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The influence of light on temperature preference in *Drosophila*

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Abstract

Ambient light affects multiple physiological functions and behaviors, such as circadian rhythms, sleep-wake activities, and development from flies to mammals [1–6]. Mammals exhibit a higher body temperature when exposed to acute light compared to when they are exposed to dark, but the underlying mechanisms are largely unknown [7–10]. The body temperature of small ecotherms, such as *Drosophila*, rely on the temperature of their surrounding environment and these animals exhibit a robust temperature preference behavior [11–13]. Here, we demonstrate that *Drosophila* prefer a one-degree higher temperature when exposed to acute light rather than dark. This acute light response, light dependent temperature preference (LDTP), was observed regardless of the time of day, suggesting that LDTP is regulated separately from the circadian clock. However, screening of eye and circadian clock mutants suggests that the circadian clock neurons, posterior dorsal neurons 1 ($DN1_p$s) and *pigment-dispersing factor receptor (pdfr)* play a role in LDTP. To further investigate the role of $DN1_p$s in LDTP, *pdfr* in $DN1_p$s was knocked down, resulting in an abnormal LDTP. The phenotype of the *pdfr* mutant was sufficiently rescued by expressing *pdfr* in $DN1_p$s, indicating that *pdfr* expression in $DN1_p$s is responsible for LDTP. These results suggest that light positively influences temperature preference via the circadian clock neurons, $DN1_p$s, which may result from the integration of light and temperature information. Given that both *Drosophila* and mammals respond to acute light by increasing their body temperature, the effect of acute light on temperature regulation may be conserved evolutionarily between flies and humans.

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Contributions

F.N.H., L.M.H., and X.T designed the research and F.N.H., L.M.H., X.T., Y.U., J.R.L., M.F., T.G., and S.E.H. performed the behavioral experiments. X.T performed immunostaining. F.N.H., E.C.C., and P.A.G. created DH31 mutants. F.N.H., L.M.H., and X.T. wrote the manuscript.

Keywords

Temperature preference; body temperature; circadian rhythm; light; pdfr; *Drosophila*

RESULTS

Acute light positively influences temperature preference *in Drosophila*

Drosophila exhibit robust temperature preference behavior. Not only do flies avoid noxious temperatures [11, 12, 14–17], they also exhibit a temperature preference rhythm (TPR) in which preferred temperature is lower in the morning and higher in the evening [18]. We previously observed that flies entrained with light and dark (LD) cycles prefer a higher temperature than flies in free-run (constant darkness (DD)) during the daytime [18], suggesting that acute light may affect the temperature preference in flies.

To determine whether acute light influences the selection of preferred temperature in *Drosophila*, we performed behavioral experiments to compare their preferred temperatures when ambient light was ON verses when ambient light was OFF. We found that wild type (w^{1118}: WT) flies preferred ~1 °C higher temperature in the light compared to their temperature preference in the dark (Fig. 1A), suggesting that acute light positively influences the selection of preferred temperature. We refer to this behavior as light-dependent temperature preference (LDTP) and investigated the neural circuits that regulate this behavior.

LDTP is controlled separately from the circadian clock

To determine whether LDTP is observed regardless of the time of day, we tested the temperature preference behavior at different time points throughout the day (Fig. 1A and B). We found that the flies consistently preferred a higher temperature throughout the day when the behavioral assays were performed in the light (Fig. 1A and B). In the same way, the flies consistently preferred a lower temperature throughout the day when the behavioral assays were performed in the dark, although preferred temperatures at ZT 19–21 were similar (Fig. 1B). These data suggest that LDTP occurs irrespective of the circadian clock.

To confirm that LDTP is independent of circadian clock function, we examined LDTP in mutants for *period* (*per*) and *timeless* (*tim*), which disrupt the circadian clock. If LDTP is independent of the circadian clock, per^{01} and tim^{01} mutants should still exhibit LDTP. We found that per^{01} and tim^{01} mutants exhibited a normal LDTP and preferred a higher temperature in the light than in the dark at all time points throughout the daytime (Fig. 1C and D), with the exception of the tim^{01} mutants at ZT4-6. At this time point, the tim^{01} mutants preferred a slightly higher temperature in the light, but the difference was not statistically significant. Thus, we concluded that LDTP is regulated separately from the circadian clock and is dependent solely on light.

glass is required for LDTP

To investigate the neural circuits that regulate LDTP, we first examined the effect of eye components on LDTP. Flies have seven eye components: two compound eyes, three ocelli,

and two Hofbauer-Buchner (H-B) eyelets [19]. Subsets of eye components are abnormal in the mutant fly strains *eyes absent* (*eya*^{1}), *sine oculis* (*so*^{1}), *histidine decarboxylase* (*hdc*^{JK910}), and *glass* (*gl*^{60j}), and in flies in which the proapoptosis gene *hid* is expressed under the control of a *glass* multimer response element (*GMR-hid)* [20, 21] (Fig. 2G). We found that *eya*^{1}, *so*^{1}, *hdc*^{JK910}, and *GMR-hid* mutants all showed normal LDTP, preferring a higher temperature in the light compared to the dark (Fig. 2A). These data suggest that abnormalities in the compound eyes, ocelli and H-B eyelets do not disrupt LDTP and thus, these eye components are not essential for LDTP.

However, we found that a *null* allele of *glass*, *gl*^{60j}, had abnormal LDTP, preferring a higher temperature in the dark than in the light. Even the weak loss-offunction alleles of *glass*, *gl*^{1}, *gl*^{2} and *gl*^{3} [22], had abnormal LDTP, in which the flies preferred a similar temperature in the light and dark (Fig. 2A). To determine whether *glass* is responsible for LDTP, we used the 10 KB genomic *glass* mini-gene to rescue the *glass* mutants [22]. Both of the *gl(10kb); gl*^{3} and *gl(10kb); gl*^{60j} flies preferred significantly higher temperatures in the light than in the dark, indicating that the normal LDTP was restored and that *glass* function is required for LDTP.

Interestingly, the *gl*^{60j} mutants not only have abnormal eye components but also lack a subset of circadian clock cells, the posterior dorsal neurons 1 (DN1$_p$s) (Fig. 2G). Previous studies show that *glass* is expressed in DN1$_p$s but not in the anterior dorsal neurons 1, DN1$_a$s [21, 23]. To confirm that *glass* is expressed in DN1$_p$s, we used the DN1$_p$s driver, *Clk4.5F-Gal4* [24, 25], to label DN1$_p$s in the brain (Fig. 2B). We performed immunostaining on the *UAS-mCD8::GFP;Clk4.5F-Gal4* (*Clk4.5FGal4>UAS-mCD8::GFP*) flies using the Glass antibody and confirmed that Glass is expressed in the DN1$_p$s (Fig. 2B). Conversely, we found that *Clk4.5F-Gal4>UASmCD8:: GFP* signals were not detected in *gl*^{60j}/ *gl*^{60j} mutants (Fig. 2D) but were still present in the *gl*^{60j} /+ heterozygous control (Fig. 2C), indicating that DN1$_p$s were ablated in the *gl*^{60j} mutants. If DN1$_p$s are key neurons for LDTP, DN1$_p$s should be restored in the *gl(10kb); gl*^{60j} flies given that *gl(10kb); gl*^{60j} flies exhibit a normal LDTP (Fig. 2A). To determine this, we performed immunostaining using the Timeless (TIM) antibody and found that DN1$_p$s were restored in *gl(10kb); gl*^{60j} (Fig. 2E). In addition, as a control, we confirmed that DN1$_p$s were present in *GMR-hid* flies (Fig. 2F). These data suggested that the DN1$_p$s may be critical for LDTP.

TrpA1 and *Rhodopsin 1* are not necessary for LDTP

Transient receptor potential A 1 (TrpA1) is important for temperature preference behavior as flies use *TrpA1* to detect and avoid warm temperatures. *TrpA1* is not only a warm sensor in both larvae and adult flies [12, 26], but also is involved in light-sensing behavior in the body wall of larvae [5]. Furthermore, *Rhodopsin 1* (*Rh1*), encoded by the *neither inactivation nor afterpotential E (ninaE*) gene, is a molecular light sensor and has been suggested to regulate temperature-sensing behavior in larvae [27]. Therefore, we sought to determine whether *TrpA1* and *Rh1* were involved in LDTP by using strong loss-of-function mutants for *TrpA1* (*TrpA1*^{ins})[12] and *null* mutants for *Rh1 (ninaE*^{17})[28](Fig. 3A). However, both mutants showed normal LDTP, indicating that *TrpA1* and *Rh1* are not necessary for LDTP.

LN_vs are dispensable for LDTP

The clock neurons, small ventrolateral neurons (sLN_vs), project to DN1s [29, 30]. sLN_vs not only contact $DN1_p$s but also receive information from the light sensors, large ventrolateral neurons (lLN_vs)[31–33], and receive light inputs from the optic lobe [34]. To determine whether LN_vs are involved in LDTP, we used a mammalian inward rectifier K^+ channel (*UAS-Kir*) to genetically inhibit sLN_vs with *R6-Gal4* [34] as well as sLN_vs and lLN_vs with *Mz520-Gal4* [35, 36]. Because *R6-Gal4*/*UAS-Kir* flies did not survive to adult, we used a temperature dependent conditional repressor of *Gal4*, $tubGal80^{ts}$, to transiently inhibit the LN_vs depending on the permissive temperature (18°C) and the restrictive temperature (29°C). However, at both permissive and restrictive temperatures, the *R6-Gal4* and *Mz520-Gal4* with *UAS-Kir; tub-*$Gal80^{ts}$ flies exhibited normal LDTP (Fig. 3B), suggesting that LNvs are not important for LDTP. As positive control using locomotor activity, we showed that *Mz520-Gal4* with *UAS-Kir; tub-*$Gal80^{ts}$ flies exhibited abnormal rhythmicity at 29°C but normal rhythmicity at 18°C (Supplemental Figure S1 and Table S1).

PDFR acts in $DN1_p$s to control LDTP

Our data suggest that $DN1_p$s are critical for LDTP. Although the persistence of LDTP in *per* and *tim* mutants indicates that this behavior does not require a functional circadian clock (Fig. 1C and D), the $DN1_p$s participate in circadian clock function and thus, express many clock genes. To determine which molecules might act within the $DN1_p$ cells to control LDTP, we examined the involvement of additional clock genes, including *cryptochrome (cry)*, *Clock (Clk)*, and *pigment-dispersing factor receptor (pdfr)*, and tested LDTP of mutations in these genes: cry^b, cry^{01}, cry^{02}, Clk^{Jrk}, $pdfr^{5304}$ and $pdfr^{3369}$ (Fig. 4A). Like per^{01} and tim^{01} mutants, cry^b, cry^{01}, cry^{02} and Clk^{Jrk} mutants all preferred a higher temperature in the light than in the dark, although cry^{01} preferred a much lower temperature in the dark. Interestingly, we found that $pdfr^{5304}$ and $pdfr^{3369}$ mutants displayed abnormal LDTP, in which they preferred similar temperatures in the light and the dark, suggesting that *pdfr is* required for LDTP (Fig. 4A). PDFR is a G-protein coupled receptor and is critical for locomotor activity and synchronization of the circadian clock [37–40].

To determine whether *pdfr* expression in $DN1_p$s is necessary for LDTP, we knocked down *pdfr* in $DN1_p$s by using *UAS-pdfr-RNAi* with *Clk4.5F-Gal4*, which is selectively expressed in subsets of $DN1_p$s (Fig. 4B). *Clk4.5F-Gal4/UAS-pdfr-RNAi* flies, the flies exhibited an abnormal LDTP, showing similar preferred temperatures in the light and the dark. However, each *Gal4* and *UAS* control fly line exhibited a normal LDTP, indicating that *pdfr* expression in $DN1_p$s is necessary for LDTP (Fig. 4B).

To determine whether PDFR expression in $DN1_p$s is sufficient to rescue the $pdfr^{5304}$ mutants' phenotype, we expressed *UAS-pdfr* using *Clk4.5F-Gal4* in the $pdfr^{5304}$ mutants. The $pdfr^{5304}$ flies that expressed *pdfr* in $DN1_p$s preferred a higher temperature in the light than in the dark, while the control flies did not, indicating that *pdfr* expression in $DN1_p$s restored LDTP of $pdfr^{5304}$ mutants. Thus, PDFR expression in $DN1_p$s is necessary and sufficient to support pdfr's role in LDTP (Fig. 4C).

Because Pigment-dispersing factor (PDF) and Diuretic hormone 31 (DH31) activate PDFR *in vitro* [37], we examined whether PDF and DH31 are involved in LDTP. We used the *pdf* null mutant, pdf^{01}, and the *Dh31*mutant, $Dh31^{\#51}$, which was generated by P-element excision. $Dh31^{\#51}$ is a strong loss-of-function mutation, as it contains a deletion of the entire active peptide of DH31 (Supplemental Figures S2 and S3). Nonetheless, both pdf^{01} and $Dh31^{\#51}$ and even the double mutant of $Dh31^{\#51}$; pdf^{01} exhibited a normal LDTP, indicating that PDF and DH31 are not required for LDTP. These results suggest that LDTP mediated by PDFR in $DN1_p$s is not due to the pathway activated by these known neuropeptides.

DISCUSSION

Here, we show that acute light positively affects temperature preference in *Drosophila.* LDTP is controlled by *Pdfr* expressing $DN1_p$s independently from the circadian clock, suggesting $DN1_p$s play an important role in integrating light and temperature information.

Although we tested several eye component mutants, abnormal light or temperature sensing mutants and *cry* mutants, these mutants still exhibited LDTP behavior. Because light sensors can be redundant in the eye and body wall, partial disruption of these light sensors may not be sufficient for abnormal LDTP (Fig. 2). In fact, the double mutants of *GMR-hid/+*; cry^{01}, which lack the functions of the compound eye, ocelli, H-B eyelet and CRY, exhibited an abnormal LDTP (Fig. 4A). This result suggests that at least two pathways, such as the visual system and *cry*, act together to mediate light detection and play an important role in LDTP. Notably, in humans, 460nm light is important for an increase in body temperature during the night [10]. Therefore, it would be interesting to examine which light pathway and wavelengths are critical for LDTP.

While we show that LDTP is circadian clock independent, PDFR expression in $DN1_p$s is critical for LDTP (Fig. 4). However, it is unclear how PDFR is activated because neither PDF nor DH31, the ligands of PDFR, are important for LDTP (Fig. 4). Therefore, our data suggest that PDFR in $DN1_p$s is activated by other unknown mechanisms responsible for LDTP. One possible mechanism is CRY, because CRY is expressed in the clock cells, including $DN1_p$s, and have convergent roles with PDFR for the circadian rhythm of locomotor activity [41]. CRY also antagonizes the temperature synchronization in the dorsal neurons, suggesting that CRY may be involved in the integration of light and temperature [42]. Therefore, it is possible that CRY and PDFR work to regulate LDTP. For example, $DN1_p$s may directly receive light input via CRY, which regulates the signal cascade of PDFR.

Light is critical not only for entraining the circadian clock, but also for a behavior termed masking, in which the flies exhibit a robust increase of locomotor activity after light is turned ON or OFF [43]. The masking effect is controlled separately from the circadian clock [20, 24] and $DN1_p$s are involved in a masking effect for locomotor activity when light is ON [24]. Given that light positively affects preferred temperature separately from the circadian clock, LDTP could be part of the masking effect. However, the light input pathways for the masking effect in locomotor activity and LDTP are not the same. This is demonstrated through evidence that shows that disruption of the compound eye is sufficient for the

masking effect of locomotor activity [16], but not for LDTP (Fig. 2). Furthermore, the molecular mechanisms controlling the masking effect in locomotor activity and LDTP are different, as *Pdfr* mutants exhibit a normal masking effect for locomotor activity [20] but an abnormal LDTP (Fig. 4). Therefore, our data indicate that the masking effect of locomotor activity and LDTP are controlled differently.

Here, we show the positive effect of acute light on the preferred temperature in flies. Given that *Drosophila* adapt their body temperature to ambient temperature [13], the flies' body temperature increases in light as a result of their temperature preference behavior. In humans, light exposure increases body temperature during the nighttime [7–9] and is dependent on light intensity [10]. While humans control body temperature through the generation of heat, ectotherms use behavioral strategies to regulate body temperature [13]. Although the mechanism of heat generation is different between humans and flies, the body temperature of both humans and *Drosophila* increases when exposed to light. Thus, we propose that the effect of light on temperature regulation may be evolutionarily conserved from flies to humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Sehgal A, Mignot E. Genetics of sleep and sleep disorders. Cell. 2011; 146:194–207. [PubMed: 21784243]
2. Rao S, Chun C, Fan J, Kofron JM, Yang MB, Hegde RS, Ferrara N, Copenhagen DR, Lang RA. A direct and melanopsin-dependent fetal light response regulates mouse eye development. Nature. 2013; 494:243–246. [PubMed: 23334418]
3. LeGates TA, Altimus CM, Wang H, Lee HK, Yang S, Zhao H, Kirkwood A, Weber ET, Hattar S. Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. Nature. 2012; 491:594–598. [PubMed: 23151476]
4. Yuan Q, Xiang Y, Yan Z, Han C, Jan LY, Jan YN. Lightinduced structural and functional plasticity in Drosophila larval visual system. Science. 2011; 333:1458–1462. [PubMed: 21903815]
5. Xiang Y, Yuan Q, Vogt N, Looger LL, Jan LY, Jan YN. Light-avoidance-mediating photoreceptors tile the Drosophila larval body wall. Nature. 2010; 468:921–926. [PubMed: 21068723]
6. Mazzoni EO, Desplan C, Blau J. Circadian pacemaker neurons transmit and modulate visual information to control a rapid behavioral response. Neuron. 2005; 45:293–300. [PubMed: 15664180]
7. Myers BL, Badia P. Immediate effects of different light intensities on body temperature and alertness. Physiol Behav. 1993; 54:199–202. [PubMed: 8327605]

8. Cajochen C, Zeitzer JM, Czeisler CA, Dijk DJ. Dose-response relationship for light intensity and ocular and electroencephalographic correlates of human alertness. Behav Brain Res. 2000; 115:75–83. [PubMed: 10996410]

9. Dijk DJ, Cajochen C, Borbely AA. Effect of a single 3-hour exposure to bright light on core body temperature and sleep in humans. Neurosci Lett. 1991; 121:59–62. [PubMed: 2020391]

10. Cajochen C, Munch M, Kobialka S, Krauchi K, Steiner R, Oelhafen P, Orgul S, Wirz-Justice A. High sensitivity of human melatonin, alertness, thermoregulation, and heart rate to short wavelength light. J Clin Endocrinol Metab. 2005; 90:1311–1316. [PubMed: 15585546]

11. Hong ST, Bang S, Hyun S, Kang J, Jeong K, Paik D, Chung J, Kim J. cAMP signalling in mushroom bodies modulates temperature preference behaviour in Drosophila. Nature. 2008; 454:771–775. [PubMed: 18594510]

12. Hamada FN, Rosenzweig M, Kang K, Pulver SR, Ghezzi A, Jegla TJ, Garrity PA. An internal thermal sensor controlling temperature preference in Drosophila. Nature. 2008; 454:217–220. [PubMed: 18548007]

13. Stevenson RD. The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. The American Naturalist. 1985; 126

14. Garrity PA, Goodman MB, Samuel AD, Sengupta P. Running hot and cold: behavioral strategies, neural circuits, and the molecular machinery for thermotaxis in C. elegans and Drosophila. Genes Dev. 2010; 24:2365–2382. [PubMed: 21041406]

15. Tomchik SM. Dopaminergic neurons encode a distributed, asymmetric representation of temperature in Drosophila. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013; 33:2166–2176a. [PubMed: 23365252]

16. Ueno T, Tomita J, Kume S, Kume K. Dopamine modulates metabolic rate and temperature sensitivity in Drosophila melanogaster. PloS one. 2012; 7:e31513. [PubMed: 22347491]

17. Bang S, Hyun S, Hong ST, Kang J, Jeong K, Park JJ, Choe J, Chung J. Dopamine signalling in mushroom bodies regulates temperature-preference behaviour in Drosophila. PLoS Genet. 2011; 7:e1001346. [PubMed: 21455291]

18. Kaneko H, Head LM, Ling J, Tang X, Liu Y, Hardin PE, Emery P, Hamada FN. Circadian Rhythm of Temperature Preference and Its Neural Control in Drosophila. Current biology : CB. 2012; 22:1851–1857. [PubMed: 22981774]

19. Buschbeck EK, Friedrich M. Evolution of Insect Eyes: Tales of Ancient Heritage, Deconstruction, Reconstruction, Remodeling, and Recycling. Evolution: Education and Outreach. 2008; 1:448–462.

20. Rieger D, Stanewsky R, Helfrich-Forster C. Cryptochrome, compound eyes, Hofbauer-Buchner eyelets, and ocelli play different roles in the entrainment and masking pathway of the locomotor activity rhythm in the fruit fly Drosophila melanogaster. Journal of biological rhythms. 2003; 18:377–391. [PubMed: 14582854]

21. Klarsfeld A, Malpel S, Michard-Vanhee C, Picot M, Chelot E, Rouyer F. Novel features of cryptochrome-mediated photoreception in the brain circadian clock of Drosophila. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004; 24:1468–1477. [PubMed: 14960620]

22. Moses K, Ellis MC, Rubin GM. The glass gene encodes a zinc-finger protein required by Drosophila photoreceptor cells. Nature. 1989; 340:531–536. [PubMed: 2770860]

23. Shafer OT, Helfrich-Forster C, Renn SC, Taghert PH. Reevaluation of Drosophila melanogaster's neuronal circadian pacemakers reveals new neuronal classes. The Journal of comparative neurology. 2006; 498:180–193. [PubMed: 16856134]

24. Zhang L, Chung BY, Lear BC, Kilman VL, Liu Y, Mahesh G, Meissner RA, Hardin PE, Allada R. DN1(p) circadian neurons coordinate acute light and PDF inputs to produce robust daily behavior in Drosophila. Current biology : CB. 2010; 20:591–599. [PubMed: 20362452]

25. Zhang Y, Liu Y, Bilodeau-Wentworth D, Hardin PE, Emery P. Light and temperature control the contribution of specific DN1 neurons to Drosophila circadian behavior. Current biology : CB. 2010; 20:600–605. [PubMed: 20362449]

26. Rosenzweig M, Brennan KM, Tayler TD, Phelps PO, Patapoutian A, Garrity PA. The Drosophila ortholog of vertebrate TRPA1 regulates thermotaxis. Genes Dev. 2005; 19:419–424. [PubMed: 15681611]
27. Shen WL, Kwon Y, Adegbola AA, Luo J, Chess A, Montell C. Function of rhodopsin in temperature discrimination in Drosophila. Science. 2011; 331:1333–1336. [PubMed: 21393546]
28. O'Tousa JE, Baehr W, Martin RL, Hirsh J, Pak WL, Applebury ML. The Drosophila ninaE gene encodes an opsin. Cell. 1985; 40:839–850. [PubMed: 2985266]
29. Helfrich-Forster C. The neuroarchitecture of the circadian clock in the brain of Drosophila melanogaster. Microsc Res Tech. 2003; 62:94–102. [PubMed: 12966496]
30. Cavanaugh DJ, Geratowski JD, Wooltorton JR, Spaethling JM, Hector CE, Zheng X, Johnson EC, Eberwine JH, Sehgal A. Identification of a circadian output circuit for rest:activity rhythms in Drosophila. Cell. 2014; 157:689–701. [PubMed: 24766812]
31. Sheeba V, Gu H, Sharma VK, O'Dowd DK, Holmes TC. Circadian- and light-dependent regulation of resting membrane potential and spontaneous action potential firing of Drosophila circadian pacemaker neurons. J Neurophysiol. 2008; 99:976–988. [PubMed: 18077664]
32. Fogle KJ, Parson KG, Dahm NA, Holmes TC. CRYPTOCHROME is a blue-light sensor that regulates neuronal firing rate. Science. 2011; 331:1409–1413. [PubMed: 21385718]
33. Parisky KM, Agosto J, Pulver SR, Shang Y, Kuklin E, Hodge JJ, Kang K, Liu X, Garrity PA, Rosbash M, et al. PDF cells are a GABA-responsive wake-promoting component of the Drosophila sleep circuit. Neuron. 2008; 60:672–682. [PubMed: 19038223]
34. Helfrich-Forster C, Shafer OT, Wulbeck C, Grieshaber E, Rieger D, Taghert P. Development and morphology of the clock-gene-expressing lateral neurons of Drosophila melanogaster. The Journal of comparative neurology. 2007; 500:47–70. [PubMed: 17099895]
35. Grima B, Chelot E, Xia R, Rouyer F. Morning and evening peaks of activity rely on different clock neurons of the Drosophila brain. Nature. 2004; 431:869–873. [PubMed: 15483616]
36. Fujii S, Amrein H. Ventral lateral and DN1 clock neurons mediate distinct properties of male sex drive rhythm in Drosophila. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:10590–10595. [PubMed: 20498055]
37. Mertens I, Vandingenen A, Johnson EC, Shafer OT, Li W, Trigg JS, De Loof A, Schoofs L, Taghert PH. PDF receptor signaling in Drosophila contributes to both circadian and geotactic behaviors. Neuron. 2005; 48:213–219. [PubMed: 16242402]
38. Lear BC, Merrill CE, Lin JM, Schroeder A, Zhang L, Allada R. A G protein-coupled receptor, groom-of-PDF, is required for PDF neuron action in circadian behavior. Neuron. 2005; 48:221–227. [PubMed: 16242403]
39. Hyun S, Lee Y, Hong ST, Bang S, Paik D, Kang J, Shin J, Lee J, Jeon K, Hwang S, et al. Drosophila GPCR Han is a receptor for the circadian clock neuropeptide PDF. Neuron. 2005; 48:267–278. [PubMed: 16242407]
40. Taghert PH, Nitabach MN. Peptide neuromodulation in invertebrate model systems. Neuron. 2012; 76:82–97. [PubMed: 23040808]
41. Im SH, Li W, Taghert PH. PDFR and CRY signaling converge in a subset of clock neurons to modulate the amplitude and phase of circadian behavior in Drosophila. PloS one. 2011; 6:e18974. [PubMed: 21559487]
42. Gentile C, Sehadova H, Simoni A, Chen C, Stanewsky R. Cryptochrome antagonizes synchronization of Drosophila's circadian clock to temperature cycles. Current biology : CB. 2013; 23:185–195. [PubMed: 23333312]
43. Page TL. Masking in invertebrates. Chronobiol Int. 1989; 6:3–11. [PubMed: 2650895]

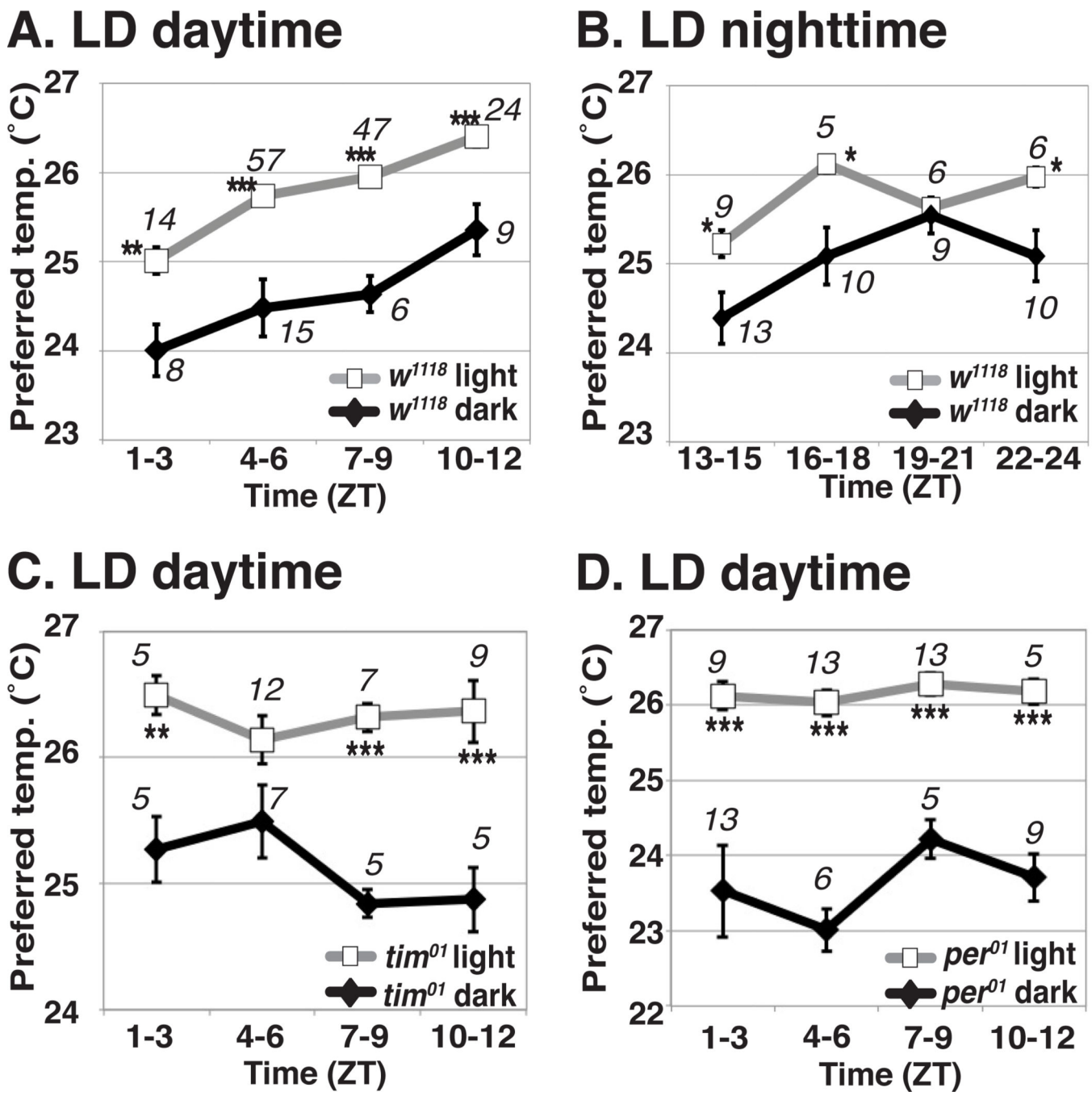

Figure 1. Acute light positively influences temperature preference *in Drosophila*

(A and B) Comparison of preferred temperature between light (gray line) and dark (black line) conditions for w^{1118} flies during the daytime (A) or nighttime (B). w^{1118} flies were raised in LD (light (12 h)-dark (12 h)) cycles. Ambient light was either ON or OFF when the behavioral experiments were performed for 30 min.

(C and D) Comparison of preferred temperature between light (gray line) and dark (black line) conditions for tim^{01} (C) and per^{01} (D) during the daytime. tim^{01} (C) and per^{01} (D) flies were raised in LD. Italicized numbers represent the number of assays. ZT, Zeitgeber Time

(ZT0 is lights-on, ZT12 is lights-off). The same behavioral data in light (A), dark (B), light (C) and light (D) from [18] are used. t-test compared preferred temperature between light and dark conditions: ***$P < 0.001$, **$P < 0.01$ or *$P < 0.05$.
Error bars are the SEM.

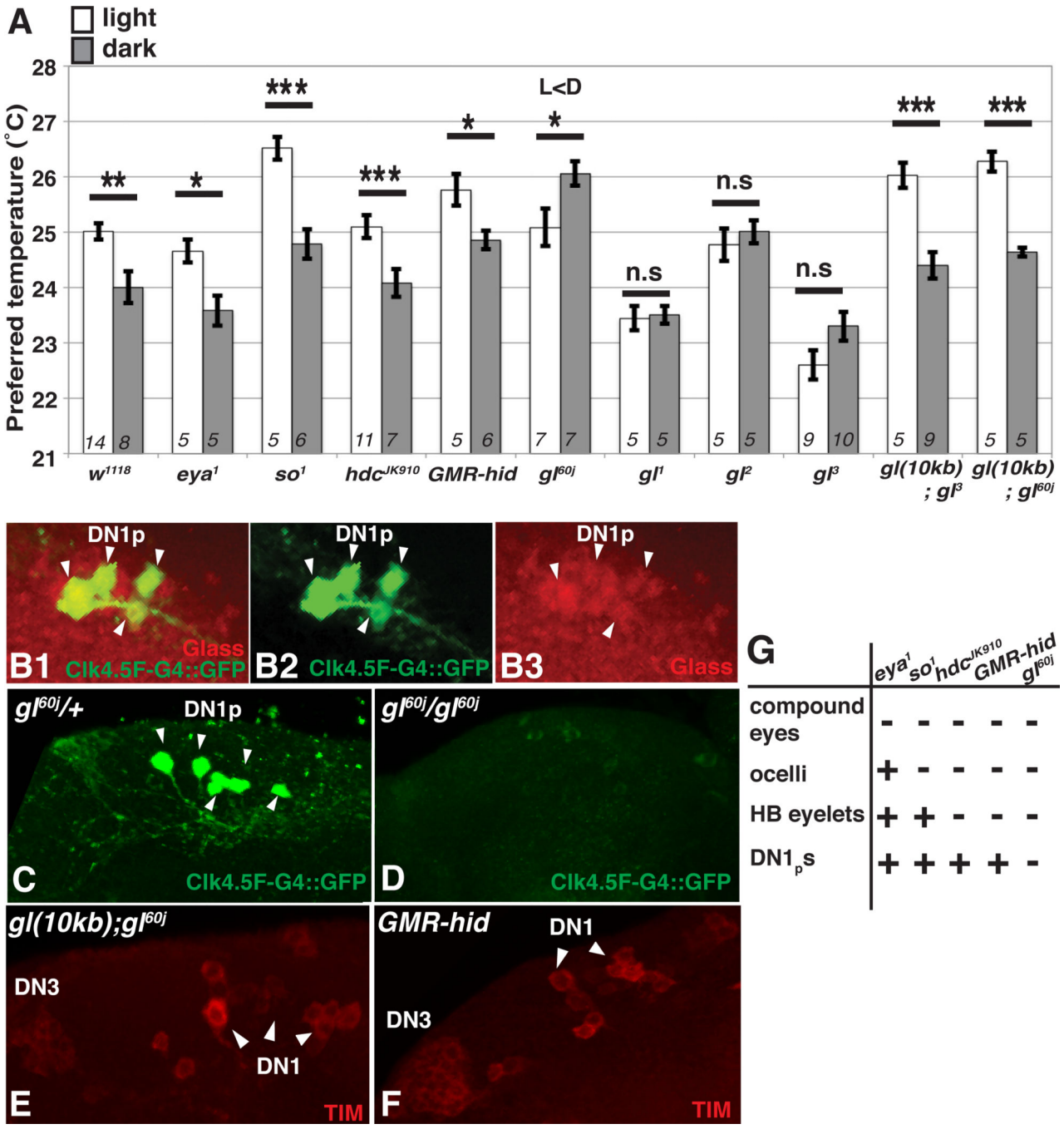

Figure 2. *glass* is required for LDTP

(A) Comparison of preferred temperature of each genotype in light (white bar) and dark conditions (gray bar). The behavioral experiments were performed at ZT1-3. t-test compared preferred temperature between light and dark conditions: ***P < 0.001, **P < 0.01 or *P < 0.05. Italicized numbers represent the number of assays. Error bars are the SEM.

(B) The *Clk4.5F-Gal4>UAS-mCD8::GFP* brains were stained with anti-GFP (green) and anti-Glass (red). The GFP signals, labeled by *Clk4.5F-Gal4>UAS-mCD8::GFP*, overlap

with the signals labeled by anti-Glass (B1). The cells for which anti-GFP and anti-Glass overlapped are shown at the arrow heads (B1-3).

(C–D) The $DN1_p$s labeled by *Clk4.5F-Gal4>UAS-mCD8::GFP* with anti-GFP (green) were still present in the $gl^{60j}/+$ heterozygous control (C) but were not detected in gl^{60j}/gl^{60j} mutants (D).

(E–F) The $gl(10kb);gl^{60j}$ (E) and *GMR-hid* (F) brains were stained with anti-TIM (red). DN1s are shown at the arrow heads.

(G) A summary of eye and $DN1_p$s phenotypes for each eye mutant fly line. +: normal; −: abnormal.

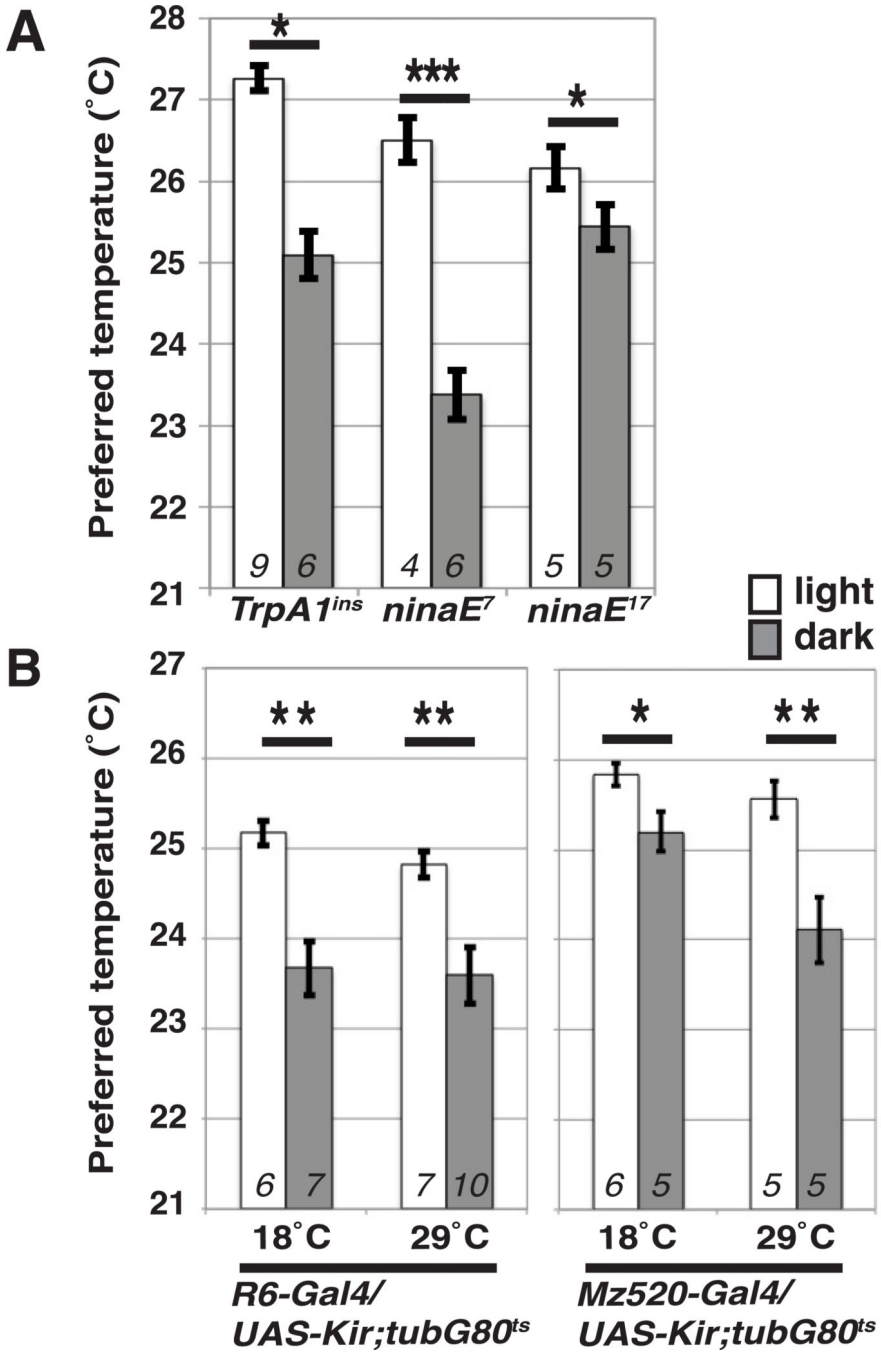

Figure 3. *TrpA1, Rhodopsin 1* and sLNvs are not critical for LDTP

(A) Comparison of preferred temperature of each genotype in light (white bar) and dark (gray bar) conditions. The behavioral experiments were performed at ZT1-3. t-test compared preferred temperature between light and dark conditions: ***P < 0.001, **P < 0.01 or *P < 0.05. Italicized numbers represent the number of assays. Error bars are the SEM.

(B) Comparison of preferred temperature of each genotype in light (white bar) and dark (gray bar) conditions. *R6-Gal4* is expressed in sLNvs and *Mz520-Gal4* is expressed in both sLNvs and lLNvs. A temperature dependent conditional repressor of *Gal4, tub-Gal80^{ts}* and a

mammalian inward rectifier K^+ channel, *Kir*, were used to transiently inhibit the sLNvs depending on permissive temperature (18°C) and restrictive temperature (29°C) as adults. The behavioral experiments were performed at ZT1-3. t-test compared preferred temperature between light and dark conditions: ***P < 0.001, **P < 0.01 or *P < 0.05. Italicized numbers represent the number of assays. Error bars are the SEM.

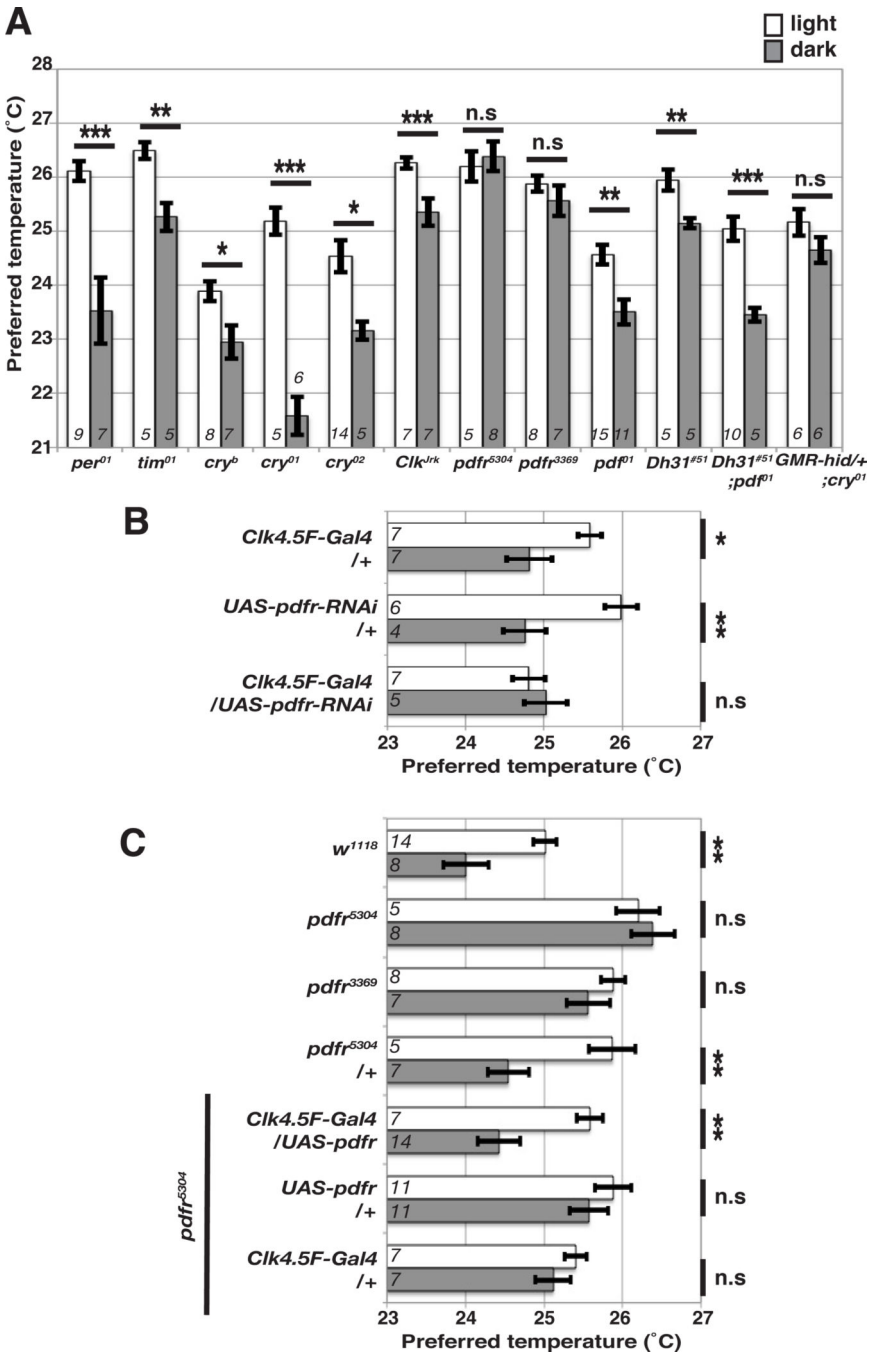

Figure 4. PDFR in $DN1_p$s is necessary and sufficient for LDTP

(A) Comparison of preferred temperature of circadian clock mutants in light (white bar) and dark (gray bar) conditions. The behavioral experiments were performed at ZT1-3. ttest compared preferred temperature between light and dark conditions: ***P < 0.001, **P < 0.01 or *P < 0.05. Italicized numbers represent the number of assays. Error bars are the SEM.

(B) PDFR expression in $DN1_p$s is necessary for LDTP.

Comparison of preferred temperature for flies with *UAS-pdfr-RNAi* in a subset of clock cells using a $DN1_p$s driver, *Clk4.5F-Gal4*, and its controls. The behavioral experiments were performed at ZT1-3. *t*-test compared preferred temperature between light and dark conditions: ***P < 0.001, **P < 0.01 or *P < 0.05. Italicized numbers represent the number of assays. Error bars are the SEM.

(C) PDFR expression in $DN1_p$s is sufficient for LDTP.

Comparison of preferred temperature of $pdfr^{5304}$ mutants with *UAS-pdfr* in a subset of clock cells using a $DN1_p$s driver, *Clk4.5F-Gal4*, and its controls. The behavioral experiments were performed at ZT1-3. t-test compared preferred temperature between light and dark conditions: ***P < 0.001, **P < 0.01 or *P < 0.05. Italicized numbers represent the number of assays. Error bars are the SEM.