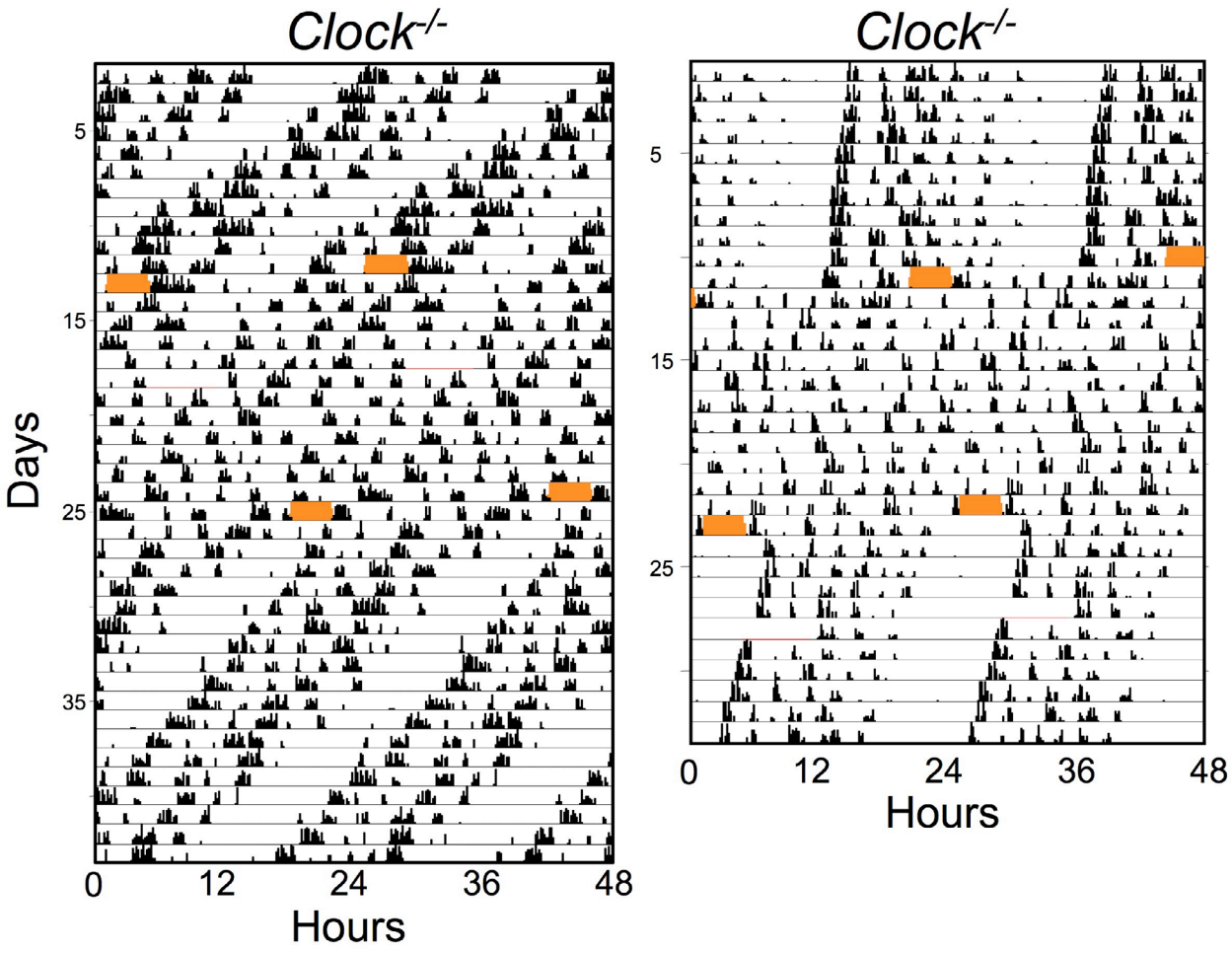
Supplemental Online Material

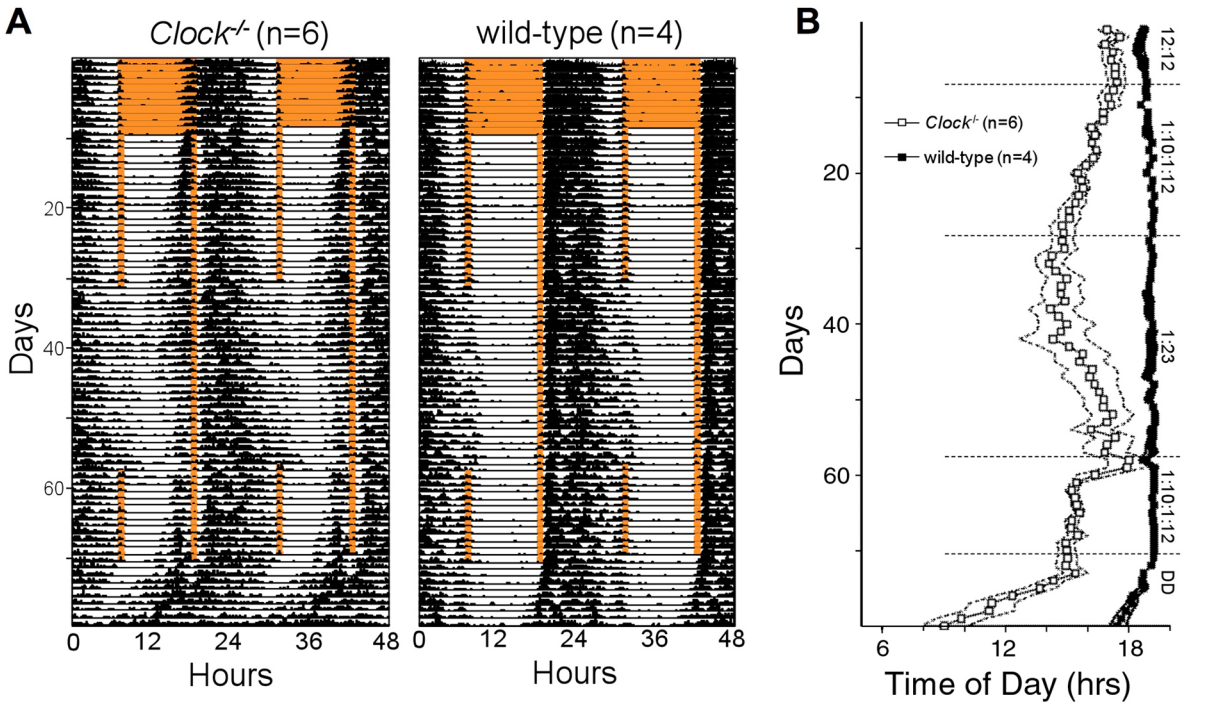
"Photic Resetting and Entrainment in CLOCK-Deficient Mice"

Robert Dallmann, Jason P. DeBruyne, and David R. Weaver

Contents:

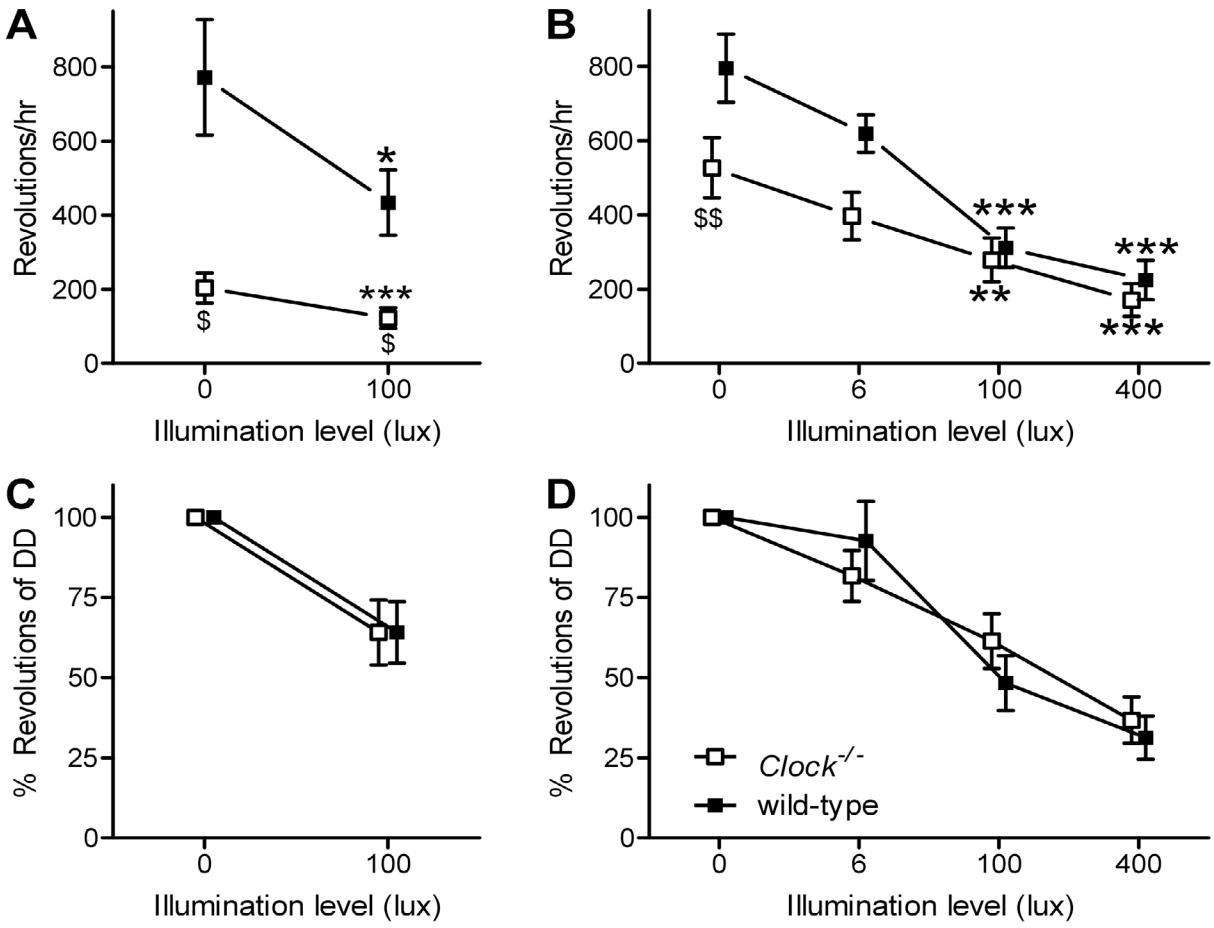


Supplemental Figure S1: Double-plotted actograms of two *Clock*$^{-/-}$ mice that became arrhythmic after a light pulse and reverted back to a rhythmic pattern after the next light pulse. Orange signifies light pulse. Red baseline signifies a short gap in data collection.

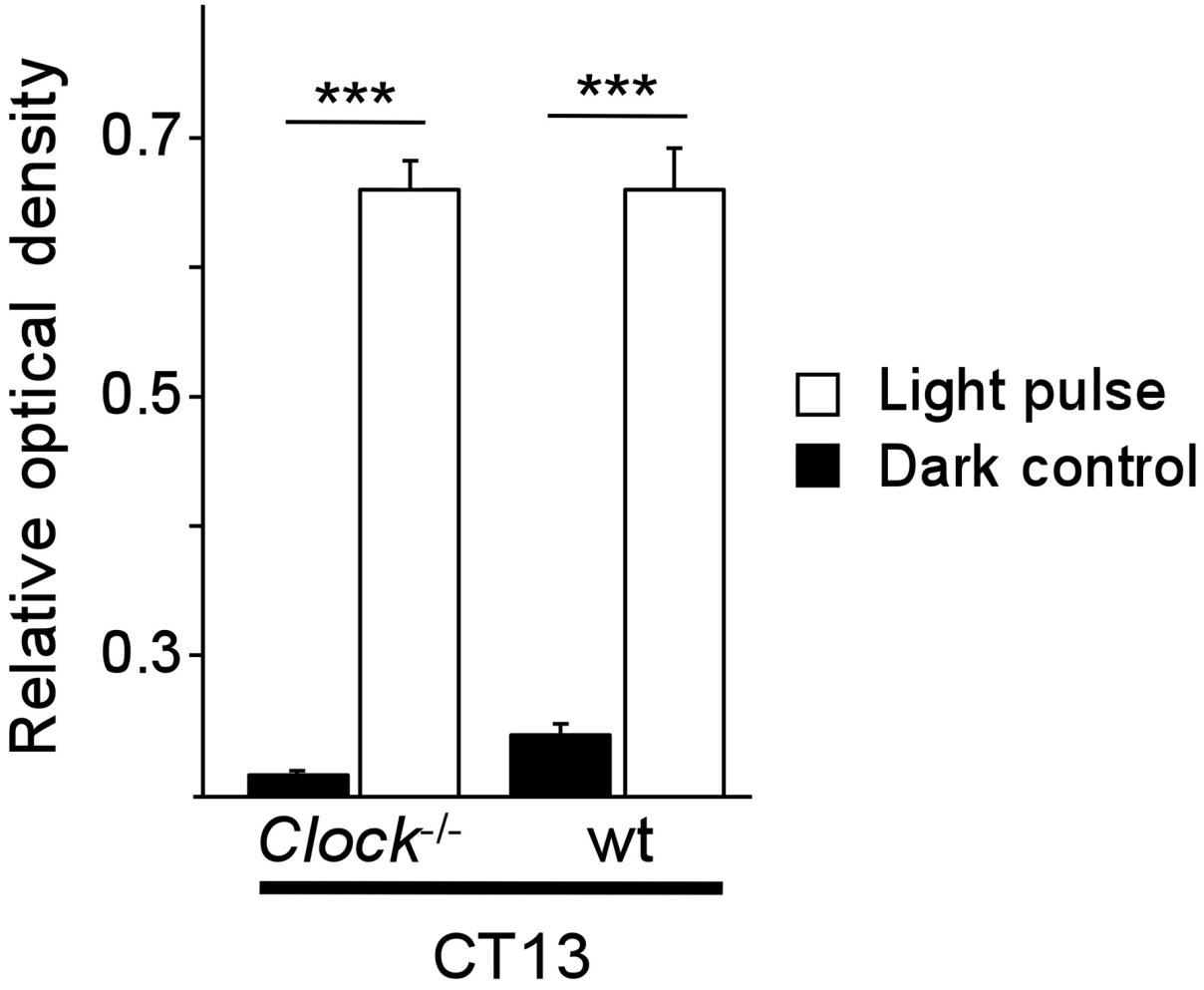


Supplemental Figure S2: Skeleton photoperiod experiment with different order and duration of lighting schedules. (A) Double-plotted group actograms of 6 *Clock*$^{-/-}$ mice (5 males, 1 female) and 4 wild-type mice (2 males, 2 females) during exposure to LD and skeleton photoperiods. (B) Average activity onset for each genotype during each phase of study. Values represent the average for 4-6 mice per genotype.

Methods: Animals were recorded for 10 days in LD 12:12 (lights on at 07:00), and then the light cycle was changed to a skeleton photoperiod consisting of the first and last 1-hour intervals from the previous lighting cycle (lights on from 07:00 to 08:00 and from 18:00 to 19:00 (1:10:1:12)). After 21 days, the morning light pulse was discontinued, i.e., lights on 18:00 – 19:00 (1L:23D). The morning light pulse was reintroduced 25 days later, for 13 days, and then the animals were released into constant darkness (DD). The waveform of activity and onset of activity were determined using ClockLab software.



Supplemental Figure S3: Activity levels during LL experiments. Average absolute and relative running wheel activity levels are shown for LL experiment 1 (Panels A & C; n = 9 per genotype) and LL experiment 2 (Panels B & D; n = 14 per genotype). Values represent mean ± SEM. Significant differences vs. wild-type in the same light condition are depicted as $ $p < 0.05$ and $$ $p < 0.01$. Significant differences vs. DD of the same genotype are depicted as * $p < 0.05$, ** < 0.01 and *** < 0.001. Statistical analysis was performed on the absolute data (revolutions/hr) in Panels A and B; the percent data in Panels C and D were not analyzed.



Supplemental Figure S4. Quantification (mean ± SEM) of *c-fos* RNA in the SCN following a 30 min (~ 60 lux) light pulse at CT13 on the first day in DD. *** $p < 0.001$, t-test. Abbreviation: wt, wild-type.

Methods: Thirty minutes after the light pulse started, mice (n = 4 per group) were euthanized by CO_2 asphyxiation and brains were collected. Cryostat sections were processed for *in situ* hybridization for c-*fos* expression and film autoradiograms were quantitated, as previously described (Shearman LP and Weaver DR (1999) Photic induction of *Period* gene expression is reduced in *Clock* mutant mice. *NeuroReport* 10: 613-618).

Supplemental Table S1. Raw data for the phase response curve shown in Fig. 3. Values represent mean ± SEM phase shift in hours for each genotype within each 2-hr CT bin. Values in parentheses are number of light pulses per bin. Data are derived from n = 12 mice per genotype. Significant differences vs. wild-type are indicated as * $p < 0.05$, ** < 0.01 and *** < 0.001.

CT	*Clock*$^{-/-}$	*Npas2*$^{m/m}$	wild-type
2	1.55 ± 0.44 (7)	0.91 ± 0.54 (2)	0.36 ± 0.19 (3)
4	0.74 ± 0.50 (7)	0.12 ± 0.17 (9)	0.15 ± 0.15 (8)
6	0.14 ± 0.16 (5)	0.03 ± 0.09 (7)	0.13 ± 0.17 (13)
8	-0.65 ± 0.80 (3)	-0.18 ± 0.16 (8)	-0.41 ± 0.40 (4)
10	-1.36 ± 0.50 (4)	-0.83 ± 0.31 (4)	-1.40 ± 0.31 (4)
12	-3.69 ± 0.60 (4)	-2.41 ± 0.46 (7)	-2.55 ± 0.19 (4)
14	-3.77 ± 0.20 (6)	-3.17 ± 0.33 (8)	-2.61 ± 0.53 (6)
16	-4.64 ± 0.37 (9)*	-3.71 ± 0.14 (12)	-3.48 ± 0.19 (8)
18	-9.46 ± 0.88 (7)**	-0.86 ± 0.78 (9)	-2.21 ± 0.39 (13)
20	8.00 ± 0.46 (5)***	1.41 ± 0.84 (7)	0.46 ± 0.55 (3)
22	5.15 ± 0.65 (12)**	1.85 ± 0.66 (5)	1.33 ± 0.16 (4)
24	3.19 ± 0.32 (9)*	0.45 ± 0.40 (6)	1.65 ± 0.42 (6)
Total	78	84	76