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## Supporting Online Material

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Materials and Methods  
SOM Text  
Fig. S1  
References

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# The Transcriptional Repressor DEC2 Regulates Sleep Length in Mammals

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Sleep deprivation can impair human health and performance. Habitual total sleep time and homeostatic sleep response to sleep deprivation are quantitative traits in humans. Genetic loci for these traits have been identified in model organisms, but none of these potential animal models have a corresponding human genotype and phenotype. We have identified a mutation in a transcriptional repressor (hDEC2-P385R) that is associated with a human short sleep phenotype. Activity profiles and sleep recordings of transgenic mice carrying this mutation showed increased vigilance time and less sleep time than control mice in a zeitgeber time- and sleep deprivation-dependent manner. These mice represent a model of human sleep homeostasis that provides an opportunity to probe the effect of sleep on human physical and mental health.

Although sleep is an essential process for life, the brain circuits regulating sleep and the cellular and/or molecular mechanisms involved in this complex process are still enigmatic (1–3). Sleep or a “sleeplike” behavior is present in virtually every animal species where it has been studied. Total sleep deprivation can be fatal, and partial deprivation of sleep has serious consequences on cognition, mood, and health (4–6). It is obvious that situational increases in behavioral drive can transiently delay sleep, but very little is known about chronic partial sleep curtailment as a possible consequence of a persistent elevation in waking behavioral drive. The latter trait, sometimes referred to as a “hyperthymic” temperament (7), is a theoretical third influence on sleep habits.

Murine Dec2 (mDec2) is a negative component of the circadian clock (8–10). It belongs to a basic helix-loop-helix (bHLH) protein family in which members can dimerize with each other and

can affect gene transcription by binding to specific DNA sequences (11). While performing candidate gene resequencing in DNAs from human families, segregating alleles for extremely early wake up times, we identified an hDEC2 point mutation in a small family with two affected individuals (Fig. 1A) (12). Subjects carrying this mutation had lifelong shorter daily sleep times than normal individuals (Table 1). The self-reported nonworkday habitual sleep-offset times of the mutation carriers were much earlier than those of the noncarriers (including noncarrier family members and general controls). However, these two individuals have sleep-onset times that are similar to that of conventional sleepers. The habitual self-reported total sleep time per 24-hour day was much shorter in mutation carriers (average 6.25 hours) compared with the noncarriers (average 8.06 hours) in this family. Thus, they represent “natural short sleepers” who routinely sleep less than individuals with familial advanced sleep-phase syndrome (FASPS) or general controls (Table 1). The average total sleep time for American adults on nonworkdays is ~7.4 hours (www.sleepfoundation.org). The mutation changes a C to G in the DNA sequence of DEC2, which is predicted to cause a proline-to-arginine alteration at amino acid position 385 of DEC2 (Fig. 1B). This change was not found in over 250 control DNA samples. The proline at position 385 of DEC2 (P385) is conserved in mammals but not invertebrates. P385 is located in a highly conserved region within a proline-rich domain of unknown function and is close to the C-terminal

histone deacetylase (HDAC)-interacting region of DEC2 (Fig. 1B). Activity-rest recording in one mutation carrier using 10-day sleep logs with coincident wrist actigraphy demonstrated an extended active period each day (Fig. 1C).

To examine the effect of the P385R mutation on Dec2 repressor activity, a wild-type (WT) or a P385R mDec2 construct was used in a luciferase assay, and the results showed that P385R attenuated Dec2 repressive activity of Clk/Bmal1-mediated transactivation (fig. S1A). The reduction in Dec2 repressive activity was moderate compared with that of the R57A/K mutations (in which arginine 57 was replaced by alanine or lysine) reported before (13). Dec2 was previously shown to preferentially bind to class B E-box elements (CACGTG) as a homodimer and to repress the transcription of target genes in an HDAC-dependent manner (13). The effect of HDAC on the mutant Dec2 repression was then analyzed by monitoring mPer2 promoter-driven luciferase activity with or without a general HDAC inhibitor trichostatin A (TSA) (fig. S1B). HDAC inhibition resulted in similar increases in luciferase activity for both WT and mutant Dec2. Coimmunoprecipitation was then performed for mDec2 (WT or P385R) and human sirtuin-1 (hSIRT1). HEK293 cells were transiently cotransfected with green fluorescent protein (GFP)-tagged (WT or mutated) mDec2 and FLAG-tagged hSIRT1, followed by FLAG-peptide pull-down and detection of GFP with antibodies on Western blots. The results showed similar physical interactions between WT or P385R mDec2 and hSIRT1 (fig. S1C). Taken together, these results suggest that the P385R mutation affects Dec2 transcriptional repression activity independently from its interaction with HDAC/SIRT.

Because there are only two human mutation carriers in this study, the question remained whether the natural short sleep phenotype was caused by the DEC2 mutation. Thus, we generated WT and P385R DEC2 transgenic (Tg) mice using a human bacterial artificial chromosome (BAC) clone (RP11-288E19) carrying the entire hDEC2 gene to test this hypothesis. As DEC2 has been established as a component of circadian clock (9, 14), we first set out to determine the circadian period ( $\tau$ ) of DEC2-P385R mice. Mice with Dec2 deleted [knockout (KO) mice] (10) and WT littermates were tested in parallel as controls. No significant differences in  $\tau$  were detected among mice of different genotypes (table S1).

Because the mutation was identified in human short sleepers who, presumably, have cor-

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respondingly longer total daily activity periods, we next determined the duration of the activity period ( $\alpha$ ) for these mice. *DEC2-P385R* mice retained the WT pattern of rest and activity (running primarily during the dark phase). However,  $\alpha$  was ~1.2 hours longer for *DEC2*-mutant transgenic mice (Fig. 2A) than for wild-type mice, *DEC2-WT* Tg mice, and *Dec2* KO mice, which suggests that the expression of the *DEC2-P385R* allele leads to a dominant increase in the quantity of wakefulness in mice. In agreement with this notion, the  $\alpha$  was lengthened further (~2.5 hours) when the endogenous *Dec2* alleles were removed by crossing *DEC2-P385R* mice onto the *Dec2* KO background.

To study sleep directly (versus activity rhythms) and to investigate a possible role for *DEC2* in sleep-quantity regulation, electroencephalography (EEG) and electromyography (EMG) were performed. Because we did not observe a change in  $\alpha$  for *DEC2-WT* Tg mice (Fig. 2A) and because human mutation carriers have one normal allele with one mutant allele, we chose to perform EEG and EMG on *DEC2-P385R* mice and their WT littermates. Mice of both genders (female:male/1:1) were included in all EEG studies to exclude the possibility of sex differences noticed in other reports (15). *DEC2-P385R* mice were awake (as defined by EEG) ~8% longer than WT mice in the light phase (Fig. 2B, table S2). The short-sleep phenotype of these mice was reflected in

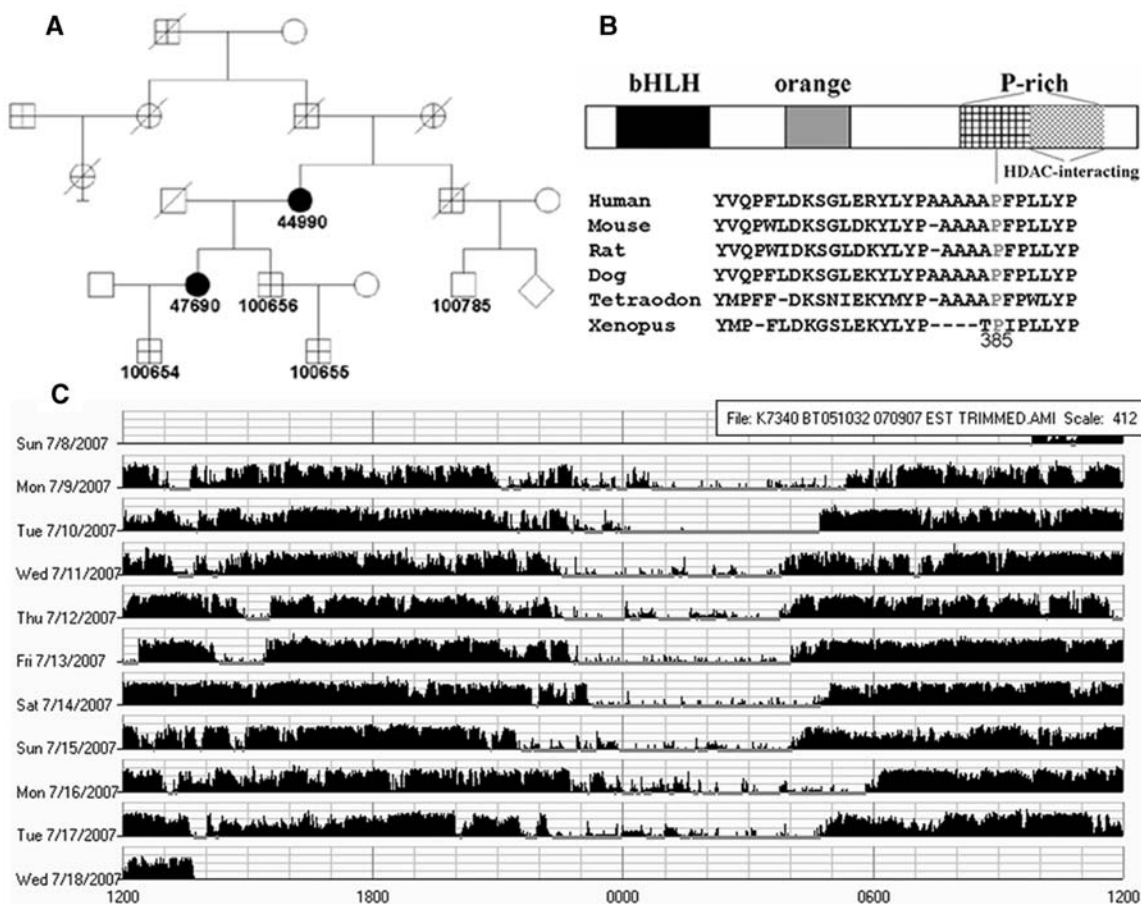
the significant shortening of both non-rapid eye movement (NREM) and rapid eye movement (REM) during sleep in the light phase for *DEC2-P385R* when compared with control mice (Fig. 2C and table S2). NREM sleep was ~6% less and REM sleep was ~2% less in *DEC2-P385R* versus WT mice during the light phase. Sleep architecture was further characterized by counting sleep and wakefulness episodes. Over a 12-hour period, *DEC2-P385R* mice showed more episodes of wakefulness than WT mice ( $193 \pm 12$

versus  $133 \pm 10$ ,  $P < 0.05$ ), but the mean duration of each episode was slightly shorter during the light phase ( $97 \pm 10$  s versus  $116 \pm 12$ ,  $P < 0.05$ ) (Fig. 2D and table S3). Consistent with this, *DEC2-P385R* mice also showed more NREM episodes during light periods ( $190 \pm 10$  versus  $139 \pm 9$ ,  $P < 0.05$ ) but each episode was shorter ( $118 \pm 3$  s versus  $184 \pm 5$ ,  $P < 0.05$ ) (Fig. 2E and table S3). REM episodes were similar in abundance ( $41 \pm 5$  versus  $53 \pm 6$ ) and duration ( $63 \pm 3$  s versus  $64 \pm 3$ ) for *DEC2-P385R*

**Table 1.** Sleep schedule comparison for human subjects. Age refers to when data were collected. Status: C, mutation carrier; NC, nonmutation carrier. Sleep offset is local standard clock time of "average" final morning awakening, and sleep onset is evening time of first falling asleep as stated by individuals recalling extended vacations based on structured interviews. Values are  $\pm$  SD.

Subjects	Age	Status	Sleep offset	Sleep onset	Sleep length (hour)
44990	69	C	4:00	22:00	6.0
47690	44	C	4:30	22:00	6.5
101174	51	NC	6:00	22:35	7.4
100785	51	NC	7:00	22:45	8.3
100656	44	NC	5:00	21:30	7.5
100654	16	NC	7:45	24:00	7.7
100655	10	NC	7:00	21:35	9.4
FASPS			$4:30 \pm 1.33$	$19:45 \pm 1.33$	$8.66 \pm 0.80$ (n = 16)
Control			$6:12 \pm 2.45$	$21:50 \pm 1.76$	$8.37 \pm 1.67$ (n = 15)

**Fig. 1.** A *DEC2* point mutation was identified in a short sleep family. (A) Pedigree of K7430 family carrying *DEC2* mutation (P385R). (B) P385 is localized in the C-terminal proline-rich domain and its flanking sequences are highly conserved among mammalian *DEC2* orthologs. (C) Activity recording by wrist actigraphy for one mutation carrier demonstrates the extended active period each day.

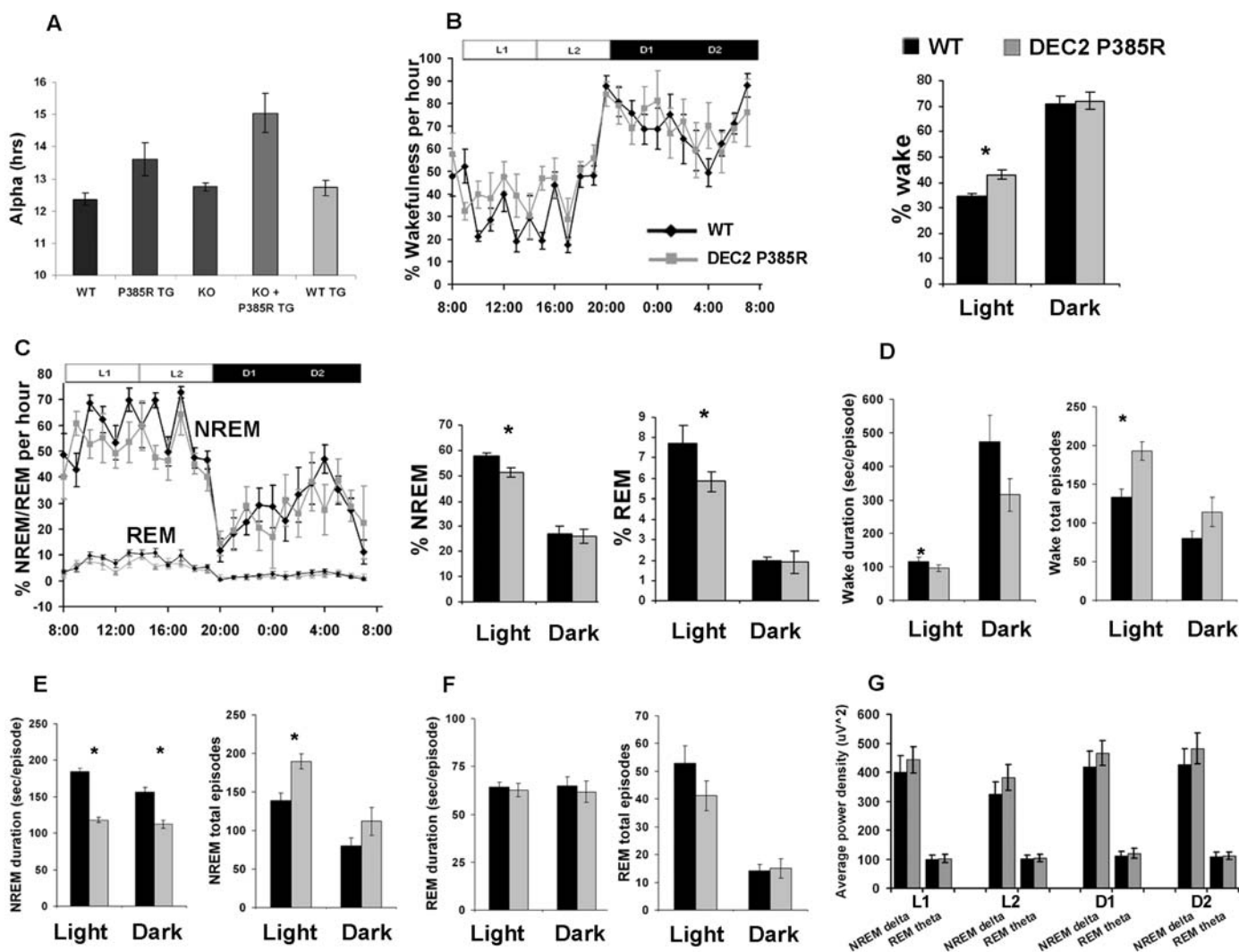


and WT mice (Fig. 2F and table S3). These results suggest that the sleep structure of *DEC2-P385R* mice is more fragmented than that of WT mice (particularly NREM sleep). Although a slightly increased NREM delta power in the EEG is visible in *DEC2-P385R* mice, we did not see a statistically significant difference in NREM delta or REM theta power during daytime or nighttime sleep, which suggested that, despite decreased sleep duration and continuity, sleep depth under baseline conditions is not significantly affected in mutant transgenic mice (Fig. 2G). These results indicate that the amount and consolidation of wakefulness, NREM, and REM in *DEC2-P385R* mice are affected mainly during the light phase. The only significant difference observed in the dark phase was the mean duration of NREM. Together, these results strongly

suggest that the changes in sleep measurements for *DEC2-P385R* mice are caused by the P385R point mutation.

We next examined the response of *DEC2-P385R* mice to sleep deprivation to further probe the role of *DEC2* in sleep regulation. *DEC2-P385R* and WT littermate mice were subjected to 6 hours of continuous sleep deprivation. Both genotypes showed a rebound in NREM and REM sleep in the remaining 6 hours of the light period and the subsequent 12 hours of darkness (Fig. 3, A and B). During the dark phase (D1 and D2), NREM sleep was increased less in *DEC2-P385R* ( $\Delta D1$ , 17.1% and  $\Delta D2$ , 2.0%) versus WT mice ( $\Delta D1$ , 71.8% and  $\Delta D2$ , 27.2%) (Fig. 3B and table S2). Simi-

larly, REM sleep was increased less for *DEC2-P385R* ( $\Delta D1$ , 74.4%,  $\Delta D2$ , 82.3%) than for WT mice ( $\Delta D1$ , 175.2%,  $\Delta D2$ , 122.4%). As seen in Fig. 3C, cumulative sleep loss and recovery data relative to the baseline hours of sleep across the deprivation and recovery periods show that WT mice lost more sleep during the L1 sleep deprivation period, probably because they had a baseline of more sleep during L1 periods. The slower recovery of acutely lost sleep in the *Dec2-P385R* mice is therefore even more remarkable because they started the experimental sleep deprivation with chronically less sleep. Immediately after sleep deprivation, mutant *DEC2-P385R* mice also had less compensatory gain in NREM compared with WT mice (Fig. 3C), consistent with a role for *Dec2* in sleep homeostasis. A significantly lower NREM delta power density change after sleep deprivation



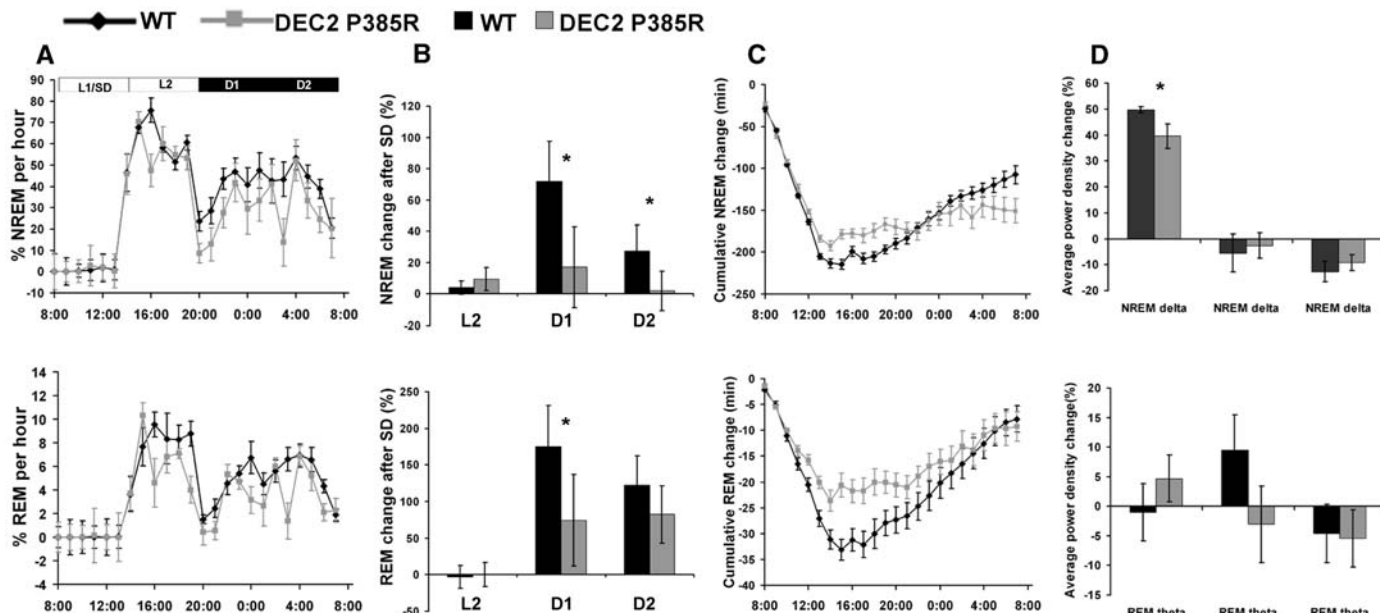
**Fig. 2.** Baseline sleep-wake characterizations of *DEC2-P385R* and WT littermate mice. (A) Duration of the active phase ( $\alpha$ ) for mice of the indicated genotype. (B) Percentage of time in a wakeful state was significantly increased in *DEC2-P385R* ( $n = 5$ ) compared with their WT littermates ( $n = 8$ ), especially in light phase (ZT 0–12, ZT 0 = 8 A.M.). Horizontal bar indicates light and dark phases. L1, ZT 0–6; L2, ZT 6–12; D1, ZT 12–18; D2, ZT 18–24. (C) Percentage of time spent in NREM and REM during the light phase was shortened in *DEC2-P385R* mice. Comparisons of

mean duration time and episode number of wakefulness (D), NREM (E), and REM (F) for *DEC2-P385R* (gray bar) and WT littermates (dark bar). (G) Average spectral power was calculated for each of the 6-hour periods (from left to right: L1, L2, D1, and D2) as indicated by the horizontal bar in (B). NREM delta and REM theta power were compared for the two genotypes. Significant differences were marked with asterisks.  $P < 0.05$ , by one-tailed and two-tailed Student's  $t$  test. All data are expressed as means  $\pm$  SEM. Error bars represent SEM.

was found in *DEC2-P385R* compared with WT mice, which further confirmed the altered NREM homeostasis in mutant mice (Fig. 3D). The differ-

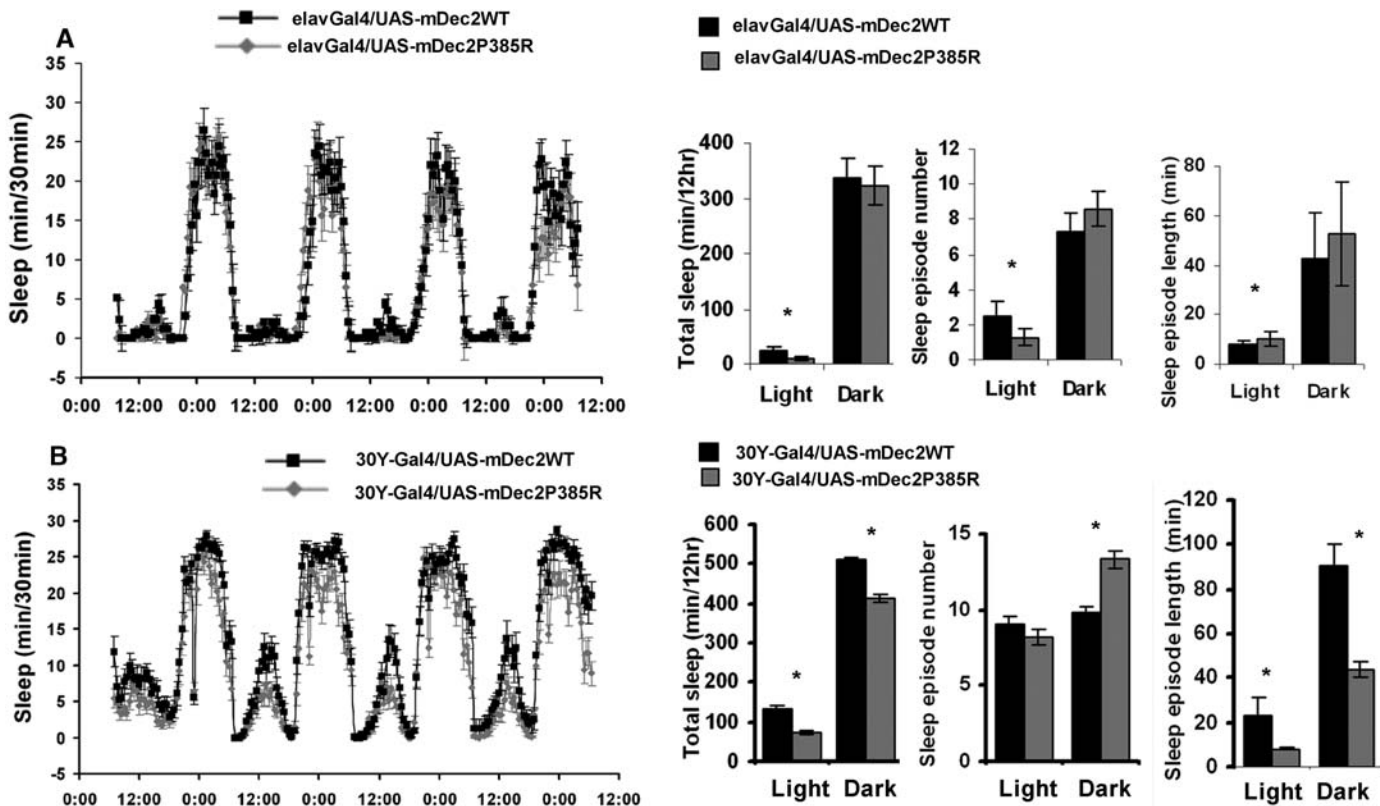
ences of REM qualities between WT and *DEC2-P385R* mice were much less than those of the NREM and did not reach statistical significance.

These results demonstrate that the P385R mutation leads to alterations in sleep rebound and NREM intensity after sleep deprivation in mice.



**Fig. 3.** Altered sleep regulation in *DEC2-P385R* mice. Data for NREM sleep are shown in top panels and for REM sleep are shown in bottom panels. (A) Time course of NREM and REM sleep as percentage of time spent every hour during and after sleep deprivation for one day. (B) Percentage changes of time after sleep deprivation in comparison with the baseline condition for NREM and REM

in L2, D1, and D2 for *DEC2-P385R* and WT mice. (C) Cumulative NREM and REM sleep loss and gain compared with baseline conditions for the sleep deprivation experiment. (D) Analysis of spectral sleep power changes compared with baseline conditions for L2, D1, and D2. Significant differences are marked with asterisks.  $P < 0.05$ , by one-tailed and two-tailed Student's  $t$  test. Error bars represent SEM.



**Fig. 4.** Characterization of sleeplike behavior in transgenic flies overexpressing WT (black) or P385 (gray) *mDec2* with *elav-GAL4* (A) or *30Y-GAL4* (B) drivers. Profile of daily sleep-like behavior (amount of sleep during each 30-min period),

total sleep time, sleep episode number, and mean sleep episode length in the light and dark periods of the day were plotted. Significant differences by one-tailed Student's  $t$  test are marked with asterisks ( $P < 0.05$ ). Error bars represent SEM.

These changes in sleep homeostasis in the mutant mice provide a testable hypothesis for future work examining why human subjects with the mutation lead such active lives despite their persistently shorter sleep.

We performed EEG and EMG on *Dec2* KO mice and their WT littermates. Baseline wakefulness, NREM, and REM percentages showed that *Dec2* KO mice sleep slightly more than the WT mice and that the difference is mostly in the dark period (table S4 and fig. S2). During the light period, only the NREM sleep of *Dec2* KO mice was more abundant than that of WT mice and only slightly so. NREM rebound after sleep deprivation for *Dec2* KO mice was much slower, which implied that *Dec2* is an important factor regulating sleep recovery.

We next set out to test whether *mDec2P385R* can cause a similar rest-sleep phenotype in *Drosophila*. The closest homologous protein to DEC2 in *Drosophila* [CG17100, clockwork orange (16, 17)] shares <18% amino acid sequence similarity, <11% identity, and P385 is not conserved. We therefore generated transgenic flies with expression-inducible UAS-*mDec2WT* and UAS-*mDec2P385R* on the *w1118* background. When these flies were crossed with *elav-GAL4*, driving pan-neuronal overexpression (18), *mDec2P385R* flies showed significantly lower daytime sleep-like behavior with reduced rest bout number and lengthened rest bout durations compared with WT flies (Fig. 4A). Because mushroom bodies were shown to be the likely sleep-rest behavior center in *Drosophila* (19), we also overexpressed P385R and WT *mDec2* under the control of a mushroom body *30Y-GAL4* driver (20). It was noteworthy that *mDec2P385R* transgenic flies driven by the *30Y-GAL4* showed significantly less sleep-like behavior with significantly shorter sleep bout duration in both light and dark phases than *mDec2WT* flies (Fig. 4B). However, rest or sleep bout number was significantly higher only in the dark phase for *mDec2P385R* transgenic flies. These results indicate that the behavior of flies with mushroom body driver expression of *mDec2P385R* echoed those of the *DEC2-P385R* transgenic mice (Fig. 2, B, C, and E, and Fig. 4B).

The power of human genetics in studying human behavioral traits was demonstrated in the identification of mutations and the subsequent molecular characterization of FASPS (21–23). As currently understood, FASPS is primarily a circadian rhythm variant leading to altered phase; total daily sleep time is normal (21, 23, 24). We have applied a similar approach and identified a gene involved in regulation of sleep quantity. This provides a unique opportunity for exploring human sleep quantity regulation. *DEC2-P385R* mutation gave a short sleep phenotype, which was recapitulated in transgenic mouse and fly models but was not found in *Dec2* KO mice. In addition, this phenotype was enhanced by the absence of endogenous *Dec2* alleles, which suggested that P385R leads to a dominant-negative

mutation. Our results demonstrate that *DEC2* plays an important role in regulating daily total sleep time in mammals and that the control of sleep-like behavior may be conserved and regulated in a similar manner as far back in evolution as invertebrates.

We did not see statistically significant differences in the NREM delta or REM theta power in the *DEC2-P385R* mice during day- or night-time sleep, although there is a trend toward increased NREM delta in these mice. It is possible that a small deficit of sleep in the short term does not significantly affect sleep power, whereas a long-term accumulation will. Alternatively, the attenuated NREM delta power enhancement, together with slow and incomplete NREM sleep recovery after sleep deprivation in the *DEC2-P385R* mice, suggests that these mice are able to cope with shorter sleep because of altered sleep homeostasis. It is noteworthy that recent data on human sleep emphasizes the importance of cumulative sleep debt even if it is only due to partial sleep deprivation. Understanding the regulatory mechanisms of sleep quality and quantity will facilitate the development of interventions to alleviate pathologies associated with sleep disturbance.

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#### Supporting Online Material

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References

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## Protein Friction Limits Diffusive and Directed Movements of Kinesin Motors on Microtubules

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Friction limits the operation of macroscopic engines and is critical to the performance of micromechanical devices. We report measurements of friction in a biological nanomachine. Using optical tweezers, we characterized the frictional drag force of individual kinesin-8 motor proteins interacting with their microtubule tracks. At low speeds and with no energy source, the frictional drag was related to the diffusion coefficient by the Einstein relation. At higher speeds, the frictional drag force increased nonlinearly, consistent with the motor jumping 8 nanometers between adjacent tubulin dimers along the microtubule, and was asymmetric, reflecting the structural polarity of the microtubule. We argue that these frictional forces arise from breaking bonds between the motor domains and the microtubule, and they limit the speed and efficiency of kinesin.

**F**riction is the force that resists the relative motion of two bodies in contact. Contact is mediated by adhesive bonds between individual molecules, and friction arises from the forces necessary to deform and break these bonds

(1). When a bond breaks, the energy stored in its deformation is dissipated. Adhesive interactions occur between proteins (2, 3), and how they might give rise to protein friction has been discussed theoretically (4). Protein friction is expected to be