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Morning and Evening Oscillators Cooperate to Reset Circadian Behavior in Response to Light Input

Pallavi Lamba,[1,2] Diana Bilodeau-Wentworth,[1] Patrick Emery,[1,2,*] and Yong Zhang[1,*]
[1]Department of Neurobiology, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605, USA
[2]Program in Neuroscience, Graduate School of Biomedical Sciences, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605, USA
*Correspondence: patrick.emery@umassmed.edu (P.E.), yong.zhang@umassmed.edu (Y.Z.)
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SUMMARY

Light is a crucial input for circadian clocks. In *Drosophila*, short light exposure can robustly shift the phase of circadian behavior. The model for this resetting posits that circadian photoreception is cell autonomous: CRYPTOCHROME senses light, binds to TIMELESS (TIM), and promotes its degradation, which is mediated by JETLAG (JET). However, it was recently proposed that interactions between circadian neurons are also required for phase resetting. We identify two groups of neurons critical for circadian photoreception: the morning (M) and the evening (E) oscillators. These neurons work synergistically to reset rhythmic behavior. JET promotes acute TIM degradation cell autonomously in M and E oscillators but also nonautonomously in E oscillators when expressed in M oscillators. Thus, upon light exposure, the M oscillators communicate with the E oscillators. Because the M oscillators drive circadian behavior, they must also receive inputs from the E oscillators. Hence, although photic TIM degradation is largely cell autonomous, neural cooperation between M and E oscillators is critical for circadian behavioral photoresponses.

INTRODUCTION

In *Drosophila*, the self-sustained pacemaker that generates molecular and behavioral circadian rhythms is a negative transcriptional feedback loop: PERIOD (PER) and TIMELESS (TIM) repress CLOCK (CLK) and CYCLE (CYC), which are activators of *per* and *tim* transcription (Zhang and Emery, 2012). This mechanism is present in approximately 150 brain neurons (Nitabach and Taghert, 2008). In a standard 12-hr-light:12-hr-dark (LD) cycle, *Drosophila* exhibits two peaks of activity. The morning (M) peak is driven by the Pigment Dispersing Factor (PDF) positive small ventrolateral neurons (s-LNvs), also referred to as the M oscillators (Grima et al., 2004; Stoleru et al., 2004). The evening (E) peak is driven by six dorsolateral neurons (LNds), two PDF negative s-LNvs called "fifth s-LNvs," and perhaps a few Dorsal Neurons (DN1s) (Cusumano et al., 2009; Grima et al., 2004; Picot et al., 2007; Stoleru et al., 2004). These cells are known as the E oscillators. The M oscillators also function as pacemaker neurons: they maintain behavioral rhythms under constant darkness (DD) and control their pace and phase (Renn et al., 1999; Stoleru et al., 2005).

Circadian rhythms are only beneficial if they are synchronized with the day/night cycle. Light is a crucial cue to entrain the circadian clock. In *Drosophila*, a brief light pulse in the early night, mimicking a delayed dusk, leads to a phase delay, whereas a late-night light pulse resembling an early dawn causes a phase advance (Levine et al., 1994). Light promotes rapid TIM degradation, which is critical to reset the circadian pacemaker and behavioral rhythms (Suri et al., 1998; Yang et al., 1998). Upon light exposure, the intracellular blue-light photoreceptor CRYPTOCHROME (CRY) changes its conformation, binds to TIM, and triggers its proteasomal degradation by recruiting a JETLAG (JET)-containing E3 ubiquitin ligase (Busza et al., 2004; Koh et al., 2006; Ozturk et al., 2011; Peschel et al., 2009).

Loss of CRY results in severe photoreception defects: light-induced TIM degradation and behavioral phase shifts are abolished (Dolezelova et al., 2007; Lin et al., 2001; Stanewsky et al., 1998). *cry* mutant flies also remain rhythmic in constant light (LL), whereas wild-type flies are arrhythmic under these conditions (Emery et al., 2000). Two *jet* mutants (jet^c and jet^r) are also rhythmic in LL (Koh et al., 2006; Peschel et al., 2006). However, this and other circadian photoresponse phenotypes are only observed in flies carrying the long-short *tim* variant (*ls-tim*) (Rosato et al., 1997). The long TIM isoform encoded by this variant has reduced affinity for CRY, making flies much less sensitive to light compared to flies carrying the short *tim* allele (*s-tim*) (Sandrelli et al., 2007). Thus, although JET promotes TIM degradation, whether it is actually required for TIM degradation and circadian photoresponses remains to be determined.

Although strong evidence supports a cell-autonomous model for circadian photoreception, recent studies indicate that such a mechanism is not sufficient to explain photic resetting of circadian behavior. Indeed, TIM degradation in M oscillators appears to be neither necessary nor sufficient for phase delays (Tang et al., 2010). Based on the pattern of TIM degradation at Zeitgeber Time (ZT) 15, it was proposed that the DN1s would be important for phase delays (Tang et al., 2010). Moreover, the large (l)-LNvs have been implicated in phase advances (Shang et al., 2008). Ultimately, the DN1s and the l-LNvs would

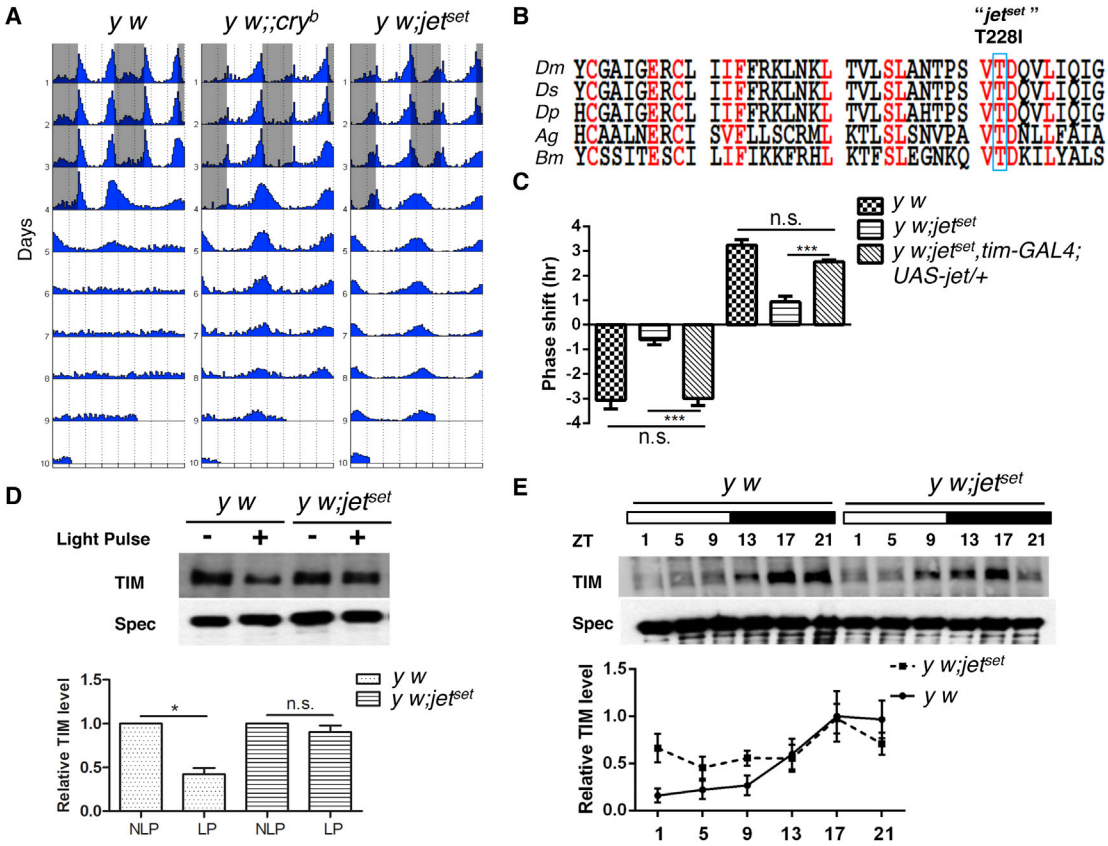

Figure 1. Identification and Characterization of *jet*^{set}

(A) *y w; jet*^{set} flies are rhythmic under LL. Representative double-plotted actograms of *y w*, *cry*^{b}, and *y w; jet*^{set} flies. (White indicates the light phase, and gray indicates the dark phase.)

(B) Sequence alignment of the LRR region of insect JET proteins. The blue box indicates the *jet*^{set} mutation.

(C) Behavioral phase shifts after short light pulses are profoundly disrupted in *jet*^{set} mutants. Phase delays and advances are plotted as negative and positive values, respectively. Phase shifts were almost completely abolished compared to control (*y w*) flies. Phase shifting defects were fully rescued by expression of *UAS-jet* with *tim-GAL4*. For each experiment, sixteen flies were used per genotype, n = 3. Error bars correspond to SEM. ***p < 0.001, n.s., not significant at the 0.05 level as determined by one-way analysis of variance (ANOVA) coupled to post hoc Tukey's test for multiple comparisons, $F(5, 12) = 121.9$ with $p < 0.0001$.

(D) *jet*^{set} is defective for acute TIM degradation in response to short light pulses. Upper panel: representative western blot showing TIM degradation after light pulse in *y w* and *y w; jet*^{set}. A light pulse (LP) was given at ZT21 and nonlight pulsed (NLP) flies were used as controls. Lower panel: quantification of TIM levels. Upon light pulse, *y w* flies showed about 50% TIM degradation, whereas *jet*^{set} did not show any obvious TIM degradation. n = 3. For each genotype the LP values are normalized to their NLP control values. Data are plotted as mean ± SEM, *p < 0.05; n.s., not significant as determined by comparing the LP and NLP groups for each genotype by Student's t test.

(E) TIM oscillations in *jet*^{set} are dampened under LD conditions. Upper panel: representative western blots showing TIM oscillation in whole heads at indicated ZT times under a LD cycle. The white bars represent the day, and the black bars represent the night. TIM levels were normalized to the SPECTRIN levels. n = 5. Lower panel: quantification of TIM levels. TIM expression levels for *y w* at ZT17 were set to 1, and other values were normalized to it. Data represent mean ± SEM.

have to communicate with the M oscillators, because these cells drive circadian behavior in DD, the condition in which phase is measured after exposing flies to a light pulse. Neuronal circuits would thus be important for circadian behavioral photoresponses. Acute TIM degradation in CRY-negative LNds also indicates the existence of nonautonomous photoreceptive mechanisms in the brain (Yoshii et al., 2008).

We used a severe *jet* mutant and *jet* RNAi to map the neuronal circuits controlling circadian photoreception. Our results indicate that both cell-autonomous and nonautonomous photoreception take place within the circadian neural network, and that the M and E oscillators are crucial for sensing light and resetting circadian locomotor behavior.

RESULTS

The *jet*^{set} Mutation Profoundly Disrupts Circadian Photoresponses

In a screen for mutants affecting *Drosophila* circadian behavior, we identified a strain that remains robustly rhythmic in LL (Figure 1A; Table S1). This mutant did not complement *jet*^{c} and *jet*^{r} (Table S1), and a point mutation causing a threonine to isoleucine substitution in JET's leucine-rich repeats (LRR) was identified (Figure 1B). However, although *jet*^{c} and *jet*^{r} show circadian light response defects only with *ls-tim* (Koh et al., 2006; Peschel et al., 2006), our mutant carries the highly light-sensitive *s-tim* allele (Sandrelli et al., 2007). It is thus a much more severe