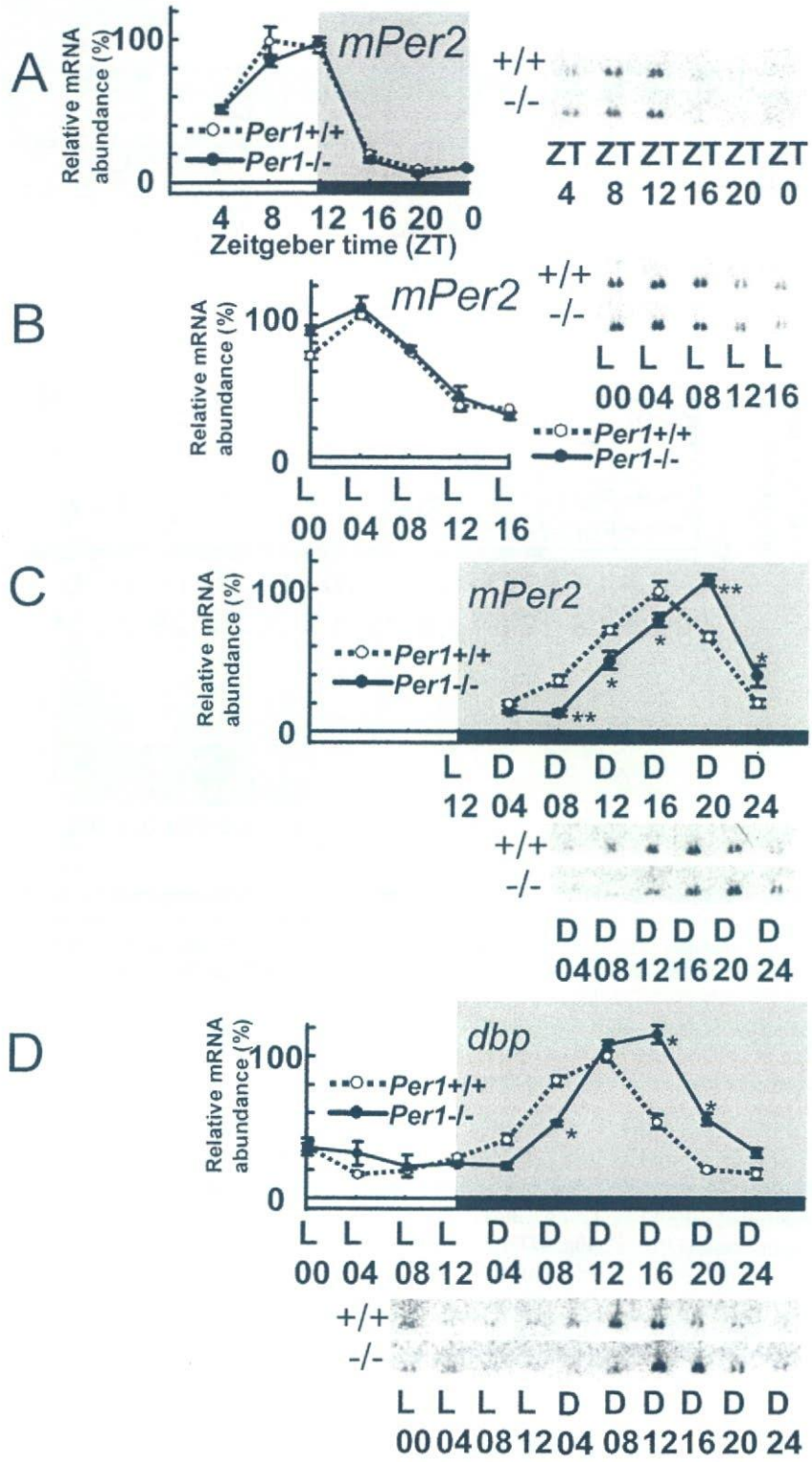

Figure 2. ***A***, Expression profiles of *mPer2* mRNA in the SCN of *mPer1* $^{+/+}$ and *mPer1* $^{-/-}$ mice in LD. Open circles (*mPer1* $^{+/+}$) and filled circles (*mPer1* $^{-/-}$) indicate quantified values of *mPer2* mRNA (mean ± SEM; $n = 4$). ***B***, Expression profiles of *mPer2* mRNA in the SCN of *mPer1* $^{+/+}$ and *mPer1* $^{-/-}$ mice during the prolongation of light for 16 h. Open circles (*mPer1* $^{+/+}$) and filled circles (*mPer1* $^{-/-}$) indicate quantified values of *mPer2* mRNA (mean ± SEM; $n = 4$). ***C***, Expression of *mPer2* mRNA in the SCN of *mPer1* $^{+/+}$ and *mPer1* $^{-/-}$ mice after the 12 h prolongation of light. Open circles (*mPer1* $^{+/+}$) and filled circles (*mPer1* $^{-/-}$) indicate quantified values of *mPer2* mRNA (mean ± SEM; $n = 4$). The values indicated by asterisks are statistically significant. $^{**}p \leq 0.0005$; $^{*}p < 0.005$ *mPer1* $^{+/+}$ versus *mPer1* $^{-/-}$ (Bonferroni/Dunn). ***D***, Expression of *dbp* mRNA in the SCN of *mPer1* $^{+/+}$ and *mPer1* $^{-/-}$ mice during and after the 12 h prolongation of light. Open circles (*mPer1* $^{+/+}$) and filled circles (*mPer1* $^{-/-}$) indicate quantified values of *dbp* mRNA (mean ± SEM; $n = 3$). The values indicated by asterisks are statistically significant. $^{*}p < 0.0001$, *mPer1* $^{+/+}$ versus *mPer1* $^{-/-}$ (Bonferroni/Dunn). A representative autoradiogram of the SCN is attached to each graph.

examined *mPer2* expression profiles in the SCN during the light-prolongation task. In the usual 12 h LD regimen (ZT0, ZT4, ZT8, ZT12, ZT16, ZT20), in both *mPer1* $^{-/-}$ and *mPer1* $^{+/+}$ mice, *mPer2* mRNA began to increase before dawn, peaked at ZT12, and then steadily decreased by ZT16–20 (two-way ANOVA; *mPer1* $^{-/-}$ vs *mPer1* $^{+/+}$; $p = 0.094$) (Fig. 2*A*). In 16 h of elongated light exposure (from L00 to L16) from lights off at ZT12 (L00), which induces a larger phase delay in *mPer1* $^{-/-}$ mice, *mPer2* transcript levels increased with 4 h of light prolongation (L04); thereafter, expression decreased during extended light exposure in both *mPer1* $^{-/-}$ and *mPer1* $^{+/+}$ mice in a time-dependent manner (one-way ANOVA; $p < 0.0001$) (Fig. 2*B*). Because *mPer1* $^{-/-}$ and *mPer1* $^{+/+}$ mice display analogous profiles of *mPer2* expression (two-way ANOVA; $p = 0.128$) (Fig. 2*B*), light-induced transient increase of *mPer2* transcription may occur similarly in these mice. These results indicated that the cause of the enhanced delay of behavioral onset by light prolongation in *mPer1* $^{-/-}$ mice was not attributable to changes at the *mPer2* transcription level.

Core clock oscillation and its output show a larger phase delay in the second cycle after extended light exposure in *mPer1* $^{-/-}$ mice

We then examined *mPer2* expression profiles after the end of a 12 h light prolongation (LP12 task) with 4 h intervals (D04, D08, D12, D16, D20, and D24). A LP12 task (12 h of light prolongation) was chosen because the magnitude of the behavioral phase shift was largest in both genetic backgrounds (Fig. 1*B*). *mPer1* $^{+/+}$ mice displayed an increase at D08 and a peak at D16 followed by a decrease (Fig. 2*C*). The increase of *mPer2* expression in *mPer1* $^{-/-}$ mice began at D12 and peaked at D20. Thus, in contrast to similar expression profiles observed during the long light prolongation in *mPer1* $^{-/-}$ and *mPer1* $^{+/+}$ mice (Fig. 2*B*), *mPer2* mRNA peaks 4 h later in *mPer1* $^{-/-}$ mice (two-way ANOVA; $p < 0.0001$) (Fig. 2*C*). The magnitude of the behavioral phase shift (4–6 h) did not correlate with the first *mPer2* mRNA expression profiles in light but correlated with the phases of *mPer2* expression in the next cycle. As an indicator of clock output levels, we next examined the expression profiles of a clock-controlled gene, *dbp*, the transcription of which is directly regulated by clock genes (Ripperger et al., 2000; Yamaguchi et al., 2000). As shown in Figure 2*D*, *dbp* expres-

sion was equivalent in *mPer1*$^{-/-}$ and *mPer1*$^{+/+}$ mice during the extended-light task (from L00 to L12; two-way ANOVA; $p = 0.2696$). However, after the task, *dbp* expression in *mPer1*$^{-/-}$ mice displayed a 4 h delay when compared with *mPer1*$^{+/+}$ mice (from D04 to D24; two-way ANOVA; $p < 0.0001$). These findings suggest that expression of core clock genes and clock-controlled genes did not reflect the behavioral phase shift seen during extended light exposure without *mPer1*. But after the task, the expression of these genes does reflect the behavioral rhythms; this occurs after the cessation of the lighting task.

Delayed disappearance of mPER2 protein in the SCN of *mPer1*$^{-/-}$ mice after the extended light exposure

Because there is a dissociation between clock gene expression during the extended-light task and the observed behavioral phase shifts, we examined the expression profile of clock protein levels to know whether there is a dissociation between protein expression patterns and behavioral rhythms. In the usual 12 h LD regimen, in both *mPer1*$^{-/-}$ and *mPer1*$^{+/+}$ mice, mPER2 protein began to increase at ZT8, peaked at ZT16, and then steadily decreased by ZT0 (Fig. 3*A*). We examined the expression of mPER2 protein in the SCN during (L00, L04, L08, and L12) and after light prolongation (D04, D08, and D12) (Fig. 3*B*, left). At L00 (ZT12), mPER2 protein levels were similarly high in both *mPer1*$^{-/-}$ and *mPer1*$^{+/+}$ mice. In *mPer1*$^{+/+}$ mice, mPER2 levels began to decrease at L08 and reached minimal expression at D08, to increase thereafter (D12) (Fig. 3*B*, left). In contrast, the high expression of mPER2 did not decrease until L08 in *mPer1*$^{-/-}$ mice, to reach minimal levels at L12 (4 h later than *mPer1*$^{+/+}$ mice) and then to steadily decrease until D12. From L04 to D08, mPER2 levels are clearly higher in *mPer1*$^{-/-}$ mice than that in *mPer1*$^{+/+}$ mice (two-way ANOVA; $p < 0.01$). We then examined mPER2 protein expression profiles in the next cycle at 4 h intervals (D16, D20, D24, D28, D32, and D36). In *mPer1*$^{+/+}$ mice, mPER2 at D16 steadily decreased until D32. In *mPer1*$^{-/-}$ mice, however, mPER2 protein increased until D24 and then decreased thereafter (Fig. 3*B*, right). Clear phase difference (4–8 h) of mPER2 expressions between *mPer1*$^{-/-}$ mice and *mPer1*$^{+/+}$ mice was observed in the second cycle (two-way ANOVA; $p < 0.0001$) (Fig. 3*B*, right). These findings reveal that *mPer1* regulates mPER2 protein levels without affecting *mPer2* transcription and that the alteration is likely to induce profound changes within transcription of core clock components and clock-controlled genes for the next cycle, which is then reflected at behavioral level.

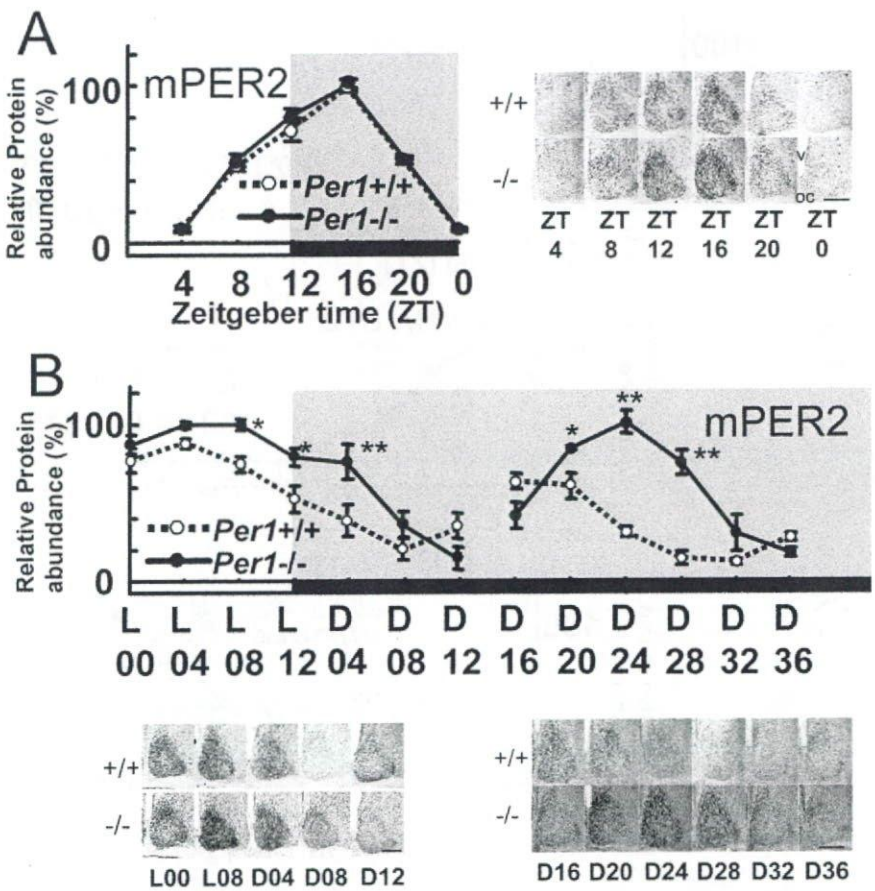

Figure 3. *A*, Quantified immunostaining values of mPER2 in the SCN of *Per1*$^{-/-}$ and *Per1*$^{+/+}$ mice in LD (mean ± SEM; $n = 4$). *B*, Quantified immunostaining values of mPER2 in the SCN of *Per1*$^{-/-}$ and *Per1*$^{+/+}$ mice during and after the 12 h prolongation of light (mean ± SEM; $n = 4$). Representative immunohistochemistry of the SCN is attached to each graph. Open circles (*mPer1*$^{+/+}$) and filled circles (*mPer1*$^{-/-}$) indicate relative number of mPER2-positive cells with the mean number of *mPer1*$^{-/-}$ at L00 being adjusted to 100. oc, Optic chiasma; v, third ventricle. Scale bar, 100 μm. The values indicated by asterisks are statistically significant. $^{**}p < 0.0005$; $^{*}p < 0.01$, *mPer1*$^{+/+}$ versus *mPer1*$^{-/-}$ (Bonferroni/Dunn).

mPer1-deficient mice cannot adapt to environmental light/dark cycles consisting of long complete photoperiods of dim light or long skeleton photoperiods

The larger phase resetting that we observed after several hours of light prolongation raised a possibility that *mPer1*$^{-/-}$ mice have a difficulty in entraining to a long-day environment. It is known that expression profiles of *Per* genes alter in response to photoperiodic change (Steinlechner et al., 2002). First, we put animals in a long-day schedule: we exposed *mPer1*$^{-/-}$ and *mPer1*$^{+/+}$ mice to LD 20/4 h (light on from ZT0 to ZT20; *mPer1*$^{-/-}$, $n = 14$; *mPer1*$^{+/+}$, $n = 12$; 200 lux illumination) (Fig. 4*A–C*) and LD 17/7 h (*mPer1*$^{-/-}$, $n = 8$; *mPer1*$^{+/+}$, $n = 8$; 200 lux illumination) (Fig. 4*D–F*). During the period of observation (3–10 weeks), all mice entrained to the both long-day tasks. Because this entrainability is also influenced by the intensity of light, we decrease the intensity of luminescence to ~0.3 lux, adding the long-day schedules in the first group (LD 20/4 h group). It is possible that low light intensity (0.3 lux) (Fig 4 *A–C*,*G*) conditions are more natural for nocturnal rodents than the classically high light intensity (200–300 lux) in which usual laboratory animals are housed, because nocturnal rodents live in dim light or darkness in daytime. Nine of 14 *mPer1*$^{-/-}$ mice could not entrain to the dim long-day photoperiod in 35 d (Fisher's exact probability test; $p < 0.005$) (Fig. 4*G*) by phase delaying (Fig. 4*A*) and advancing (Fig. 4*B*). In contrast, all *mPer1*$^{+/+}$ mice kept entrained to the long complete photoperiod for >35 d (Fig. 4*C*,*G*). However, low light conditions per se are not enough to entrain the rhythm, because all control mice (*mPer1*$^{-/-}$, $n = 8$; *mPer1*$^{+/+}$, $n = 6$) of the first group kept entrained to LD 12 h lighting cycle in 0.3 lux for >35 d (data not shown; Fisher's exact probability test; $p = 0.65$).

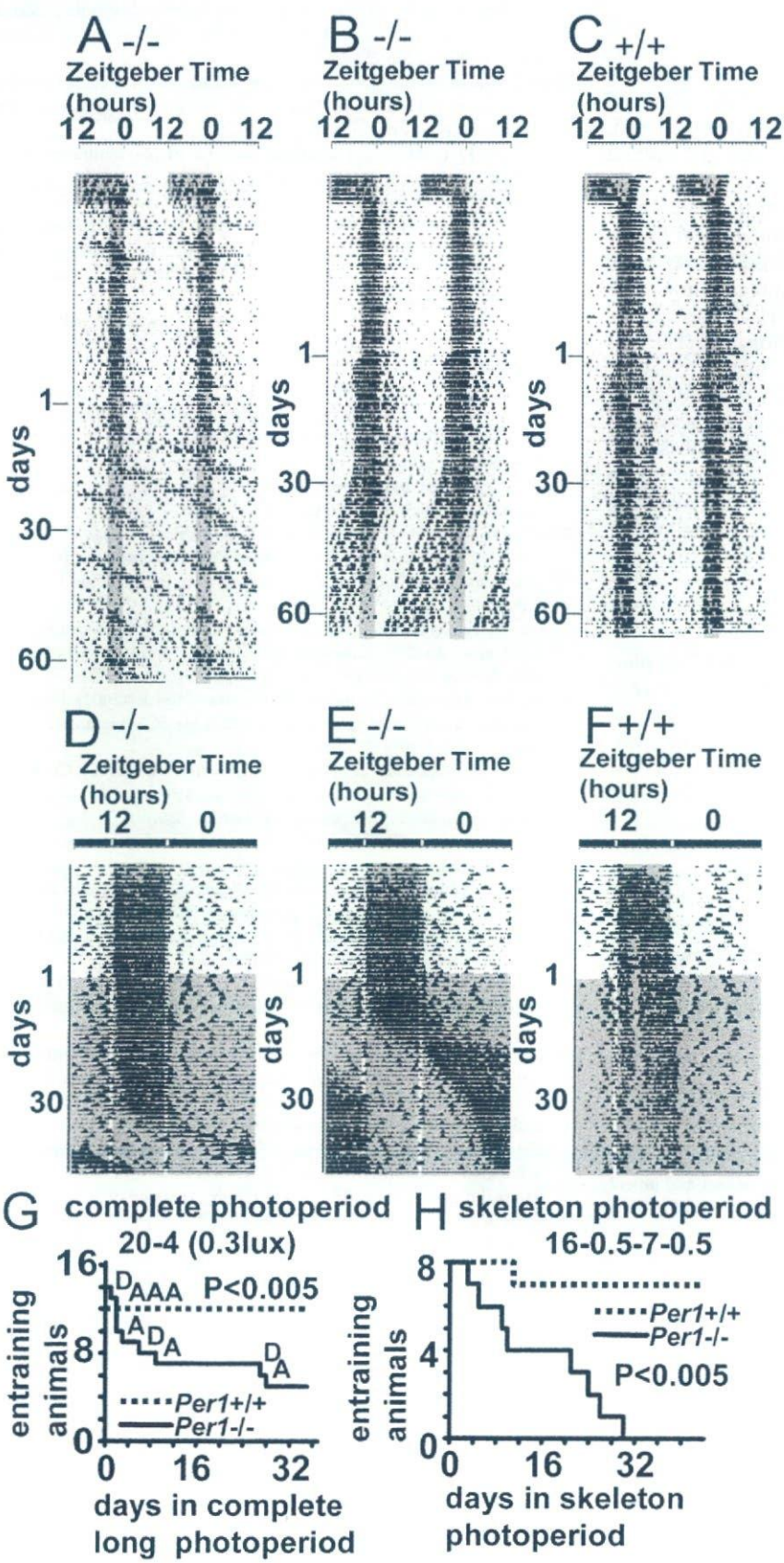

Figure 4. ***A–C***, Representative actograms of *Per1* $^{-/-}$ (***A***, ***B***) and *Per1* $^{+/+}$ (***C***) mice in complete photoperiod with dim light (double plot). Mice were moved (day 1, ZT12) to complete photoperiod with dim light (LD 20/4 h, 0.3 lux) after the same photoperiod with bright light (200 lux). Periods of dim light and complete darkness are indicated by light and dark gray backgrounds, respectively. ***D–F***, Representative actograms of *Per1* $^{-/-}$ (***D***, ***E***) and *Per1* $^{+/+}$ (***F***) mice housed in skeleton photoperiod (single plot). Mice were moved (day 1, ZT19) to skeleton photoperiod (L/D/L/D, 0.5/16/0.5/7 h) after complete photoperiod (LD 17/7 h). Periods of darkness are indicated by gray backgrounds. ***G***, Time course of the number of entrained animals in complete photoperiod. *mPer1* $^{-/-}$ mice desynchronized the activity rhythm from light cycle by phase delaying (D) or advancing (A). ***H***, Time course of the number of entrained animals in the skeleton photoperiod. The day of desynchronization is determined when the activity onsets began to advance or delay.

Next, a second group (LD 17/7 h group; light on, ZT19; lights off, ZT12) was exposed to skeleton photoperiods (Fig. 4*D–F*,*H*). Johnson et al. (2003) speculated that in the case of nocturnal animals, a skeleton photoperiod mimics natural seasonal lighting changes better than complete photoperiods. DeCoursey (1986) suggests that nocturnal rodents in nature reset their clocks by short light exposures. In her simulation experiments, nocturnal rodents (flying squirrels) housed in cages containing dark nest boxes returned to their nest during daytime, and therefore they reset their clocks by short periods (only several minutes per day) of daily light sampling. After entrainment to a LD 17/7 h lighting cycle for 3 weeks, mice were moved to skeleton photoperiods [L/D/L/D, 0.5/16/0.5/7 h; light on from ZT19 to ZT19.5 and from ZT11.5 to ZT12 (the beginning of the skeleton photoperiod being ZT19)]. As shown in Figure 4, *D* and *E*, all eight *mPer1* $^{-/-}$ mice could not entrain to the skeleton photoperiod during 30 d. In contrast, as shown in Figure 4*F*, seven of eight *mPer1* $^{+/+}$ mice kept entrained to the skeleton photoperiod for 44 d (Fisher's exact probability test; $p < 0.005$) (Fig. 4*H*). In this experimental condition, all desynchronized mice moved to another light interval by delaying the clock. In contrast, after entrainment to a LD 12 h lighting cycle for >3 weeks, control mice (*mPer1* $^{-/-}$, $n = 8$; *mPer1* $^{+/+}$, $n = 8$) of the second group were moved to a skeleton photoperiod [L/D/L/D, 0.5/11/0.5/12 h; light on from ZT0 to ZT0.5 and from ZT11.5 to ZT12 (the beginning of skeleton photoperiod being ZT0]. In this skeleton photoperiod, seven of eight *mPer1* $^{-/-}$ mice and all eight *mPer1* $^{+/+}$ mice remained entrained for 44 d (data not shown; Fisher's exact probability test; $p = 0.5$). Thus, whether synchronization occurs is a result of the difficulty of the task, and we speculate that *mPer1* $^{-/-}$ mice have weaker adaptation ability to environmental light/dark cycle.

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Discussion

In the present study, we adopted a strong-lighting task to test the role of *mPer1* in clock resetting. The prolongation of the lighting period clearly induces a larger-delay phase shift of the behavioral rhythm in *mPer1*$^{-/-}$ mice. Compared with wild-type mice, in *mPer1*$^{-/-}$ mice, long light exposure initially changed mPER2 protein levels, although the expression profiles of *mPer2* at mRNA level was not altered. This result suggests that *mPer1* suppresses synthesis or enhances decay of light induced mPER2 in the SCN. In *mPer1*$^{-/-}$ mice, this increased mPER2 may cause larger behavioral phase delay, because *mPer2* mutant show attenuated phase delay by light pulse (Spoelstra et al., 2004). It is possible that desynchronization from the photoperiod (Fig. 4) is caused by the stability of mPER2 in *mPer1*$^{-/-}$ mice.

These findings raise the attractive possibility that mPER1 represses light-induced mPER2 at the protein level and attenuates phase resetting by light. Because this process is abolished in *mPer1* knock-out mice, environmental light information readily changes the phases of these animals. Although the molecular process involved is unknown at present, the dimerization of mPER1 and mPER2 (Zylka et al., 1998; Field et al., 2000; Yagita et al., 2000) may provide a likely explanation, because it could indeed influence the phosphorylation and/or ubiquitine proteasome-dependent mPER2 degradation.

mPer1$^{-/-}$ mice could not entrain to experimental long photoperiod with dim light or to skeleton photoperiods. These conditions (dim light, skeleton photoperiod) may mimic the natural lighting schedule for nocturnal rodents. From the present investigation, therefore, it is possible that a nocturnal animal that has *mPer1* has an advantage for entrainment to environmental light/dark cycles.

One essential issue is whether the duplication of clock genes in mammals has happened only to ensure functional redundancy or whether it has a physiological significance in the context of evolution. Our study underscores the importance of *mPer1* in entrainment to dim long photoperiods and long skeleton photoperiods, conditions that mimic seasonal changes of day/night (long-day condition) for nocturnal rodents living in nature. Our results demonstrate that *mPer1* appears to perform a function completely distinct from *mPer2*, because it is involved in the plasticity of the circadian system, allowing it to adapt to changing photoperiodic cycles.

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The 3111T/C Polymorphism of *hClock* Is Associated with Evening Preference and Delayed Sleep Timing in a Japanese Population Sample

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Sleep timing is influenced by the circadian system. Morningness-eveningness (ME) preference in humans is affected by the free-running period, which is determined by circadian clock-relevant genes. In this study, we investigated association between the 3111T/C polymorphism in the 3′-flanking region of *hClock (Homo sapiens Clock* homolog) and ME preference in 421 Japanese subjects. The Horne–Ostberg ME questionnaire (MEQ) scores showed normal distribution, with mean score of 51.2 ± 1.4 (range, 25–73), and scores were positively correlated with sleep onset time ($r = 0.541$, $P < 0.001$) and wake time ($r = 0.513$, $P < 0.001$). MEQ scores were significantly lower in subjects with 3111C/C (n = 12) than in subjects with 3111T/C (n = 106, $P < 0.001$) or 3111T/T (n = 303, $P < 0.001$), suggesting a stronger eveningness preference in 3111C/C homozygotes. This group also showed significantly delayed sleep onset ($P < 0.001$), shorter sleep time ($P < 0.001$), and greater daytime sleepiness ($P < 0.001$) in comparison to parameters in the subjects with the 3111T allele. There was no significant difference in any of these parameters between the 3111C/T and 3111T/T genotypes. The influence of the 3111T/C polymorphism on ME preferences in Caucasian populations remains controversial. The present findings in a Japanese population sample, which should have a relatively low risk of population stratification effects, suggest the significance of the association of the 3111C/C allele of *hClock* with evening preference.

KEY WORDS: clock gene; *hClock*; morningness-eveningness preference; circadian rhythm; sleep

Studies during the past decade have suggested that genetic polymorphisms/variants could affect circadian properties or susceptibility to circadian rhythm sleep disorders. Pedigrees showing Mendelian inheritance patterns for circadian sleep rhythm disorders such as advanced sleep phase syndrome (ASPS) or delayed sleep phase syndrome (DSPS) have been reported [Jones et al., 1999; Ancoli-Israel et al., 2001; Satoh et al., 2003]. A mutation in the clock gene *hPer2* (*Homo sapiens* period homolog 2) has been found to be associated with ASPS in a family of Northern European descent, and this trait segregates as an autosomal dominant with high penetrance [Toh et al., 2001]. One affected member of this family suffered from extraordinarily short free-running period (tau; τ) of 23.3 hr for both sleep-wake and core body temperature rhythms [Jones et al., 1999], which could contribute to extreme advanced shift in the entrained sleep phase under light-dark cycles. Researchers have also reported that some genotypes or haplotypes of *hPer3* (*Homo sapiens* period homolog 3) were associated with DSPS [Ebisawa et al., 2001; Archer et al., 2003]. These polymorphisms/variants are located within or adjacent to the region containing putative Casein kinase Iε (CKIε) phosphorylation sites in *hPER2* and *hPER3* [Ebisawa et al., 2001; Toh et al., 2001; Archer et al., 2003], and it is thought that altered CKIε-dependent phosphorylation of the target clock proteins alters the length of τ, which causes an advanced or delayed shift in sleep phase under entrained conditions.

Altered τ has also been found to affect morningness-eveningness (ME) preferences in humans. The length of τ was shown to correlate significantly with ME scores and sleep phases, with shorter periods corresponding to morningness and earlier wake times [Duffy et al., 2001]. Two previous reports have discussed whether a polymorphism in the 3′-flanking region of the human *Clock* homolog (3111T/C, *hClock*; GenBank Accession No. NM_004898) is correlated with ME preferences in humans. Katzenberg et al. [1998] reported that individuals homo- or hetero-zygous for the 3111C allele had increased evening preference in comparison to subjects with the 3111T/T genotype. In contrast, Robilliard et al. [2002] found no association between the 3111C allele and ME preferences in their study population. The inconsistencies in the results of these previous studies were due, at least in part, to population stratification effects. Ninety-five percent of the subjects in the Katzenberg et al. study were derived from five Caucasian ethnic groups (German, British, Scandinavian, Central European, and Southern European), whereas information about the ethnic background(s) of the research population in the Robilliard et al.'s study was not available. In the present study, we attempted to confirm whether the 3111T/C polymorphism of *hClock* is correlated with ME preference in a Japanese population sample, which could be expected to have a relatively low risk of population stratification effects in comparison to Caucasian populations that have a geographically broader-based inheritance.

Study subjects were 421 healthy Japanese adults (male/female ratio = 116/305; mean age, 35.5 years; range, 20–58 years) who were employees of several medical facilities in Akita prefecture (Table I). Each volunteer completed the Horne–Ostberg ME questionnaire (MEQ) [Horne and Ostberg, 1976] and the Pittsburgh Sleep Quality Index (PSQI) [Buysse et al., 1989] to assess ME preference and sleep properties including

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TABLE I. Demographical Population Characteristics of the Three *hClock* Genotype Groups

	Whole subjects	3111T/T	3111T/C	3111C/C	*P*-value
Number	421	303	106	12	
Age (yrs)	35.5 ± 0.5	35.6 ± 0.6	35.6 ± 0.5	36.1 ± 0.7	0.950
% Male (M/F)	27.6% (116/305)	27.1% (82/221)	29.2% (31/75)	25.0% (3/9)	0.892 (χ^2)
% Shift worker (shift/non-shift)	64.6% (272/149)	64.4% (195/108)	66.0% (70/36)	58.3% (7/5)	0.857 (χ^2)

Data are expressed as mean ± SEM.

mean sleep onset time, mean wake time, and mean daytime sleepiness during the prior month. Approximately two-thirds of the study subjects were shift workers, and these subjects were asked to answer the questionnaire as if they were in day-shift conditions.

Each subject donated 5 ml of whole blood for later genetic analysis. Genomic DNA was extracted from blood samples with the Wizard Genomic DNA Purification Kit (Promega, Madison, WI). The target region of *hClock* containing the 3111T/C polymorphism was amplified with forward (TCCAGCAGTTTCATGAGATGC) and reverse (GAGGTCATTTCATAGCTGAGC) primers as described by Katzenberg et al. [1998]. Amplification conditions were 94°C for 4 min followed by 5 cycles of 95°C for 30 sec, 58°C for 30 sec, and 72°C for 60 sec; then 30 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 60 sec. PCR products were sequenced directly with an ABI Prizm 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) to determine 3111C/T genotypes. The study was approved by the Institutional Ethics Committee in Akita University School of Medicine, and written informed consent was obtained from all subjects.

Observed genotype and allele frequencies were compared with expected Hardy-Weinberg equilibrium values. To compare 3111T/C allele frequencies in subjects with the most extreme morningness and eveningness irrespective of age, linear regression analysis between the MEQ score and age was performed as described by Robilliard et al. [2002]. Subjects farthest from the regression line (7% from each extreme; $n = 30$ each group) and an equal number of subjects whose MEQ scores fell on the regression line were selected. Differences in genotype and allele frequencies, male/female distribution or shift/non-shift worker distribution between different ME preference or genotype groups were assessed using χ^2 test of independence. Multiple linear regression analysis and one-way ANOVA followed by Bonferroni's post hoc test were properly used to assess differences in MEQ scores and sleep properties between shift and non-shift worker groups, between male and female groups or between different genotype groups, adjusted for the possible confounding factors of age, sex, and presence of shift work. Relations between MEQ scores and age or sleep phase parameters were examined by Pearson's correlation coefficient. Results are reported as mean ± standard error of the mean (SEM). $P < 0.05$ was considered statistically significant.

Table I shows demographical characteristics of the three genotype groups in the present Japanese study population. There were no significant differences in age, male/female distribution, and shift/non-shift worker distribution between three genotype groups. Although it has been suggested that adaptation to the shift work could be easier for subjects with evening preference, the present shift and non-shift worker groups showed no significant differences in the MEQ scores and sleep parameters (Table II). Similarly, there was no significant difference in genotype distribution between shift and non-shift worker groups (Table II).

Table III shows MEQ scores adjusted for age, sex, and shift work in the three 3111T/C genotype groups. Genotype frequency of 3111T/C were consistent with Hardy-Weinberg equilibrium (χ^2, $P = 0.90$). MEQ scores showed normal distribution with a mean score of 51.2 ± 1.4 (range, 25–73; Fig. 1a). There was a significant difference in MEQ scores ($F = 25.4$, $df = 2$, $P < 0.001$) such that a significantly lower MEQ score was found in subjects with the 3111C/C genotype than in subjects with the 3111T/C ($P < 0.001$) or 3111T/T ($P < 0.001$) genotype. Similarly, there was a significant difference in MEQ scores ($F = 45.4$, $df = 1$, $P < 0.001$) such that a significantly lower MEQ score was found in subjects with the 3111C/C ($P < 0.001$) genotype than in subjects with the 3111T positive genotype. This means 3111C/C homozygotes showed evening preference in comparison to 3111T/T homozygotes or 3111T/C heterozygotes. Katzenberg et al. [1998] reported that 3111C/C homozygotes and 3111T/C heterozygotes showed

TABLE II. Genotypes, Morningness-Eveningness Preferences, and Sleep Properties in Shift and Non-Shift Workers

	Whole subjects	Shift worker	Non-shift worker	*P*-value (shift vs. non-shift)
Number	421	272	149	
Age	35.5 ± 0.5	35.5 ± 0.6	35.6 ± 0.8	0.603
Sex (% male)	27.6	28.7	25.5	0.486 (χ^2)
Genotypes				0.857 (χ^2)
3111T/T	303	195	108	
3111T/C	106	70	36	
3111C/C	12	7	5	
MEQ score	50.9 ± 0.4	50.7 ± 0.5	51.2 ± 0.7	0.144
Sleep onset time (h:m)	23:41 ± 00:02	23:42 ± 00:04	23:37 ± 00:05	0.545
Wake time (h:m)	6:28 ± 00:03	6:28 ± 00:03	6:23 ± 00:03	0.332
Total sleep time (min)	404.2 ± 3.9	404.9 ± 4.6	399.4 ± 4.8	0.496
Daytime sleepiness	1.57 ± 0.05	1.58 ± 0.05	1.48 ± 0.06	0.269

Data are adjusted for age and sex, and are expressed as mean ± SEM.
MEQ: Horne–Ostberg morningness-eveningness questionnaire.

TABLE III. Morningness-Eveningness Preference and Sleep Properties of the Three *hClock* Genotype Groups

Genotype	3111T positive	3111T/T	3111T/C	3111C/C	3111C positive
MEQ score	51.0 ± 0.2	50.9 ± 0.2	51.6 ± 0.3	44.5 ± 1.5*	50.9 ± 0.8
Sleep onset time	23:39 ± 00:02	23:37 ± 00:02	23:44 ± 00:03	0:27 ± 00:07*	23:59 ± 00:03#
Wake time	6:28 ± 00:01	6:28 ± 00:02	6:25 ± 00:02	6:35 ± 00:07	6:26 ± 00:02
Total sleep time	407.3 ± 0.8	409.7 ± 0.9	399.6 ± 1.1	345.0 ± 9.0*	391.6 ± 2.6#
Daytime sleepiness	1.53 ± 0.01	1.58 ± 0.02	1.39 ± 0.01	2.10 ± 0.13*	1.50 ± 0.04

Data are adjusted for age, sex, and shift work and are expressed as mean ± SEM.
MEQ: Horne–Ostberg morningness-eveningness questionnaire.
Symbols mean $P < 0.001$ compared to the corresponding values in subjects with 3111T positive (*), and 3111T/T (#), respectively.

similar MEQ scores that were lower than those of 3111T/T homozygotes, suggesting that the 3111C allele has a greater influence on evening preference. In contrast, we found no difference in MEQ scores between 3111T/T homozygotes and 3111T/C heterozygotes in our sample. In the present study, the difference in the mean MEQ score between 3111C/C homozygotes and either 3111T/T homozygotes or 3111T/C heterozygotes was 6 to 7 points. The difference is approximately two-times higher than that reported by Katzenberg et al. in their predominantly Caucasian population.

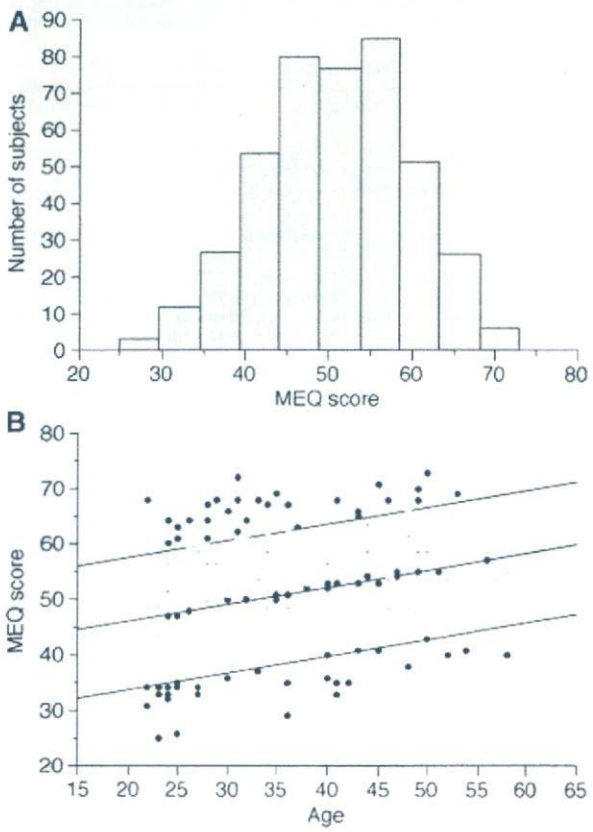

Fig. 1. **A**: Distribution of the Horne–Ostberg morningness-eveningness questionnaire (MEQ) scores in our Japanese population sample. **B**: Correlation between age and MEQ scores. Black circles indicate subjects with the most extreme morningness and eveningness irrespective of age (7% from each extreme; $n = 30$ each group) and subjects with intermediate preference. Several subjects with identical age and MEQ scores overlap each other.

A significant positive correlations were found between age and MEQ score ($r = 0.34$, $P < 0.0001$, Fig. 1b). We further analyzed genotype and allele frequencies in subjects with extreme morningness or eveningness to examine the influence of 3111C/C allele on ME preference irrespective of age. The MEQ scores in the subjects with extreme morningness ($n = 30$), eveningness ($n = 30$), and intermediate ME preference (n = 30) were 66.5 ± 0.6, 52.1 ± 0.5, and 35.0 ± 0.8, respectively. Genotype and allele frequencies in subjects with extreme morningness, eveningness, and intermediate ME preferences are shown in Table IV. There were marked differences in genotype (χ^2, $P = 0.055$) and allele (χ^2, $P < 0.001$) distributions between the three ME preference groups. In contrast with the results in Robilliard et al. [2002] study, there was a significant difference in the distribution of 3111T-positive and -negative subjects (χ^2, $P < 0.02$) such that the 3111C/C allele was also found more frequently in our Japanese subjects with extreme eveningness than in other ME preference groups. Population stratification effects may explain these discrepancies between the present and previous studies. Our present findings in a Japanese population sample, which should have a relatively low risk of population stratification effects, support the significance of the association of the 3111C/C allele of *hClock* with evening preference.

Significant positive correlation was observed between MEQ score and sleep onset time ($r = 0.541$, $P < 0.001$) and wake time ($r = 0.513$, $P < 0.001$), suggesting that MEQ score is a predictable marker of sleep phases under light-dark entrained conditions in the present study subjects. Sleep properties adjusted for age, sex, and shift work in three 3111T/C genotype groups were shown in lower panel of Table III. Significant differences were observed among the three genotypes for sleep onset time ($F = 23.1$, $df = 2$, $P < 0.001$), total sleep time ($F = 144.0$, $df = 2$, $P < 0.001$), and daytime sleepiness ($F = 67.4$, $df = 2$, $P < 0.001$). Subjects with 3111C/C genotype showed significantly delayed sleep onset ($P < 0.001$), shorter sleep time ($P < 0.001$), and greater daytime sleepiness ($P < 0.001$) in comparison to parameters in subjects with 3111T/T, 3111T/C, or 3111T positive genotype. The 3111C/C homozygotes experienced a delayed shift of sleep onset time of 40–50 min on average compared to that in subjects with the 3111T allele. The delayed timing of sleep onset phase in subjects with the 3111C/C genotype was consistent with their marked evening preference. In contrast, the delay in wake time in this genotype group did not reach statistical significance in comparison to that in subjects with the 3111T allele (7–10 min on average). It was assumed that the wake times were affected by work times. These sleep phase characteristics resulted in constricted sleep time and consequent daytime sleepiness in 3111C/C homozygotes. Interestingly, differences in sleep onset time ($F = 14.9$, $df = 1$, $P < 0.001$) and total sleep time ($F = 70.7$, $df = 1$, $P < 0.001$) between subjects with 3111C positive genotype (3111C/C plus 3111C/T) and 3111T/T genotype also reached statistically significant level ($P < 0.001$), suggesting that the 3111C allele could possess semi-dominant influence on

TABLE IV. *hClock* 3111T/C Genotype and Allele Frequencies in Japanese Subjects With Extreme Morning and Evening Preferences

	Genotypes			Allele frequency		3111T	
	3111T/T	3111T/C	3111C/C	3111T	3111C	Positive	Negative
Morning	24/30	6/30	0/30	0.894	0.106	30	0
Intermediate	25/30	5/30	0/30	0.913	0.087	30	0
Evening	19/30	7/30	4/30	0.796	0.204	26	4
P-value (χ^2)			0.055		0.001		0.015

sleep phases but not on ME preferences in humans. Physiological cross relationship between ME preferences, sleep phases and clock genotypes should be further assessed in future studies.

Iwase et al. [2002] reported that the 3111C allele frequency was lower in patients with DSPS than in controls. The finding that the 3111C allele was associated with evening preference in Caucasian and Japanese populations did not support the hypothesis that DSPS is simply an extreme form of evening preference. As was suggested in previous studies [Ozaki et al., 1996; Duffy et al., 1999; Uchiyama et al., 2000; Iwase et al., 2002], patients with DSPS could suffer from different pathological phase relationship between endogenous circadian phase and sleep timing from that for people with strong evening preference. Although the present study contained shift workers and the sample size for 3111C/C genotype was small (n = 12), our results suggest that a single nucleotide polymorphism may influence a human behavioral pattern and possibly lead to altered daytime brain performance.

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Passive Body Heating Ameliorates Sleep Disturbances in Patients With Vascular Dementia Without Circadian Phase-Shifting

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Objective: *This study investigated the sleep-promoting, thermoregulatory, and circadian phase-shifting actions of passive body heating (PBH) in elderly insomniac patients (IPs) with mild-to-moderate vascular dementia.* **Methods:** *Thirteen elderly IPs with vascular dementia (mean age 76.9 years; male/female ratio 2/11) were subjected to a PBH trial session. This session comprised a 3-day baseline period, 2-day PBH period, and 1-day post-PBH period. In the PBH period, the subjects received PBH (immersion in hot water about 40.0°C to mid-thorax level) for 30 minutes beginning 2 hours before bedtime. Sleep–waking, estimated by actigraph, core body temperature (cBT), and heart rate variability were continuously monitored. Dim-light melatonin-onset time (DLMO) was determined in the baseline and post-PBH periods.* **Results:** *PBH significantly improved subjects' sleep quality; sleep latency decreased; sleep efficiency increased; and wake time after sleep onset decreased. These trends were more prominent in the latter half of the sleep time. PBH induced a rapid cBT elevation of approximately 0.80°C, on average, followed by enhanced heat loss (ΔcBT: difference in cBT between just after the PBH and bedtime), lasting 1.5 hours before sleep. There was a significantly positive correlation between ΔcBT and sleep latency. PBH induced no significant phase shift in DLMO. Heart-rate variability data showed that PBH induced parasympathomimetic action during sleep time in the subjects.* **Conclusion:** *PBH may have a sleep-promoting effect by intervening in the thermoregulatory and autonomic systems in elderly IPs with vascular dementia.* (Am J Geriatr Psychiatry 2005; 13:369–376)

The prevalence of sleep disturbances increases with age, especially in elderly patients with dementia.[1] Passive body heating (PBH), accomplished by immersion in hot water in the afternoon or several hours before bedtime, has been reported to promote sleep by reducing sleep latency, increasing slow-wave sleep, and enhancing sleep-maintenance ability in both young and older adults.[2–6]

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It has been hypothesized that PBH achieves its sleep-promoting effects by modifying the thermoregulatory process. A series of previous studies demonstrated that the acute heat-loss process after various thermoregulatory manipulations plays a critical role in inducing sleepiness in humans.[2,3,6–9] PBH is one of the convenient and effective interventional techniques to modify the human thermoregulatory process. PBH induces an acute elevation in core body temperature (cBT) of 0.7°C–1.6°C, followed by a rapid cBT decline lasting several hours toward the sleep-time.[2–6] Another research group also reported that PBH improved sleep quality accompanied by phase-delay of cBT rhythm in elderly insomniac patients (IPs).[3] The occurrence and continuity of human sleep show characteristic profiles intimately related to circadian cBT rhythm;[10,11] sleep propensity increases on the falling limb and decreases on the rising limb of the cBT curve. It has been proposed that phase advance of the cBT rhythm relative to sleep timing could cause the decline in sleep-maintenance ability observed in elderly people.[12–14] Theoretically, this age-related cBT shift advance loads the latter part of sleep-time on the rising limb of the BT curve in elderly persons, during which time sleep propensity generally decreases, thereby increasing the vulnerability to sleep-maintenance disturbances such as decreased sleep efficiency and increased nocturnal awakening. It is possible that rapid and steep fall of cBT, as well as realignment of the acrophase between cBT rhythm and sleep timing after PBH, improves sleep initiation and sleep-maintenance disturbances with advancing age.

In the present study, we aimed to confirm the sleep-promoting and thermoregulatory actions of PBH in elderly IPs with mild-to-moderate vascular dementia. We simultaneously estimated the circadian phase-shifting effect of PBH by measuring dim-light melatonin onset time (DLMO),[15] another reliable circadian phase-marker, which is less subject to the masking effects of PBH, to verify whether PBH induced a phase-realignment action in our subjects.

METHODS

Participants

Subjects of the present study were 2 men and 11 women over age 70 (mean age, 76.9 years). Informed consent to participate was obtained from each subject or his/her family. All subjects had been residents of the same facility for elderly patients for at least 3 months before participating in the study. They all met DSM-IV criteria for vascular dementia. All subjects underwent brain magnetic resonance imaging; all subjects exhibited multiple lacunes, mainly occurring in basal ganglia, and deep white-matter lesions. All subjects were capable of independent ambulation; patients suffering from apparent pyramidal as well as extrapyramidal motor symptoms, such as abnormal gait, intention tremor, bradykinesia, hemiparesis, or myoclonus, were excluded from the study. Their mean Mini-Mental State Exam score (standard deviation [SD]) was 17.4 (3.2); range: 13–22), suggesting a mild-to-moderate degree of dementia.

Subjects were required to satisfy Lushington et al.'s criteria,[16] based on the International Classification of Sleep Disorders (ICSD) criteria for psychophysiological insomnia and Waters et al.'s criteria[17] for sleep-maintenance insomnia. Thus, subjects were selected from among patients who met these criteria on the basis of the Pittsburgh Sleep Quality Index[18] and a 7-day sleep diary. Inclusion criteria were a reported mean wake time after sleep onset (calculated from the accumulated time awake after the onset of sleep) >30 minutes, total sleep time <6 hours, and sleep efficacy (the total time asleep as a percentage of the total time in bed) <85%. For those participants whose self-estimation of sleep time seemed not to be sufficiently reliable, we also referred to a 7-day sleep diary of estimated times recorded by trained caregivers. Before enrollment in the study, all subjects underwent overnight polysomnography to rule out sleep apnea or periodic limb movement as defined by the ICSD criteria.

All subjects underwent rigorous physical and psychological evaluations by physicians. Subjects were screened to ensure the absence of moderate-to-severe physical disease (including arthritis, asthma, chronic obstructive pulmonary disease, heart disease) that could significantly affect sleep states and tolerance of the PBH procedure (described later); history of psychiatric disease, including depression and alcohol-related disorders, as defined by DSM-IV; and abnormal hematology and urinalysis findings. With the exception of four elderly subjects who were taking short-acting benzodiazepines, no subject took any medication such as a beta-blocker or antidepressant that

has been reported to modify sleep states or melatonin-secretion levels during the 3 months before the study. The four patients who took benzodiazepines had been on the medication continuously at fixed dosages for at least 6 weeks before participating in the study, and they continued the treatment. All female subjects had reached menopause.

Study Design

The study consisted of a 7-day pre-study session, followed by a 6-day PBH trial session. The PBH trial session comprised a 3-day baseline period, followed by a 2-day PBH period and a 1-day post-PBH period. The facility's corridor and shared-space lights were turned off at 21:00 h (9:00 P.M.), the time assigned for bedtime. Average bedtime (clock time) per subject in the pre-study session was referred to as her/his 00 hour (relative time), and each subject maintained bedtime at 00 hours during the PBH trial session. Sleep was forbidden outside the bedroom, and compliance with this rule was confirmed by the disciplined care-giving staff and actigraph data (described later). At –04 hours to –03 hours, 08 hours to 09 hours, and 12 hours to 13 hours, a 500–750-calorie meal and as much water as desired were given to each subject.

Laboratory illumination was maintained at less than 10 lux during time in bed throughout the study and at less than 100 lux in the post-PBH period, except during the melatonin sampling (described later), when it was maintained at less than 50 lux. To ensure the dim-light condition, luminous intensity was simultaneously monitored at 1-minute intervals for each subject by means of a thin, patch-type photosensor (Matsushita Electric Works, Ltd., Osaka, Japan) calibrated to measure light intensities from 1 to 10,000 lux ($r = 0.99999$) that was applied to each subject's clavicle and connected to a small, ambulatory illuminorecorder (Gram Ltd., Tokyo, Japan). The ambient temperature in the bedroom was maintained at 24 (± 1)°C.

PBH Procedure

The subjects received PBH for 2 consecutive days in the PBH trial session. The PBH procedure, which was similar to that previously report by Dorsey et al.,[3] involved immersing the subjects to mid-thorax level in bath water of 40.0 ± 0.5°C, beginning at –02 hours for 30 minutes (2 – 1.5 hours before bedtime). The bath water temperature was controlled automatically by a circulating hot-water supply system equipped with a thermosensor, and temperature was confirmed by extra thermoprobes positioned in the water. After the body heating intervention ended, subjects drank 100 ml of water to ensure adequate hydration, as they did at the same time each day during the baseline and post-PBH periods. All subjects were asked to urinate immediately before bedtime every night to avoid the potentially sleep-disruptive effects of increased urinary frequency due to water intake. Before the study, all subjects were accustomed to taking a bath (immersion to neck level) or shower 2 to 5 times weekly, and they were allowed to maintain their bathing habits during the pre-study period. During the baseline period, the subjects were allowed to take only a blanket bath every night.

Evaluation of Sleep Properties

Throughout the present study, sleep quality was continuously assessed with an actigraph (AMI Inc., Ardsley, NY) fitted to each subject's non-dominant wrist. We collected actigraph data and estimated sleep–wake status, using Cole's algorithm with optimal parameters.[19] Nighttime sleep parameters were determined per subject as follows: bedtime (clock time for going to bed; i.e., 00 hours); sleep-onset time (clock time for the onset of sleep); wake time (clock time for the end of major night sleep after which the subject did not fall asleep again); sleep latency (SL; time from bedtime to sleep-onset time); sleep-period time (SPT; time from sleep-onset to wake time); total sleep time (TST; total time asleep); midpoint of sleep (the midpoint between sleep onset and wake time); sleep efficiency (SE; TST as a percentage of SPT); and wake time after sleep-onset (WASO; the accumulated time awake after the onset of sleep). Sleep time was divided at the midpoint into two segments, and SE and WASO were calculated separately for the first and second halves of sleep time.

Evaluation of Thermoregulatory Properties

A rectal thermistor (polyethylene-covered thermoprobe, accuracy within 0.01°C) was continuously inserted 10 cm into the subject's rectum throughout the PBH trial session except during bathing and defecation. The thermistor was connected to an ambulatory temperature-monitoring system (Kohden

Medical Inc., Tokyo, Japan), and sampling occurred at 1-minute intervals. All cBT recordings in the baseline and PBH periods were collapsed into 15-minute bins for later analysis.

The magnitude of heat loss after PBH (ΔcBT) was defined as the difference between cBT just after PBH (1.5 hours before bedtime) and cBT at bedtime in each subject. The larger the negative value, the greater the heat loss during the 1.5-hour period before bedtime.

Evaluation of Melatonin-Secretion Properties

Blood samples for serum-melatonin assessment were painlessly collected every 20 minutes from –03 hours to 00 hour on Day3 in the baseline period and in the post-PBH period via intravenous catheter under dim light (<50 lux at eye-level) to reduce the masking effect on melatonin levels as much as possible. The intravenous catheter was withdrawn immediately before the subject went to bed. Blood samples were centrifuged (3,000 rpm for 15 minutes), and serum was separated and frozen (at less than –80°C) before radioimmunoassay. DLMO,[15] a reliable melatonin rhythm-base marker, was defined per subject as the evening time at which serum melatonin concentration reached 5.4 pg/mL, which was twice the detectable level for the radioimmunoassay kit used in this study.

Urine samples were collected in the morning in the post-PBH period. The volume of each urine sample was recorded, and a 5-mL aliquot was frozen for later radioimmunoassay of concentration of the melatonin metabolite 6-sulphatoxymelatonin (6-STM).

Evaluation of Heart-Rate Variability

An Active Tracer AC-301 (Arm Electronics, Tokyo, Japan) was applied to obtain a surface electrocardiogram (ECG) from the precordial region throughout the PBH trial session. We obtained the RR interval (msec) as the time domain index of heart rate variability during the night-time. Digitized signals were analyzed with the MemCalc system (Suwa Trust, Tokyo, Japan) for assessing both the low-frequency components (LF; 0.04 Hz–0.15 Hz) and high-frequency components (HF; 0.15 Hz–0.40 Hz) obtained from the power spectral analysis.[25] The LF, HF, and LF/HF ratio were averaged for each night and were expressed relative to the mean value of the baseline period (defined as 100%).

Statistical Analysis

One-way analysis of variance (ANOVA) for repeated measures, followed by Bonferroni post-hoc analysis, was used to identify significant differences between sleep, cBT, and heart-rate variability indices on the first (PBH-1) and second (PBH-2) PBH night, versus the averaged preceding baseline nights, as well as to identify significant difference in the DLMO and urine 6-STM concentration between the baseline and PBH periods or post-PBH periods. Relationships between ΔcBT and SL were determined by Pearson correlation coefficient for the first and second nights in the baseline and PBH periods (4 data-points for 13 subjects). Data for the third night in the baseline period were excluded because of the possible effect of the DLMO measurement procedure on SL. Results are shown as mean and SD values. A p value of <0.0167 in Bonferroni post-hoc analysis and a p value of <0.05 on paired *t*-tests and Pearson correlation coefficient were considered to indicate significance.

RESULTS

Effects of PBH on Sleep Quality

Properties of sleep quality in the baseline and PBH periods are shown in Table 1. PBH for 2 consecutive days induced a significant reduction in SL compared with the corresponding values in the baseline period. Changes in TST, SE, and WASO did not reach statistically significant levels. However, when sleep parameters were analyzed separately for the first and second halves of sleep time, repeated-measures ANOVA showed that PBH induced a significant increase in SE ($F_{[2, 36]} = 4.42$; $p = 0.020$) and WASO ($F_{[2, 36]} = 4.41$; $p = 0.019$) in the second half of the sleep-time period (Figure 1).

Magnitude of Heat Loss Before Bedtime and Sleep Latency

Daily profiles of the cBT rhythm in the baseline and PBH periods are illustrated in Figure 2. The average ΔcBT values in the baseline period and on Day 1 and Day 2 in the PBH period were –0.195 (0.15)°C, –0.783 (0.22)°C, and –0.855 (0.24)°C, respectively. Repeated-measures ANOVA showed that PBH induced a significantly greater magnitude of ΔcBT ($F_{[2, 36]} = 39.1$; p

TABLE 1. Sleep, Melatonin, and Heart-Rate Variability Indices in the PBH Trial Session

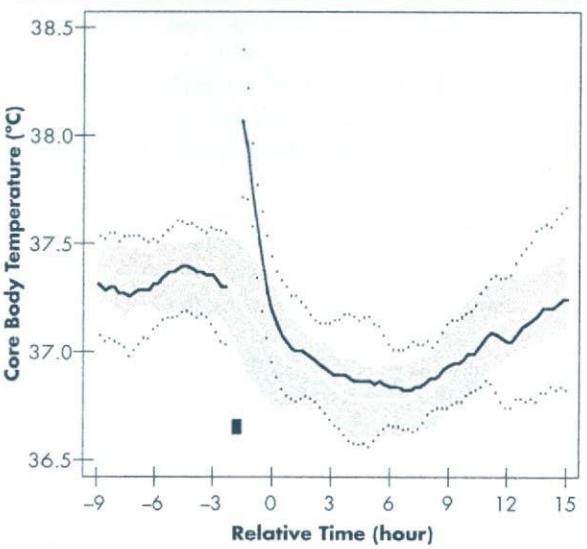
Parameters	Baseline	PBH		$F_{[df]}$	p
		Night 1	Night 2		
Sleep parameters					
Bedtime, h:m	20:47 (00:34)	20:46 (00:25)	20:42 (00:16)	$0.15_{[2, 36]}$	0.865
Waketime, h:m	05:34 (00:31)	05:28 (00:43)	05:25 (01:11)	$0.09_{[2, 36]}$	0.919
Sleep latency, minutes	27.3 (20.9)	14.2 (7.2)*	16.2 (8.0)	$4.61_{[2, 36]}$	<0.02
Total sleep time, minutes	401.5 (74.1)	437.4 (49.0)	429.2 (46.2)	$1.38_{[2, 36]}$	0.265
Sleep efficacy, %	79.4 (9.7)	86.2 (8.2)	86.6 (7.5)	$2.95_{[2, 36]}$	0.065
WASO, minutes	101.9 (45.0)	69.9 (42.3)	69.6 (41.7)	$2.43_{[2, 36]}$	0.103
Melatonin-rhythm parameters					
DLMO, h:m	20:16 (00:35)	—	20:13 (00:33)[a]	$0.03_{[1, 20]}$	0.867
6-STM, ng/mL	11.6 (10.5)	—	9.2 (10.4)	$0.33_{[1, 24]}$	0.572
Heart-rate variability parameters					
HF component, %	100.0	131.5 (47.5)	129.1 (51.0)	$2.47_{[2, 36]}$	<0.05
LF component, %	100.0	124.8 (26.3)	138.4 (55.1)	$3.96_{[2, 36]}$	<0.05
LF/HF ratio, %	100.0	112.1 (23.4)	119.7 (43.9)	$1.56_{[2, 36]}$	0.137

Note: Data are expressed as mean (standard deviation); h:m: hour:minute. PBH: passive body heating; WASO: wake time after sleep onset; DLMO: dim-light melatonin onset time (expressed as clock time); 6-STM: urinary 6-sulphatoxymelatonin concentration; HF: high-frequency components, LF: low-frequency components of ECG variability.

[a] measured in the post-PBH period.

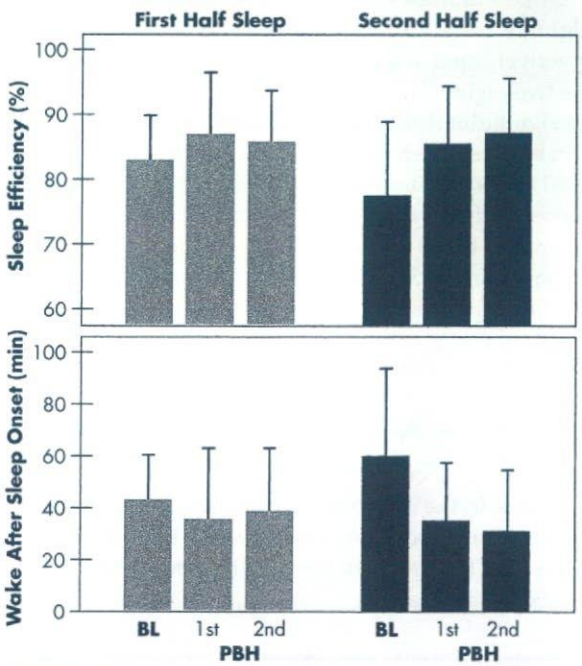
*p <0.0167 versus baseline by Bonferroni post-hoc analysis.

FIGURE 1. Daily Profiles of the Core Body Temperature (cBT) in the Baseline and Passive Body Heating (PBH) Periods



Note: The horizontal bar indicates time relative to 00 hour, the average bedtime for each subject. cBT data were collapsed into 15-minute bins. The white line and shaded area cover mean (standard deviation [SD]) of the cBT profile in the baseline period. The black thick line and small dots above and below the line represent mean and SD values of the cBT profile on the second day in the PBH period. The black square indicates the time for PBH.

FIGURE 2. Sleep Efficiency (SE) and Wake Time After Sleep Onset (WASO) in the First and Second Halves of Sleep Time in the Baseline (BL) and Passive Body Heating (PBH) Periods



Note: Columns with bars represent mean and standard deviation (SD) values in the first and second halves of sleep time. Improvement in both SE and WASO is more prominent in the second half of sleep time than the first.

<0.001), such that ΔcBT for both the first and second PBH nights was significantly increased in comparison to the corresponding value for the baseline period ($F_{[1, 24]} = 39.1$; $p < 0.001$). There was a significant positive correlation between ΔcBT and SL ($r = 0.478$; $F_{[1, 24]} = 15.6$; $p < 0.001$), suggesting that enhanced heat loss induced by PBH was correlated with shortened SL.

The Melatonin-Rhythm Phase

Two subjects showed no evening increases in serum-melatonin concentration sufficient to enable estimation of DLMO (all samples were less than 5.4 pg/mL before bedtime) on both the baseline and second PBH nights. The analyses were performed for the remaining 11 subjects. The phase difference in DLMO between the baseline and post-PBH periods was only 3 minutes, on average, and there was no significant difference in DLMO between the two periods.

Urinary 6-Sulphatoxymelatonin (6-STM) Excretion

Urine volumes on the baseline and second PBH nights were 511.1 (269) mL and 461.1 (236) mL, respectively, and did not differ significantly between the two nights ($F_{[1, 24]} = 0.25$; $p = 0.735$). There was also no significant difference in urinary 6-STM concentration between the baseline and second PBH nights (Table 1); this finding was confirmed when the 6-STM concentration was adjusted by urine creatinine concentration (crea) per sample to exclude the effect of urine volume (23.7 [24] versus 18.8 [16] ng/mL/g • crea; $F_{[1, 24]} = 0.28$; $p = 0.622$).

Heart-Rate Variability

There was a significant change in both the HF and LF components during the PBH trial session, such that PBH for 2 consecutive days induced significant increases in the HF and LF components, compared with the corresponding values for the baseline night (Table 1). The change in the LF/HF ratio was not statistically significant.

DISCUSSION

This study provides data indicating that PBH exhibited therapeutic effects on both difficulty in sleep-initiation and decreased sleep-maintenance ability in our study subjects. Despite the shortcoming that this was an open study, the present findings support the notion that PBH could be an effective tool for sleep disturbance in elderly persons with mild vascular dementia, as it was shown to be in elderly IPs without dementia.[3] PBH induced an acute and transient cBT elevation of approximately 0.80°C, on average, resulting in a significantly larger drop in cBT before bedtime on PBH nights, as compared with the baseline night. The magnitude of cBT elevation achieved by the PBH procedure was equivalent to that in a previous study by Dorsey et al.[3] We observed a significant positive correlation between ΔcBT and SL, indicating that enhanced heat loss after PBH could have contributed to the improvement in sleep-initiation in our study subjects. This finding is consistent with previous reports that sleep-producing manipulations act partly by enhancing the heat-loss mechanism.[2,3,6–8] There is an intimate temporal relationship between increasing sleepiness and the degree of heat loss induced by various thermoregulatory manipulations,[7,8] and the magnitude of heat loss before sleep predicts the reduction in sleep latency in healthy adults.[7] Time interval between PBH and bedtime, which determines magnitude of heat loss before sleep, could be an important factor that influences therapeutic efficacy of PBH at least on sleep-initiation disturbances. PBH also improved sleep-maintenance ability in the present subjects. SE values for the second half of the sleep time on the first and second PBH nights reached 86.10%–87.29%, values that were 7% to 8% higher than those on the baseline nights before PBH. The same trend was observed in WASO in the second half of the sleep time during the PBH period. Interestingly, neither the wake time nor the SPT changed significantly during the PBH nights, suggesting that PBH effectively consolidated nocturnal sleep in our subjects.

This study also aimed to confirm whether the "phase-alignment hypothesis" as the therapeutic mechanism of PBH,[3] is valid for elderly IPs with vascular dementia. The timing and duration of human sleep is regulated by the balance between the circadian timing system and homeostatic influences.[20] Human sleep quality is markedly deteriorated when the mutual phase relationship between sleep and various physiological functions that promote or inhibit human sleep is out of adequate acrophase range. Grow-

ing evidence suggests that a shift advance in the circadian timing of various physiological functions, including autonomic, neuroendocrine, metabolic, immune, and behavioral functions, occurs with advancing age.[21–24] It has been proposed that the age-related phase advance of physiological function rhythms relative to sleep timing could cause the decline in sleep-maintenance ability in elderly people. Actually, some investigators reported that aging altered the phase relationship between cBT rhythm and sleep timing, in that the cBT nadir appeared at an earlier point relative to the sleep period in elderly versus younger subjects.[12–14] This theory was supported in part by the finding that evening bright-light exposure, which induced a remarkable phase-delay of cBT and melatonin-secretion rhythms relative to sleep timing, alleviated sleep-maintenance disturbances in elderly IPs.[25,26] Dorsey et al.[3] recently reported that PBH also induced a delay in the cBT rhythm phase. They found that elderly subjects who experienced an increased delay in the cBT after PBH benefited from significantly reduced wakefulness and improved sleep continuity, suggesting that sleep propensity in the latter half of the sleep time could be improved by realignment of the acrophase between cBT rhythm and sleep timing after PBH. However, it remains debatable whether masking effects by PBH on the cBT rhythm could lead to incorrect circadian phase estimation in study subjects. The present study showed that PBH for 2 consecutive days induced no significant change in the DLMO (3-minute difference on average), another reliable circadian phase-marker,[15] which is less subject to masking effects by thermoregulatory intervention, suggesting that PBH exhibited sleep-promoting effects without circadian phase-shifting in our study subjects.

The heart-rate variability data provide some information concerning the parasympathomimetic action of PBH as a physiological pathway enhancing sleep propensity in subjects with dementia. Despite the technical limitations of recording heart-rate variability under ad lib sleep conditions without strict measurement settings, including respiration or physical movements, the present data suggest that PBH could induce non-reciprocal sympathetic and parasympathetic reactions. We observed significant increases in both the LF and HF components on the PBH nights, compared with the corresponding values on the non-PBH nights. The LF component reflects the confounding effect of both the sympathetic and parasympathetic functions, whereas the HF component more accurately reflects cardiac vagal tone.[27] Thus, the present findings support the assumption that PBH before bedtime could, at least in part, enhance parasympathetic tone during the night. Excessive physiological activation has been proposed as a cause of nocturnal sleep disturbances. Numerous previous studies indicate that poor sleepers suffer from physiological arousal symptoms in autonomic, neuroendocrinological, or metabolic systems, including cBT and heart-rate indices (see the review in a study by Lushington et al.[28]). A recent well-controlled study clearly showed that elderly poor sleepers suffered from elevated cBT levels during the usual sleep time under conditions established by the constant-routine method (sleep deprivation condition with strict demasking procedure),[30] supporting the assertion that excessive activation of sympathetic tone could underlie the decreased sleep-maintenance ability in elderly IPs. Elderly persons with vascular dementia who show disturbed sleep–waking patterns have been reported to also suffer from severely disorganized autonomic functioning.[29,30] If this is the case, the parasympathomimetic action of PBH could play an important role in alleviating the decreased sleep-maintenance ability of elderly IPs with vascular dementia.

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