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Multiple phototransduction inputs integrate to mediate UV light-evoked avoidance/attraction behavior

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Abstract

Short wavelength light guides many behaviors that are crucial for an insect's survival. In *Drosophila melanogaster*, short wavelength light induces both attraction and avoidance behaviors. How light cues evoke two opposite valences of behavioral responses remains unclear. Here we comprehensively examine the effects of (1) light intensity, (2) timing of light (duration of exposure, circadian time-of-day), (3) phototransduction mechanisms processing light information that determine avoidance versus attraction behavior assayed at high spatiotemporal resolution in *Drosophila.* External opsin-based photoreceptors signal for attraction behavior in response to low intensity UV light. In contrast, cell-autonomous neuronal photoreceptors, CRYPTOCHROME (CRY) and RHODOPSIN 7 (RH7) both signal avoidance responses to high intensity UV light. In addition to binary attraction versus avoidance behavioral responses to UV light, flies show distinct clock-dependent spatial preference within a light environment coded by different light input channels.

Introduction

Light guides many important behaviors in insects, including positive phototaxis, circadian entrainment, arousal, and sleep (Miller et al., 1981; Sheeba et al., 2008; Fogle et al., 2015; Garbe et al., 2015; Baik et al., 2017; Baik et al., 2018). Flies display both attraction and avoidance to short wavelength light. Attraction to light (positive phototaxis) is a well known behavior. In contrast, avoiding short wavelength light minimizes desiccation, UV-induced DNA damage, and excessive heat exposure. Adult flies exhibit a circadian-modulated avoidance to chronic high intensity UV light over a 12 hour period that coincides with the peak of light intensity and heat in the middle of the day (Baik et al., 2018). In contrast, low intensity light evokes immediate attraction to light at light onset within minutes (Heisenberg and Buchner, 1977; Gao et al., 2008; Yamaguchi et al., 2010; Baik et al., 2017). This suggests UV light-evoked attraction/avoidance responses may depend on intensity and/or duration of exposure. However, it is unclear which phototransduction channels modulates these two opposite behavioral responses to light stimuli, and their time-of-day variance in response. The question still remains, how does an animal spatially navigate and choose between different light environments? Here we comprehensively examine the external and

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internal factors influencing the animal's spatiotemporal choices in response to different light environments. External factors include the spectral quality and quantity of light, while internal factors include circadian timing and different phototransduction signaling channels (external opsins in the eyes and other structures and internal neuronal and eye structure CRYPTOCHROME and RHODOPSIN 7) in flies. We examined contribution(s) of (1) the duration of exposure vs. circadian time-of-day, (2) the intensity, and (3) different phototransduction mechanisms on UV light-evoked attraction/avoi dance behavior.

Both attraction and avoidance in insects are evoked by short wavelength light (Heisenberg and Buchner, 1977; Miller et al., 1981; Gao et al., 2008; Yamaguchi et al., 2010; Baik et al., 2017; Baik et al., 2018). In *Drosophila*, short wavelength light is processed through multiple phototransduction mechanisms: (i) External opsin-based photoreceptors, and direct neuronal phototransduction pathways: (ii) CRY/HK-based phototransduction, and (iii) RH7-mediated phototransduction (Feiler et al., 1992; Chou et al., 1996; Salcedo et al., 1999; Fogle et al., 2011; Fogle et al., 2015; Saint-Charles et al., 2016; Ni et al., 2017). All three phototransduction pathways mediate circadian entrainment to light (Emery et al., 1998; Stanewsky et al., 1998; Helfrich-Forster et al., 2001; Helfrich-Forster et al., 2002; Malpel et al., 2002; Rieger et al., 2003; Klarsfeld et al., 2004; Veleri et al., 2007; Sheeba et al., 2008; Kistenpfennig et al., 2012; Schlichting et al., 2014; Schlichting et al., 2015; Saint-Charles et al., 2016; Schlichting et al., 2016; Ni et al., 2017). Both CRY/HK- and RH7-mediated phototransduction evokes rapid increase in neuronal action potential firing and resting membrane potential (Fogle et al., 2011; Fogle et al., 2015; Baik et al., 2017; Ni et al., 2017). This suggests that the integration of multiple phototransduction inputs may be crucial in coordinating a complex light-evoked behavior, such as avoidance and attraction.

Materials and methods

Fly Strains

The following fly strains were obtained from Bloomington Stock Center: w^{1118} (BL5905) and gl^{60j} (BL509). cry^{01} was from Jeff Hall (Brandeis University), $timeless^{0}$ and clk^{OUT} were from Joanna Chiu (University of California Davis). $norpA^{P24}$ and $rh7^{1}$ were provided by Craig Montell (University of California Santa Barbara). $hk^{-/-}$ was provided by Ming Zhou (Baylor College of Medicine). $glass^{60j}$-cry^{01} double mutant fly line was generated in (Baik et al., 2017) by standard recombination genetic crosses of gl^{60j} and cry^{01} lines.

Standard Light Choice Assay

Standard LD Light choice assays were conducted as outlined in [(Baik et al., 2017)]. Locomotor activity of individual flies was measured using the TriKinetics Locomotor Activity Monitoring System via infrared beam-crossing recording total crosses in 1 min bins. % activity and statistics were measured using Microsoft Excel and Sigma Plot. Philips TL-D Blacklight UV source with narrow peak wavelength of 365 nm and intensity of 400 $\mu W/cm^2$ was used for high intensity, and 10 $\mu W/cm^2$ was used for low intensity by using neutral density filters. To determine acute light responses, 15 min pulses of UV light (365 nm, 400 $\mu W/cm^2$ for high intensity pulses or 10$\mu W/cm^2$ for low intensity pulses) throughout the 12 hr day on hourly intervals instead of 12h:12h UV light:dark. The experiment was

carried out under a constant dark condition (DD), and light pulses were administered only during the subjective daytime in order to maintain the prior circadian entrainment.

Multibeam Light Choice Assay

We adapted the standard LD Light choice assay outlined in [(Baik et al., 2017)] using Trikinetics MultiBeam Monitors. Two monitors were aligned parallel to each other with each tube containing a single fly measured by both monitors simultaneously. One of the two monitors was covered, providing the flies with a choice of a shaded environment (covered monitor) versus UV-exposed environment (uncovered monitor) during the 12 hours of UV light in daytime (ZT0-12). Each of the infrared beams are spaced every 3 mm. By using two monitors, each fly had 17 infrared beams on each light exposed- and on shaded-side with a total of 34 beams per fly. Locomotor activity, preference percentile, and statistics were measured using Microsoft Excel and Sigma Plot. Data are represented as mean ± S.E.M. *p < 0.05; **p < 0.01; ***p < 0.001.

Statistics

Data are presented as mean ± S.E.M. Values of “n” refer to total number of tested flies. In all cases the “n” values were obtained from at least 3 separate experiments. All statistical tests were performed with SigmaPlot 11. Comparisons of two sets of normally distributed variables, paired t-tests were performed; for non-normally distributed variables, values were compared using signed rank test.

Results

UV light-evoked attraction/avoidance behavioral response is intensity-dependent.

Previously reported avoidance to high intensity UV light was observed over a 12 hr light phase simulating daytime at 1 hr resolution (Baik et al., 2018), while UV evoked positive phototaxis events occur within a few minutes (Baik et al., 2017). If UV light behavioral attraction/avoidance depends on the duration of UV exposure, 1 hr resolution binning could miss rapid positive phototaxis responses. To determine whether if flies exhibit rapid positive phototaxis response at different intensities of UV light, we subjected adult flies to light choice assays collected and analyzed in 1 min bins. Wild-type control flies exhibit little-to-no attraction to high intensity UV light (365 nm, 400 μW/cm^2) in the first few minutes of light phase (Fig. 1). Mutant flies lacking all external opsin-based photoreceptors (*glass*^{60j} flies and *norpA*^{P24} flies) similarly lack clear positive phototaxis responses to the high intensity UV light (Fig. 1A, B). In contrast, mutant flies lacking blue/UV-specific CRY/HK-mediated (*cry*$^{-/-}$ and *hk*$^{-/-}$) or violet-peak RH7-mediated (*rh7*^{1}) phototransduction show strong, fast kinetic attraction responses to high intensity UV light that slowly attenuate with time (Fig. 1D–F, and Fig. S1C).

To test if UV light intensity impacts the choice between positive phototaxis versus avoidance, we measured the choice behavior under low intensity UV light (10 μW/cm^2). Wild-type control flies show strong attraction to dim UV light that slowly attenuates (Fig. 2). Thus attraction versus avoidance behavioral responses are UV light intensity-dependent. Mutant flies lacking CRY or HK also show attraction to low intensity UV light (Fig. 2B, C,

F, G). Similarly, mutant flies lacking RH7 show strong attraction to low intensity UV light (Fig. 2D, H). In contrast, mutant flies lacking all external opsin-based photoreceptors (*glass*^{60j}) are not attracted to low intensity UV light (Fig. 2A). Flies lacking CRY/HK- or RH7-mediated phototransduction exhibit positive phototaxis, while flies lacking external opsin-based phototransduction exhibit very slight avoidance, regardless of the intensity of UV light intensity exposure. Over the 12-hr UV exposure during the daytime-simulating light phase, CRY-, HK- or RH7-null mutant flies still exhibit time-of-day dependence (Fig. 2F–H), while external opsin-based photoreceptor mutant flies (*glass*^{60j} and *norpA*^{P24}) show relative lack of circadian modulation in attraction/avoidance to UV light (Fig. 2E, and Fig. S1A). This suggests that CRY/HK and RH7 mediates phototransduction is important for deciphering the intensity of UV light, and thus signaling for avoidance in response to high intensity UV light. In contrast, external opsin-based phototransduction is necessary for evoking fast positive phototaxis response to low intensity UV light and time-of-day dependent modulation of attraction/avoidance. Double mutant flies lacking both opsin-based external photoreceptors and CRY-mediated phototransduction almost completely lack any preference for UV-exposed vs. shaded environment (Fig. 1C, and Fig. S1B).

High intensity UV light evokes avoidance in a clock-dependent and exposure duration-independent manner, while low intensity UV light evokes fast attraction response in an external opsin photoreceptor-dependent manner.

Behavioral attraction to low intensity UV light is observed most clearly in the first few minutes following light onset, followed by gradual decrease in the degree of attraction within 15 minutes. Under constant 12 hr UV light phase, time-of-day dependent changes in overall attraction/avoi dance response behaviors are modulated by the circadian clock (Baik et al., 2018). Due to adaptation, it is difficult to determine the acute light responses using this chronic light choice assay. To determine acute light responses distributed throughout the day, we adapted the light choice assay to administer acute 15 min pulses of UV light (365 nm, 400 μW/cm^2 for high intensity pulses or 10 μW/cm^2 for low intensity pulses) throughout the 12 hr light phase on hourly intervals. The acute light response was measured in flies already entrained to 12:12 LD prior to experiment. The experiment was carried out under dark condition (DD) and light pulses were only administered during the subjective light phase to maintain prior circadian entrainment.

Using this acute repeated pulse light choice assay, wild-type control flies still exhibit robust avoidance to high intensity UV light in the early-mid daytime that shifts valence to light attraction towards the end of the light phase (Fig. 3A, and Fig. S2A). In contrast, circadian mutant flies, *timeless-null* (*tim*^{0}) and *clock-null* (*clk*^{OUT}) show strong acute attraction to high-intensity UV pulses that do not vary with time-of-day (Fig. 3E–F). This shows that avoidant behavioral response to acute high intensity UV light pulses is modulated by the circadian clock, rather than by the simple duration of UV light exposure. Wild-type control flies exhibit fast positive phototaxis response to acute low-intensity UV light pulses throughout the entire light phase (Fig. 4A and Fig. S2A). Similarly, circadian mutants *tim*^{0} and *clk*^{OUT} also show rapid attraction to low-intensity UV light at all times of the light phase (Fig. 4E–F). This suggests the valance of UV light choice is gated by light intensity: avoidance is a chronic circadian clock-modulated response to high intensity UV light, while

attraction is an acute circadian clock-independent behavioral response to low intensity UV light.

CRY-mediated electrical responses signal through an HK-dependent pathway that couples light activation to potassium channel modulation in a redox-dependent mechanism (Fogle et al., 2015). Both mutant flies null for CRY/HK-mediated phototransduction show rapid acute attraction to both high- and low-intensity UV pulses that do not vary with time-of-day (Fig. 3B, C, Fig. 4B, C, and Fig. S2C, D), supporting earlier results showing that CRY codes for UV light avoidance responses (Baik et al., 2017; Baik et al., 2018). In contrast, mutant flies lacking external opsin-based photoreceptors ($glass^{60j}$) lack clear preference (no clear attraction nor avoidance) throughout the light phase for both high- and low-intensity UV pulses (Fig. 3D, Fig. 4D, and Fig. S2B). We conclude that high-intensity UV light-evoked avoidance signals via CRY/HK-mediated phototransduction mechanism, which is modulated by the circadian clock, and appears to be independent of the duration of UV light exposure. In contrast, low-intensity UV light evokes acute attraction in a circadian clock-independent manner via external opsin-based photoreceptors. Thus, UV light intensity responses signal through different phototransduction channels in an intensity-dependent manner.

External and internal phototransduction mechanisms modulate distinct time-of-day dependent spatial preference of light environment.

To resolve the behavioral responses to UV light at high spatial resolution, we used multibeam locomotor activity monitors (with infrared beams spaced every 3 mm, 17 beams on each light exposed versus shaded side with a total of 34 beams per fly) (Fig. S3). Wild-type control flies strongly prefer shaded locations at the end of tubes that are the most distant from the UV exposed light area. This behavior is subject to circadian-modulated time-of-day driven preferences with UV avoidance behavior that peaks during the middle of the light phase (Fig. 5A). Wild-type control flies venture out of the shade to UV-exposed areas at the beginning (ZTO-1) and the end (ZT10-12) of the light phase, even though UV light intensity does not vary throughout the 12-hr light phase (Fig. 5A). During the simulated midday (ZT1-10), control flies strongly prefer to be at the edge furthest away from the UV-exposed environment, close to the food. However, food location does not dictate this behavioral preference for location in wild-type flies. The time at which wild-type flies prefer the furthest edge away from the UV-exposed environment, coincides with the trough of circadian feeding rhythm (Barber et al., 2016), and the other end of the tube on the UV exposed side is not a strongly preferred location at any time of the light phase.

In marked contrast to wild-type flies, *cry-null* flies strongly prefer the UV exposed area (Fig. 5B) and avoid the mid-region between both the shaded- and the UV-light exposed environments. In additional detail, *cry-null* flies favor the edge closest to the food (Fig. 5B). With striking similarity, *rh7-null* flies strongly prefer the UV exposed area, although not as close to the food, and like the *cry-null* flies, avoid the mid-region between both the shaded- and the UV-light exposed environments at all times of the day (Fig. 5D). Clk^{OUT} circadian mutant flies prefer UV-light exposed environment regardless of the time-of-day, and prefer the edge closest to the food in the UV exposed area. Unlike *cry-* or *rh7-null* flies, clk^{OUT} mutants do not avoid the mid-region between the shaded- and the UV-light exposed

environments (Fig. 5E). *Glass*^{60j} mutant flies, which lack all external opsin-based photoreceptors but still express CRY and HK, prefer the shaded environment at all times of the light phase and lack clear time-of-day dependent modulation. Even though they cannot "see" UV light with image forming vision, *glass*^{60j} flies sense and avoid UV light. Image forming vision does have some spatio-behavioral impact. Unlike control flies, *glass*^{60j} flies prefer the mid-region within the shaded environment at all times of the light phase rather than the edge furthest away from the UV light (Fig. 5C).

Discussion

Insect behavior, including circadian rhythmic behavior, is subject to the integration of multiple sensory inputs. The combination of these sensory cues in natural environments is sufficiently powerful to mask major clock mutations, which yield severe behavioral impairments in less complex laboratory conditions (Vanin et al., 2012). We demonstrate that flies complex spatiotemporal behavioral light responses integrate multiple photic inputs to navigate their light environment. Low intensity UV evokes rapid attraction, which diminishes quickly. This confirms its importance as a survival mechanism for escape behavior. Here we show that this fast attraction behavior is induced by external opsin-based photoreceptors at all times of the day. High intensity UV evokes avoidance via internal/direct neuronal photoreceptors, CRY and RH7. In addition to intensity of light, circadian time and light spectra are important for avoidance/attraction signaling (Baik et al., 2018). In contrast to fast kinetics of short wavelength light acute attraction, CRY- and RH7-evoked avoidance is slowly modulated by the circadian clock, changing over hours span during the daytime-simulating light phase (Baik et al., 2017; Baik et al., 2018) and Fig. S1).

External and internal photoreceptors all contribute to light entrainment of the circadian locomotor activity rhythm (Emery et al., 1998; Stanewsky et al., 1998; Helfrich-Forster et al., 2001; Helfrich-Forster et al., 2002; Malpel et al., 2002; Rieger et al., 2003; Klarsfeld et al., 2004; Veleri et al., 2007; Sheeba et al., 2008; Kistenpfennig et al., 2012; Schlichting et al., 2014; Schlichting et al., 2015; Saint-Charles et al., 2016; Schlichting et al., 2016; Ni et al., 2017). Both internal photoreceptors, CRY and RH7, are expressed in the circadian pacemaker neurons in the *Drosophila* brain, including lateral ventral neurons (LNv) (Benito et al., 2008; Yoshii et al., 2008; Ni et al., 2017). LNv circadian neurons have been shown to be necessary for having a normal short wavelength light avoidance (Baik et al., 2018). Double knockout mutant flies lacking both external and internal photoreceptors, *glass*^{60j}-*cry*$^{-/-}$, almost completely lack any preference of light environment, and thus do not display neither avoidance nor attraction to UV light (Fig. 1 and Fig. S1). The similarity of their behavioral responses supports the notion that CRY- and RH7- phototransduction signals may converge to mediate avoidance/attraction behavior. In addition to circadian neurons, CRY is expressed in the eyes and plays a role in visual sensitivity (Mazzotta et al., 2013).

Here we implemented multibeam activity monitors to acquire high temporal and spatial resolution of UV-evoked avoidance/attraction behavior. Interestingly, both wild type control and *glass*^{60j} flies both show minimal behavioral preference for the shaded food-containing edge towards end of the light phase, even though it coincides with the time of peak in feeding rhythm (Barber et al., 2016). In contrast, *cry-null* and *clk*^{OUT} mutant flies show

strong positional preference in the UV-exposed area close to food at all times of light phase with overall less time-of-day modulated positional preference as compared to wild-type controls. This suggests that observed spatial preference in the far edge in simulated midday is not due to the presence of food. We show that there are multiple levels of sensory processing even for a single type of input such as light. We conclude that multiple phototransduction mechanisms modulate a complex behavioral output depending on its spectra, intensity, and time/duration of exposure.

At the organismal level, integration of multiple types of sensory inputs, including light and temperature, are crucial to synchronize to the changing environment. Both light and temperature cycles shape complex behavioral output, including locomotor activity and sleep in *Drosophila* (Currie et al., 2009; Yoshii et al., 2009; Head et al., 2015; Harper et al., 2016; Parisky et al., 2016; Harper et al., 2017; Lamaze et al., 2017; Chen et al., 2018; Yadlapalli et al., 2018). Multisensory entrainment of the circadian clock occurs by integrating different sensory cues, including light inputs via CRY and thermal inputs from transient receptor potential A1 (TrpA1) (Das et al., 2015; Green et al., 2015; Das et al., 2016; Harper et al., 2017). Rhythmic preference of both light and temperature environment further reinforces an intricate and complex behavioral output (Figs. 1–5; (Stevenson, 1985; Kaneko et al., 2012; Head et al., 2015; Baik et al., 2017; Tang et al., 2017).

In conclusion, complex spatiotemporal behavioral responses to UV light are mediated through multiple phototransduction pathways. Acute high intensity UV light exposure (minutes) in wild type flies evokes a transient phototaxis attractive response, which is mediated by the opsin-based phototransduction in the eyes. With longer high intensity UV light exposure (tens of minutes), avoidance is the dominant response, which is mediated by the CRY/HK and RH7 pathways. Both rapid/acute and long term/chronic CRY/HK mediated behavioral responses vary by time of day, while external photoreceptor/opsin mediated responses do not appear to vary by time of day. Acute low intensity UV light exposure (minutes) in wild type flies evokes a longer lasting transient phototaxis attractive response that resolves to mostly neutral responses. Spatial responses to UV light are strongly influenced by both neuronal cell autonomous phototransduction (CRY/HK) and external opsin-based photoreceptor phototransduction, indicating integrated contributions from both major UV sensing photosystems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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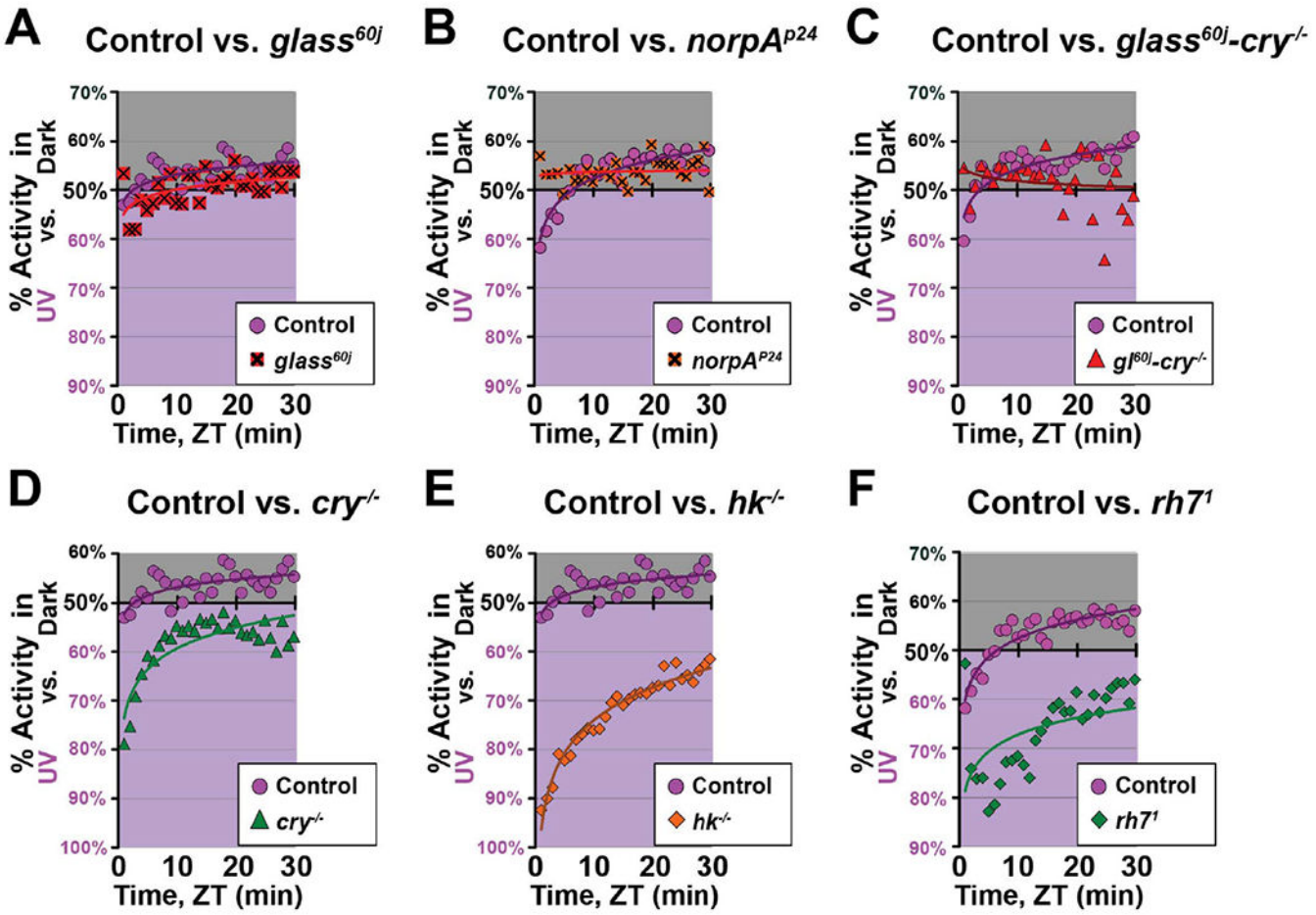

Figure 1. Internal neuronal photoreceptors are necessary for avoidance behavioral response to high intensity UV light.

UV attraction/avoidance behavior measured by preference for shaded environment vs. high intensity UV-exposed environment (365 nm, 400 μW/cm^2) during daytime of standard 12hr:12hr light:dark cycle. Preference is calculated by percent of activity in each environment over total activity for each time bin. **(A-F)** Preferences of wild-type control (w^{1118}) vs. mutant flies to high-intensity UV (365 nm, 400 μW/cm^2) in ZT 0-30 min shown in 1 min bin. Similar to wild-type flies that show little-to-no attraction in the first few minutes of UV light exposure, **(A)** *glass*^{60j} (n=76 vs. control, n=76), **(B)** *norpA*^{P24} (n=80 vs. control, n=79), and **(C)** *glass*^{60j}-*cry*$^{-/-}$ (n=55 vs. control, n=95) mutant flies lack clear positive phototaxis responses. In contrast, **(D)** *cry*$^{-/-}$ (n=78 vs. control, n=76), **(E)** *hk*$^{-/-}$ (n=77 vs. control, n=76), and **(F)** *rh7*^{1} (n=42 vs. control, n=79) mutant flies show strong fast positive phototaxis responses to high intensity UV light.

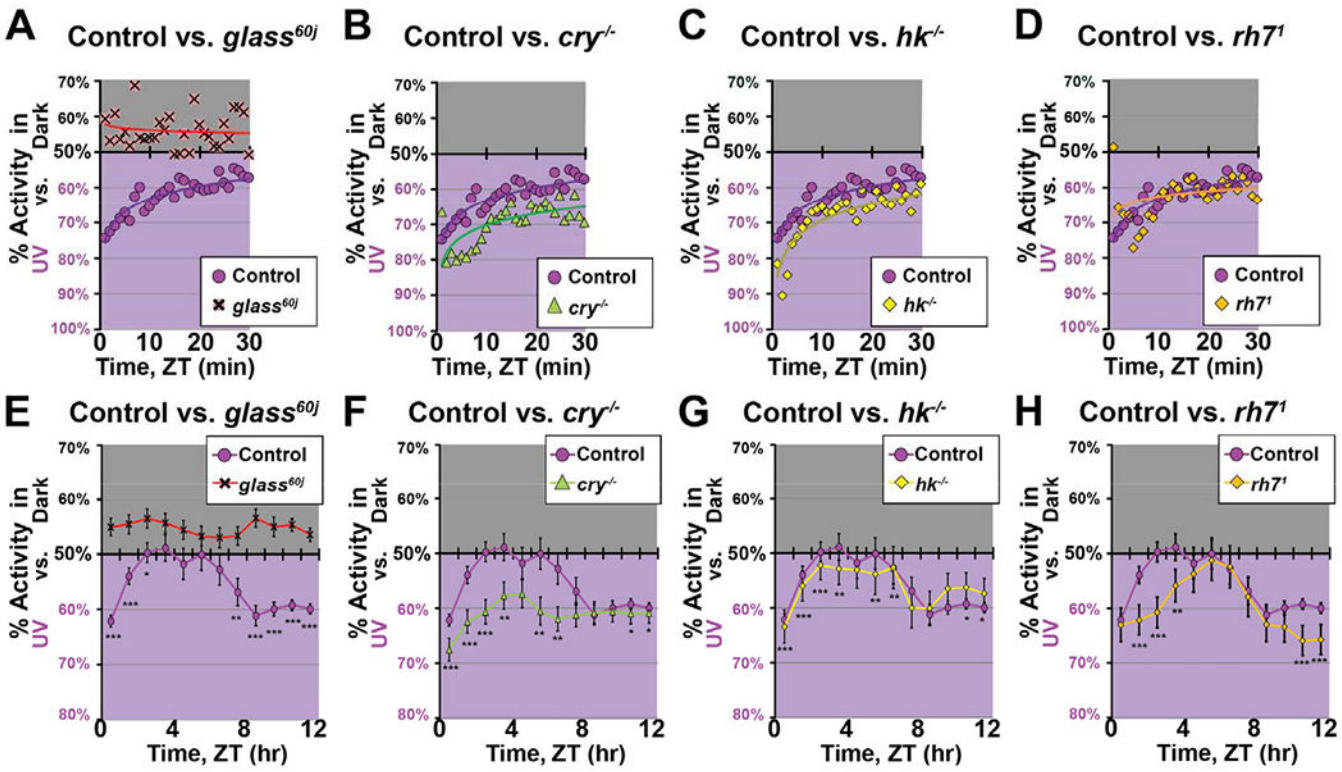

Figure 2. UV light-evoked attraction/avoidance behavioral response is intensity-dependent.

UV attraction/avoidance behavior measured by preference for shaded environment vs. low intensity UV-exposed environment (365 nm, 10 μW/cm^2) during daytime of standard 12hr:12hr light:dark cycle. Preference is calculated by percent of activity in each environment over total activity for each time bin. Preference wild-type control (w^{1118}; n=47) vs. mutant flies to low-intensity UV (10 μW/cm^2) in **(A-D)** ZT 0-30 min shown in 1 min bin and **(E-H)** in the daytime (ZT 0-12 hr) shown in 1 hr bin. Unlike wild-type control flies that show strong attraction to low intensity UV light, **(A)** $glass^{60j}$ flies (n=31) lack attraction to low intensity UV light in ZT 0-30 min. **(B)** $cry^{-/-}$ (n=44), **(C)** $hk^{-/-}$ (n=31), and **(D)** $rh7^1$ (n=105) mutant flies show strong attraction to low intensity UV light in ZT 0-30 min. **(E)** $glass^{60j}$ flies (n=31) sustain slight avoidance to low intensity UV light throughout ZT 0-12 hr. In contrast, wild-type control, **(F)** $cry^{-/-}$ (n=44), **(G)** $hk^{-/-}$ (n=31), and **(H)** $rh7^1$ (n=105) mutant flies exhibit attraction to low intensity UV light in ZT 0-12 hr shown in 1 hr bins.

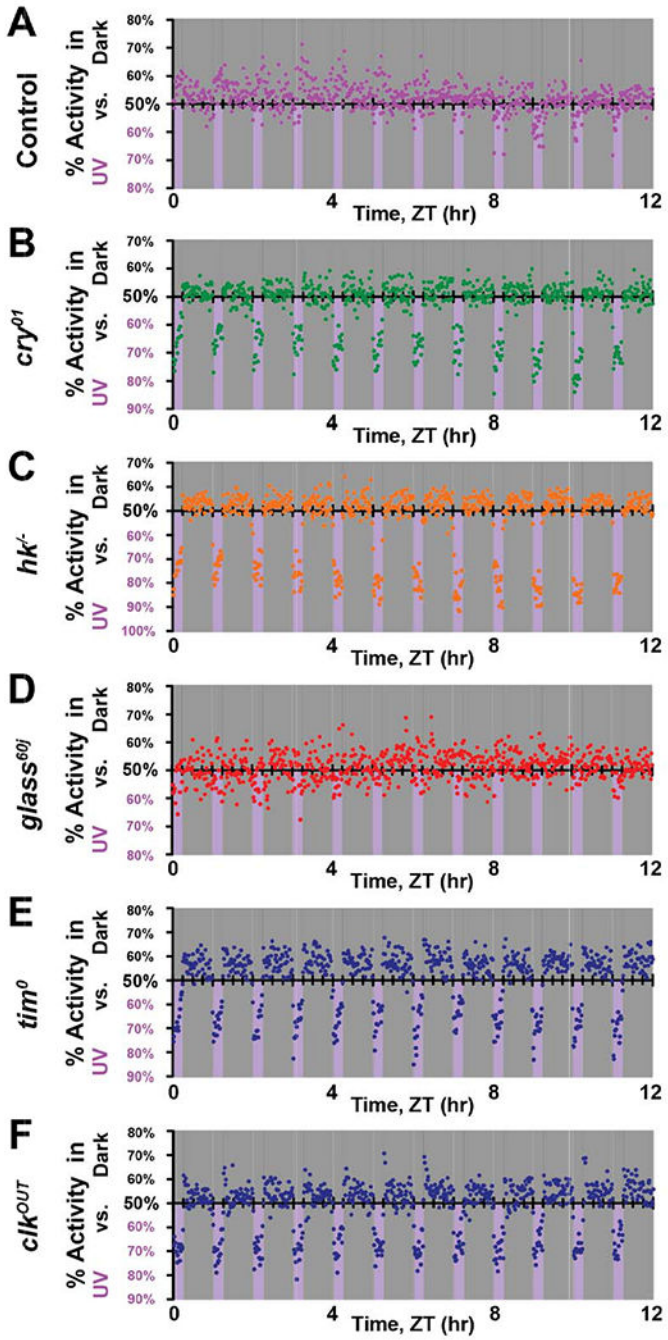

Figure 3. High intensity UV light evokes avoidance modulated by the circadian clock that is independent of duration of light exposure.

UV attraction/avoidance behavior measured by preference for shaded environment vs. high intensity UV-exposed environment (365 nm, 400 μW/cm^2) during acute 15 min pulses of light throughout the 12 hr day on hourly intervals. Preference is calculated by percent of activity in each environment over total activity for each 1min bin. **(A)** Wild-type control (w^{1118}; n=61) flies show avoidance to high intensity UV light in the early-mid daytime. In contrast, **(B)** $cry^{-/-}$ (n=63) and **(C)** $hk^{-/-}$ (n=61) mutant flies show attraction to high-

intensity 15 min UV light exposures at all time-of-day. **(D)** *glass*^{60j} (n=58) mutant flies lack clear preference at all times of the daytime. **(E)** *timeless*^{0} (n=100) and **(F)** *clock*^{OUT} (n=102) circadian mutant flies show attraction to high-intensity 15 min UV light exposures regardless of time-of-day.

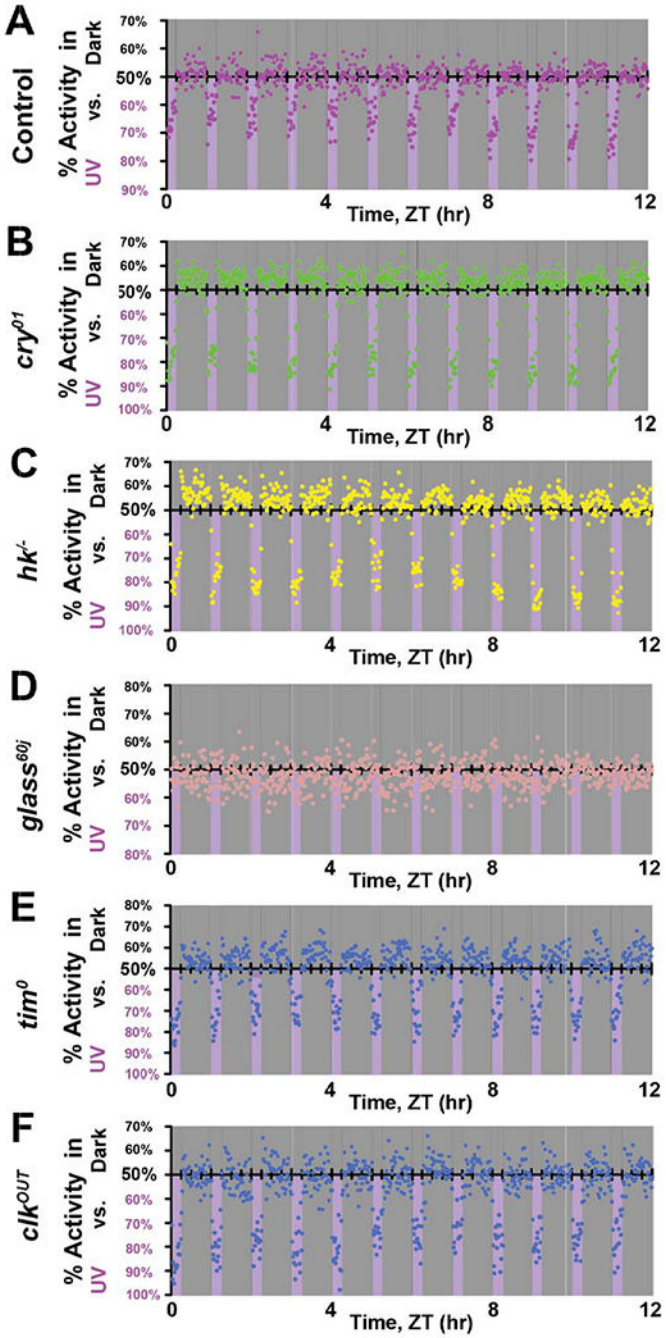

Figure 4. External opsin-based photoreceptors modulate attraction behavior in response to low intensity UV light.

UV attraction/avoidance behavior measured by preference for shaded environment vs. low intensity UV-exposed environment (365 nm, 10 μW/cm^2) during acute 15 min pulses of light throughout the 12 hr day on hourly intervals. Preference is calculated by percent of activity in each environment over total activity for each 1 min bin. **(A)** Wild-type control (*w*^{1118}; n=61), **(B)** *cry*$^{-/-}$ (n=60) and **(C)** *hk*$^{-/-}$ (n=62) flies show attraction to low-intensity 15 min UV light exposures at all time-of-day. In contrast, **(D)** *glass*^{60j} (n=62) mutant flies lack

attraction. **(E)** *timeless*^{0} (n=44) and **(F)** *clock*^{OUT} (n=40) circadian mutant flies show attraction to low-intensity 15 min UV light exposures regardless of time-of-day.

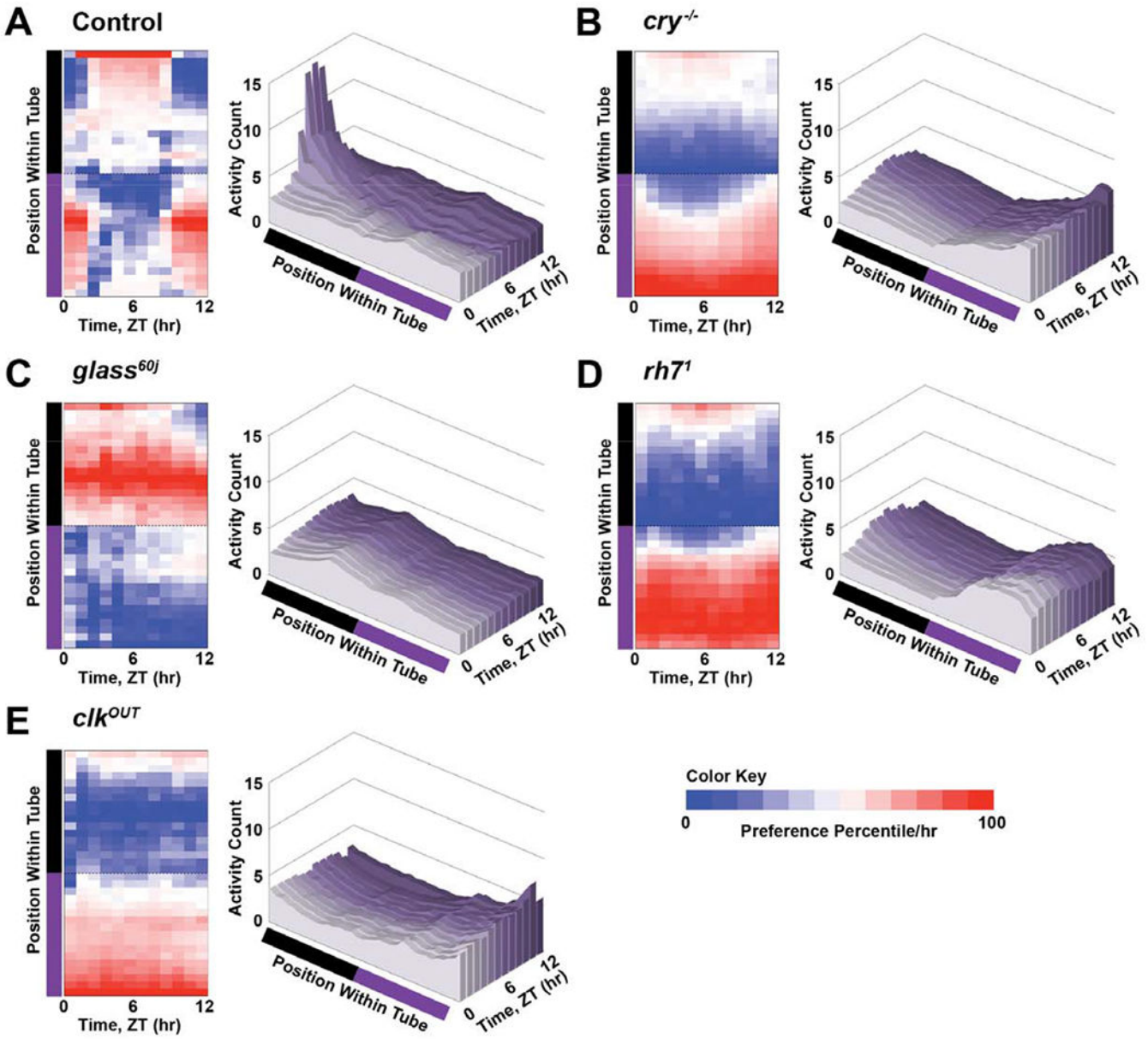

Figure 5. Avoidance/attraction behavior has distinct time-of-day dependent spatial preference.

(A-C) Time-of-day dependent spatial preference of avoidance/attraction behavior in response to high-intensity UV (365 nm, 400 μW/cm^2) vs. shade was monitored with high spatial and temporal resolutions using multibeam locomotor activity monitors. Heat map of preference for each 1hr bin (left) and activity count at each position for every 1hr bin (right) are shown. **(A)** Wild-type control (*w*^{1118}; n=144) flies prefer the mid-area of UV-exposed environment during the early morning and hours before simulated dusk. But during the midday prefer the edge of the shaded environment, furthest away from the UV-environment and close to the food. In contrast, **(B)** *cry*$^{-/-}$ (n=61) flies strongly prefer area furthest out in UV-exposed environment at all times of the day. **(C)** *glass*^{60j} (n=64) flies prefer the mid-area within the shaded environment, regardless of the time of day. **(D)** *rh7*^{1} (n=46) flies strongly prefer the outer edge of UV-exposed environment at all times of the day. **(E)** *clk*^{OUT} (n=48)

flies prefer the area furthest out in UV-exposed environment close to the food, at all times of the day.