melanogaster*, where even low-intensity constant light reduces survival (McLay et al. 2017), and in beetles, where extended photoperiods shorten lifespan (Singh et al. 2016).

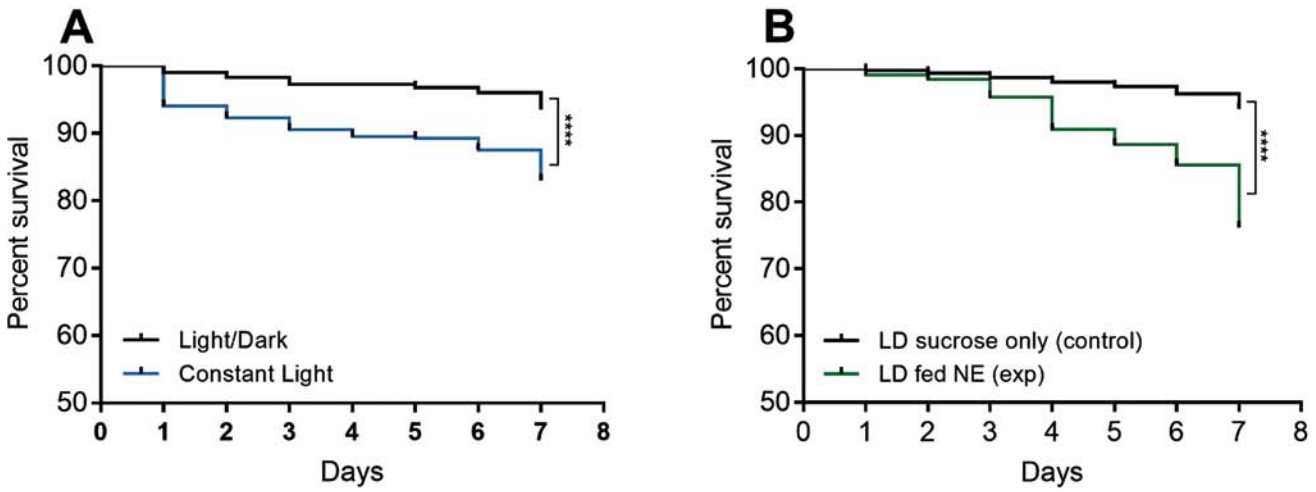

Fig. 4. Constant light or feeding bee NE increased bee mortality. (A) In the olfactory learning experiment, bees that experienced constant light had significantly lower survival and bees exposed to a 14/10 light / dark cycle (Log Rank Mantel-Cox tests, $p < 0.0001$****, Light/Dark $_{n = 90\ bees}$, constant light $n = 90\ _{bees}$). In this experiment, bees were not fed NE. (B) In the NE feeding experiment, all bees were exposed to the same 14/10 light / dark cycle (LD), but bees fed NE had significantly lower survival than bees fed pure sucrose solution (Log Rank Mantel-Cox tests $p < 0.0001$****, LD only $_{n = 90\ bees,\ NE\ fed}$ LD $n = 90\ _{bees}$).

All treatments may have experienced some stress, as the bees were housed outside their colonies. Elevated NE levels were detected in bees subjected to continuous light, continuous darkness, and even normal conditions, with a pronounced peak at midnight. However, under continuous light, NE levels were significantly higher, especially by day seven. Notably, feeding NE to bees kept under normal light conditions impaired cognition to a similar degree as continuous light exposure. Learning scores in NE-treated bees were 3.9 times lower than in controls, and memory scores were reduced by a factor of 3.5 at the 2-hour test. These results are consistent with earlier findings that NE injection impairs learning and memory in bees (Michelsen 1988).

The mechanisms driving these NE-induced cognitive deficits remain unknown and merit further study. In mammals, NE is critical in the locus coeruleus, a brain region tied to olfactory learning (Giustino & Maren 2018). In insects, NE levels fluctuate with photoperiod, as seen in the linden bug *Pyrrhocoris apterus*, where extended daylight hours during reproduction correlate with increased NE levels (Chvalova et al. 2014). NE also enhances locomotor activity through adipokinetic hormone release in firebugs (Socha et al. 2008). Circadian fluctuations in NE have been recorded in cockroaches (Natsukawa et al. 1996), suggesting that altered light cycles could similarly disrupt circadian rhythms and NE regulation in honey bees.

While octopamine levels often rise in insects under stress (Mezheritskiy et al. 2024) and can influence memory retention in *A. mellifera* exposed to toxins (Khooshe-Bast et al. 2024), our results suggest that the low-intensity illumination (2.3 µmol/s/m^2) may have fallen below the threshold needed to trigger octopamine-mediated responses. Instead, our findings point toward constant light exposure primarily disrupting circadian processes and sleep, both of which appear more closely tied to NE regulation than to octopamine signaling. These patterns highlight the complexity of neuromodulator responses to environmental stress and underscore the importance of examining multiple neurotransmitters under varying light intensities. Further studies are necessary to clarify how different levels and durations of light exposure interact with multiple neuromodulatory systems to shape honey bee stress physiology and cognition.

Future studies could delve more deeply into the mechanistic links between NE dysregulation, circadian disruption, and the cognitive and survival deficits observed in honey bees under altered light conditions. Our findings suggest that even low-intensity light can trigger physiological stress and impair key behaviors, highlighting the need to identify thresholds of exposure and potential interventions. Recognizing that light pollution is increasingly prevalent, such research could investigate how this stressor interacts with bee neurobiology to compromise their foraging efficiency, communication, and overall colony health. Clarifying these pathways will help inform evidence-based conservation strategies to safeguard honey bee populations and maintain the essential ecological services they provide.

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