Fig. S1. Light pulse-induced phase-delay and phase-advance shifts in circadian rhythms in the SCN and in behavioral rhythms in freely moving mice. (A) Other examples of the phase response at CT11.5 are illustrated for the circadian Per1-luc (Upper) and Bmal1-ELuc (Lower) behavioral rhythm plotted in a double-plotted manner (Left) and sequentially (Right). Also see the legends of Fig. 1 A and B. (B) Other examples of the phase response at CT21.5 are illustrated for the Per1-luc (Upper) and Bmal1-ELuc (Lower) circadian rhythm with a behavioral rhythm plotted in a double-plotted manner (Left) and sequentially (Right). Also see Figs. 1 and 2.
**Fig. S2.** Separation of F-luc and E-Luc bioluminescence from the SCN slice. (A and B) *Per1-luc* (A) or *Bmal1-ELuc* (B) bioluminescence from a cultured SCN slice from a single-transgenic mouse (Upper) and the percentage of transmittance via a long-pass filter (Lower). Data during days 1–4, 7–10, and 14–17 are shown in different colors. Transmittance of *Per1-luc* and *Bmal1-ELuc* through a long-pass filter was kept constant during culture for at least 17 d. (C, Upper) Time-series data of bioluminescence intensity in the cultured SCN from a double-transgenic mouse (*Per1-luc/Bmal1-ELuc*) with or without a long-pass filter. (Lower) Data after separation of *Per1-luc* and *Bmal1-ELuc.*
Fig. S3. Other examples of simultaneous measurement of Per1-luc and Bmal1-ELuc rhythms in the cultured SCN slice. (A) Three additional examples of dissociation between Per1-luc and Bmal1-ELuc rhythms in neonatal (Upper) and adult (Lower) SCN slices. The circadian rhythms are double-plotted. The time zone in which bioluminescence is larger than the mean of detrended data (0 value in the ordinate) is indicated together with acrophases by blue bars and circles, respectively, for Per1-luc and green blue bars and circles, respectively, for Bmal1-ELuc. (B) The mean circadian periods ± SD for Per1-luc and Bmal1-ELuc rhythms calculated by the slope of a regression line fitted to consecutive acrophases (Left) or to daily peak phases (Right). (C) The mean phase differences ± SD in terms of acrophase (Left) and peak phase (Right) between Per1-luc and Bmal1-ELuc rhythms in the cultured SCN slice. (D) Normalized circadian rhythms of Per1-luc or Bmal1-ELuc in the SCN slices on day 3 and day 15 of culture. Data are shown as the mean ± SD. There was no significant difference, in terms of the skewness, in the shapes of Per1-luc and Bmal1-ELuc circadian rhythm in the two stages of culture. Double asterisks indicate a statistically significant difference (P < 0.01, paired t test).
**Fig. S4.** Effects of medium exchange on rhythm amplitude and the phase relation between Per1-luc and Bmal1-ELuc rhythms. (A) Representative Per1-luc (blue) and Bmal1-ELuc (green) rhythms in a neonatal SCN slice before and after medium exchange. Medium was replaced with fresh medium at the time indicated by the yellow arrow. (B) Double-plotted rhythms. The time zone in which bioluminescence is larger than the mean of detrended data (0 value in the ordinate) is indicated together with the acrophases by blue bars and circles, respectively, for Per1-luc and green bars and circles, respectively, for Bmal1-ELuc. Medium exchange was done at the time indicated by a yellow star.
Fig. S5. Other examples of simultaneous measurement of Per1-luc and Bmal1-ELuc together with spontaneous firing in the cultured SCN. (A) Sequential plots of Per1-luc (blue, Upper) and Bmal1-ELuc (green, Lower) circadian rhythms together with spontaneous firing rates (black). (B) Double plots of Per1-luc (Upper) and Bmal1-ELuc (Lower) circadian rhythms together with circadian firing rhythms. The time zone in which bioluminescence or firing rates are larger than the mean values of detrended data in a series is indicated by colored bars with respective acrophases. (C and D) Bioluminescence images of Per1-luc (C) and Bmal1-ELuc (D) in the SCN slice. Yellow lines indicate the border between the dorsal and ventral regions in the SCN. Rayleigh plots of acrophase of circadian rhythms in the dorsal (Upper) and ventral (Lower) SCN on days 2–3 (Left) and 13–14 (Right) of culture. The mean vector $r$ is indicated inside the Rayleigh plots.

Fig. S6. Per1-luc and Bmal1-ELuc rhythms in the cultured neonatal SCN slice. (A) Experimental schemes of Per1-luc or Bmal1-ELuc measurement in the cultured SCN slice. (B) Sequential plots of the Per1-luc and Bmal1-ELuc circadian rhythms of neonatal SCN slices from different mice. Blue and green indicate Per1-luc and Bmal1-ELuc, respectively. (C) Bars indicate the mean circadian periods ± SD of Per1-luc ($n = 7$) and Bmal1-ELuc ($n = 7$). The period was measured by $\chi^2$ periodogram. Double asterisks indicate a statistically significant difference between Per1-luc and Bmal1-ELuc ($P < 0.01$, Student’s t-test).
Fig. S7. Another example of simultaneous measurement of Per1-luc, Bmal1-ELuc, intracellular calcium, and spontaneous firing in the cultured SCN slice. (A) Sequential plots of circadian Per1-luc, Bmal1-ELuc, GCaMP6s, and firing rhythms in a cultured SCN slice. Spontaneous firing was expressed as the mean firing rate at the electrodes covered by the bilateral SCN. (B) Double plots of the circadian rhythms of four measures. The time zone in which respective measure is larger than the mean of detrended data in a series is indicated by a colored bar: Per1-luc (blue), Bmal1-ELuc (green), GCaMP6s (orange), neural activity (gray). Circles of the respective colors are acrophases of circadian rhythm. (C) Rayleigh plots indicate the distribution of phase difference between Per1-luc and Bmal1-ELuc from the dorsal (Left) and ventral (Right) SCN at days 2–3 (Upper) and days 13–14 (Lower) in culture. The direction and length of a red line in the circle indicates the mean phase difference and its stability. (D) The mean vector r of acrophase for the circadian rhythms of calcium (Left), Per1-luc (Center), and Bmal1-ELuc (Right) on days 2–3 and days 13–14 of culture (n = 3). The mean vector was not significantly different between different days in culture but was significantly different in the circadian rhythms examined [two-way ANOVA; days, not significant (n.s.); rhythms, P < 0.01].

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Movie S1. Representative images of circadian rhythms of *Per1-luc* (light blue), *Bmal1-ELuc* (green), GCaMP6s (yellow), and spontaneous firing rhythms (heat map color) in the cultured SCN slice.