Intense light unleashes male courtship behavior in wild-type *Drosophila*

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Studies of the *Drosophila* courtship repertoire have elucidated several principles of the neurogenetic organization of complex behavior. Based on a multi-modal integration of olfactory, gustatory, visual, and acoustic cues, males perform stereotypic patterns of chasing, courtship song production, and copulation attempts (1, 2). Here we report a serendipitous finding that intense light (∼18 klx, comparable to direct sunlight) induces courtship-associated motor activity in wild-type (WT) male flies, including chasing with wing extension and song production. The intensified male courtship behavior was directed to both male or female targets, as shown in both male-only and mixed sex arenas. However, in male-only chambers, we found extreme behavioral manifestations such as "chaining" and "wheeling", which resembled previously reported homosexual behavior in certain mini-*white*$^+$ constructs (3, 4) and *fruitless* mutants (5). Investigations into the required sensory systems, yielded results of strong implications. In visual system mutants with defective photo-sensitive or screening pigments (*norpA*), the phenomenon was absent. Even in *sevenless* mutants, where the high-acuity blue/UV channels are disrupted but motion sensitivity is preserved (6), light-triggered courtship was greatly attenuated. However, light-induced courtship was unhampered in flies lacking acoustic signals (with wings cut to disrupt song production). Strikingly, we observed unrestrained male-male courtship behavior in olfactory mutants (*orco* and *sbl*). Particularly, *orco* males displayed maximum courtship scores regardless of light intensity. Together our observations support the notion that male courtship is restrained by olfactory cues and can be unleashed by strong visual stimulation in *Drosophila*.

We modified the semi-automated *Drosophila* tracking system, IowaFLI Tracker to analyze activity in an open arena (7). The tracking system consisted of a clear polyacrylic sheet containing four arenas which were surrounded by an LED strip (Figure 1A). A webcam mounted above captured fly behaviors. In male-only arenas, we unexpectedly discovered a high frequency of courtship behaviors at the highest light intensity setting. Seconds after activating the lights, we regularly observed individuals chasing and attempting to court (Figure 1B, Movie 1). Strikingly, we often saw chains of courting males (Figure 1B, Movie 1), reminiscent to phenotypes found in mini-*white*$^+$ transgenic lines (3) and *fruitless* mutants (5). In a modified arena, we recorded light-induced pulse and sine courtship songs (Figure 1B), correlated with courtship-related wing extensions in the arena.

To quantify light-induced courtship, we developed a protocol where the eight flies in the arena were subjected to 2-min illumination of relatively low intensity light (0.4 klx) followed by 2-min of high intensity light (18 klx) and another low intensity light period (Figure 1C). For each successive 10-s interval, we manually scored the occurrence of chasing, wing extensions and chaining in the arena. The total number of intervals during which the respective behaviors were observed in the arena was reported as the "total score" (ranging from 0 to 12, Figure 1D; see Methods). During the high-light period the total chasing score rose more than 700% ($p = 0.0001$), the total wing extension score increased by 400%. ($p = 0.0002$), and the chaining score increased by more than 800% ($p = 0.045$). This light-associated increase in courtship activity showed a monotonic relationship between light intensity and the incidence of the respective behaviors (Figure S1A). Furthermore, the exact light source did not appear to matter as incandescent light and sunlight sources also triggered the phenomenon (Figure S1B-C).

We found intense light induced courtship across WT strains (e.g. *Berlin*, Figure S1D). Furthermore, in mixed sex arenas (4 males and 4 females), we also observed an increase in courtship behaviors during high light conditions (Figure S1F), suggesting intense light generally intensifies courtship drive in males rather than a change in mate preference. To determine the sensory systems required for the light-induced courtship, we examined males with disrupted visual, wing mechanosensory and courtship song production or chemosensory systems. In blind *norpA* mutants, we found intense light did not induce courtship activity (Figure 1E, (Figure S1F, Movie 1). We then refined our analysis by examining *sevenless* mutants in which the central R7 and R8 photoreceptors (sensitive to UV and blue light respectively) in each facet are functionally impaired while the high sensitivity channel, consisting of the outer photoreceptors R1-R6 (green light sensitive) are intact (6). In *sevenless* mutants, we found light-evoked courtship is absent (Figure 1E), indicating a critical role for the R7/R8 system in generating light-triggered courtship.

In contrast to the visual system, manipulations of mechanosensory or olfactory signaling capability revealed drastically different effects. In groups of males, courtship behaviors can be triggered by playback of courtship song (8). However, we found that in cut-wing flies, which had

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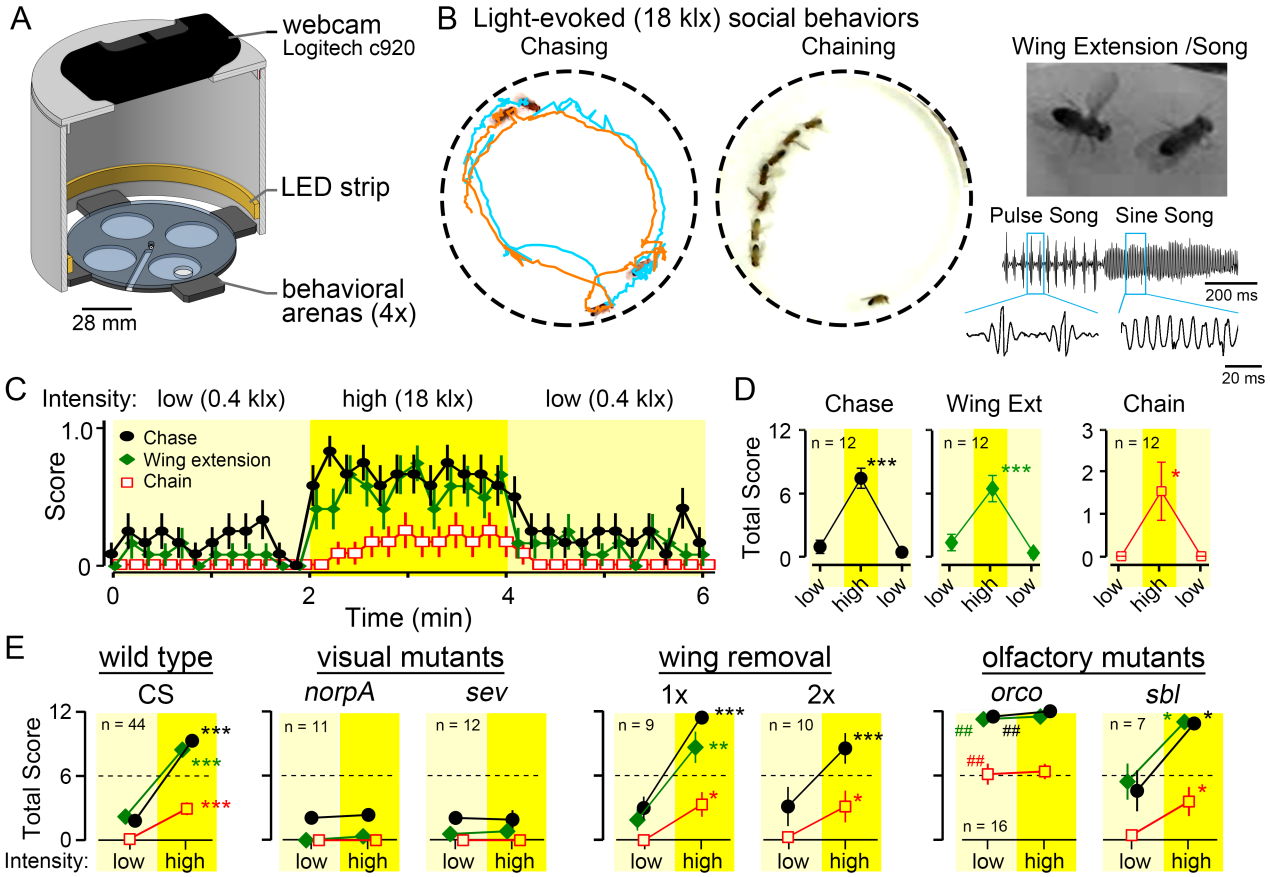

Fig. 1. Light-evoked courtship behavior in *Drosophila* (A) Schematic drawing of the behavioral arena, LED strip lighting and webcam configuration. (B) Intense light triggers indiscriminate male courtship behaviors including chasing (left, orange track chasing cyan track), chaining (middle), wing extensions and courtship song (right). Courtship songs were recorded in a modified arena equipped with a microphone (see Methods). (C) Quantification of light-induced courtship behaviors. Eight males were subjected to 2-min low light intensity period, followed by 2-min of high intensity light, and a 2-min low-light recovery period. The presence or absence of respective behaviors was scored during successive 10-s intervals (see Methods). (D) The average total score over the 2-min periods for the respective behaviors shown in C. (E) Light-triggered courtship behaviors in WT flies, visual mutants (*norpA* and *sev*), wing-removed flies and olfactory mutants (*orco* and *sbl*). Note the light-induced increase in courtship behavior is absent in visual mutants, but unimpaired in flies with one or both wings removed. Olfactory mutants show greatly enhanced courtship activity, with orco attaining maximum scores even at low-light intensity. Sample sizes indicate number of arenas. For (D-E) data are shown as mean +/- SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, paired t-test; ## $p < 0.01$, one way ANOVA, post-hoc t-test compared to WT.

disrupted wing mechanosensory inputs and were unable to produce courtship song, intense light reliably triggered chasing and chaining (Figure 1E). To determine the role of olfactory systems, we examined light-evoked courtship in two mutants, *orco* (encoding a requisite olfactory receptor subunit) and *smellblind* (*sbl*, a *para* Na^+ channel allele with disrupted odor-evoked behaviors). Surprisingly, at low light intensities, both mutants displayed strikingly elevated courtship behaviors compared to WT males (Figure 1E). Remarkably, recurrent male-male courtship in *orco* flies was so prevalent (Movie 1, Figure S1F), reaching the maximum score of 12, such that we could not discern increased courtship during the intense light periods. However, in *sbl* males, intense-light further promoted courtship activities to attain the maximum score, indicating a strong interaction between intense light and olfactory processes in gating male courtship behaviors (Figure 1E).

Together, our findings indicate light plays at least two roles in courtship behavior in *Drosophila melanogaster*. Besides facilitating visually-guided behaviors (9), light serves as a potent modulator promoting courtship. In a minimal, but straightforward model previously proposed by Wang et al. (10), male flies have an intrinsic drive to court that is normally restrained by proper olfactory processing of signals from other males (e.g. cis-vaccenyl acetate (2)). Intense light, likely involving the UV/blue visual channels, serves to unleash the intrinsic courtship repertoire by supercharging the courtship motor program to overcome olfactory inhibition. Alternatively, intense light may impact olfactory processing to nullify its inhibitory action. Although neuronal circuitry underlying courtship behavior in flies has been intensely examined, further studies are required to map out the precise functional circuitry generating intrinsic courtship drive and integrating inhibitory olfactory or excitatory light-triggered cues.

Methods

***Drosophila* stocks.** All flies were reared at 23 C (room temperature) under a 12:12 light-dark cycle in vials containing cornmeal media (11, 12). All flies studied came from the Wu Lab collection with ages specified. Throughout the report, WT strain used was *Canton-S* (except for Figure S1D,

where WT-*Berlin* was used). Other mutant lines studied included: *smellblind* (sbl^1, sbl^2) mutant alleles of the voltage-gated sodium channel gene *paralytic* (gift from J. Carlson (13, 14)); $orco^1$ encoding the olfactory co-receptor channel (Bloomington #23129 (15)); $norpA^{P12}$ encoding a phospholipase C required for visual transduction (gift from W.L. Pak (16)); and $sevenless^{LY3}$ (sev^{LY3}) lacking R7 photoreceptors (gift from W.L. Pak (17)).

Courtship behavioral recording. Fly courtship behavior was observed in a custom-built chamber which consisted of an arena piece, and arena cap, and an enclosed outer light chamber (Figure 1A). The arena piece consisted of a plexiglass sheet (2 mm thick) with four milled circular chambers referred to as "Regions of Interest" or ROIs, (28 mm diameter) seated on filter paper (Whatman #1) forming individual 2 mm-high behavioral arenas. The arena cover was constructed from a plexiglass sheet of the same specification. The light chamber was constructed from a 4" PVC pipe coupler (NIBCO #4801) with a custom milled PVC lid. A 21 LED strip (STN-A30K80-A3A-10B5M-12V, SuperBrightLEDs.com) was glued along the inner rim next to the base. LEDs were powered by a custom-built current-controlled power supplies. Fly behaviors were captured by a Logitech c920 webcam connected to a PC. For microphone recordings (Figure 1B), a modified chamber was constructed with a hole drilled in the side of the chamber. A high-gain microphone (PA-3-IL, see (18)) was placed in the opening to pick up fly sounds.

Behavioral recordings were performed by aspirating flies (4 or 8, as specified) into two ROIs of behavior arena (without anesthesia). Following an acclimatization period ($\sim$4 min), flies were recorded for 2 min under 'low light' conditions, immediately followed by 2-min of 'high light' conditions. In some experiments (Figure 1C-D), we included a 2-min 'low light' recovery period.

Quantification and Statistical Analysis. Behavioral scoring was carried out in groups of 8 males confined in the arena for the 2-min observation periods. (For male-female courtship, 4 males and 4 females were used). Criteria for chasing and wing extension behaviors were based on (Hall 1994 (1)), and we define chaining behavior as the formation of a mobile chain of 4 or mor flies (cf. Zhang & Odenwald (3) and Hing & Carlson (4)). Scoring was performed by observers who were blind to the genotype. For each successive 10-s intervals of a video, the presence/absence of each of these behaviors within an ROI was scored during a 5-s window (i.e., 5-s observation of left ROI followed by 5-s observation of right ROI). The total number of 5-s windows (out of 12 possible windows) in which the respective behaviors were observed, reported as the "total score," ranging from 0-12. Automated analysis of walking kinematics was performed offline with IowaFLI Tracker as described in Iyengar et al. (7). IowaFLI Tracker is freely available at https://github.com/IyengarAtulya/IowaFLI_tracker/releases

All statistical analyses were performed in MATLAB r2021b. Unless otherwise indicated, all significance values are reported from paired t-tests comparing the 'low' vs 'high' light conditions.

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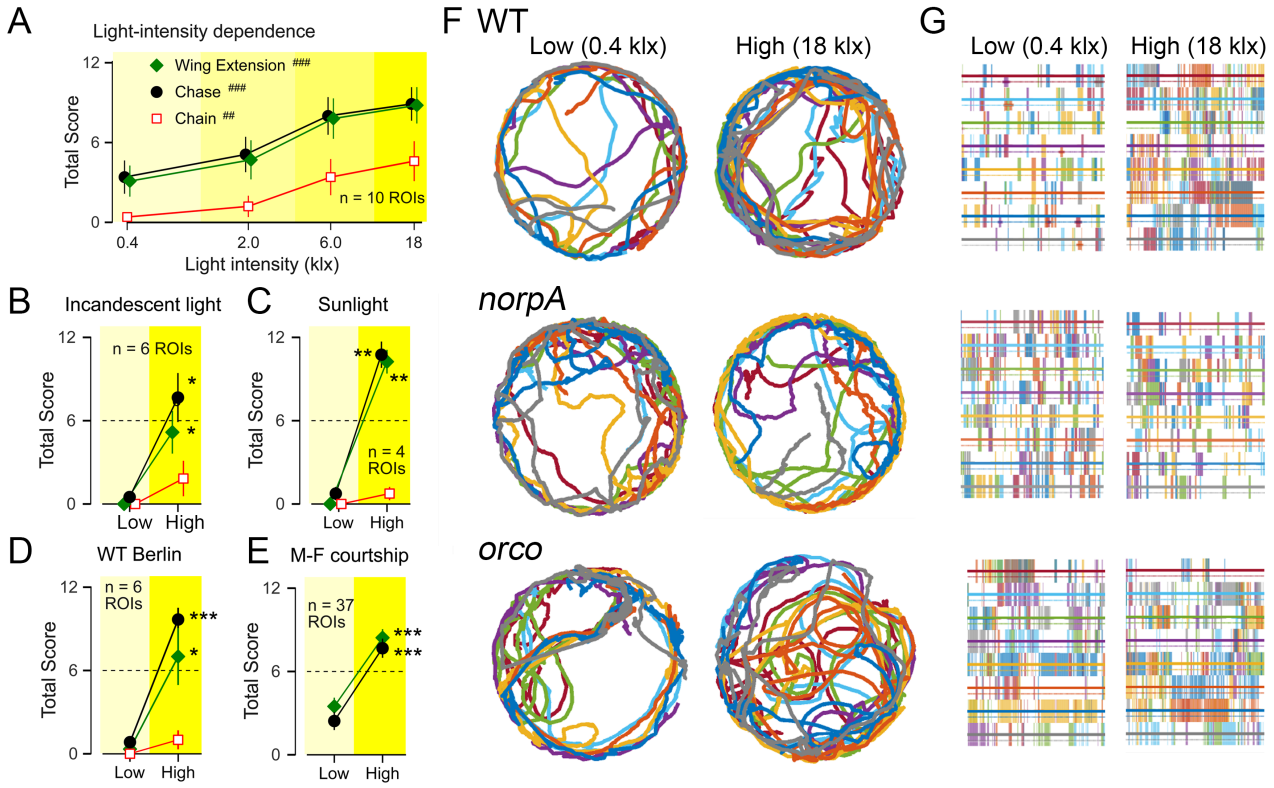

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	WT		norpA		orco	
Intensity:	**Low**	**High**	**Low**	**High**	**Low**	**High**
Distance (mm)	132 (17)	190 (10)	510 (20)	629 (36)	372 (22)	420 (13)
Velocity (mm/s)	4.1 (0.2)	4.5 (0.1)	5.7 (0.2)	6.8 (0.3)	9.0 (0.4)	9.7 (0.3)
% Time Moving	63 (8)	83 (2)	89 (1)	92 (2)	91 (2)	96 (1)

Fig. S1. Supplemental findings regarding light-evoked courtship behavior in *Drosophila* (A) Light intensity versus courtship activity. The total score over a 2-min period at the respective light intensity. The three behaviors scored varied monotonically against light intensity; ## $p < 0.01$, ### $p<0.001$ Page's L Trend Test. (B) Courtship induced by incandescent light. The LED strip was replaced by an incandescent source (USHIO EKZ 10.8V 30W; 'low' intensity: 0.4 klx; 'high' intensity: 25 klx). (C) Courtship induced by sunlight. Behavioral arena was placed on the ground in direct sunlight (∼90 klx). During the 'low' phase the arena was moved to a shaded area (∼0.4 klx). Experiments were done in the Biology Building courtyard on the University of Iowa Campus on 05\19\22 at 1500 CDT. (D) Light-triggered courtship in WT Berlin males. (E) Light-triggered courtship in arenas with 4 male and 4 female flies. Note both wing extension frequency and chasing increased during intense light. Chaining was not observed during either condition. (F) Representative tracks from IowaFLI Tracker of fly movement during low and high light condition from WT, *norpA*, and *orco* mutants. Track duration: 24 s. Note the chasing activity in WT under high light conditions, and in *orco* mutants under both low and high light conditions. (G) Representative intractograms during low and high light periods in the respective genotypes. Note the increase in sustained interactions in WT during high light and in *orco* during low and high light conditions. (H) Table of aggregate kinetic and interaction parameters during 2-min low and high light periods in the respective genotypes. Parameters were quantified by IowaFLI Tracker as described in Iyengar et al. (7).