Acute Light Exposure Suppresses Circadian Rhythms in Clock Gene Expression

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LETTER

Acute Light Exposure Suppresses Circadian Rhythms in Clock Gene Expression

Brian P. Grone,* Doris Chang,* Patrice Bourgin,† Vinh Cao,* Russell D. Fernald,* H. Craig Heller,* and Norman F. Ruby*,[1]

*Department of Biology, Stanford University, Stanford, CA, USA, and †CNRS UPR 3212, Institute for Cellular and Integrative Neurosciences and University Sleep Clinic, Hospital Civil, Strasbourg, France

Abstract Light can induce arrhythmia in circadian systems by several weeks of constant light or by a brief light stimulus given at the transition point of the phase response curve. In the present study, a novel light treatment consisting of phase advance and phase delay photic stimuli given on 2 successive nights was used to induce circadian arrhythmia in the Siberian hamster (*Phodopus sungorus*). We therefore investigated whether loss of rhythms in behavior was due to arrhythmia within the suprachiasmatic nucleus (SCN). SCN tissue samples were obtained at 6 time points across 24 h in constant darkness from entrained and arrhythmic hamsters, and *per1*, *per2*, *bmal1*, and *cry1* mRNA were measured by quantitative RT-PCR. The light treatment eliminated circadian expression of clock genes within the SCN, and the overall expression of these genes was reduced by 18% to 40% of entrained values. Arrhythmia in *per1*, *per2*, and *bmal1* was due to reductions in the amplitudes of their oscillations. We suggest that these data are compatible with an amplitude suppression model in which light induces singularity in the molecular circadian pacemaker.

Key words suprachiasmatic nucleus, desynchrony, singularity, amplitude suppression, Siberian hamster

Circadian arrhythmia can be induced in mammals by several weeks of constant light (Daan and Pittendrigh, 1976) or by a brief light stimulus given at the transition point of the phase response curve (i.e., the singularity point) (Winfree, 1980). More recently, some laboratories have employed novel light treatments that induce circadian arrhythmia in the Siberian hamster (*Phodopus sungorus*). In these studies, circadian arrhythmia was induced by using a phase-advancing light pulse on one night followed by a phase-delaying signal on the next night (Steinlechner et al., 2002; Ruby et al., 2004). Arrhythmia was reported for locomotor activity, body temperature, sleep/wake cycles, and melatonin levels (Ruby et al., 2004; Steinlechner et al., 2002). The light treatment consistently caused a marked shortening of the active phase (i.e., alpha compression), resulting in arrhythmia within 2 to 5 circadian cycles that persisted despite the continued presence of the light-dark (LD) cycle.

We hypothesized that the loss of overt rhythms was due to light-induced arrhythmia in the suprachiasmatic nucleus (SCN) because it is the central circadian

1. To whom all correspondence should be addressed: Norman F. Ruby, Department of Biology, 371 Serra Mall, Stanford University, Stanford, CA 94305-5020; e-mail: ruby@stanford.edu.

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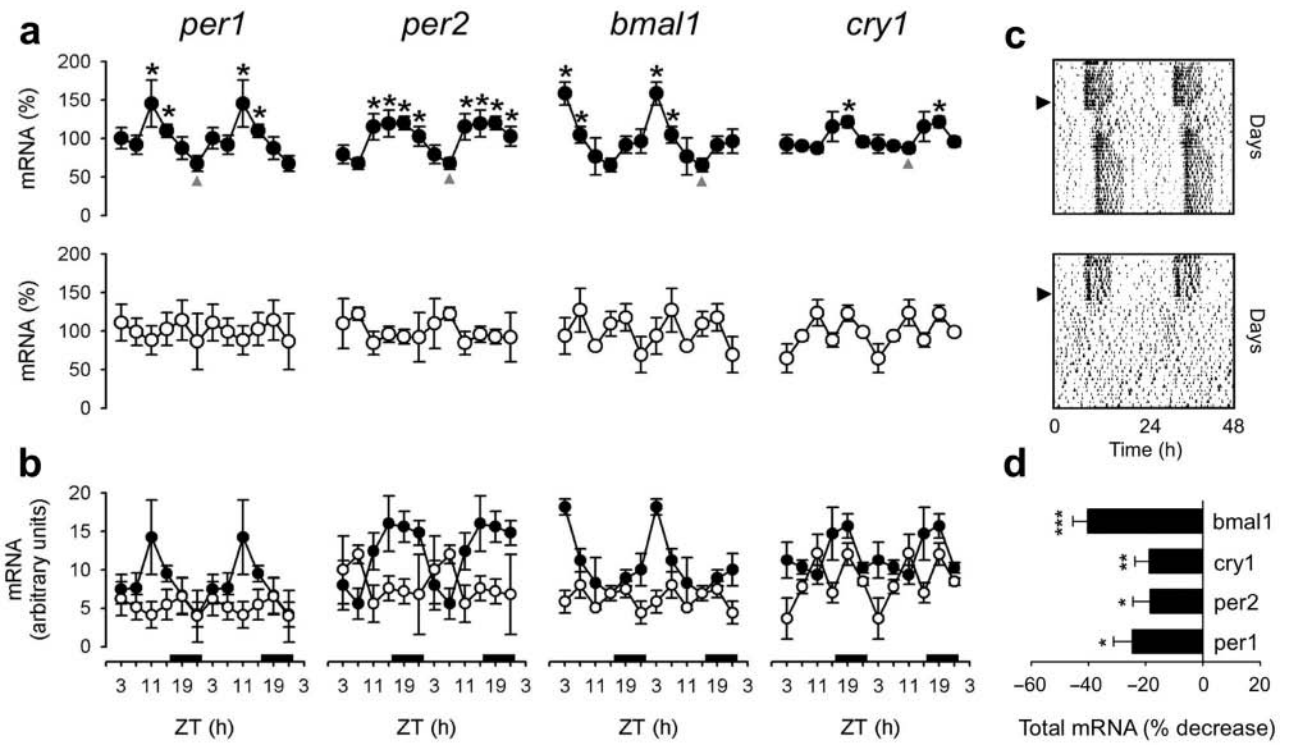

Figure 1. Clock gene expression of entrained and arrhythmic animals. (A) mRNA levels (mean ± SEM; the value for each sample was divided by the mean of all samples from all 6 time points and expressed as a percentage of the group mean) of *per1*, *per2*, *bmal1*, and *cry1* from control (●) and arrhythmic (○) hamsters. Data are double plotted. Zeitgeber time (ZT) refers to the light-dark cycle prior to DD, where ZT 0 = time of lights-on; black bar indicates the 8-h dark phase (ZT 16-24) prior to DD. Asterisks (*) indicate time points that differed significantly from the lowest time point within each condition (▲), which served as the control value for post hoc *t* tests (i.e., Dunnett post hoc correction following ANOVA for time of day effect; $*p < 0.05$). ANOVA for time of day was not significant ($p > 0.05$) for arrhythmic hamsters. (B) mRNA levels of genes from control (●) and arrhythmic (○) animals expressed in quantitative relationship to each other. Because mRNA abundance was 8 to 25 times greater for *per1* compared to the other genes, the scale was adjusted for each gene so that differences between controls and arrhythmic animals could be seen. (C) Representative double-plotted actograms of locomotor activity of hamsters that re-entrained or became arrhythmic after the light treatment. Arrhythmia occurred in approximately 65% of the hamsters. The day of the light treatment is indicated (▶). Time is given as elapsed time rather than ZT due to the phase shift of the LD cycle. (D) Total mRNA of arrhythmic animals calculated from the mean values from all 6 time points and then expressed as the percentage difference from control animals. All genes were significantly reduced after the light treatment (*t* test, control v. arrhythmic); reductions were greatest for *bmal1* (40%), followed by *per1* (25%), *per2* (18%), and *cry1* (18%); $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

pacemaker in mammals and receives direct retinal input. To determine the basis of light-induced arrhythmia, we measured clock gene mRNA expression in the SCN of arrhythmic and entrained hamsters. Circadian arrhythmia was induced by housing animals in a 24-h LD cycle (16 h light/day) and then exposing them once to a 2-h light pulse at night that began 5 h after normal dark onset. On the next day, the dark period was delayed by 3 h, and animals remained on a 16:8 LD cycle thereafter (Ruby et al., 2004). At 4 to 8 weeks after this light treatment, hamsters were classified as being rhythmic or arrhythmic based on periodogram analysis of their locomotor activity patterns. All hamsters were then moved to constant darkness (DD) for 48 h. Separate groups of animals (n = 4-5) were then killed at 6 different time points across hours 48 to 72 in DD, SCN tissue removed, and mRNA levels of *per1*, *per2*, *cry1*, and *bmal1* measured by quantitative reverse transcription–PCR (qRT-PCR) (Bio-Rad, Hercules, CA). For detailed methods, see the supplementary online material.

Circadian rhythms in locomotor activity were normal in control animals but eliminated in the arrhythmic light-treatment group (Fig. 1C). Periodogram analyses confirmed a complete absence of circadian locomotor rhythms ($p > 0.05$) for animals in which the actograms revealed an obvious loss of circadian timing. Correspondingly, there were robust circadian oscillations in *per1* ($p < 0.05$), *per2* ($p < 0.01$), *cry1* ($p < 0.05$), and *bmal1* ($p < 0.01$) mRNA abundance in SCN tissue of control animals (1-way ANOVA for each gene) (Fig. 1A). In control animals, rhythms in *per1*

and *bmal1* had discrete peaks, while oscillations in *per2* and *cry1* were of low amplitude and appeared sinusoidal. In contrast to controls, however, clock gene expression levels in arrhythmic hamsters did not change significantly across 24 h (1-way ANOVA, $p > 0.05$ for all genes) (Fig. 1A). Arrhythmic expression of the clock genes *per1*, *per2*, and *bmal1* was due to suppression of daily peak mRNA levels down to the basal levels observed in controls (Fig. 1B). The total amount of clock gene transcripts expressed over 24 h was also significantly reduced in arrhythmic animals as compared to controls (Fig. 1D). Total mRNA abundance decreased by 18% to 40% for individual genes with the greatest reduction occurring in *bmal1* (Fig. 1D). After the light treatment, mean daily locomotor activity in arrhythmic animals was reduced by 40% (paired *t* test, $p < 0.001$) but unchanged in control animals (paired *t* test, $p > 0.05$). Reduced activity in arrhythmic animals was mainly due to reductions in nighttime activity levels (data not shown).

These data show that light-induced circadian arrhythmia observed in the behavior of these hamsters extends to the level of the SCN. Our findings suggest that the LD cycle is unable to either passively drive diurnal rhythms in gene expression or synchronize cells in the SCN, even though the arrhythmic hamster SCN remains sensitive to light (Barakat et al., 2005). Broadly speaking, there are two models that may explain how light induces arrhythmia in the SCN: the desynchrony and amplitude suppression models. The desynchrony model is based largely on the observation that arrhythmia induced by constant light desynchronizes a population of oscillating SCN neurons so that the collective output of the SCN is arrhythmic (Yamaguchi et al., 2003; Ohta et al., 2005). The amplitude suppression model proposes that a single brief light stimulus induces arrhythmia by suppressing the amplitude of the pacemaker's oscillation to its singularity point (Winfree, 1980; Jewett et al., 1991). In the desynchrony model, clock gene expression and neuronal firing rates of the population of SCN cells are maintained at a median level between the circadian peak and nadir (Mason, 1991; Yamaguchi et al., 2003; Ohta et al., 2005), whereas they are reduced to basal levels in the amplitude suppression models (Leloup and Goldbeter, 2001; Huang et al., 2006).

The amplitude suppression model has received less attention so there are few studies that have addressed whether singularity occurs at the level of the SCN. A molecular model of amplitude suppression, however, predicts that a single light pulse would induce arrhythmia by suppressing the amplitude of *per* gene oscillations to basal levels (Leloup and Goldbeter, 2001). Although the model only predicts the behavior of light-inducible clock genes (i.e., *per1*, *per2*), we also observed amplitude suppression of *bmal1* (Fig. 1B), the oscillation of which is essential for SCN molecular rhythms (Bunger et al., 2000). Similarly, in the *Neurospora* circadian system, an acute light stimulus induced circadian arrhythmia in behavior by suppressing the gene *frq* to levels that were equivalent to those expressed by controls at the nadir of their circadian oscillation (Huang et al., 2006).

These two models may not be mutually exclusive. It is entirely possible that our light treatment reduced the amplitude of clock gene oscillations, which led to desynchrony among the population of cells, similar to the way SCN cells respond to continuous perifusion with tetrodotoxin (Yamaguchi et al., 2003). If such low amplitude oscillations were present, they were not sufficient to drive rhythms in behavior and are unlikely to be physiologically significant. Conversely, desynchrony may possibly lead to suppression of molecular rhythms (Ukai et al., 2007). Regardless of which model one favors, the present data cannot be explained by a pure desynchrony model because desynchrony alone does not result in basal levels of gene expression as we observed. We therefore believe that the most parsimonious explanation for our data is one in which light induced arrhythmia by driving the SCN pacemaker to singularity (cf. Steinlechner et al., 2002). Future studies may reveal that small amplitude oscillations persist in individual cells, but such findings would confirm that those oscillations are insufficient to produce coherent rhythms throughout the SCN. Furthermore, this issue cannot be fully resolved by single cell recordings because only a relatively small number of cells need to oscillate to drive rhythms in behavior (Ruby et al., 2002), and such cells could easily go undetected in an otherwise arrhythmic cell population. Nevertheless, the finding that 2 successive light signals can induce arrhythmia, even though each has no adverse effect on the pacemaker when presented alone (Ruby et al., 2004), may reveal a novel vulnerability of the circadian system.

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NOTE

Supplementary material for this article is available on the journal's Web site: http://jbr.sagepub.com/supplemental.

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