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Neuroendocrine Control of *Drosophila* Larval Light Preference

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Abstract

Animal development is coupled with innate behaviors that maximize chances of survival. Here we show that the prothoracicotropic hormone (PTTH), a neuropeptide that controls the developmental transition from juvenile stage to sexual maturation, also regulates light avoidance in *Drosophila melanogaster* larvae. PTTH, through its receptor Torso, acts on two light sensors, the Bolwig's organ and the peripheral class IV dendritic arborization neurons, to regulate light avoidance. We find that PTTH concomitantly promotes steroidogenesis and light avoidance at the end of larval stage, thereby driving animals towards a darker environment to initiate the immobile maturation phase. Thus, PTTH controls the decisions of when and where animals undergo metamorphosis, optimizing conditions for adult development.

Animal development is associated with multiple primitive, innate behaviors, allowing inexperienced juveniles to choose an environment that maximizes their survival fitness before the transition to adulthood. In insects this transition is timed by a peak of ecdysone production induced by the prothoracicotropic hormone (PTTH) (1). In the larval brain of *Drosophila*, PTTH is produced by two pairs of neurosecretory cells projecting their axons onto the prothoracic gland (PG) where ecdysone is produced (2, 3). Transition to adulthood is associated with drastic changes in larval behavior: feeding larvae remain buried in the food, whereas wandering larvae (at the end of larval development) crawl out and find a spot where they immobilize and pupariate (4, 5). Mechanisms allowing proper coordination of these behavioral changes with the developmental program remain elusive.

Two pairs of neurons in the central brain were recently reported to control larval light avoidance (6). Using specific anti-PTTH antibodies, we established that these neurons labeled by the *NP0394-Gal4* and *NP0423-Gal4* lines correspond to the PTTH-sexpressing neurons (Fig. 1A and S1). Moreover, silencing the *ptth* gene using *NP0423-Gal4* or a ubiquitous driver (*tub-Gal4*) impaired light avoidance (Fig. 1B and S2), indicating that PTTH itself controls this behavior. PTTH activates Torso, a receptor tyrosine kinase whose

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knockdown in the PG prevents ecdysone production and induces a developmental delay ((7) and Fig. S3A–C). By contrast, knocking down *torso* in the PG (as shown in (7)) did not cause any change in light avoidance (Fig. 1B), indicating that the role of PTTH in ecdysteroidogenesis is functionally distinct from its role in light avoidance behavior.

Since in *Drosophila* the PTTH-producing neurons only innervate the PG (2, 3) (see also Fig. 1A and S2), we reasoned that PTTH is secreted into the hemolymph and reaches the cells or organs involved in light avoidance. Consistent with this, inactivation of PTTH-expressing neurons affects light avoidance with 8–10 h delay (Fig. 2A and S4), arguing against PTTH neurons projecting directly on their target cells to control light avoidance. PTTH peptide is present in the PTTH-expressing neurons throughout larval development (Fig. 2B and S5) and shows a significant increase before wandering (Fig. 2B), correlating with the rapid increase of ecdysteroidogenesis at this stage (3, 8). Using an ELISA assay, we found that PTTH is readily detected in the hemolymph with a fluctuation pattern similar to that of its accumulation in the PTTH-expressing neurons (Fig. 2C). Furthermore, hemolymph PTTH levels were significantly decreased upon RNAi-mediated knockdown of *ptth* in the PTTH-expressing neurons (Fig. 2C), suggesting that, in addition to the paracrine control of ecdysteroidogenesis in the PG, PTTH also carries endocrine function.

Pan-neuronal knockdown of *torso* (*elav>torso-RNAi*^{GD}) recapitulated the loss of light avoidance observed upon *torso* ubiquitous knockdown (*tub>torso-RNAi*^{GD}) (Fig. 3A), suggesting that PTTH acts on neuronal cells to control light avoidance. We specifically tested the potential role of torso in two neuronal populations previously identified as light sensors in *Drosophila* larvae (5): the Bolwig's organ (BO) (9, 10) and the class IV dendritic arborization (da) neurons tiling the larval body wall (11). An enhancer trap analysis of *torso*, as well as *in situ* hybridization using a *torso* antisense probe, confirmed *torso* expression in class IV da neurons (Fig. S6A). In parallel, *torso* transcripts were detected by quantitative RT-PCR in larval anterior tips containing the BO, and their levels were efficiently knocked down using the BO-specific drivers *Kr5.1-Gal4* (12) and *Rh5-Gal4* (10), demonstrating *torso* expression in the BO (Fig. S6B and C). The knockdown of *torso* in the BO (*Kr5.1>torso-RNAi*^{GD} and *GMR>torso RNAi*^{GD}) or in the class IV da neurons (*ppk>torso-RNAi*^{GD}) (11, 13) abolished larval light avoidance (Fig. 3A, motoneurons serve as a negative control: *OK6>torso-RNAi*^{GD} (14)). Knocking down *torso* in both neuronal populations (*ppk>, GMR>torso-RNAi*^{GD}) mimicked the effect observed with the BO driver or class IV da neuron driver alone. A similar loss of light avoidance was observed when these neurons were separately inactivated by expressing the hyperpolarizing channel Kir2.1 (*GMR>Kir2.1* and *ppk>Kir2.1*) (Fig. 3A), suggesting that both of these light sensors are necessary for light avoidance behavior. Downregulation of PTTH/Torso signaling did not lead to any neuronal morphology or locomotion defect (Fig. S7, S8A and B), further indicating its direct effect on light sensing. Importantly, the knockdown of *torso* in class IV da neurons or in the BO had no effect on the pupariation timing (Fig. S3A). Taken together, these results indicate that PTTH/Torso signaling is required for light avoidance behavior in two distinct populations of light-sensing neurons, and that this function is separate from its role in controlling developmental progression.

Drosophila light-sensing cells use photosensitive opsins that, upon exposure to light, activate transient receptor potential (TRP) cation channels, thus depolarizing the membrane and triggering neural activation (15). Although the BO and class IV da neurons use different photosensitive molecules and TRP channels (5), one can assume that PTTH/Torso signaling regulates the phototransduction pathway through a similar mechanism in both types of neurons. Immunohistochemical detection of Rh5, the opsin involved in light avoidance behavior in the BO (5), showed no difference in protein level in *torso* mutant background (Fig. S8A–C). PTTH/Torso signaling knockdown did not change the expression level of

Gr28b, a gustatory receptor family gene that plays an opsin-like role in class IV da neurons (11) (Fig. S8D). These results strongly suggest that PTTH affects signaling components downstream of the photoreceptors.

We next investigated the neural activity of the light sensors using the calcium indicator GCaMP3 for live calcium imaging. *torso* mutant class IV da neurons showed a 25% reduction of their response to light compared to control (Fig. 3B and C). This was accompanied by a loss of light avoidance (Fig. 3D), indicating that such partial reduction of the GCaMP3 signal corresponds to a reduction of neural activity strong enough to exert a behavioral effect. Indeed, blocking the firing of class IV da neurons using *TrpA1-RNAi* (11) caused a similar 25% reduction of the GCaMP3 signal (Fig. 3B and C) and behavioral effect (Fig. 3A). This suggests that in da neurons, PTTH/Torso signaling exerts its action upstream of TrpA1 channel activation. Accordingly, we observed a strong genetic interaction between *torso* and *TrpA1* mutants for light preference (Fig. 3D). We also detected a genetic interaction between *torso* and *Rh5* mutants (Fig. 3D), further supporting that PTTH/Torso signaling affects a step in phototransduction between the photoreceptor molecule and the TRP channel. Collectively, these data are consistent with the notion that PTTH/Torso signaling acts to facilitate TRP activation downstream of photoreceptor-dependent light sensing.

A previous study suggested that larval photophobic behavior diminishes at the end of larval development, perhaps facilitating larval food exit and entry into the wandering phase (16). Our present finding and the increase of PTTH at the beginning of wandering stage (Fig. 2B and C) appears to contradict such hypothesis. Indeed, we detected a sustained larval light avoidance mediated by PTTH that persisted through the wandering stage (Fig. 4A). These results imply that wandering behavior is triggered by a signal distinct from light preference. Consistent with this notion, the timing of wandering initiation in *ppk>torso-RNAi*^{GD} or *Kr5.1>torso-RNAi*^{GD} larvae was found comparable to that of control animals (Fig. S3D), despite the fact that these animals are not photophobic (Fig. 3A).

As found in other insects, wandering is either directly or indirectly triggered by PTTH-induced ecdysone production (17). Therefore, concomitant PTTH-mediated photophobicity could ensure that wandering larvae maintain a dark preference for pupariation site, thereby providing better protection from predators and dehydration during the immobile pupal stage. To test this hypothesis, we developed a light/dark preference assay for pupariation (Fig. S9). When exposed to a light/dark choice, larvae indeed showed a strong preference to pupariate in the dark (Fig. 4B). This behavior was abolished either by inactivating PTTH-expressing neurons (*ptth>Kir2.1*), silencing *ptth* in the PTTH-expressing neurons (*NP0423>ptth-RNAi, dicer2*) or in a *torso* mutant background (*torso[e00150]/[1]*) (Fig. 4B). Importantly, dark site preference for pupariation was observed in *Drosophila* populations collected in the wild (Fig. 4C), confirming that this innate behavior was selected in a natural environment.

In conclusion, our work illustrates the use of a single biochemical messenger, PTTH, for the concomitant control of two major functions during larval development (Fig. 4D). PTTH establishes a neuroendocrine link between distinct neuronal components previously shown to be involved in light avoidance. In contrast to previous interpretations (16) but consistent with another study (18), we show that wandering is independent of light preference and that PTTH maintains a strong light avoidance response through to the time of pupariation. High-levels of circulating PTTH during the wandering stage could reinforce the robustness of light avoidance, which might otherwise be compromised by active roaming. This eventually promotes larvae to pupariate in the dark, a trait potentially beneficial for ecological selection. PTTH is thus at the core of a neuroendocrine network, promoting developmental progression and appropriate innate behavioral decisions to optimize fitness and survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References and Notes

1. Yamanaka N, Rewitz KF, O'Connor MB. Ecdysone control of developmental transitions: lessons from Drosophila research. Annu Rev Entomol. 2013; 58:497. [PubMed: 23072462]
2. Siegmund T, Korge G. Innervation of the ring gland of Drosophila melanogaster. J Comp Neurol. 2001; 431:481. [PubMed: 11223816]
3. McBrayer Z, et al. Prothoracicotropic hormone regulates developmental timing and body size in Drosophila. Dev Cell. 2007; 13:857. [PubMed: 18061567]
4. Bodenstein, D. Biology of Drosophila. Demerec, M., editor. John Wiley & Sons, Inc; New York: 1950. p. 275-367.
5. Keene AC, Sprecher SG. Seeing the light: photobehavior in fruit fly larvae. Trends Neurosci. 2012; 35:104. [PubMed: 22222349]
6. Gong Z, et al. Two pairs of neurons in the central brain control Drosophila innate light preference. Science. 2010; 330:499. [PubMed: 20966250]
7. Rewitz KF, Yamanaka N, Gilbert LI, O'Connor MB. The insect neuropeptide PTTH activates receptor tyrosine kinase torso to initiate metamorphosis. Science. 2009; 326:1403. [PubMed: 19965758]
8. Warren JT, et al. Discrete pulses of molting hormone, 20-hydroxyecdysone, during late larval development of Drosophila melanogaster: correlations with changes in gene activity. Dev Dyn. 2006; 235:315. [PubMed: 16273522]
9. Hassan J, Iyengar B, Scantlebury N, Rodriguez Moncalvo V, Campos AR. Photic input pathways that mediate the Drosophila larval response to light and circadian rhythmicity are developmentally related but functionally distinct. J Comp Neurol. 2005; 481:266. [PubMed: 15593374]
10. Mazzoni EO, Desplan C, Blau J. Circadian pacemaker neurons transmit and modulate visual information to control a rapid behavioral response. Neuron. 2005; 45:293. [PubMed: 15664180]
11. Xiang Y, et al. Light-avoidance-mediating photoreceptors tile the Drosophila larval body wall. Nature. 2010; 468:921. [PubMed: 21068723]
12. Schmucker D, Taubert H, Jackle H. Formation of the Drosophila larval photoreceptor organ and its neuronal differentiation require continuous Kruppel gene activity. Neuron. 1992; 9:1025. [PubMed: 1463605]
13. Grueber WB, Jan LY, Jan YN. Tiling of the Drosophila epidermis by multidendritic sensory neurons. Development. 2002; 129:2867. [PubMed: 12050135]
14. Sanyal S. Genomic mapping and expression patterns of C380, OK6 and D42 enhancer trap lines in the larval nervous system of Drosophila. Gene Expr Patterns. 2009; 9:371. [PubMed: 19602393]
15. Montell C. Drosophila visual transduction. Trends Neurosci. 2012; 35:356. [PubMed: 22498302]
16. Sawin-McCormack EP, Sokolowski MB, Campos AR. Characterization and genetic analysis of Drosophila melanogaster photobehavior during larval development. J Neurogenet. 1995; 10:119. [PubMed: 8592272]
17. Dominick OS, Truman JW. The physiology of wandering behaviour in Manduca sexta. II. The endocrine control of wandering behaviour. J Exp Biol. 1985; 117:45. [PubMed: 4067505]

18. Markow TA. Light-dependent pupation site preferences in Drosophila: behavior of adult visual mutants. Behav Neural Biol. 1981; 31:348. [PubMed: 6784709]

19. Mirth C, Truman JW, Riddiford LM. The role of the prothoracic gland in determining critical weight for metamorphosis in Drosophila melanogaster. Curr Biol. 2005; 15:1796. [PubMed: 16182527]

20. Marques G, et al. The Drosophila BMP type II receptor Wishful Thinking regulates neuromuscular synapse morphology and function. Neuron. 2002; 33:529. [PubMed: 11856528]

21. Ainsley JA, Kim MJ, Wegman LJ, Pettus JM, Johnson WA. Sensory mechanisms controlling the timing of larval developmental and behavioral transitions require the Drosophila DEG/ENaC subunit, Pickpocket1. Dev Biol. 2008; 322:46. [PubMed: 18674528]

22. Pichaud F, Desplan C. A new visualization approach for identifying mutations that affect differentiation and organization of the Drosophila ommatidia. Development. 2001; 128:815. [PubMed: 11222137]

23. Kitamoto T. Conditional modification of behavior in Drosophila by targeted expression of a temperature-sensitive shibire allele in defined neurons. J Neurobiol. 2001; 47:81. [PubMed: 11291099]

24. Yamaguchi S, Wolf R, Desplan C, Heisenberg M. Motion vision is independent of color in Drosophila. Proc Natl Acad Sci U S A. 2008; 105:4910. [PubMed: 18353989]

25. Gong Z. Behavioral dissection of Drosophila larval phototaxis. Biochem Biophys Res Commun. 2009; 382:395. [PubMed: 19285485]

26. Chou WH, et al. Identification of a novel Drosophila opsin reveals specific patterning of the R7 and R8 photoreceptor cells. Neuron. 1996; 17:1101. [PubMed: 8982159]

27. Pasco MY, Leopold P. High sugar-induced insulin resistance in Drosophila relies on the lipocalin neural lazarillo. PLoS ONE. 2012; 7:e36583. [PubMed: 22567167]

28. Chavez VM, et al. The Drosophila disembodied gene controls late embryonic morphogenesis and codes for a cytochrome P450 enzyme that regulates embryonic ecdysone levels. Development. 2000; 127:4115. [PubMed: 10976044]

29. Macleod GT, Hegstrom-Wojtowicz M, Charlton MP, Atwood HL. Fast calcium signals in Drosophila motor neuron terminals. J Neurophysiol. 2002; 88:2659. [PubMed: 12424301]

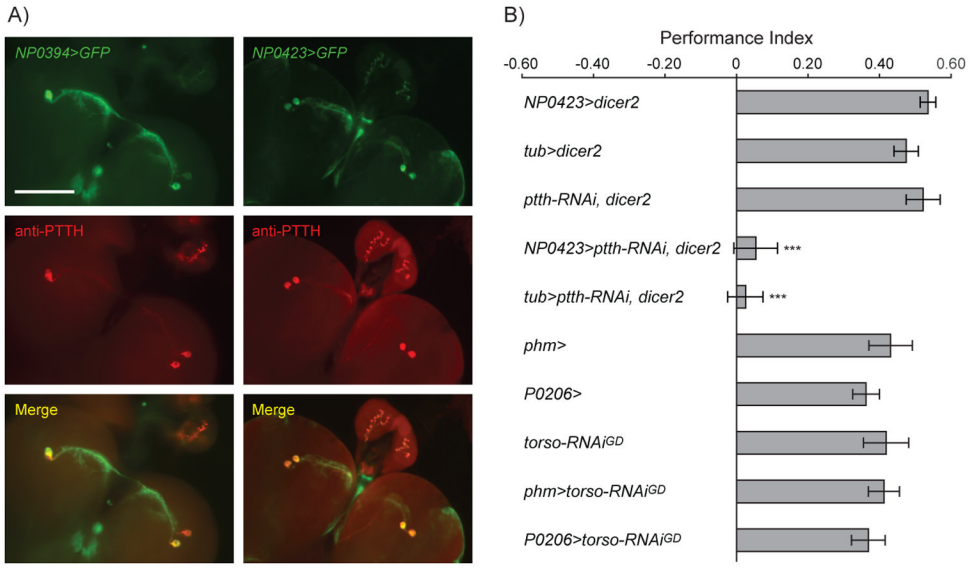

Fig. 1. PTTH controls *Drosophila* light preference

(A) Wandering third instar larval brain expressing *CD8-GFP* under the control of *NP0394-Gal4* or *NP0423-Gal4* drivers (green) were stained with anti-PTTH antibody (red). Scale bar, 100μm. **(B)** Foraging larvae were tested in a light/dark assay. n ≥ 15 tests. For statistical analyses in all figures, see Supplementary Materials.

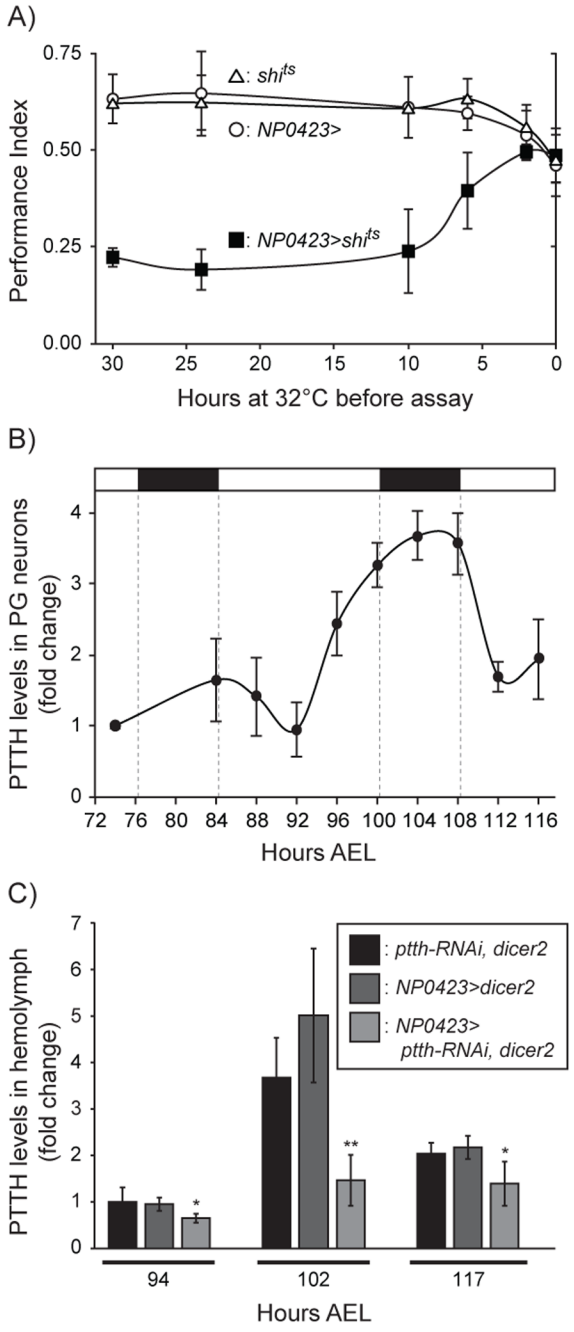

Fig. 2. Endocrine function of PTTH

(A) *NP0423>*, *UAS-shi^{ts}* and *NP0423>shi^{ts}* third instar larvae were tested in a light/dark assay after being exposed to *Gal80^{ts}* restrictive temperature (32°C) for the indicated times. n ≥ 9 tests for each time point. **(B)** PTTH protein levels in the cell bodies of PTTH-expressing neurons, measured from confocal images of wild type larval brains stained with anti-PTTH. Larvae were raised on 16/8h LD cycles, with dark periods highlighted in black. Quantification is in arbitrary units relative to the 74 h after egg laying (AEL) time point. **(C)** Hemolymph level of PTTH at given time points AEL was determined by an ELISA assay using anti-PTTH.

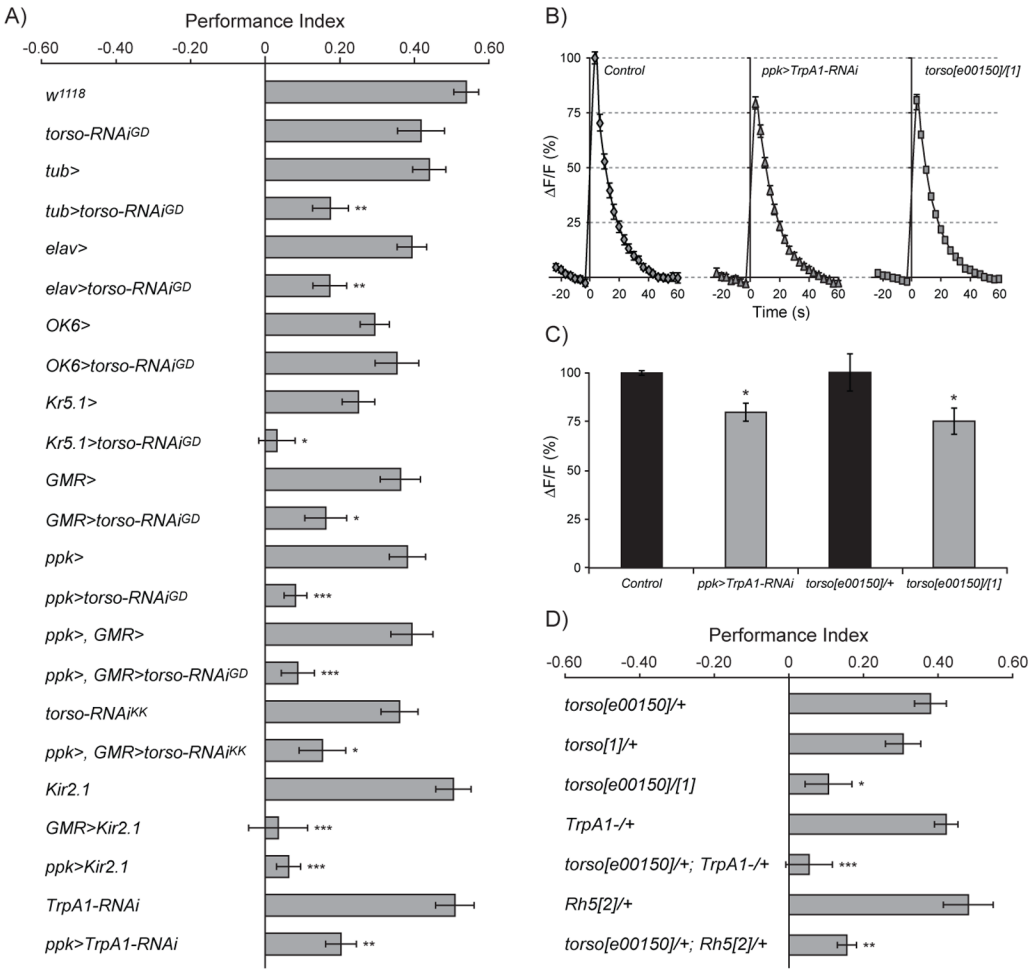

Fig. 3. PTTH/Torso signaling promotes light sensing in the BO and the class IV da neurons

(A) Light/dark preference of third instar larvae assayed in tissue-targeted RNAi or *Kir2.1* overexpression larvae. n ≥ 15 tests. **(B)** Activation of GCaMP3-expressing class IV da neurons after 2-second blue light (470 nm) illuminations (t=0). **(C)** Quantification of the maximum somatic fluorescence (ΔF/F) of GCaMP3-expressing class IV da neurons. n > 20 neurons. **(D)** Light/dark preference of third instar larvae carrying *torso*, *TrpA1* and/or *Rh5* mutations. n ≥ 15 tests.

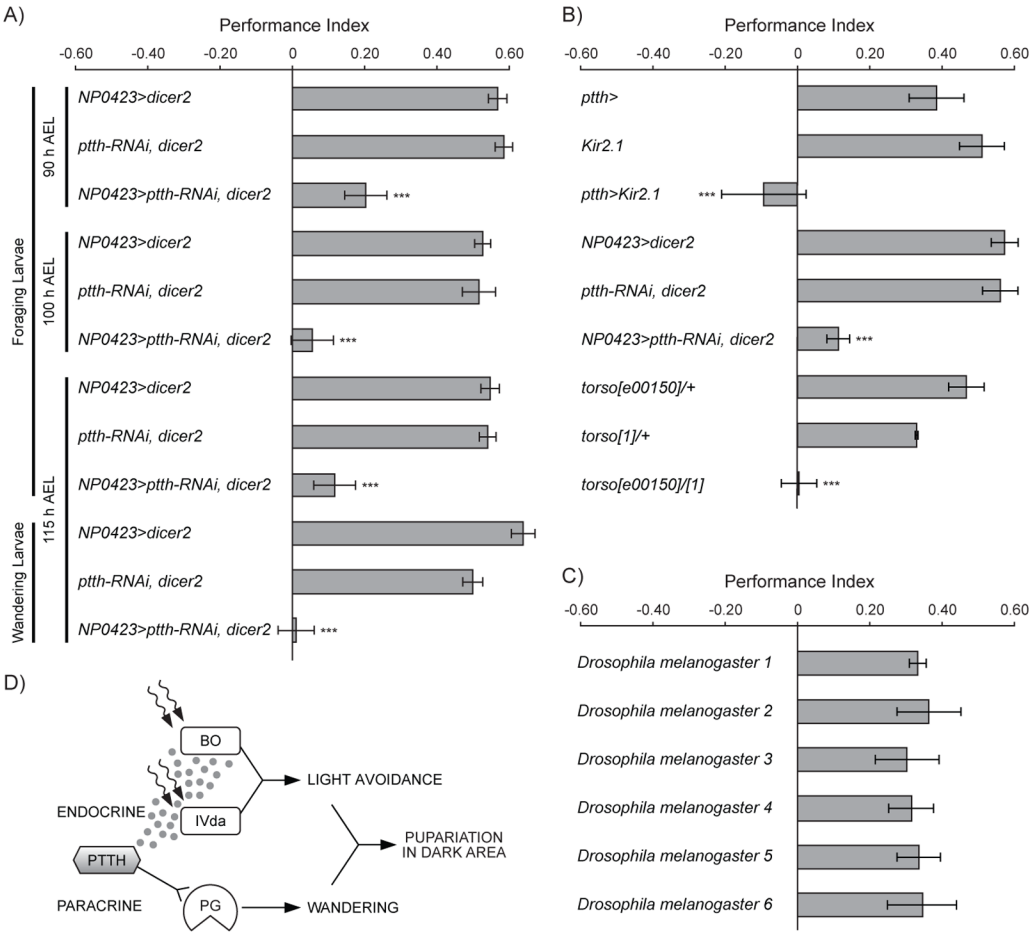

Fig. 4. PTTH/Torso signaling promotes light avoidance during the entire larval period, allowing animals to move to a dark place for pupal development

(A) Foraging and wandering larvae with indicated genotypes were tested in a light/dark assay. n ≥ 9 tests. **(B)** Light/dark preference for pupariation assayed on animals with indicated genotypes. n ≥ 9 tests. **(C)** Light/dark preference for pupariation in six different isogenic wild *Drosophila melanogaster* lines. n ≥ 7 tests. **(D)** Schematic representation of the two separate PTTH/Torso signaling functions at pupariation.