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CRYPTOCHROME mediates behavioral executive choice in response to UV light

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Drosophila melanogaster **CRYPTOCHROME (CRY) mediates behavioral and electrophysiological responses to blue light coded by circadian and arousal neurons. However, spectroscopic and biochemical assays of heterologously expressed CRY suggest that CRY may mediate functional responses to UV-A (ultraviolet A) light as well. To determine the relative contributions of distinct phototransduction systems, we tested mutants lacking CRY and mutants with disrupted opsin-based phototransduction for behavioral and electrophysiological responses to UV light. CRY and opsin-based external photoreceptor systems cooperate for UV light-evoked acute responses. CRY mediates behavioral avoidance responses related to executive choice, consistent with its expression in central brain neurons.**

cryptochrome | phototransduction | UV | neural decision making | *Drosophila*

For nearly a century, it has been assumed that insect behavioral responses to UV light are exclusively mediated by UV-sensitive opsins expressed in eyes and other external photoreceptors. However, organisms express other nonopsin photoreceptors, including the blue-light-sensitive flavoprotein CRYPTOCHROME (CRY). In *Drosophila melanogaster*, CRY mediates rapid membrane depolarization and increased spontaneous action potential firing rate in the lateral ventral neurons (LNvs), which are involved in arousal and circadian behavioral responses (1–7). Blue-light-activated CRY couples to membrane depolarization in *Drosophila* LNv neurons by a redox-based mechanism involving potassium channel heteromultimeric complexes, consisting of the downstream redox sensor cytoplasmic potassium beta (Kvβ), HYPERKINETIC (HK), and ion-conducting voltage-gated potassium alpha (Kvα) *ether-a-go-go* family subunits (8). Electrical activity in the LNvs contributes to circadian rhythms (9–11), and, reciprocally, LNv neuronal firing rate is circadian-regulated (1, 2, 12). Circadian regulation of firing rate is widely conserved in other invertebrate and vertebrate species (13, 14). For insects, CRY is characterized as the primary photoreceptor for blue–light-activated circadian entrainment (15–21). CRY-expressing large LNvs (l-LNvs) also mediate acute behavioral arousal responses to blue-light-containing spectra (4–7). Arousal and circadian functions are not strictly segregated between the LNv subsets because the small LNvs (s-LNvs) also contribute to arousal (5), and clock cycling is robustly altered in the l-LNv in response to light entrainment cues (22, 23).

Many insects, including *Drosophila*, display strong spectral sensitivity for short-wavelength light. The ability to sense and respond to UV light is important because it guides physiological and behavioral responses to sunlight that are crucial for survival. The absorbance spectra of purified CRY from *Drosophila* at the oxidized baseline state of the flavin dinucleotide chromophore show a strong UV peak near 365 nm, in addition to the 450-nm blue-light peak (24–26). Furthermore, UV light triggers CRY degradation in cultured cells that heterologously express fly CRY (27). To test the in vivo functional significance of these findings, we measured behavioral and electrophysiological responses to UV light near the CRY UV peak.

Results

CRY Mediates Opsin-Independent Electrophysiological Responses to UV Light in l-LNvs. Light-modulated behaviors are driven by the modulation of membrane excitability in contributing neurons, such as the l-LNvs (5, 6, 8–11). We tested whether similar l-LNv electrophysiological response (firing frequency light on/light off) was observed in response to UV light. Control fly l-LNvs respond to UV light with varying degrees to different intensities (Fig. 1 *A* and *E*; 365 nm; low, 20 μW/cm^2; intermediate, 150 μW/cm^2; and high, 640 μW/cm^2). The l-LNv response to UV light is significantly attenuated in *cry*-null mutant flies ($cry^{-/-}$; Fig. 1 *B* and *E*) and *hk*-null mutant flies ($hk^{-/-}$; Fig. 1 *C* and *E*) relative to control (Fig. 1 *A* and *E*).

To determine whether CRY-mediated l-LNv UV light responses are cell-autonomous, we performed genetic rescue experiments. Genetic rescue of LNv-targeted expression of CRY in $cry^{-/-}$ genetic background cell-autonomously rescues the l-LNv UV light response at low and intermediate intensities, but incompletely at high intensity (Fig. 1 *D* and *F*). Similarly, LNv-targeted expression of WT-HK in $hk^{-/-}$ genetic background rescues l-LNv UV light response at low and intermediate intensities, but again not at high intensity (Fig. 1*G*). Expression of redox sensor-disabled HK point mutant, HK-D260N, does not rescue l-LNv response to UV light at all intensities (Fig. 1*G*). Thus, electrophysiological responses to UV light are specifically mediated by light-activated CRY coupled to the membrane via HK redox sensor, consistent with previous findings using blue light (8).

Significance

Many animals exhibit behavioral responses to UV light, including harmful insects. Recently, the explosive spread of diseases carried by mosquitoes has increased motivation to better understand insect UV phototransduction. CRYPTOCHROME (CRY) is a highly conserved nonopsin photoreceptor expressed in a small number of brain circadian and arousal neurons in *Drosophila melanogaster* that mediates cell-autonomous electrophysiological membrane excitability in response to UV light. CRY signaling modulates multiple fly behaviors evoked by UV light, including acute nighttime arousal responses to light flashes and phototaxis toward low-intensity UV light. Loss of CRY or the redox sensor HYPERKINETIC (HK) leads to the loss of ability to avoid high-intensity UV light; thus, CRY signaling exhibits novel features of behavioral executive choice.

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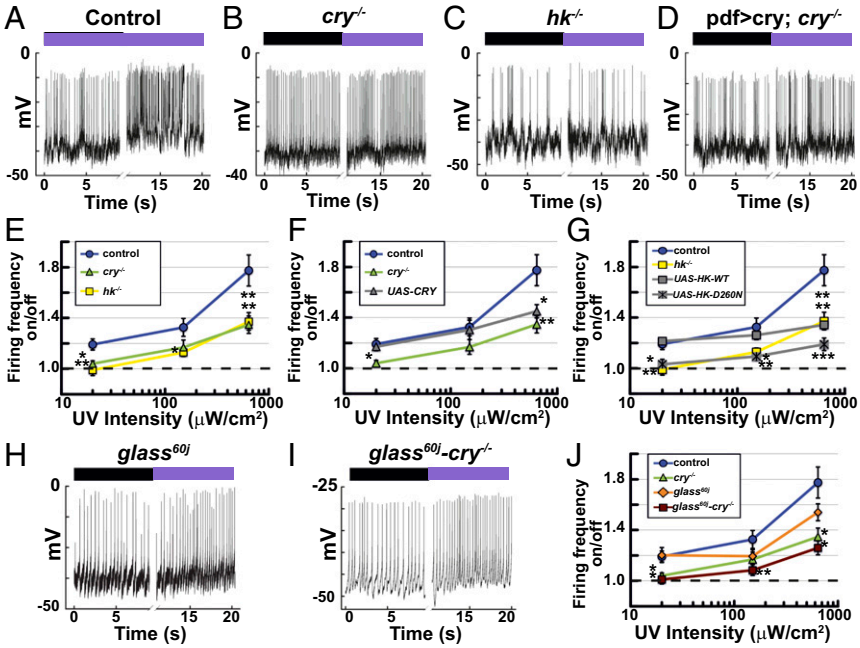

Fig. 1. l-LNv electrophysiological response to UV light is attenuated in flies lacking CRY-based phototransduction. (*A–D*) Representative trace for control l-LNv UV light response (*A*) (365 nm, 640 μW/cm^2, violet bar; lights off, <0.01 μW/cm^2, black bar; the gap in the *x* axis removes <1 s, wherein a noise transient is caused by manual opening of the shutter to expose the prep to light) vs. representative traces for *cry*$^{-/-}$ (*B*), *hk*$^{-/-}$ (*C*), and "*cry* rescue" (*D*) flies (*pdf-GAL4*–driven LNv *UAS-CRY* expression in a *cry*$^{-/-}$ background). (*E–G*) Dose–response quantification of l-LNv firing frequency (FF) response (FF on/FF off) to UV light at low (20 μW/cm^2), intermediate (150 μW/cm^2), and high (640 μW/cm^2) intensities. (*E*) Electrophysiological response of control flies increase with increasing intensities of UV light (1.19 ± 0.04, $n = 17$, low; 1.33 ± 0.07, $n = 15$, intermediate; 1.77 ± 0.12, $n = 15$, high intensity). The significantly attenuated UV light responses of *cry*$^{-/-}$ (1.04 ± 0.02, $n = 17$, $P = 0.01$, low; 1.17 ± 0.06, $n = 15$, $P = 0.129$, intermediate; 1.35 ± 0.07, $n = 15$, $P = 0.005$ vs. control, high intensity) and *hk*$^{-/-}$ (0.99 ± 0.04, $n = 15$, $P = 0.002$, low; 1.13 ± 0.03, $n = 14$, $P = 0.049$, intermediate; 1.37 ± 0.07, $n = 26$, $P = 0.008$ vs. control, high intensity) flies do not differ from each other ($P = 0.622$, low; $P = 0.879$, intermediate; $P = 0.978$, high intensity). (*F*) Dose–response quantification of FF for control vs. *cry*$^{-/-}$ and *cry* rescue flies. Full rescue is achieved at low (1.18 ± 0.03, $n = 15$, $P = 0.99$ vs. control) and intermediate intensities (1.24 ± 0.03, $n = 15$; $P = 0.14$ vs. control), but is incomplete at high-intensity UV light (1.45 ± 0.05, $n = 15$, $P = 0.03$ vs. control and $P = 0.68$ vs. *cry*$^{-/-}$). (*G*) Dose–response quantification of FF for control vs. *hk*$^{-/-}$ and *pdf-GAL4*–driven rescue of WT-HK (*UAS-HK-WT*) or of redox sensor-disabled point mutant HK-D260N (*UAS-HK-D260N*), both in *hk*$^{-/-}$ genetic background. WT-HK rescue flies also achieve rescue at low (1.21 ± 0.03, $n = 16$, $P = 0.97$ vs. control) and intermediate (1.26 ± 0.03, $n = 16$, $P = 0.732$ vs. control) intensities, but not at high intensity (1.34 ± 0.04, $n = 16$, $P = 0.001$ vs. control, and $P = 0.99$ vs. *hk*$^{-/-}$). The redox sensor-disabled point mutant HK-D260N fails to rescue the light response at all UV light intensities (1.03 ± 0.04, $n = 13$, $P = 0.033$, low; 1.09 ±0.03, $n = 15$, $P = 0.004$, intermediate; 1.19 ± 0.05, $n = 11$, $P \leq 0.001$ vs. control, high intensity; $P \geq 0.417$ vs. *hk*$^{-/-}$ all intensities). (*H*) Representative trace for *glass*^{60j} (*gl*^{60j}) mutant l-LNv UV light response. (*I*) Representative trace for *gl*^{60j}- *cry*$^{-/-}$ double-mutant l-LNv UV light response. (*J*) Dose–response quantification FF for *gl*^{60j} and *gl*^{60j}- *cry*$^{-/-}$ double-mutant flies. *gl*^{60j} flies response do not significantly differ from control (1.20 ± 0.06, $n = 18$, $P = 0.87$, low; 1.19 ± 0.05, $n = 14$, $P = 0.15$, intermediate; 1.54 ± 0.07, $n = 16$, $P = 0.098$ vs. control, high intensity). *gl*^{60j}- *cry*$^{-/-}$ double mutant has significantly attenuated UV response compared with control (1.01 ± 0.03, $n = 20$, $P = 0.002$, low; 1.08 ± 0.04, $n = 32$, $P = 0.003$, intermediate; 1.26 ± 0.05, $n = 28$, $P \leq 0.001$ vs. control, high intensity) and do not differ from *cry*$^{-/-}$ response ($P = 0.499$, low; $P = 0.252$, intermediate; $P = 0.157$ vs. *cry*$^{-/-}$, high intensity). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Because low levels of CRY are expressed in the R7 and R8 photoreceptors (28), we recorded from l-LNv of *sevenless* mutant flies, which lack all R7 photoreceptors (29). The l-LNv UV light responses of *sevenless* flies do not significantly differ from control flies (Fig. S1). To determine whether opsin-based photoreceptors also contribute to the l-LNv electrophysiological response to UV light, we recorded from the l-LNv neurons of *glass*^{60j} (*gl*^{60j}) flies, which lack eyes and other external photoreceptors (and DN1p circadian cells). The l-LNv UV-light responses of *gl*^{60j} flies are qualitatively lower, but do not significantly differ from control flies (Fig. 1 *H* and *J*). The l-LNv UV light response of *glass*^{60j}-*cry*$^{-/-}$ (*gl*^{60j}-*cry*$^{-/-}$) double-mutant flies is indistinguishable from that of *cry*$^{-/-}$ flies (Fig. 1 *I* and *J*). These results suggest that CRY mediates electrophysiological responses to UV in the l-LNvs in an opsin-independent manner. *gl*^{60j}-*cry*$^{-/-}$ double-mutant flies show some residual electrophysiological UV response at higher intensities, indicating the presence of a yet-to-be identified third photoreceptor for the l-LNvs, consistent with earlier findings (17).

Acute Arousal Behavioral Response to UV Light Is CRY-Dependent. CRY is expressed in circadian, arousal, and photoreceptor neurons (3, 11, 28, 30), including l-LNvs, which mediate acute arousal behavioral responses to blue light at physiological intensities that transmit the head and eye cuticles (8). We measured the proportion of UV light transmittance through eye and head cuticle tissue using a 365-nm LED light source using procedures described in ref. 3. Eye cuticles are >85% transparent, and head cuticles are nearly 50% transparent to 365-nm UV light (Fig. S2). We then measured acute behavioral responses to 5-min pulses of 365-nm UV (3 mW/cm^2) or 595-nm orange (7 mW/cm^2) LED light in the middle of the subjective night at zeitgeber time (ZT) 18, ZT19, and ZT20 for three consecutive nights in control and *cry*$^{-/-}$ mutants, as well as *no receptor potential A* null mutant (*norpA*^{P24}) and *gl*^{60j} mutant flies, which, respectively, have defects in opsin phototransduction in the eyes and the ocelli and external photoreceptor development. A representative averaged behavioral actogram of control flies ($n = 32$) responding to UV light pulses is shown (Fig. 2*A*). We examined flies that were asleep immediately before the light pulse (4, 5). The percentage of flies that awaken in response to 365-nm UV light is significantly lower in *cry*$^{-/-}$, *norpA*^{P24}, and *gl*^{60j} mutant flies compared with control (Fig. 2*B*). In response to 595-nm orange light, the percentage of flies that awaken is comparable between control and *cry*$^{-/-}$ flies. However, a significantly higher percentage of *norpA*^{P24} flies awaken, whereas a significantly lower percentage of *gl*^{60j} flies awaken in response to orange light relative to controls, indicating that the

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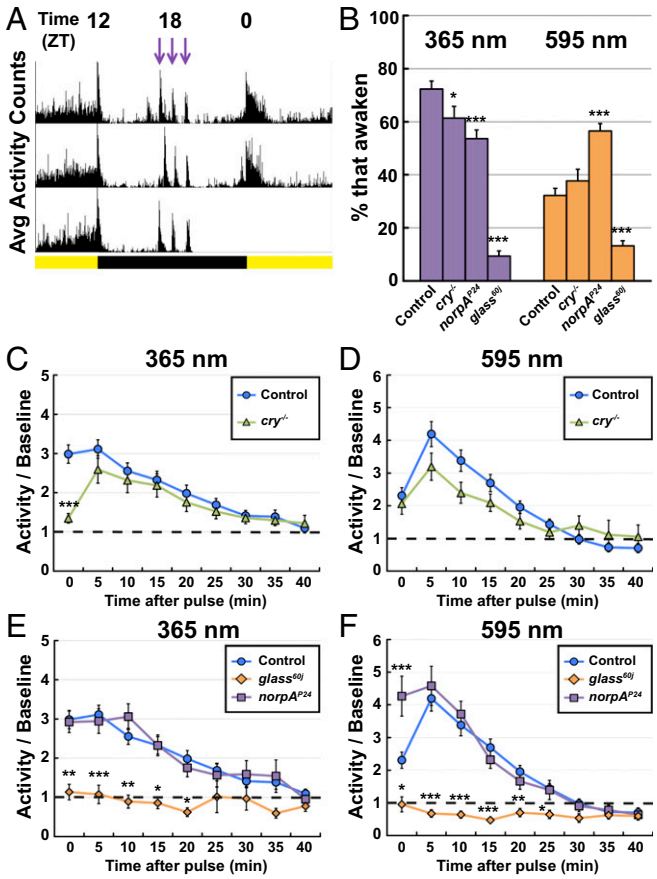

Fig. 2. *Drosophila* acute arousal response to UV light is CRY-dependent. (*A*) Representative averaged double-plotted actogram of $n = 32$ flies given three 5-min light pulses (365 nm, 3 mW/cm^2) during three consecutive nights. Flies respond acutely to light pulses, but remain entrained to the LD 12:12 environmental cues. (*B*) Percentage of sleeping flies that awaken during the light pulse for UV (365 nm, 3 mW/cm^2) and orange light (595 nm, 7 mW/cm^2). Compared with control flies (0.72 ± 0.03, $n = 384$ flies for UV), a significantly lower percentage of $cry^{-/-}$ flies (0.61 ± 0.04, $n = 192$ flies, $P = 0.039$ vs. control) awaken in response to UV light pulse. Percentage of sleeping $cry^{-/-}$ flies that awaken during orange light pulse does not differ from percentage of sleeping control flies that awaken (0.32 ± 0.03, $n = 224$ flies for control; 0.38 ± 0.04, $n = 128$ flies, for $cry^{-/-}$; $P = 0.264$ $cry^{-/-}$ vs. control). Both $norpA^{P24}$ and gl^{60j} flies have a significantly lower percentage of flies that awaken in response to UV light pulses (0.54 ± 0.03, $n = 192$ flies, $P \leq 0.001$ for $norpA^{P24}$ vs. control; 0.09 ± 0.02, $n = 64$ flies, $P \leq 0.001$ for gl^{60j} vs. control). A significantly higher percentage of $norpA^{P24}$ flies awaken (0.56 ± 0.03, $n = 160$ flies, $P \leq 0.001$ for vs. control), whereas a significantly lower percentage of gl^{60j} flies awaken in response to orange light pulses (0.13 ± 0.02, $n = 64$ flies, $P \leq 0.001$ vs. control). (*C–F*) Time course of activity of awake flies during and after UV (*C* and *E*) or orange (*D* and *F*) light pulse. Each point on the graph represents a bin of 5 min, with the first bin collected during the pulse. (*C*) During the UV light pulse, control flies show a dramatic increase in arousal activity (activity/baseline is 2.98 ± 0.23, $n = 384$ flies), whereas $cry^{-/-}$ flies remain relatively inactive (1.35 ± 0.12, $n = 192$ flies, $P \leq 0.001$ vs. control), only responding after the pulse ($cry^{-/-}$ vs. control, $P > 0.213$ for all bins, after the light pulse). (*D*) $cry^{-/-}$ and control fly activities do not differ during and after the orange light pulse ($n = 128$ flies, $cry^{-/-}$ vs. control, $n = 224$ flies, $P > 0.064$ for all bins). (*E*) Activity of awake $norpA^{P24}$ flies does not differ from that of control flies ($n = 192$ flies, $norpA^{P24}$ vs. control, $n = 384$ flies, $P > 0.174$ for all bins). gl^{60j} flies show a significantly lower arousal response both during and after the UV light pulse ($n = 64$ flies, gl^{60j} vs. control, $P < 0.010$ for bins during and 5–20 min after the light pulse). (*F*) gl^{60j} flies show a significantly lower arousal response during and after orange light pulse ($n = 64$ flies, gl^{60j} vs. control, $n = 224$ flies, $P < 0.018$ for bins during and 5–25 min after the light pulse). $norpA^{P24}$ flies have significantly higher activity than control flies during the orange light pulse ($n = 160$ flies, $norpA^{P24}$ vs. control, $P \leq 0.001$), but do not differ in activity after the orange light pulse ($norpA^{P24}$ vs. control, $P < 0.332$ for all bins, after the light pulse). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

$norpA^{P24}$ and gl^{60j} flies differ in their light responses (Fig. 2*B*). Recently, *Drosophila* transient receptor potential A1 (TRPA1) channel has been implicated as an H_2O_2-sensitive high-intensity UV (600–5,000 mW/cm^2) sensor (31). We tested acute arousal responses of *trpA1*-null ($trpA1^1$) flies to UV light (3 mW/cm^2) and orange light (7 mW/cm^2). Acute arousal response of $trpA1^1$ flies to either UV or orange light pulses is indistinguishable from that of control flies (Fig. S3), indicating that acute arousal responses to UV light pulse is not mediated by TRPA1 at the light intensities tested.

We also examined the behavioral responses of flies that were awake immediately before the light pulse. During the UV light pulse (just after 0 min), awake control flies show increased arousal activity, whereas awake $cry^{-/-}$ flies remain relatively inactive during the UV light pulse, then show a delayed response minutes later after the UV light pulse (Fig. 2*C*). CRY-mediated acute arousal activity is specific to UV light, because the $cry^{-/-}$ response to orange light does not differ from that of control flies (Fig. 2*D*). Acute arousal activity of awake $norpA^{P24}$ flies in response to UV light pulse is indistinguishable from that of control flies (Fig. 2*E*). $norpA^{P24}$ flies show increased acute arousal responses during the orange light pulse, but after the orange light pulse, their activity does not differ from that of control flies (Fig. 2*F*). gl^{60j} awake flies do not respond to either UV or orange light pulses (Fig. 2 *E* and *F*). This finding suggests that acute arousal behavioral response to UV may be modulated by DN1 cells and/or Hofbauer–Buchner (HB) eyelet, which is functionally defective in the gl^{60j} mutants, but not in $norpA^{P24}$ mutants (17, 32–35).

CRY Mediates Executive Choice Attributes of Positive Phototaxis and Avoidance Behaviors to Different Intensities of UV Light. Light can serve as either a repellent or an attractive signal for an animal's behavior, depending on intensity and spectra. Many insects exhibit an innate spectral attraction to low-intensity UV light, as shown by phototaxis behavioral assays (36–38). In contrast, high-intensity UV light induces avoidance behavior, particularly in larvae and egg-laying females (39, 40), and reduces mating activity in adult male *Drosophila* (41).

Positive phototaxis behavior of adult male flies in response to very low-intensity UV light (3 μW/cm^2 365-nm LED, 5 min per exposure) was measured by using a retrofitted Trikinetics DAM5 *Drosophila* Activity Monitor attached to a light-tight chamber holding a population of 40 flies (Fig. 3*A*). Positive phototaxis is measured by increased activity levels (counts per min) from flies migrating to the light-transparent activity monitor in the front (Fig. 3*A*). WT control flies show robust attraction in response to 5-min pulses of very low-intensity UV light (Fig. 3 *B* and *D*). Positive phototaxis to UV light is significantly attenuated in $cry^{-/-}$ flies compared with control flies (Fig. 3 *B* and *D*). Interestingly, control flies choose to linger in the previously light-exposed region after the light-pulse long after the light has been turned off, compared with $cry^{-/-}$ flies, which leave the previously light-exposed region quickly (Fig. 3*D*). This finding suggests that CRY potentially mediates aspects of executive choice, specifically in choosing to linger in a previously light-exposed region, in addition to simple acute sensory function (Fig. 3 *B* and *D*). Both $norpA^{P24}$ and gl^{60j} flies show little attraction toward very low-intensity UV light (Fig. 3 *B* and *F*). Thus, external photoreceptors have a primary acute sensory role for UV phototaxis, whereas CRY modulates the magnitude and duration of the response. $trpA1^1$ mutant flies do not exhibit attenuated positive phototaxis in response to 5-min pulses of very–low-intensity UV light, but, surprisingly, show significantly higher positive phototaxis compared with control flies (Fig. S4 *A* and *C*). Orange light (3 μW/cm^2 595-nm LED, 5 min per exposure) fails to evoke strong positive phototaxis (Fig. 3 *C*, *E*, and *G* and Fig. S4 *B* and *D*).

CRY potentially contributes to executive choice evoked by UV light. To test this hypothesis directly, we measured behavioral avoidance responses to high-intensity UV light (400 μW/cm^2). A

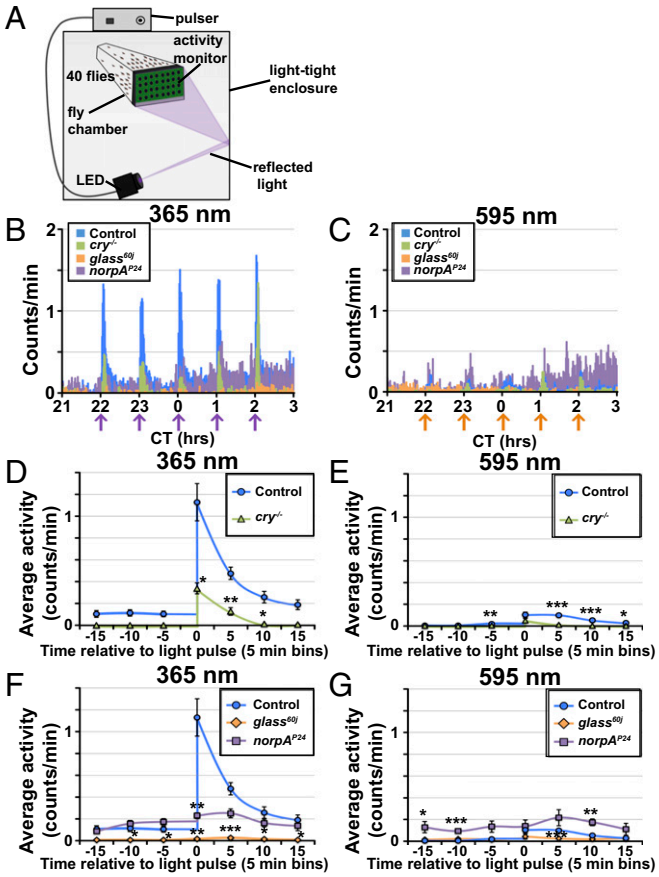

Fig. 3. *Drosophila* positive phototaxis behavior toward UV-light is attenuated in mutants lacking CRY- and in mutants lacking external photoreceptors. (*A*) A DAM2 *Drosophila* Activity Monitor (32 channels with dual infrared beams; Trikinetics) was mounted to the front of the light-tight chamber holding a population of 40 flies and sealed with a glass cover on the outer face. (*B*) Average phototaxis activity counts per min toward a very low-intensity UV light pulse (365 nm, 3 μW/cm^2, five exposures of 5-min light; indicated by violet arrows) followed by 55 min of darkness starting at circadian time (CT) 21 to CT 3 for control (nine experimental repeats, $n = 40$ flies per experiment), $cry^{-/-}$ (three experimental repeats, $n = 40$ flies per experiment), $norpA^{P24}$ (four experimental repeats, $n = 40$ flies per experiment), and gl^{60j} flies (four experimental repeats, $n = 40$ flies per experiment). (*C*) Average phototaxis activity counts per min toward five 5-min orange light pulses (595 nm, 3 μW/cm^2, indicated by orange arrows) followed by 55 min of darkness starting at CT 21 to CT 3 for control (four experimental repeats, $n = 40$ flies per experiment), $cry^{-/-}$ (five experimental repeats, $n = 40$ flies per experiment), $norpA^{P24}$ (three experimental repeats, $n = 40$ flies per experiment), and gl^{60j} flies (six experimental repeats, $n = 40$ flies per experiment). (*D*–*G*) Average phototaxis activity in 5-min bins relative to the UV (*D* and *F*) or orange (*E* and *G*) light pulses averaged from *B* and *C*. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

modified Trikinetics *Drosophila* Activity Monitor system was fitted for long tubes with two infrared photobeams separated by 8.4 cm that measure locomotor activity at different zones for a single fly, with food and air holes placed equally on either side of the long tube to prevent food and air spatial preferences (Fig. 4*A*). Adult male flies were 12:12 light–dark (LD)-entrained in standard white light (3 d), followed by 12:12 LD entrainment in UV light (3 d). Then, an opaque screen was placed covering one side of each long tube, so that half the length of the tube was exposed to high-intensity UV light, and the other half of the tube was shaded, thus blocking all direct UV light (Fig. 4*B*). Each infrared photobeam on either side of the long tube allowed us to measure the fly's choice of locomotor activity, either in the zone of the tube exposed to high-intensity UV light or in the zone of the tube shaded from direct UV light. To measure potential time-of-day effects, high-intensity UV light was on for 12 h, matching the entrained daytime (ZT0–12), followed by all lights off (ZT12–24). This schedule presented flies with a choice between activity in the high-intensity UV light-exposed environment vs. escape to the covered environment shaded from direct UV light at all times during daytime for 10 d (Fig. 4*B*). We refer to this as the "Mad Dogs and Englishmen" experiment [after Noel Coward, 1931 (42)].

Control and gl^{60j} flies significantly avoid UV light and strongly prefer to be in the shaded environment, including during the midday (Fig. 4 *C*–*E* and Fig. S5 *A* and *D*). The gl^{60j} flies are not as effective as control flies for UV avoidance, but show the same pattern of avoidance. In contrast, $cry^{-/-}$ and $hk^{-/-}$ flies significantly prefer the high-intensity UV light environment over the shaded environment during the daytime, particularly during the early morning and all afternoon hours, and exhibit significantly attenuated avoidance behavior to high-intensity UV light compared with controls at all times of day (Fig. 4 *C* and *D* and Fig. S5 *B* and *C*). To control for potential olfactory cues deposited by flies during daytime activity, we analyzed for environmental preference for both sides of the monitor (ZT12–24) when the UV light is off. No differences in preferences are detected between all four genotypes during subjective nighttime (Fig. 4*F*). However, on an hour-by-hour basis, $cry^{-/-}$ and $hk^{-/-}$ flies show small, but significant, preferences for the covered side during all of the night (Fig. S5 *B* and *C*). Similarly, control and gl^{60j} flies show small, but significant, preferences for the covered side during half or nearly half of the night (Fig. S5 *A* and *D*). This nighttime activity might reflect residual olfactory cues left during daytime activity or differences in food quality on the covered side. Thus, results show clearly that CRY and its downstream redox sensor HK mediate choice in avoidance behavior in response to high-intensity UV light during day. This territorial preference does not extend in the absence of UV light.

Discussion

The results above show that both CRY- and opsin-based photoreceptors contribute to UV light-sensing and behaviors. The l-LNv electrophysiological UV light responses increase monotonically with increasing UV light intensity. The l-LNv electrophysiological response to UV light is severely attenuated in $cry^{-/-}$ and $hk^{-/-}$ null mutants, along with qualitative decreases seen in gl^{60j} mutants (Fig. 1). There is a small residual l-LNv electrophysiological light response even in gl^{60j}-$cry^{-/-}$ double mutants (Fig. 1*J*), suggesting that there is another short-wavelength light photoreceptor that has yet to be identified. Subtleties in our data suggest potential circuit-level effects for encoding light. Gene-replacement rescue experiments in $cry^{-/-}$ and $hk^{-/-}$ null backgrounds show intensity-dependent degrees of rescue, for which rescue is complete for lower light intensities, but incomplete for higher light intensities (Fig. 1 *F* and *G*). This result may be due to the fact that the genetic rescue is limited to the LNv, not all neurons that ordinarily express CRY.

Mutants lacking CRY show significantly altered behavioral responses to UV light by three very different assays: (*i*) acute arousal response to high-intensity UV light flashes during the night; (*ii*) positive phototaxis for very low-intensity UV light; and (*iii*) avoidance of high-intensity UV light. The ability to discern the changes in intensity, spectral content, timing, and exposure length of light provides valuable environmental information crucial to an organism's well-being and survival. UV light-avoidance behavior has been demonstrated in foraging larva and egg-laying activity in females (39, 40). We demonstrate that the CRY/HK signaling pathway mediates UV light avoidance behavior in adult male *Drosophila*. During peak UV light intensity (midday in most natural environments), flies (especially males) tend to take a "siesta" rest and thus avoid heat exposure and desiccation. UV light avoidance behavior is highest during the midday (Fig. 4), despite unvarying UV intensity for our experimental conditions

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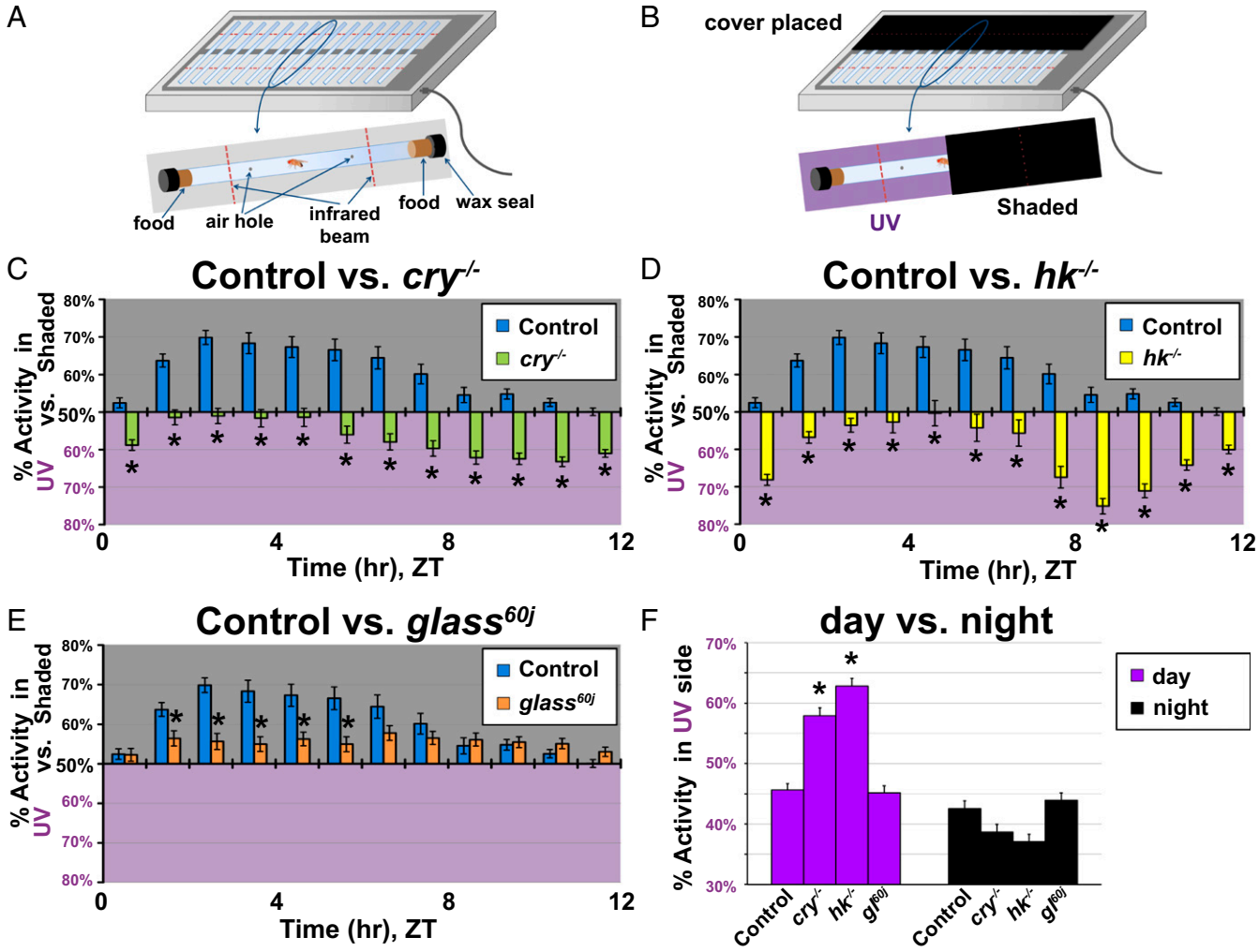

Fig. 4. CRY-based phototransduction contributes to UV light avoidance behavior in *Drosophila*. (*A*) Diagram of the "light choice" apparatus. Standard Trikinetics *Drosophila* activity monitors were modified to fit behavior tubes of 2× length, which have food and air hole on both sides of the tube. Flies are first entrained in standard 12:12 white light LD without any cover. The 12:12 white light LD is then replaced by 12:12 UV light LD. (*B*) Half of the monitor is then covered with cardboard to provide flies a choice between UV light-exposed (400 μW/cm^2) and shaded environments. UV light is turned on only during the entrained daytime (ZT0-12). (*C*–*E*) Preference for UV-exposed vs. shaded environment is measured by percent of activity in each environment over total amount of activity for each ZT. Gray shade indicates shaded environment preference (light avoidance), and violet-shade indicates UV environment preference. (*C*) *cry*$^{-/-}$ flies have a significant defect UV light avoidance behavior at all times of the day compared with control flies and prefer the UV environment over the shaded (*cry*$^{-/-}$, $n = 78$ vs. control, $n = 76$, all $P < 0.05$). (*D*) Similarly, *hk*$^{-/-}$ flies also have a significant defect in UV light avoidance behavior at all times of the day compared with control flies and prefer the UV environment over the shaded (*hk*$^{-/-}$, $n = 77$ vs. control, all $P < 0.05$). (*E*) Mutant flies lacking all opsin-based external photoreceptors (*gl*^{60j}) show significantly less UV avoidance compared with control flies only during the midday, ZT1-6 (*gl*^{60j}, $n = 76$ vs. control, all $P < 0.05$). (*F*) Average percent activity in UV-exposed environment during the day vs. night. *cry*$^{-/-}$ and *hk*$^{-/-}$ flies have significantly higher activity in the UV-exposed environment during the day compared with control flies ($P < 0.05$). Daytime percent activity in UV-exposed environment of *gl*^{60j} flies does not significantly differ from control. Percent activities in UV-exposed environment for *cry*$^{-/-}$, *hk*$^{-/-}$, and *gl*^{60j} flies during the nighttime do not differ from control. *$P < 0.05$.

during the daytime (ZT0-12). This finding suggests that CRY/HK-mediated UV light avoidance behavior may be under circadian control, comparable to larval avoidance behavior shown to be dependent on opsin-based photoreceptors and subsets of circadian pacemaker neurons and circadian genes (43, 44).

CRY dually mediates attraction and avoidance behaviors to UV light depending on UV light intensity. The differences in CRY-mediated behavioral response to varying intensities of UV light poses the interesting question of whether CRY may be important, not only for the acute sensory detection of the light, but also for modulating more complex aspects of behavior, such as executive choice. CRY-mediated behavioral responses likely depend on spectral composition, intensity, and duration of light exposure, as well as integration with other sensory cues, most notably temperature (45–49). CRY-mediated electrophysiological light responses vary monotonically depending on UV light intensity. Thus, the cell-autonomous neuronal CRY light sensor codes for graded responses to UV light intensity rather than gated on/off responses.

Opsin-based light sensing is clearly critical for behavioral light responses. The *gl*^{60j} mutant exhibits the developmental loss of all external opsin-based photoreceptors, HB eyelet, and the DN1p subset of circadian neurons (17, 32, 33). The DN1s have been implicated for light-evoked morning arousal activity (50, 51). HB eyelet cells project into the accessory medulla and to the LNvs (52, 53). The *norpA*^{P24} mutant disrupts opsin-based phototransduction in eyes without disrupting phototransduction in the HB eyelet or development of the DN1p circadian neurons; thus, the *gl*^{60j} and *norpA*^{P24} mutants are not functionally equivalent (17, 34, 35). In contrast to the dramatic loss of arousal response to UV light in *gl*^{60j} mutants, *norpA*^{P24} mutants show UV light arousal responses that

closely resemble those of controls (Fig. 2*E*), suggesting the DN1ps and/or the HB eyelet may also contribute to the UV light arousal response. CRY's contribution is functionally distinct from that of opsins, as shown by both electrophysiological and behavioral results. In conclusion, CRY is a major modulator of a wide range of fly behavioral responses to UV light.

Materials and Methods

Locomotor activity was recorded by using the TriKinetics *Drosophila* Activity Monitor system (9). l-LNv recordings were performed on acutely dissected adult fly brains in whole-cell current clamp mode (1, 2). Extended information on materials and methods is described in *SI Materials and Methods*, including protocols for electrophysiology, optics, genetics, behavioral testing, and statistical analysis.

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