NIH Public Access
Author Manuscript
Science. Author manuscript; available in PMC 2012 November 06.

Published in final edited form as:
Science. 2008 May 23; 320(5879): 1074–1077. doi:10.1126/science.1153277.

Differential Rescue of Light- and Food-Entrainable Circadian Rhythms

Patrick M. Fuller, **Jun Lu**, and **Clifford B. Saper***
Department of Neurology, Division of Sleep Medicine, and Program in Neuroscience, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA

Abstract

When food is plentiful, circadian rhythms of animals are powerfully entrained by the light-dark cycle. However, if animals have access to food only during their normal sleep cycle, they will shift most of their circadian rhythms to match the food availability. We studied the basis for entrainment of circadian rhythms by food and light in mice with targeted disruption of the clock gene *Bmal1*, which lack circadian rhythmicity. Injection of a viral vector containing the *Bmal1* gene into the suprachiasmatic nuclei of the hypothalamus restored light-entrainable, but not food-entrainable, circadian rhythms. In contrast, restoration of the *Bmal1* gene only in the dorsomedial hypothalamic nucleus restored the ability of animals to entrain to food but not to light. These results demonstrate that the dorsomedial hypothalamus contains a *Bmal1*-based oscillator that can drive food entrainment of circadian rhythms.

The circadian timing system (CTS) exerts endogenous temporal control over a wide range of physiological and neurobehavioral variables, conferring the adaptive advantage of predictive homeostatic regulation (1). When food is freely available, light signals from the retina entrain circadian rhythms to the day-night cycle (2). However, when food is available only during the normal sleep period [restricted feeding (RF)], many of these rhythms are reset so that the active phase corresponds to the period of food availability (3, 4). In light entrainment, retinal ganglion cells that contain the photopigment melanopsin provide signals to neurons in the suprachiasmatic nuclei (SCN) of the hypothalamus that generate circadian rhythms through a series of molecular transcriptional, translational, and posttranslational feedback loops (5). The SCN in turn synchronizes circadian rhythms in peripheral tissue clocks through synaptic and humoral outputs (5, 6). During RF, the SCN remains on the light cycle and SCN lesions do not prevent food entrainment, which suggests that another clock may supersede the SCN (3, 4, 7). Two recent studies have suggested that at least one food-entrainable clock may be located in the dorsomedial nucleus of the hypothalamus (DMH), but the importance of this clock for food entrainment has been disputed (3, 8-10).

The core components of the molecular clock include the activating transcription factors BMAL1 and CLOCK and the negative regulatory feedback elements encoded by the *Per* and *Cry* genes (11, 12). *Bmal1* is the only circadian clock gene without a known functional paralog and hence the only one for which a single gene deletion causes a complete loss of behavioral and molecular rhythmicity (13). Because its gene product BMAL1 is a

*To whom correspondence should be addressed. csaper@bidmc.harvard.edu.

Supporting Online Material
www.sciencemag.org/cgi/content/full/320/5879/1074/DC1
Materials and Methods
Figs. S1 to S4
References

$watermark-text
$watermark-text
$watermark-text

$watermark-text

$watermark-text

$watermark-text

transcription factor that likely influences many downstream genes, *Bmal1*$^{-/-}$ mice also exhibit other physiological defects unrelated to the circadian defect (14), including progressive arthropathy, decreased locomotor activity levels and body mass, and a shortened life span (15-18).

In this study, we used *Bmal1*$^{-/-}$ mice, which harbor a null allele at the *Bmal1* locus (19). The circadian patterns of locomotor activity (LMA) and body temperature (T_b) were monitored by telemetry (Fig. 1, A to C, and fig. S2). As previously reported, these animals showed no circadian rhythms in a 12-hour-light/12-hour-dark (LD) cycle or constant darkness (DD) when given ad libitum (AL) access to food (Fig. 1B). We also attempted to entrain *Bmal1*$^{-/-}$ mice to a 4-hour window of RF during the normal sleep period for mice, under both LD [ZT4-8 (from 4 to 8 hours after light onset)] and DD [CT4-8 (from 4 to 8 hours after presumptive light onset)] conditions. In LD and DD conditions, wild-type (WT) and heterozygous littermates showed an elevation of T_b and LMA ~2 to 3 hours before food became available (Fig. 2, A and D, and fig S2). By contrast, *Bmal1*$^{-/-}$ mice did not show a comparable elevation in T_b or increase in LMA before the window of RF in DD; T_b and LMA were, however, markedly elevated in the *Bmal1*$^{-/-}$ mice after food presentation (Fig. 2, B and D, Fig. 3C, and fig. S2). In addition to the preprandial elevation in T_b under RF, WT and heterozygous littermates demonstrated a clear circadian T_b rhythm (Fig. 2A), whereas *Bmal1*$^{-/-}$ mice demonstrated a persisting ultradian T_b pattern throughout the remainder of the day (Fig. 2B). In DD conditions, *Bmal1*$^{-/-}$ mice occasionally showed periods of torpor (T_b below 31°C), which were distributed randomly across the circadian day. Consequently, the *Bmal1*$^{-/-}$ mice not only failed to show elevation of T_b or LMA in anticipation of the RF but often slept or were in torpor through the window of RF, requiring us to arouse them by gentle handling after presentation of the food to avoid their starvation and death during RF.

After 14 days in this RF regimen, mice were killed to examine clock gene expression in the brain and were compared to mice that had been fed AL. As previously reported (8), WT animals with AL food showed peak expression of *Per1* and *Per2* mRNA at ZT5-6, and *Bmal1* at ZT18-19 in the SCN (Fig. 3, D and E), but little or no expression at other hypothalamic sites. By contrast, WT animals under RF also showed no change in this expression pattern in the SCN (Fig. 3, D and E) but did show induction of *Per1* and *Per 2* at ~ZT3-9 (preceding, during, and after the RF window) in the DMH with peak expression levels at ZT7-8 (Fig. 3F). We also saw induction of *Bmal1* mRNA in the DMH, with peak expression at ZT18-21 (Fig. 3G), consistent with neurons in the DMH showing induction of rhythmic expression of the entire suite of clock genes during RF. As previously reported for *Per1* and *Per2, Bmal1* gene expression was restricted to the compact region of the DMH. Finally, as expected in *Bmal1*$^{-/-}$ mice, no *Bmal1* mRNA and very low expression levels of *Per1* and *Per2* were detected in the SCN and DMH under any feeding or lighting condition (Fig. 1E, Fig. 2, E to G, and fig. S4).

These results suggest that a BMAL1-based circadian clock may be induced in the DMH after starvation and refeeding to drive entrainment of circadian rhythms to the time of food availability. This hypothesis is consistent with previous studies showing that the DMH is a major recipient of direct and relayed input from the SCN and that it is important in relaying circadian signals for sleep-wake cycles, LMA, feeding, and corticosteroid rhythms to other brain systems (20). c-Fos expression in the DMH, but not in the SCN, is shifted to coincide with the activation of LMA and T_b during RF, and lesions of the DMH prevented entrainment of LMA, T_b, and sleep-wake cycles to RF [(3); also see (9, 10)].

To test the role of the DMH-inducible clock in entrainment of circadian rhythms, we attempted to rescue both light and food entrainment of circadian rhythms by injecting adeno-

$watermark-text

$watermark-text

$watermark-text

associated viral vectors (AAV, serotype 8) containing the *Bmal1* gene (including both 5' and 3' promoter elements) (19) into the brains of *Bmal1*$^{-/-}$ mice. To test this construct, we first injected AAV-BMAL1 into the SCN. All mice with SCN injections ($n = 6$) (Fig. 1C), but none in which the injections missed the SCN ($n = 16$) or in which we injected a different AAV containing the gene for green fluorescent protein (GFP) into the SCN ($n = 3$), demonstrated entrainment of both LMA and T_b rhythms to a 12:12 LD cycle (Fig. 1C). When the mice were in DD conditions, this rhythm continued as a high-amplitude, free-running rhythm of 23.7 hours (Fig. 1C and fig. S1). Thus, focal bilateral injection of AAV-BMAL1 into the SCN of *Bmal1*$^{-/-}$ mice rescued the fundamental properties of the circadian oscillator, including light entrainment, free-running period, and rhythm amplitude. A previous study had shown that rescue of *Bmal1*$^{-/-}$ mice could be achieved by a transgene in which BMAL1 was placed under a constitutively active cytomegalovirus promoter (13). However, this gene construct, which was expressed continuously throughout the brain, produced a circadian period of ~22.7 hours, about 1 hour shorter than that of WT mice (13). By contrast, in our study, when we placed BMAL1 under its own promoter and restored this gene only to the SCN, the period of the circadian cycle was precisely the same as in WT animals (Fig. 1, A and C, right panel). Thus the expression of BMAL1 under its own promoter in the SCN alone is sufficient for recovery of light-entrained circadian rhythms. Our results also establish the SCN as sufficient for the generation of the circadian T_b rhythm, a point that has been in dispute (21).

On the other hand, similar to the mice with transgenic replacement of BMAL1 throughout the brain (14), locomotor activity levels in the animals with AAV-BMAL1 injections into the SCN remained significantly lower ($P < 0.001$) than those of WT and heterozygous littermate mice. Moreover, mice with AAV-BMAL1 injections into the SCN did not show improvement in any of the other physiologic deficits. Hence, these deficits are unlikely to be due to loss of circadian rhythmicity per se (14).

We next tested the animals with BMAL1 replacement in the SCN for their ability to entrain to a restricted temporal window of food availability. Previous studies had demonstrated that animals with lesions of the SCN could still entrain to food, suggesting that there was a food-entrainable oscillator elsewhere in the animals but not excluding participation of the SCN in intact animals (4, 7, 22). When animals with SCN injections of AAV-BMAL1 who had complete rescue of the light-entrained rhythms of LMA and T_b were placed into a food-restriction paradigm under DD conditions, we found that they maintained the rhythm that had been entrained by light (high-amplitude, free-running period of ~23.7 hours) and never showed an increase in LMA or T_b in anticipation of the food presentation (fig. S3). Hence, although a BMAL1-based clock is necessary to support food entrainment, restoration of clock function in the SCN alone is not able to rescue this behavior.

To test the hypothesis that the BMAL1-based clock induced in the DMH during restricted feeding might drive circadian entrainment, we performed stereotaxic bilateral delivery of AAV-BMAL1 (the same construct and vector as used in the SCN) into the DMH of *Bmal1*$^{-/-}$ mice. Mice who sustained bilateral DMH injections of the AAV-BMAL1 did not demonstrate entrainment to a 12:12 LD cycle or free-running rhythms of T_b or LMA in DD (Fig. 3A). By contrast, under conditions of food restriction in DD, they exhibited a clear anticipatory increase in T_b and LMA before food presentation (Fig. 2C and Fig. 3B). Each individual mouse showed very little day-to-day variation in the timing of the increase in T_b and LMA under DD (i.e., the phase angle of entrainment was stable). Finally, the increase in T_b and LMA before the predicted period of food presentation persisted during a 24-hour fast at the end of restricted feeding (arrow in Fig. 3B), demonstrating the circadian nature of the response.

$watermark-text

$watermark-text

$watermark-text

In both our study and the study by Mieda *et al.* (8), clock gene expression in the DMH was largely restricted to cells in the compact part of the nucleus, which consists of small, closely packed neurons that are highly reminiscent of the SCN itself. These neurons appear mainly to have local connections with the adjacent output zones of the DMH (23), suggesting that the timing signal from the compact part of the DMH may impinge upon the same output neurons in the remainder of the DMH as are used to control light-entrained rhythms directed by the SCN. This relationship may explain how the DMH clock is able to override the SCN clock input during conditions of food entrainment in an intact animal. It is unlikely that feedback from the DMH alters activity in the SCN in any major way, because the SCN remains phase-locked to the LD cycle for many weeks during food entrainment (as long as the animals are not also hypocaloric). These observations also raise the interesting possibility that the DMH may form the neuroanatomic basis of the so-called methamphetamine-sensitive circadian oscillator (MASCO), which also operates independent of the SCN and does not entrain to light [for a review, see (24)].

Our data indicate that there is an inducible clock in the DMH that can override the SCN and drive circadian rhythms when the animal is faced with limited food availability. Thus, under restricted feeding conditions, the DMH clock can assume an executive role in the temporal regulation of behavioral state. For a small mammal, finding food on a daily basis is a critical mission. Even a few days of starvation, a common threat in natural environments, may result in death. Hence, it is adaptive for animals to have a secondary “master clock” that can allow the animal to switch its behavioral patterns rapidly after a period of starvation to maximize the opportunity of finding food sources at the same time on following days.

In an intact animal, peripheral oscillators in many tissues in the body, including the stomach and liver, as well as elsewhere in the brain, may contribute to food entrainment of circadian rhythms (25, 26). Consequently, it has been difficult to dissect this system by using lesions of individual components of the pathway (3, 9, 10). However, by starting with a genetically arrhythmic mouse and using focal genetic rescue in the brain, we have identified the SCN molecular clock as sufficient for light but not food entrainment of T_b and LMA rhythms in mice, and the DMH as sufficient for food but not light entrainment of circadian rhythms of T_b and LMA. These results demonstrate the power of viral-based gene replacement in the central nervous system to dissect complex neural functions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Q. Ha and M. Ha for technical work. Support was provided by U.S. Public Health Service grants HL60292, NS33987, NS051609, NS057119, and HL07901-08.

References and Notes

1. Hastings MH, Reddy AB, Maywood ES. Nat Rev Neurosci. 2003; 4:649. [PubMed: 12894240]
2. Saper CB, Lu J, Chou TC, Gooley J. Trends Neurosci. 2005; 28:152. [PubMed: 15749169]
3. Gooley JJ, Schomer A, Saper CB. Nat Neurosci. 2006; 9:398. [PubMed: 16491082]
4. Stephan FK, Swann JM, Sisk CL. Behav Neural Biol. 1979; 25:346. [PubMed: 464979]
5. Lowrey PL, Takahashi JS. Annu Rev Genomics Hum Genet. 2004; 5:407. [PubMed: 15485355]
6. Kramer A, et al. Science. 2001; 294:2511. [PubMed: 11752569]
7. Stephan FK. Physiol Behav. 1989; 46:489. [PubMed: 2623075]
8. Mieda M, et al. Proc Natl Acad Sci U S A. 2006; 103:12150. [PubMed: 16880388]

9. Landry GJ, Simons MM, Webb IC, Mistlberger RE. Am J Physiol. 2006; 290:6.
10. Gooley JJ, Saper CB. J Biol Rhythms. 2008; 22:479. [PubMed: 18057322]
11. Gekakis N, et al. Science. 1998; 280:1564. [PubMed: 9616112]
12. Zheng B, et al. Cell. 2001; 105:683. [PubMed: 11389837]
13. Bunger MK, et al. Cell. 2000; 103:1009. [PubMed: 11163178]
14. McDearmon EL, et al. Science. 2006; 314:1304. [PubMed: 17124323]
15. Bunger MK, et al. Genesis. 2005; 41:122. [PubMed: 15739187]
16. Laposky A, et al. Sleep. 2005; 28:395. [PubMed: 16171284]
17. Shimba S, et al. Proc Natl Acad Sci U S A. 2005; 102:12071. [PubMed: 16093318]
18. Rudic RD, et al. PLoS Biol. 2004; 2:e377. [PubMed: 15523558]
19. Materials and methods are available as supporting material on *Science* Online
20. Chou TC, et al. J Neuroscience. 2003; 23:1069.
21. Fuller CA, et al. Am J Physiol. 1981; 241:5.
22. Krieger DT, Hauser H, Krey LC. Science. 1977; 197:398. [PubMed: 877566]
23. Thompson RH, Canteras NS, Swanson LW. Brain Res Brain Res Rev. 1998; 27:89. [PubMed: 9622601]
24. Hiroshige T, Honma K, Honma S. Brain Res Bull. 1991; 27:441. [PubMed: 1959043]
25. Stokkan KA, et al. Science. 2001; 291:490. [PubMed: 11161204]
26. Damiola F, et al. Genes Dev. 2000; 14:2950. [PubMed: 11114885]

$watermark-text
$watermark-text
$watermark-text

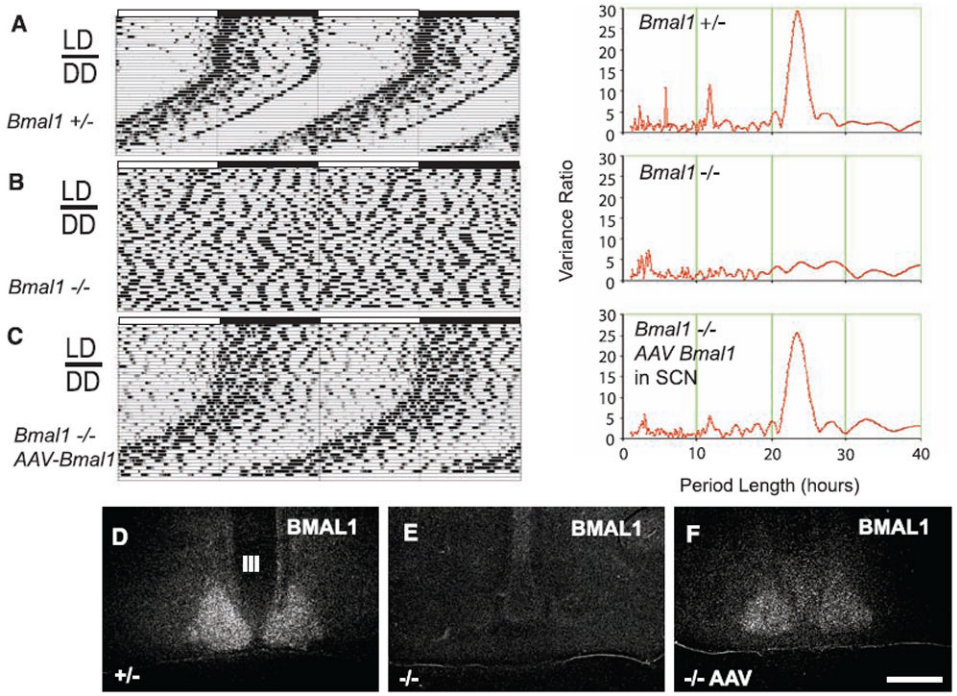

Fig. 1.

Bmal1 expression in SCN rescues circadian rhythms and light entrainment in *Bmal1*$^{-/-}$ mice. Representative body temperature (T_b) records (double-plotted actigrams) and period analyses for (**A**) *Bmal1*$^{+/-}$, (**B**) *Bmal1*$^{-/-}$, and (**C**) *Bmal1*$^{-/-}$ mice with AAV-BMAL1 injections into the SCN under both light-dark (LD) and constant darkness (DD) conditions with AL access to food. The power spectrum for period (right panel) shows a dominant frequency at 23.7 hours for both Bmal1 heterozygous littermates and mice with AAV-BMAL1 rescue in the SCN, and the lack of a ~24-hour harmonic in the *Bmal1*$^{-/-}$ mice. (**D** to **F**) *Bmal1* mRNA expression (by in situ hybridization) in the SCN in (D) *Bmal1*$^{+/-}$ mice, (E) *Bmal1*$^{-/-}$ mice, and (F) after AAV-BMAL1 rescue by injection into the SCN (all ZT18). Scale bar, 100 μm.

$watermark-text
$watermark-text
$watermark-text

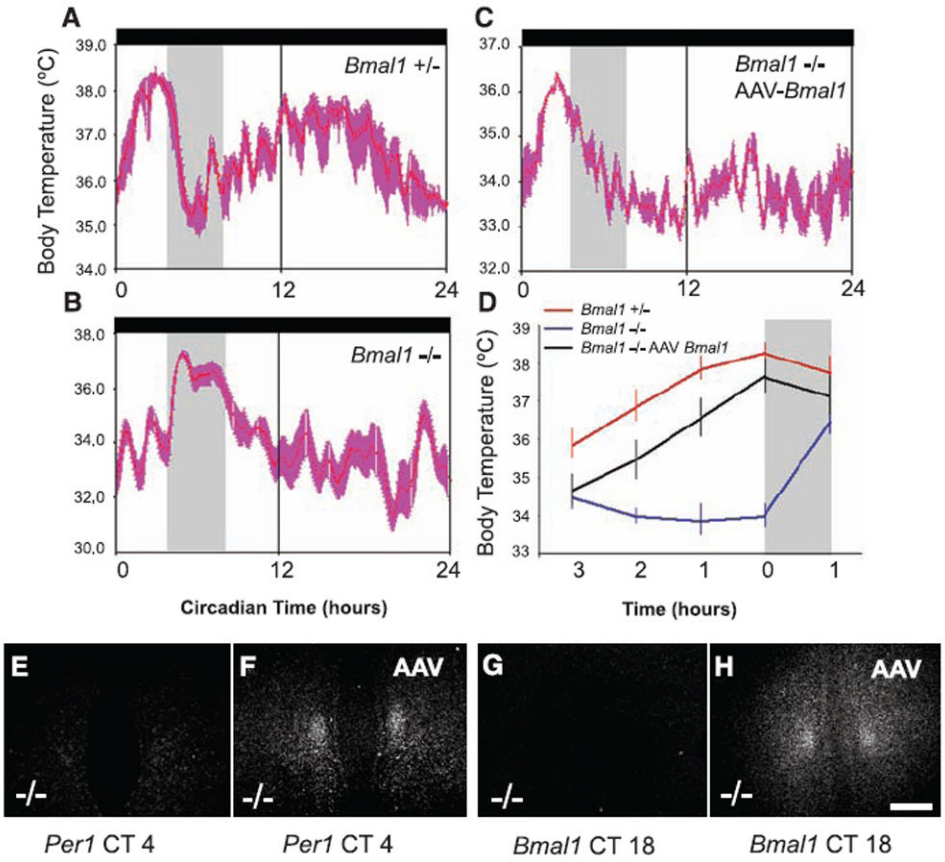

Fig. 2.

Food entrainment of the T_b rhythm is rescued by AAV-BMAL1 injection into the DMH. Under DD conditions, *Bmal1*$^{+/-}$ and *Bmal1*$^{-/-}$ mice were placed under RF (food available CT4-8, gray vertical bar). (**A**) *Bmal1*$^{+/-}$ but not (**B**) *Bmal1*$^{-/-}$ mice demonstrated a clear rise in T_b ~2 to 3 hours before food availability under RF [(A) to (C) are waveforms showing mean ± SEM T_b on days 10 to 14 of RF for an individual mouse]. (**C**) After bilateral injection of AAV-BMAL1 into the DMH of *Bmal1*$^{-/-}$ mice, there was a preprandial elevation in T_b, but not a circadian rise in T_b during the presumptive dark cycle (CT12-24) [compare (C) with (A)]. As seen in the summary data plot, during the 3-hour window preceding food availability in RF (**D**), *Bmal1*$^{-/-}$ mice with DMH AAV-BMAL1 injections (n = 4, black trace) showed a comparable preprandial elevation in T_b (mean ± SEM) to that of *Bmal1*$^{-/+}$ mice (red trace), whereas noninjected *Bmal1*$^{-/-}$ mice (blue trace) showed no preprandial elevation in T_b; an increase in T_b occurred only after food presentation in the noninjected *Bmal1*$^{-/-}$ mice (gray bar in D is first hour of food availability). Under RF, *Bmal1*$^{-/-}$ mice demonstrated very low expression levels of *Per1* mRNA (**E**) (CT4 shown) and no expression of *Bmal1* mRNA at CT18 (**G**) (near-peak expression time in the heterozygote littermates) in the DMH. By contrast, after AAV-BMAL1 injection into the DMH (**F** and **H**), *Bmal1*$^{-/-}$ mice demonstrated robust *Per1* expression in the DMH at CT4 (F) and *Bmal1* expression at CT18 (H).

$watermark-text
$watermark-text
$watermark-text

$watermark-text
$watermark-text
$watermark-text

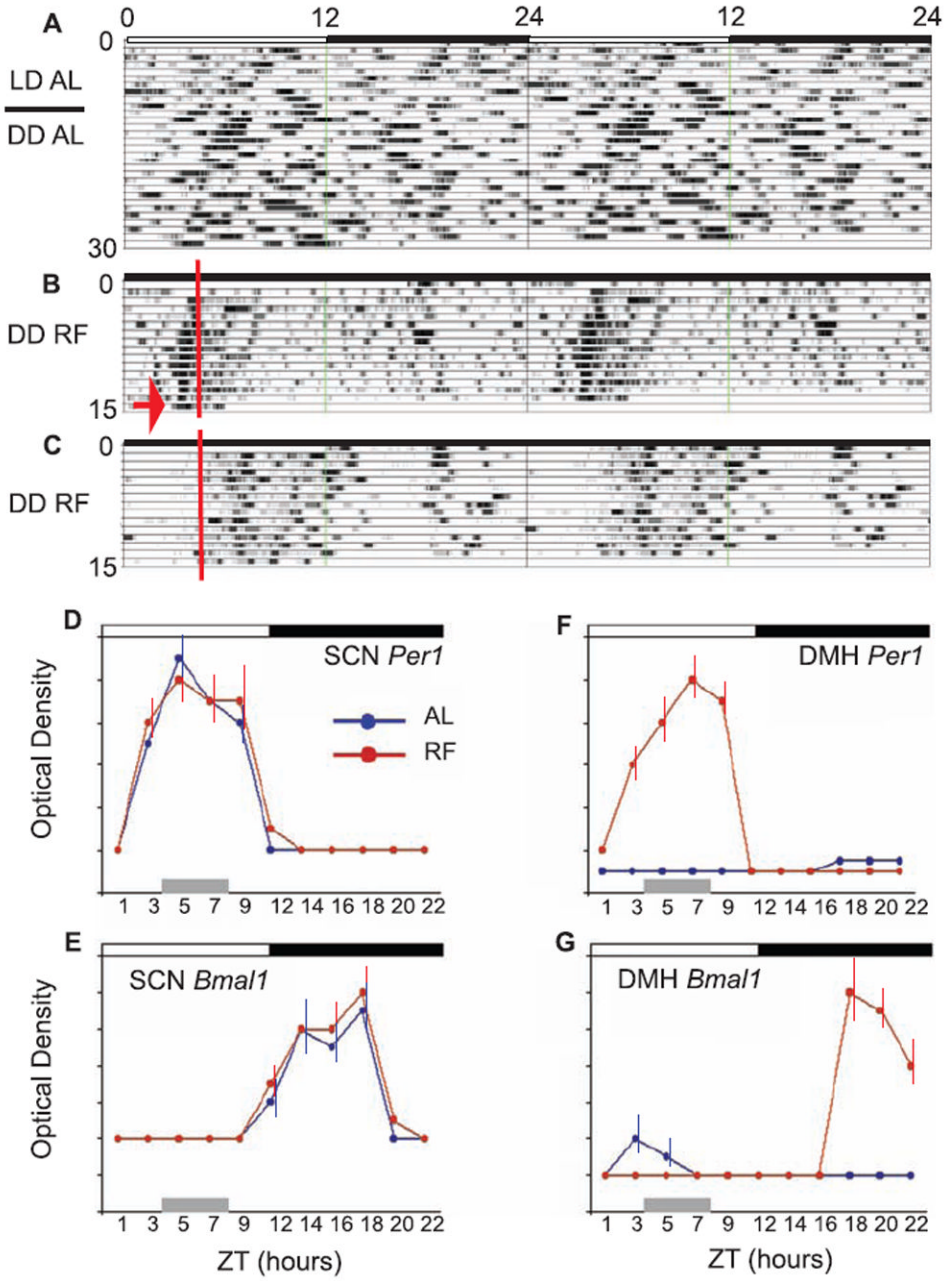

Fig. 3.

AAV-Bmal1 injection into the DMH rescues food entrainment but not light entrainment in *Bmal1*$^{-/-}$ mice. (**A**) A double-plotted actigram of T_b from a *Bmal1*$^{-/-}$ mouse after bilateral injections of AAV-Bmal1 into the DMH. These mice demonstrate the lack of entrainment to the light-dark (LD) cycle and persisting ultradian rhythmicity in constant darkness (DD) under AL feeding conditions (Fig. 1B). These same mice, however, demonstrated anticipation and entrainment (**B**) to a RF cycle (red line, food presentation) in DD conditions (arrow, last day, no food given). By contrast, noninjected *Bmal1*$^{-/-}$ mice (**C**) did not demonstrate anticipation or entrainment to the RF cycle. (**D** to **G**) *Per1* and *Bmal1* gene expression (optical density, mean ± SEM; $n = 3$ mice per time point) in WT mice across the circadian day under both AL and RF (all data 12:12 LD) at 2-hour intervals, except for a 3-

hour interval between ZT9-12. (D) *Per1* expression in the SCN showed a peak at ~ ZT6 under both AL and RF (gray bar, ZT4-8) conditions, demonstrating that the SCN remained phase-locked to the LD cycle during RF. (E) *Bmal1* expression in the SCN showed a peak at ~ZT18 under both AL and RF, further indicating that the SCN remained synchronized to the LD cycle during RF. (F) *Per1* expression in the DMH was undetectable at all ZT under AL; by contrast, *Per1* expression was sharply up-regulated by RF, with a peak at ~ZT7-8. (G) *Bmal1* expression in the DMH was also undetectable at all ZT (except very modest expression ~ZT 3-5) under AL, however, and similar to *Per1*, during RF *Bmal1* demonstrated up-regulation with a peak at ~ ZT18.

$watermark-text
$watermark-text
$watermark-text