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The Acetyltransferase CLOCK Is Dispensable for Circadian Aftereffects in Mice

Christian Beaulé[*] and **Hai-Ying M. Cheng**[*,1,2]

[*]Ottawa Institute of Systems Biology and Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, ON, Canada

Abstract

Recent demonstration of the histone acetyltransferase activity of the *Clock* gene greatly expanded the regulatory role of circadian clocks in gene transcription. *Clock* and its partner *Bmal1* are responsible for the generation of circadian oscillations that are synchronized (entrained) to the external light cycle. Entraining light often produces long-lasting changes in the endogenous period called aftereffects. Aftereffects are light-dependent alterations in the speed of free-running rhythms that persist for several weeks upon termination of light exposure. How light causes such long-lasting changes is unknown. However, the persistent nature of circadian aftereffects in conjunction with the long-term effects of epigenetic modifications on development and various aspects of brain physiology prompted us to hypothesize that the histone acetyltransferase CLOCK was required for circadian aftereffects. The authors exposed *Clock* knockout mice to 25-hour light cycles and report that these mice retain the ability to display circadian aftereffects, indicating that *Clock* is dispensable for this form of circadian plasticity.

Keywords

aftereffects; entrainment; epigenetic; plasticity; mouse

Chromatin remodeling shapes transcription profiles that underlie neuronal plasticity, learning and memory, early life modulation of adult behavior, and responses to drug exposure (reviewed in Meaney and Ferguson-Smith, 2010). Chromatin remodeling is also integral to circadian clock function. The *Clock* gene is the only canonical clock gene with histone acetyltransferase activity, and *Clock* acetylates several histone and nonhistone residues on genes including *Bmal1*, its partner in circadian transcription (Asher and Schibler, 2006; Doi et al., 2006; Grimaldi et al., 2009). Thus, the interplay between *Clock* and *Bmal1* as regulators of the circadian program and *Clock*-mediated acetylation regulates the transcriptional mechanism underlying the expression of overt circadian rhythms (Grimaldi et al., 2009).

[2]To whom all correspondence should be addressed: Hai-Ying M. Cheng, Department of Biology, University of Toronto Mississauga, 3359 Mississauga Road North, Mississauga, ON L5L 1C6, Canada; haiying.cheng@utoronto.ca.
[1]Present address: Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.

CONFLICT OF INTEREST STATEMENT
The author(s) have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Circadian aftereffects are light-induced alterations of the free-running period following exposure to non–24-hour light schedules (T cycles) (Pittendrigh and Daan, 1976). Because circadian aftereffects are a form of experience-dependent long-term phenotypic plasticity, and because *Clock* influences chromatin events involved in circadian rhythmicity, we hypothesized that circadian aftereffects are dependent on *Clock* by virtue of its effects on chromatin modifications. We tested this hypothesis by exposing *Clock* knockout (*Clock*−/−) mice (DeBruyne et al., 2006) to a 25-hour T cycle (T25) known to induce aftereffects in wild-type mice.

We measured the endogenous free-running period, entrainment to a 25-hour T cycle, and induction of aftereffects in 6 male *Clock*−/− and 6 male wild-type (*Clock*+/+) mice (Appendix). We confirmed that *Clock*−/− mice did not show staining for the CLOCK protein in the SCN (Fig. 1). As shown previously (DeBruyne et al., 2006), *Clock*−/− mice are rhythmic, free run with a period significantly shorter than *Clock*+/+ mice (Fig. 2 and Table 1), display reduced locomotor activity (average of 54% decrease, $p < 0.001$), and show significantly advanced phase angles of entrainment under both LD (LD1 *Clock*−/−: −1.70 ± 0.10 h v. *Clock*+/+: −0.05 ± 0.1 h, $p < 0.00001$; LD2 *Clock*−/−: −1.85 ± 0.22 h v. *Clock* +/+: −0.20 ± 0.17 h, $p < 0.001$) and during T25 (*Clock*−/−: −3.20 ± 0.39 h v. *Clock*+/+: −0.31 ± 0.15 h, $p < 0.0001$). Except for the initial LD condition, we report that *Clock*−/− mice display lower circadian amplitudes throughout the experiment (average of 60% decrease in amplitude, $p < 0.01$), a finding not reported by Reppert's group (DeBruyne et al., 2006). All mice entrained to the T25 (Fig. 2) and started to free run in DD with a phase predicted by their respective phase angles during entrainment to the T cycle (bottom of each actogram in Fig. 2). All mice displayed circadian aftereffects following T25 (Fig. 2 and Table 1) and had identical free-running periods following the T cycle (Table 1). Thus, we conclude that CLOCK, and by extension its acetyltransferase activity, is dispensable for the expression of circadian aftereffects.

Despite the reported role played by the acetyltransferase activity of the CLOCK protein in regulating gene transcription, including regulation of the *Bmal1* gene essential for expression of circadian rhythms (Grimaldi et al., 2009), we find that CLOCK is dispensable for circadian aftereffects. A recent report further showed that circadian aftereffects do not depend on any of the 3 *Period* genes (Pendergast et al., 2010), suggesting that aftereffects are independent from clock gene regulation. These results also suggest that if chromatin remodeling were indeed involved in aftereffects, its mechanism would depend on factors other than the *Clock* and *Period* genes.

Interestingly, aftereffects can be maternally transferred when pregnant mice are exposed to T cycles during gestation (Aton et al., 2004). Additionally, photoperiod length, another condition that induces aftereffects (Pittendrigh and Daan, 1976), experienced by neonatal mice changes (imprints) their adult free-running period, showing again that light has long-lasting effects on circadian behaviors (Ciarleglio et al., 2011). Whether maternally transferred aftereffect or photoperiod imprinting requires chromatin remodeling, and whether CLOCK is required for this type of light-induced circadian plasticity, remains to be explored.

It is interesting that *Clock−/−* mice entrain to T25, which requires daily phase delays of approximately 1.9 hours, despite a previous report showing severe reductions in their ability to phase delay (Debruyne et al., 2006). In our experiment, *Clock−/−* mice entrained to light cycles with periods significantly longer than their endogenous free-running periods and displayed large, negative phase angles of entrainment under all entrainment conditions. Advanced phase angles of entrainment in *Clock−/−* mice can be a consequence of both their reduced ability to phase delay as well as their shorter endogenous free-running period. However, this altered phase relationship also complicates the determination of phase shifts obtained by a modified Aschoff type 2 procedure, as employed previously (Debruyne et al., 2006). Determination of a complete phase-response curve for *Clock−/−* mice would confirm that these mice can indeed phase delay and characterize the shape of their phase responses to light.

The question is still open on whether the acetyltransferase activity of CLOCK is required for circadian behaviors. Finally, it is unclear how phenotypes previously observed in *Clock/Clock* mutant mice bearing a dominant-negative form of CLOCK, such as sensitivity to drugs of abuse, metabolic syndrome, or even circadian disruptions (King et al., 1997; McClung et al., 2005; Turek et al., 2005), are due to disrupted chromatin remodeling. Future research should make good use of the *Clock* knockout model to further characterize the requirements for CLOCK-mediated acetylation in the regulation of circadian and other functions.

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APPENDIX

Animals

Six male *Clock*−/− (backcrossed 11 generations on a C57Bl/6J background) and 6 male wild-type (C57BL/6J, referred as *Clock*+/+ throughout) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All procedures followed the guidelines of the Canadian Council on Animal Care (http://www.ccac.ca) and were approved by the Animal Care Committee at the University of Ottawa (Ottawa, ON, Canada). Mice were housed in cages equipped with running wheels placed into a chamber with computer-controlled light schedules (Phenome Technologies, Chicago, IL). Light intensity at cage level was approximately 80 lux. Mice had ad libitum access to food and water throughout the experiment.

Mice were first entrained to a 12-hour light:12-hour dark cycle (LD1) and released in complete darkness (DD) to measure initial free-running period. Mice were then re-entrained to LD (LD2) followed by a 12-hour light:13-hour dark T cycle (T25). Mice were kept on T25 for 30 days and then released in DD to measure aftereffects.

Immunohistochemistry

We confirmed absence of staining for CLOCK in *Clock*−/− mice by performing immunohistochemistry using a rabbit polyclonal antibody generated against the C-terminus portion of CLOCK containing the histone acetyltransferase domain (Doi et al., 2006). The sequence used to generate the antibody has been characterized previously (Lee et al., 2001). Tissue harvesting and immunohistochemistry were performed as previously described (Alvarez-Saavedra et al., 2011). The rabbit polyclonal anti-CLOCK primary was used at a 1:40,000 dilution (12- to 16-hour incubation at 4 °C), and the biotinylated anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA) was used at a 1:300 dilution (2-hour incubation at room temperature). Images were captured on a Zeiss Axio Observer Z1 microscope (Oberkochen, Germany) using the 10x objective.

Data Analysis

Behavioral analyses were performed with the ClockLab software (Actimetrics, Wilmette, IL) using the last 10 days of each condition to measure daily locomotor activity (wheel revolution per day), free-running period (in hours), circadian amplitude, and phase angles of entrainment (in hours). Period and circadian amplitude were measured with χ^2 periodogram. Phase angles of entrainment were measured by calculating the difference between the onset of activity and lights-off (a negative phase angle means that mice were active before lights-off). Data were analyzed with 2-tailed *t* tests with α set at 0.05.

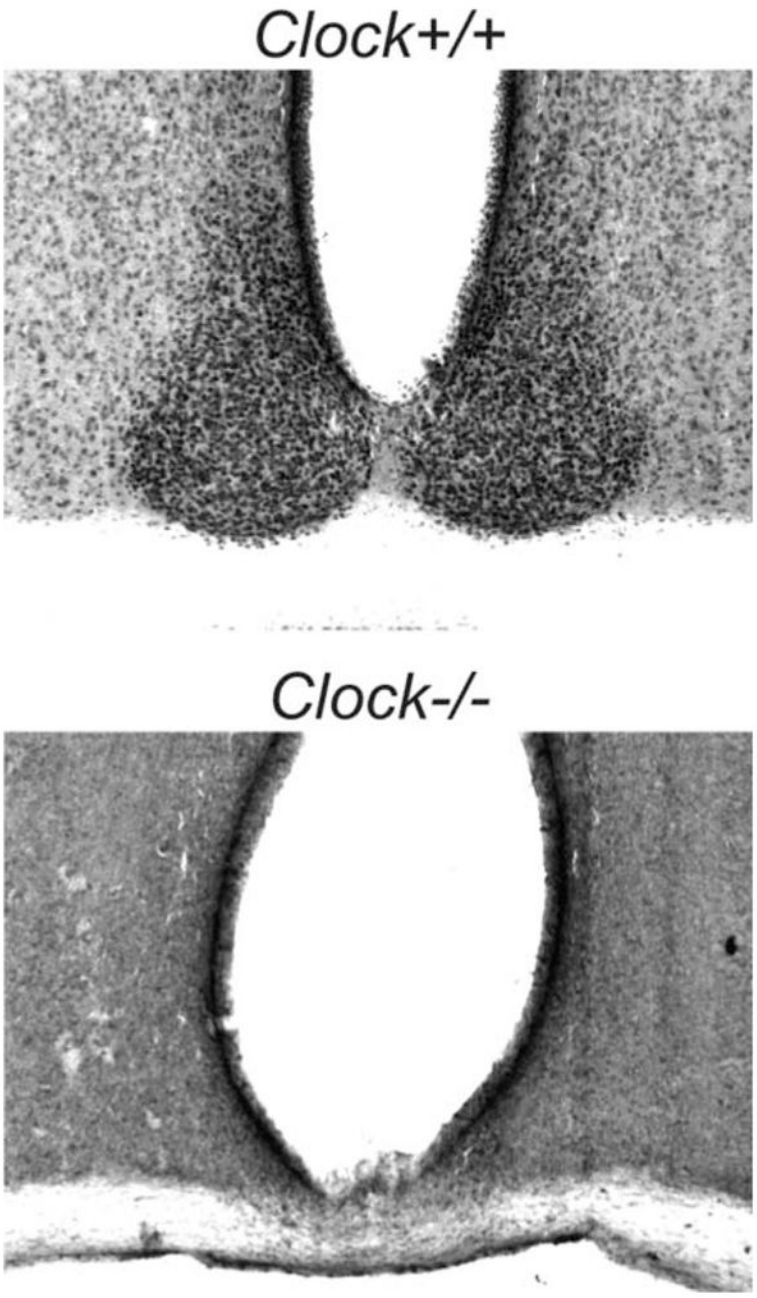

Figure 1.

Clock−/− mice do not express CLOCK. Representative photomicrographs of a *Clock*+/+ (top) and a *Clock*−/− (bottom) SCN section stained with a rabbit anti-CLOCK primary. Magnification, 10x.

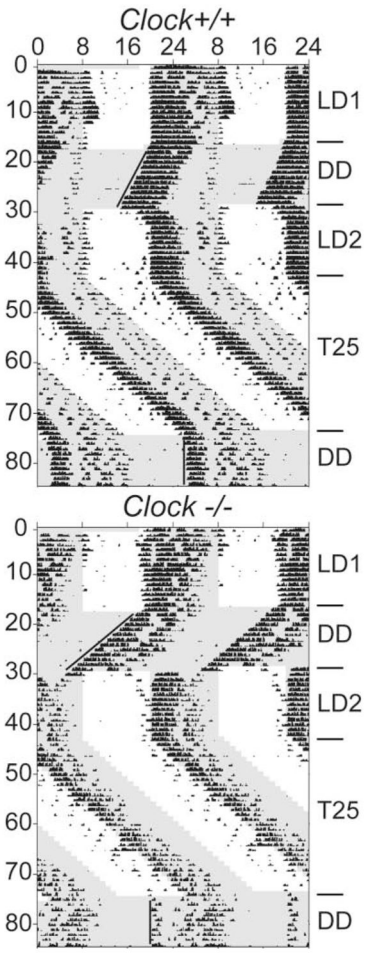

Figure 2.

Clock−/− mice entrain to 25-hour T cycles (T25) and show aftereffects. Representative double-plotted actograms for a *Clock*+/+ (top) and a *Clock*−/− mouse (bottom). Black regression lines over activity onsets in DD conditions illustrate the period of free-running rhythms and the aftereffect induced by the T cycle. Gray shaded area represents times of darkness. LD = 12-hour light:12-hour dark cycle; DD = complete darkness; T25 = 12-hour light:13-hour dark cycle.

Table 1

Circadian period in DD and during aftereffects.

	DD (h)	DD After effect (h)	*t* Test (Paired)
***Clock*+/+**	23.63 ± 0.06	24.08 ± 0.04	$p < 0.0001$
***Clock*−/−**	23.10 ± 0.06	23.97 ± 0.07	$p < 0.001$
***t* test**	$p < 0.001$	NS	

NS = not significant.